Вестник РУДН. Серия: МЕДИЦИНА
RUDN Journal of MEDICINE. ISSN 2313-0245 (Print). ISSN 2313-0261 (Online)
2025;29(4)
http://journals.rudn.ru/medicine

ТЕМА НОМЕРА: МЕДИЦИНСКАЯ ГЕНЕТИКА
THEME OF THE ISSUE: MEDICAL GENETICS

DOI 10.22363/2313-0245-2025-29-4-421-435
EDN AAFUKT

ОБЗОР
REVIEW

Single cell RNA sequencing: modern approaches and achievements

Artem E. Gusev, Petr V. Chernov, Nikolai A. Dmitriev, Ilya A. Kofiadi ✉

National Research Center Institute of Immunology of the Federal Medical-Biological Agency, *Moscow, Russian Federation*
✉ kofiadi@mail.ru

Abstract. *Relevance.* Single-cell RNA sequencing (scRNA-seq) is a modern approach to studying the diversity and heterogeneity of RNA transcripts in individual cells, as well as to identifying the composition of different cell types and functions in organisms, organs, and tissues. Based on NGS (next-generation sequencing), scRNA-seq provides a vast amount of information at high cellular resolution in various fields, enabling new discoveries in understanding the composition and interaction patterns of individual cell types in humans, animal models, and plants. Despite its rapid development, optimization, and automation worldwide over the past 15 years, scRNA-seq remains relatively new and has only recently been used in Russia. The challenge of mastering and successfully implementing this method is urgent and critical — it is a powerful tool for in-depth analysis and diagnostics, as demonstrated by the results of studies in which it has been used. *The aim* of the review was to examine the basic principles and steps of scRNA-seq implementation, both in terms of technical implementation and sample preparation as an extension of the classic NGS method, as well as in terms of the complexity and expansion of data processing, and the use of new algorithms and databases. We examined commercially available scRNA-seq technologies and technologies described in scientific literature that have served as prototypes and alternatives. We also examined examples and results of the use of such technologies in various fields of science and medicine, such as oncology, senescence, diagnostics, and clinical trials. *Conclusion.* Development and successful application of the scRNA-seq method in scientific and clinical practice will become the key to a wide range of future discoveries and successful accurate personalized diagnostics and healthcare.

Keywords: sequencing, RNA, next-generation sequencing (NGS), single-cell sequencing (sc-seq, scRNA-seq), cellular heterogeneity

Funding. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (the Federal Scientific-technical program for genetic technologies development for 2019–2030 (agreement N 15.ЦГИМУ.25.3.3.)

Author contributions. Data collection and analysis, writing the article — Gusev A.E.; analysis of literature, writing the article — P.V. Chernov; analysis of literature, editing of the article — N.A. Dmitriev; analysis of literature, scientific proofreading — I.A. Kofiadi. All authors have made significant contributions to the manuscript preparation, read, and approved final version before publication.

Conflict of interest statement. The authors declare no conflict of interests.

Ethics approval — not applicable.

Acknowledgements — not applicable.

Consent for publication — not applicable.

Received 27.08.2025. Accepted 25.09.2025.

For citation: Gusev AE, Chernov PV, Dmitriev NA, Kofiadi IA. Single cell RNA sequencing: modern approaches and achievements. *RUDN Journal of Medicine.* 2025;29(4):421–435. doi: 10.22363/2313-0245-2025-29-4-421-435. EDN: AAFUKT

Introduction

The human body is a complex system consisting of more than 400 different cell types and more than 3×10^{13} unique cells in its composition [1]. Despite almost all cells in the human body sharing the same set of genes, their transcriptome function in each type and each individual cell exhibits a unique activity for only a certain part of these genes, which increases the relevance of the issue of studying their unique properties and heterogeneity.

Sequencing technology became one of the common tools for studying cell heterogeneity, including genomics, transcriptomics, epigenomics, proteomics and metabolomics. Sequencing allows to establish the primary structure of the studied linear biopolymers, for DNA and RNA it allows to establish their nucleotide sequence, presented in simple text form. During its widespread use over the past 50 years, sequencing technology has become mundane in various fields of biology and medicine, and currently represents three main generations of this technology [2, 3] (presented in Table 1, [4–15]). The most frequently employed set of methods belongs to the second generation sequencing, which is also called short-read sequencing or next generation sequencing (NGS) is currently the "gold standard" in scientific and laboratory genetic research [3].

The main limitation of the basic sequencing approach is its non-specific character. In its classical implementation, the second-generation sequencing technology provides only average data (bulk sequencing) for the total populations of the analyzed cells; data for individual cell populations, represented in insignificant quantities, are then "lost" against the background of the total mass of recorded data. This limitation gave an impetus to the development of a technology for isolating and indexing unique cells for their subsequent mass analysis using NGS methods. Over the past fifteen years, classical approaches to RNA sequencing (bulk RNA-seq) based on cell populations had a significant role in deciphering transcriptome variations across the genome in a wide range of areas, including oncology, cell development and aging, and cellular homeostasis [16–18]. However, since bulk RNA-seq data, as described above, represent only an "average" of gene expression in individual cells, they may obscure transcriptional trends of individual subpopulations with the most common cell types or states [19].

A modification of NGS technology in the form of single-cell RNA sequencing (scRNA-seq) overcame this obstacle, providing a broad range of possibilities for gene expression profiling at single-cell resolution. With scRNA-seq, it is now possible to analyze the transcriptome at the single-cell level for more than

Table 1

Generations of sequencing methods

Generation and characteristics	Examples of technologies	Ref.
First generationSanger sequencing, used from 1975 to the present. Average read length: 600–1000 base pairs, analysis of single samples	Manual and automated protocols using radioactively or fluorescently labeled reagents and gel electrophoresis	4, 5
Second generationNext generation sequencing (NGS) or short-read sequencing or High-throughput sequencing, used from 2005 to the present.Short read length: 250–800 base pairs, multiple parallel analysis.	**"Ion Torrent"**, Thermo Fisher Scientific, USA	6, 7
	"Illumina", Illumina, Inc., USA	8, 9
	"MGI/BGI", Beijing Genomics Institute, BGI, China	10, 11
Third generationLong-read sequencing, under development and implementation since 2010.High read length: 105–107 base pairs, single and multiple parallel analysis.	**"PacBio"**, Pacific Biosciences, USA	12, 13
	"ONT", Oxford Nanopore Technologies, UK	14, 15

millions of cells in a single study. Most importantly, the development of the method has significantly reduced the cost while improving automation and throughput. This has enabled various discoveries in various fields, such as identifying new cell types [20, 21], understanding a range of cellular dynamic processes [22–25], revealing intracellular gene variations [26], and RNA processing [27]. As a result, scRNA-seq has begun to be used as a key tool in the field of precision medicine [28, 29].

Concept of scRNA-seq method

Since its first application in 2009 [30], scRNA-seq technology has opened the possibility of studying the underlying cellular heterogeneity of complex systems [31]. However, at the time of its introduction, practical procedures were labor-intensive and expensive, presented as "closed solutions" in the form of reagent kits and protocols from a single manufacturer, requiring the researcher to have a full product line of equipment from a specific manufacturer. Currently, the application of the technology is relatively unified and can be successfully applied in typical laboratories, which promotes scRNA-seq to be used as a standard procedure in an increasing number of tasks.

The main concept of scRNA-seq technology has not changed since its discovery and represents approaches for isolating individual cells, capturing and scaling their genetic material with subsequent use of NGS sequencing [32, 33]. Later, various modifications of scRNA-seq technology were proposed, supported by the development and evolution of methods for bioinformatics analysis of the final data. All scRNA-seq technologies can be described in the form of five key steps presented in Figure 1: isolation of single cells from biomaterial and their combination with reagents, isolation of the transcriptome from a single cell and its capture with a reagent, individual barcoding of the transcriptome by reverse transcription (synthesis and amplification of cDNA), library generation and NGS sequencing, additional bioinformatics analysis of the data [34]. The first three steps of the technology are the most difficult to implement, the remaining two are true for all sequencing methods.

Single cell isolation is a mechanical process of capturing cells after their dissociation from tissues and combining them into separate isolated cavities along with reagents. With classical sequencing, the transcriptome can only be examined at a general level and cannot distinguish individual cell variations. In the case of scRNA-seq technology, special attention

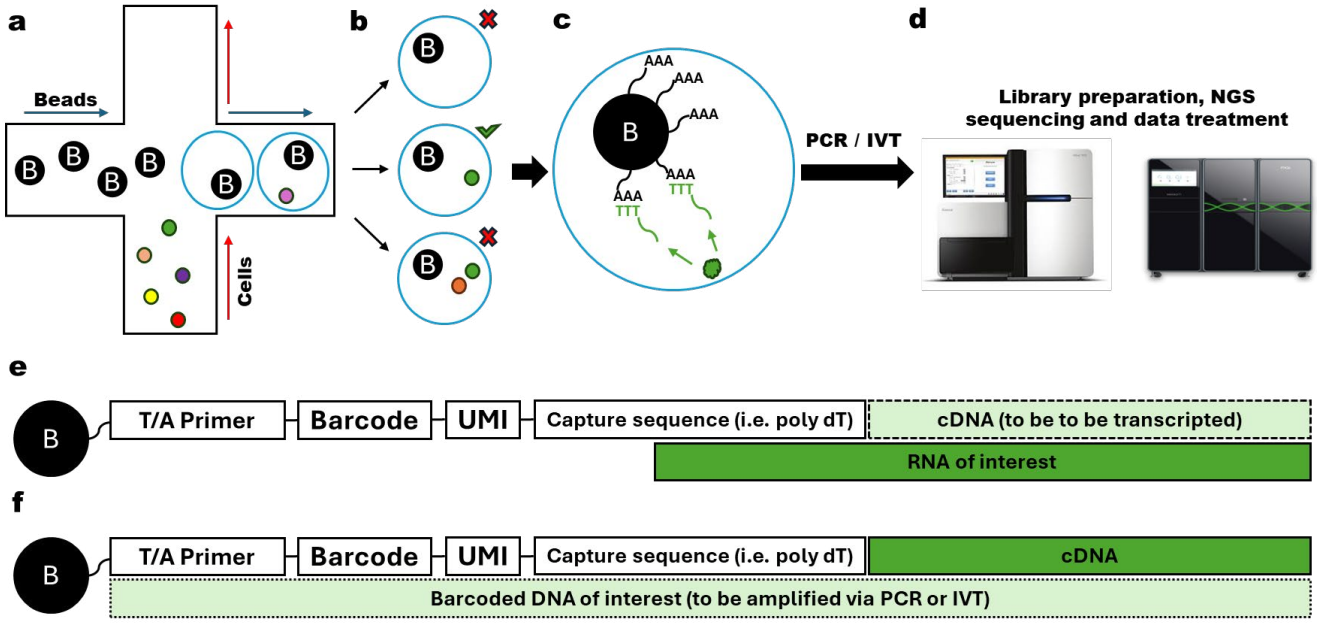

Fig. 1. Schematic of the scRNA-seq experiment. A mixture of beads, cells, and reagents is pumped through an orthogonal fluidic system to form droplets via a reverse emulsion (a). The system parameters are adjusted to maximize the ratio of single cell captures per droplet while minimizing double captures (b). Each droplet is an individual mini-reaction chamber containing all the necessary reagents for cell lysis, reverse transcription, and amplification (c), thus generating large quantities of barcoded DNA complementary to the cellular transcriptome, suitable for subsequent NGS sequencing (d). Each individual bead contains on its surface numerous complex primers, including in their composition both identical regions necessary for, for example, transcription or amplification ("T/A Primer"), unique for each specific bead regions for cell barcoding ("Barcode") and unique for each primer regions for barcoding each specific transcriptome sample extracted from the cell ("UMI"). The terminal region of the primer contains a sequence of oligonucleotides for complementary capture of the type of transcriptome of interest, for example, poly-dT regions for binding to many RNA variants. After such capture, conditions are created for reverse transcription and obtaining cDNA (e) and further, cycles of synthesis of the complete barcoded DNA variant (f).

Note: PCR — polymerase chain reaction; IVT — in vitro transcription; UMI — unique molecular identifier; cDNA — complementary DNA.

is required to obtain the genetic material from each cell in a unique, isolated form and preventing mixing.

Methods for isolating and capturing single cells vary greatly depending on the organism, tissue, or cell properties [35]. Cell isolation can be achieved for whole cells or, for example, individual elements of cells [36]. The most common methods for isolating and capturing single cells include microfluidic systems, Fluorescence-Activated Cell Sorting (FACS) or Magnetic Activated Cell Sorting (MACS), and laser microdissection [31]. The main goal of each method is to perform a single capture into an isolated reaction mixture, which will also contain reagents for the further process.

The process can be schematically considered using the example of a microfluidic system shown in Figure 1. A suspension of dissociated cells is fed to a microfluidic system with selected parameters of channel sizes and flow rates; a reaction mixture in an aqueous medium and oils are fed to orthogonal directions of the chip to form spherical capsules — droplets according to the principle of inverse emulsion (Figure 1a). With the correct selection of system parameters, conditions are achieved where the majority of single cells are enclosed in emulsion spheres, which contain only one cell and a solution of reagents for further processing, a significant part of the empty spheres and only a small part of the spheres with double inclusion of cells (Figure 1b). Each of the spheres will represent a separate reaction vessel, ensuring the isolated flow of processes. In this way, unique genetic and biochemical

information is extracted for its further transformation and study [37].

Transcriptome isolation and capture occurs due to the presence of a lysis buffer and special microparticles in the reaction mixture. The lysis buffer ensures cell lysis and the release of genetic material into the reaction mixture, the microparticle captures genetic material from the reaction mixture via oligonucleotide molecules grafted onto the surface — primers (Figure 1c).

The general structure of the primer can be divided into at least three main sections: (1) cell barcode (Cell Label / Barcode) — a short sequence common to all primers on one microparticle, for the precise identification of all transcripts belonging to the same cell; (2) unique molecular identifier (Unique Molecular Identifier, UMI) — a molecular tag for the precise identification of each read of each unique captured transcript and their number before the amplification stage [38]; (3) a capture and amplification segment designed for a specific capture target, e.g. the basic version of the capture segment for RNA is a poly-T tail to capture the 3' end of the transcript (Figure 1e). In modern scRNA-seq methods, additional sections of the primer structure may be present, e.g. for cDNA amplification or further work in a specific NGS sequencing system. Thus, a specific part of the genetic material released into the reaction mixture is captured by a microparticle designed for this type of genetic material due to complementary interaction.

Barcoding of the single-cell transcriptome is a key step of the scRNA-seq technology in all available protocols and is the main difference from mass RNA-seq. The introduction of this step allows for the unambiguous designation of each transcriptome with a unique cellular barcode. The transcript complementarily bound to the microparticle is subjected to several cycles of amplification using the polymerase chain reaction (PCR) or *in vitro* transcription (IVT) due to the presence of the necessary reagents and primers in the reaction mixture. In the first amplification cycle, DNA complementary to the transcript on the microparticle is synthesized, forming a single chain with a complex primer, and, as a result, the barcode described above (Figure 1f). Then, in subsequent amplification cycles, multiple cDNA synthesis occurs in the reaction mixture [37].

The subsequent steps of the technology are similar to classical NGS sequencing. Library preparation and quality control, the sequencing process itself and computer processing of the obtained data are carried out in accordance with the protocol and using reagents from the sequencing equipment manufacturer (Figure 1d). Historically, the most popular sequencing platforms in the majority of cases are various series of NGS sequencers from Illumina (Illumina, Inc., USA) due to the high efficiency and high quality of results obtained using this equipment. Most of the commercially available scRNA-seq solutions presented have been developed and adapted for Illumina equipment. A relatively new player in this field is the MGI/BGI (Beijing Genomics Institute, BGI, China) line of NGS sequencers, which is competitively increasing its market share and demonstrating equivalent sequencing results even in studies of individual cells [39]. Besides, the growth and development of software should not be overlooked. Recent advances enable additional processing and visualization of data obtained by the scRNA-seq method.

The process of data processing in the scRNA-seq experiment, unlike bulk analysis options, requires the use of additional tools to solve emerging problems due to the increase in the amount of individual data [40, 41]. Currently, more than 600 stand-alone tools in the R and Python programming languages are available for analyzing and exploring scRNA-seq data [42], but most of them have a high entry threshold and will be difficult for beginners to master [43, 44]. Furthermore, due to the relative novelty of the method, working with different types of cells and biological samples may require a different approach to data processing and analysis, which also complicates working with scRNA-seq data.

The obtained sequencing data undergo primary processing by the algorithms of the software of the sequencing equipment manufacturer. Then the data undergoes additional processing using specialized scRNA-seq software packages. The data are presented in the form of gene expression matrices (in the "cell — gene" format), where the number of copies of the detected transcripts is indicated for each gene. Such separation of cells is achieved

by introducing additional identifiers at the barcoding stage — Cell Label / Barcode and UMI. The presence of the Cell Label / Barcode allows to associate all readings with a specific cell, and the presence of unique molecular identifiers UMI allows to count the number of readings of specific transcripts for each gene within a single cell. Further processing involves dividing cells into populations using normalization methods (SCT / log), and then reducing the dimensionality using the Principal Component Analysis (PCA) method and the nonlinear dimensionality reduction (Uniform Manifold Approximation and Projection, UMAP) method. This allows to plot single cells on a two-dimensional graph considering their differences in gene expression for their most variable representatives. After this, individual cell clusters are isolated by detecting cells with a similar expression profile. The isolated clusters are then identified as individual cell populations with a specific phenotype (Figure 2).

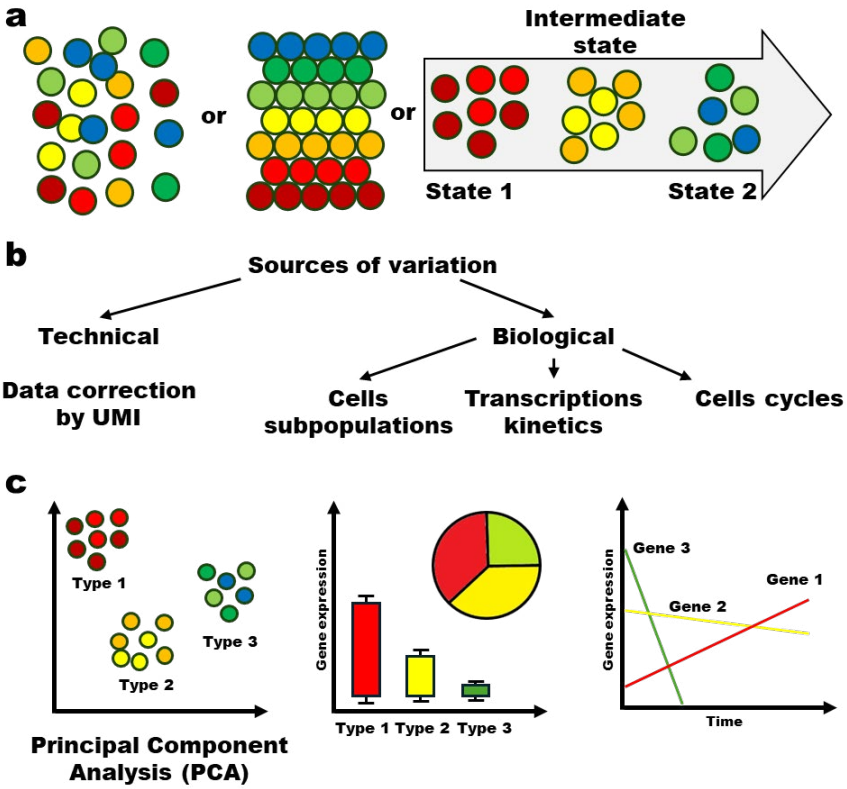

Fig. 2. Data processing in an scRNA-seq experiment. Various variants of heterogeneous cell populations can be studied: a mixture of different cell types, such as blood; a tissue with a gradient of different cells that may be associated during development; cells transitioning from one state to another, such as differentiating cells (a). The main sources of variation in the experiment are technical and biological. Technical noise is estimated and corrected for using approaches that relate the number of unique reads and labels. Sources of biological variability can be variability among different subpopulations, cell-to-cell variability in gene expression and transcription kinetics, and variability due to cell function and biological processes, such as the cell cycle, which serves as a source of data for further analysis (b). Identification of cell populations can be performed using principal component analysis (PCA) or hierarchical clustering. There are also various approaches to characterize subpopulations: searching for cell type markers by analyzing differential expression between different groups of cells; frequency of occurrence of cell populations; identification of genes that exhibit certain patterns during a process, such as development or response to stimuli: genes which expression either increases or decreases throughout the process, but, most interestingly, genes that are expressed transiently in intermediate cell types, since these genes may be important for the process (c).

Note: UMI — unique molecular identifier.

A classic example of a software package for working with scRNA-seq data regardless of the cell types studied is the approach described in [45], which proposes four ready-to-use software packages that include raw count normalization, feature selection, dimensionality reduction, and clustering, shown in Figure 2. The proposed software packages use both R and Python programming languages and include Seurat (R) [46], Scanpy (Python) [47], Monocle (R) [48, 49], and gf-icf (R) [50]. Using this approach will allow inexperienced users to analyze scRNA-seq data without complications and loss of information. An example of using this approach with a description of the steps, demonstration of graphs and command lines is available on "github" [51].

Examples of using the scRNA-seq method

During their development and application over the past 15 years, scRNA-seq technologies have grown into various solutions, including commercial ones (presented in Table 2, [52–68]), which can be classified in various ways, the most relevant of which, in our opinion, is by the cell isolation method. Similar to what was described in the section above, the technologies can be divided into microfluidic options that use chips and the formation of emulsion microdroplets for separation, options with magnetic or fluorescent sorting, and other options that use, for example, laser dissection or cytometry. For example, in the field of scRNA-seq, microfluidic technologies have recently gained popularity due to their cost-effectiveness, high performance, and moderate requirements for the budget and the size of the generated data [27, 28]. Another important parameter is the division into technologies with high throughput but low detection limits, and, conversely, low throughput but deep detection limits.

The most well-known commercial technologies currently in use are Chromium® from 10X Genomics, USA and BD Rhapsody® from BD Biosciences, USA, which provide users with a wide range of commercial solutions and equipment for sample preparation using scRNA-seq technology and compatible with NSG sequencing methods from various manufacturers. Technologies have successfully competed for a long time and are subject to constant comparison [69]. As scRNA-seq technology is mastered and distributed, more and more manufacturers are mastering the technology and offering their commercial solutions, for example, ddSEQ® Single-Cell from BioRad, DNBelab® C Series from MGI/BGI, China, Quantum Scale® from Scale Bioscience, USA and Gexscope® from Singlerion, Germany.

Table 2

scRNA-seq technologies

Technology	Isolation type	Add. parameters	Year / Ref.	Examples of commercial technologies
Smart-seq	FACS	PCR, Full-length	2012 [52]	SMART-Seq®, Takara Bio, Japan [62]
Smart-seq2	FACS	PCR, Full-length	2013 [53]	
MATQ-seq	FACS	PCR, Full-length	2017 [54]	
CEL-seq	FACS	IVT, 3' end	2012 [55]	
MARS-seq	FACS	IVT, 3' end	2014 [56]	
Fluidigm C1	Micro-fluidic	PCR, Full-length	2013 [57]	BD Rhapsody®, BD Biosciences, USA [63]Quantum Scale ®, Scale Bioscience, USA, [64]Gexscope®, Singlerion, Germany [65]
Seq-Well	Micro-fluidic	PCR, 3' end	2017 [58]	
Drop-seq	Microdroplets	PCR, 3' end	2015 [37]	Chromium®, Gem-X®, 10X Genomics, USA [66] DNBelab® C Series, MGI/BGI, China [67]ddSEQ® Single-Cell, BioRad, USA [68]
inDrop-seq	Microdroplets	IVT, 3' end	2015 [59]	
10x Genomics	Microdroplets	PCR, 3' end	2016 [60]	
DNBelab C4	Microdroplets	PCR, 3' end	2019 [61]	

Note: FACS — fluorescence-activated cell sorting; PCR — polymerase chain reaction; IVT — in vitro transcription.

However, the implementation of scRNA-seq methods is still not widely available for many laboratories due to its relatively high cost [34, 35]. At the moment, the technology is still relatively expensive, the cost of a reagent kit varies from several thousand to several hundred US dollars excluding the cost of equipment that performs operations according to the protocol. Nevertheless, it is the presence and development of competition between commercial scRNA-seq solutions that has a positive effect on this gradual reduction in the cost of using the technology. Moreover, a number of companies have already announced the development and imminent release of new cost-effective commercial solutions for scRNA-seq technology. Organizations such as Parse Biosciences, USA and Fluent BioSciences, USA announce the development of solutions that allow to scale up analysis multiple times and simplify the procedure for analyzing and processing data due to the implementation of new technologies and use of machine learning and artificial intelligence. ArgenTAG, Argentina, in turn, is preparing a commercial solution for applying scRNA-seq to third-generation sequencers, in particular Oxford Nanopore, and plans to reduce the cost due to both more productive cell barcoding technologies and the advantage of long reads of new sequencers. Also, Scipio Bioscience, France, has proposed an alternative approach to barcoding in hydrogel without the use of expensive equipment and is preparing its kits for release to the market. Such a solution will allow scRNA-seq experiments to be carried out simply at the lab table further reducing their cost.

Below are examples of the application of the technology and the results obtained in the fields of clinical diagnostics, cellular aging and oncology, obtained mainly using only commercial solutions Chromium® from 10X Genomics, USA and BD Rhapsody® from BD Biosciences, USA. The rapid development of scRNA-seq technology inspires optimism and promises growth in the prospects for future medical and biological research.

Oncology

Currently, scRNA-seq technology is widely used in oncology for profiling the transcriptomes of individual cells. The data obtained using this method have improved our understanding of the biological characteristics and dynamics within cancer lesions, including information related to malignant cell and immune cell landscapes, tumor heterogeneity, circulating tumor cells, and the underlying mechanisms of tumor biological behavior [70]. Various scRNA-seq technologies have been used to study cell hierarchy and decipher clonal heterogeneity in normal bone marrow and acute myeloid leukemia [71], and to search for new diagnostic and prognostic biomarkers among various tumors [72], such as bladder cancer and pancreatic cancer [73]. Systematic accumulation of scRNA-seq data has allowed the creation of a cancer cell atlas that covers a spectrum of cancer types [74–84] and may provide guidance for treatment of conditions where basic guidelines are lacking [85].

The use of scRNA-seq has enabled the characterization of dynamic interactions within a single cell by analyzing transcriptome variation over space and time. The sequential attachment of spatial barcodes allows the encoding and extraction of location information of individual cells, which can effectively inform research and diagnostics. Thus, tracking temporal and spatial variability allows the study of the impact on tumor heterogeneity and stress response, which in turn can be used for cancer diagnosis, classification, and treatment [86–88]. For example, a study [89] deciphered a detailed spatial map of individual cell phenotypes and cell systems, which the researchers used to demonstrate the phenotypic heterogeneity of tumor cells in breast cancer. Similar results were achieved in studies [90, 91] on pancreatic and breast cancer.

The use of scRNA-seq has enabled the identification of gene expression changes during cancer progression. Studies using single cell sequencing of adjacent normal tissues and adenomas at different stages in patients have comprehensively revealed genomic changes, clonal architecture, and metabolic dynamics during tumor development, which in turn has provided insight into tumor progression inhibition [92]. For example, a study [93] investigated the cell evolutionary pathway in salivary gland squamous cell carcinoma, including the stages of basal cell carcinogenesis, activation of Wnt

signaling cascades, and subsequent cell differentiation into luminal cells. Another example is a study [94] describing the mechanism of transformation of familial adenomatous polyposis to adenocarcinoma, indicating that malignant cells retain epithelial characteristics while undergoing rapid migration in breast cancer.

The use of scRNA-seq allows clustering of different cells. Clustering of tumor cell subsets helps to identify rare subsets or a spectrum of cell states of malignant cells [70]. For example, studies [95–99] using scRNA-seq have identified melanoma cells in intermediate states and malignant cell subsets with unique characteristics in pancreatic, ovarian, and gastric cancers. scRNA-seq can also be used to cluster subsets of immune cells that make up the tumor immune microenvironment, also allowing to assess the impact on immunotherapeutic response and clinical outcomes [70]. For example, a study [100] describes gradual replacement of resident myeloid cells by macrophages and monocyte-derived DCs upon T cell depletion in metastatic lung adenocarcinoma.

Senescence

The field of studying cellular senescence is an interesting example of the early use of scRNA-seq technology. Various factors can induce cellular senescence, and the rate of accumulation of senescent cells varies across organs. In addition, since heterogeneity is due to the spatiotemporal context of senescent cells, *in vivo* studies are required to improve the understanding of senescent cells [101]. While previous attempts have been made to find markers commonly expressed in senescent cells, researchers have been actively using bulk RNA-seq as a primary tool to study cells and their relationships over time [102–105].

The use of scRNA-seq in senescence is currently a relatively new approach to study rare senescent cells. This is because only modern scRNA-seq technologies achieve the minimum required throughput of ~10,000 cells per approach to work with rather rare senescent cells in the bulk, which constitute only 2% of specific tissues *in vivo* [106]. Technologies such as InDrops and Drop-seq can easily scale up cell numbers, thereby increasing the ability to detect rare cells [107]. Therefore, improving the throughput of scRNA-seq and ways to enrich for rare senescent cells are priority areas for development.

One of the most striking examples of the use of scRNA-seq in this area is the creation of the Tabula Muris Senis — atlas of mouse aging [108], where various tissues of male and female mice were analyzed over time. The study observed the emergence of aging-specific cell types in tissues such as the liver and bladder, as well as immune infiltration of various tissues. It was noted that the number of T and B cells in gonadal adipose tissue increased with age, and a cluster of B cells highly expressing immunoglobulin J was found specifically in old mice. Moreover, the use of the scRNA-seq data sets by the researchers [108, 109] showed a certain discrepancy between the expected results and the already established theory about the expression of the aging marker p16. In the study [108], it was noted that the fraction of cells expressing p16 doubled after 24 months compared to 3 months, and the level of p16 expression also increased approximately 2-fold. However, according to the study [109], the proportion of p16-expressing cells and the average level of p16 expression did not increase during aging. This allowed to conclude both that p16-expressing senescent cells are quite rare and are likely to be lost during tissue dissociation and sequencing library creation, and that the pattern of accumulation of p16-expressing senescent cells may differ between subjects [101].

For ethical reasons, scRNA-seq analysis for human tissues has been performed only in limited quantities. Several studies [110–115] have obtained data for the creation of aging cell atlases for muscle, pancreas, brain, retina, and blood cells. A study [116] using scRNA-seq on human lung tissue was able to show a relationship between senescent cell populations and age-related progression of pulmonary fibrosis, despite the poor representation of classical senescent markers determined by bulk RNA-seq. These data led to the conclusion that *in vivo* human senescent cells may have a different transcriptomic signature from senescent cells *in vitro* and the use of single-cell spatial transcriptomics to study

different tissues longitudinally may help to expand the understanding of the systemic effects of senescent cells [117].

Diagnostics and clinical research

The use of scRNA-seq technology paired with machine learning is a powerful tool for creating complex diagnostic systems for various diseases. For example, in the study of systemic lupus erythematosus [118], a machine learning-based pipeline was presented that was able to compare hundreds of single-cell proteome and transcriptome data points and identify several working combinations of biomarkers for diagnosis and further monitoring of the disease.

A study [119] devoted to postmenopausal osteoporosis also undertook solving the problem of discovering and screening new biomarkers for the disease, as well as attempts to identify new therapeutic targets by combining scRNA-seq and bulk RNA-seq technologies. Three potential biomarkers were identified and a correlation with the effectiveness of several potential drugs for treatment was established. These data were used for early diagnosis and targeted treatment of osteoporosis.

Another study [120] using a combination of scRNA-seq and bulk RNA-seq technologies aimed at finding biomarkers and developing methods for diagnosing sepsis. A specific biomarker has been identified that demonstrates diagnostic and prognostic value in sepsis and has been nominated for potential use in the treatment of this pathology. Single-cell sequencing is widely used to measure the clinical efficacy and safety of new drugs in clinical trials. Single-cell sequencing can be used to study dynamic regulatory networks and identify heterogeneous cellular behavior in response to different chemotherapy or radiation in cell lines or tumor samples such as esophageal squamous cell carcinoma [121]. As an example, a study [122] described the sci-Plex method used to decipher transcriptomic changes and their underlying mechanisms in millions of malignant cells in response to drug therapy.

Conclusion

The use of the scRNA-seq method allows to obtain sequencing data with greater accuracy, volume and quality. This opens up a wide field to conduct a detailed study of biological processes occurring in the central and peripheral organs of the immune system, inflammatory and tumor processes, as well as cellular aging processes, which is already confirmed by examples of the conducted studies.

In our opinion, mastering this method is one of the current professional challenges for domestic research teams. Tackling such a challenge will require increasing the level of technical equipment, gaining experience in using the current and previous versions of the technology — bulk RNA-seq / NGS, and studying modern bioinformatics tools for processing and interpreting data, which in turn implies the development of competencies in the field of immunology and biology, as well as the study and implementation of programming languages in practice.

Based on the scientific examples presented in this review, it can be concluded that the development and successful application of the scRNA-seq method in scientific and clinical practice will become the key to a wide range of future discoveries and successful accurate personalized diagnostics and healthcare.

References / Список литературы

1. Hatton IA, Galbraith ED, Merleau NSC, Miettinen TP, Smith BM, Shander JA. The human cell count and size distribution. *Proceedings of the National Academy of Sciences*. 2023;120(39). doi:10.1073/pnas.2303077120

2. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. *Journal of Applied Genetics*. 2011;52(4):413–435. doi:10.1007/s13353-011-0057-x

3. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: An overview. *Human Immunology*. 2021;82(11):801–811. doi:10.1016/j.humimm.2021.02.012

4. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology*. 1975;94(3):441–448. doi:10.1016/0022–2836 (75) 90213-2

5. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*. 1977;74(12):5463–5467. doi:10.1073/pnas.74.12.5463

6. Thermo Fisher Scientific [Internet]. Ion Torrent Next-Generation Sequencing Instruments [Accessed July 31, 2025]. Available from: https://www.thermofisher.com/ru/ru/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence.html

7. Merriman B, Rothberg JM. Progress in Ion Torrent semiconductor chip based sequencing. *Electrophoresis*. 2012;33(23):3397–3417. doi:10.1002/elps.201200424

8. Illumina [Internet]. Explore Illumina sequencing technology [Accessed July 31, 2025]. Available from: https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html

9. Illumina [Internet]. Bringing next-generation sequencing to clinical labs [Accessed July 31, 2025]. Available from: https://www.illumina.com/systems/ivd-instruments.html

10. BGI/MGI [Internet]. Introduction to MGI sequencing technology [Accessed July 31, 2025]. Available from: https://en.mgi-tech.com/products/resources

11. BGI/MGI [Internet]. Sequencer Products. Platforms [Accessed July 31, 2025]. Available from: https://en.genomics.cn/en-sequencplatform.html

12. PacBio [Internet]. Introducing the Sequel System: The Scalable Platform for SMRT Sequencing [Accessed July 31, 2025]. Available from: https://www.pacb.com/blog/introducing-the-sequel-system-the-scalable-platform-for-smrt-sequencing/

13. PacBio [Internet]. Human genomics solutions [Accessed July 31, 2025]. Available from: https://www.pacb.com/human-genomics/

14. ONT [Internet]. About [Accessed July 31, 2025]. Available from: https://nanoporetech.com/about

15. Jain M, Olsen HE, Paten B, Akeson M. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome Biology*. 2016;17(1). doi:10.1186/s13059-016-1103-0

16. Wang ET, Sandberg R, Luo S, Khrebutkova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature*. 2008;456(7221):470–476. doi:10.1038/nature07509

17. Cacchiarelli D, Trapnell C, Ziller MJ, et al. Integrative analyses of human reprogramming reveal dynamic nature of induced pluripotency. *Cell*. 2015;162(2):412–424. doi:10.1016/j.cell.2015.06.016

18. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nature Reviews Cancer*. 2007;7(4):233–245. doi:10.1038/nrc2091

19. Trapnell C. Defining cell types and states with single-cell genomics. *Genome Research*. 2015;25(10):1491–1498. doi:10.1101/gr.190595.115

20. Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J. Roma G, Klein AM, Jaffe AB. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature*. 2018;560(7718):377–381. doi:10.1038/s41586-018-0394-6

21. Suo S, Zhu Q, Saadatpour A, Fei L, Guo G, Yuan GC. Revealing the critical regulators of cell identity in the Mouse cell Atlas. *Cell Reports*. 2018;25(6):1436–1445.e3. doi:10.1016/j.celrep.2018.10.045

22. Velasco S, Kedaigle AJ, Simmons SK, et al. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature*. 2019;570(7762):523–527. doi:10.1038/s41586-019-1289-x

23. Fischer DS, Fiedler AK, Kernfeld EM, Genda RMJ, Bastidas-Ponce A, Bakhti M, Lickert H, Hasenauer J, Maehr R, Theis F. Inferring population dynamics from single-cell RNA-sequencing time series data. *Nature Biotechnology*. 2019;37(4):461–468. doi:10.1038/s41587-019-0088-0

24. Liu Z, Wang L, Welch JD, et al. Single-cell transcriptomics reconstructs fate conversion from fibroblast to cardiomyocyte. *Nature*. 2017;551(7678):100–104. doi:10.1038/nature24454

25. Cacchiarelli D, Qiu X, Srivatsan S, et al. Aligning Single-Cell developmental and reprogramming trajectories identifies molecular determinants of myogenic reprogramming outcome. *Cell Systems*. 2018;7(3):258–268.e3. doi:10.1016/j.cels.2018.07.006

26. Van Dijk D, Sharma R, Nainys J, et al. Recovering Gene Interactions from Single-Cell Data Using Data Diffusion. *Cell*. 2018;174(3):716–729.e27. doi:10.1016/j.cell.2018.05.061

27. Hayashi T, Ozaki H, Sasagawa Y, Umeda M, Danno H, Nikaido I. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. *Nature Communications*. 2018;9(1). doi:10.1038/s41467-018-02866-0

28. Savas P, Virassamy B, Ye C. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nature Medicine*. 2018;24(7):986–993. doi:10.1038/s41591-018-0078-7

29. Moghe I, Loupy A, Solez K. The Human Cell Atlas Project by the numbers: Relationship to the Banff Classification. *American Journal of Transplantation*. 2018;18(7):1830. doi:10.1111/ajt.14757

30. Tang F, Barbacioru C, Wang Y, et al. mRNA-Seq whole-transcriptome analysis of a single cell. *Nature Methods*. 2009;6(5):377–382. doi:10.1038/nmeth.1315

31. Perik-Zavodskaia OYu, Perik-Zavodskii RYu, Sennikov SV. Innovative approaches in immunology: single cell multi-omics and spatial transcriptomics. *Immunologiya*. 2024;45(4):414–426. doi:10.33029/1816-2134-2024-45-4-414-426 (In Russian). [Перик-Заводская О.Ю., Перик-Заводский Р.Ю., Сенников С.В. Инновационные подходы в иммунологии: мультиомика единичных клеток и пространственная транскриптомика // *Иммунология.* 2024; 45 (4): 414–426 DOI: https://doi.org/10.33029/1816-2134-2024-45-4-414-426]

32. Hedlund E, Deng Q. Single-cell RNA sequencing: Technical advancements and biological applications. *Molecular Aspects of Medicine*. 2017;59:36–46. doi:10.1016/j.mam.2017.07.003

33. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & Molecular Medicine*. 2018;50(8):1–14. doi:10.1038/s12276-018-0071-8

34. Ziegenhain C, Vieth B, Parekh S, Reinius B, Guillaument-Adkins A, Smets M, Leonhards H, Heyn H, Hellmann I, Enard W. Comparative analysis of Single-Cell RNA sequencing Methods. *Molecular Cell*. 2017;65(4):631–643.e4. doi:10.1016/j.molcel.2017.01.023

35. Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS. Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proceedings of the National Academy of Sciences*. 2009;106(33):13939–13944. doi:10.1073/pnas.0907143106

36. Zeb Q, Wang C, Shafiq S, Liu L. An overview of Single-Cell isolation techniques. In: *Elsevier eBooks*.; 2019:101–135. doi:10.1016/b978-0-12-814919-5.00006-3

37. Macosko EZ, Basu A, Satija R. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161(5):1202–1214. doi:10.1016/j.cell.2015.05.002

38. Islam S, Zeisel A, Joost S, La Manno G, Zajac P, Kasper M, Lönnerberg P, Linnarsson S. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nature Methods*. 2013;11(2):163–166. doi:10.1038/nmeth.2772

39. Senabouth A, Andersen S, Shi Q, et al. Comparative performance of the BGI and Illumina sequencing technology for single-cell RNA-sequencing. *NAR Genomics and Bioinformatics*. 2020;2(2). doi:10.1093/nargab/lqaa034

40. Stegle O, Teichmann SA, Marioni JC. Computational and analytical challenges in single-cell transcriptomics. *Nature Reviews Genetics*. 2015;16(3):133–145. doi:10.1038/nrg3833

41. Lähnemann D, Köster J, Szczurek E, et al. Eleven grand challenges in single-cell data science. *Genome Biology*. 2020;21(1). doi:10.1186/s13059-020-1926-6

42. scRNA-tools [Internet]. scRNA-tools page [Accessed July 31, 2025]. Available from: https://www.scrna-tools.org

43. Luecken MD, Theis FJ. Current best practices in single cell RNA seq analysis: a tutorial. *Molecular Systems Biology*. 2019;15(6). doi:10.15252/msb.20188746

44. Neu KE, Tang Q, Wilson PC, Khan AA. Single-Cell Genomics: Approaches and utility in Immunology. *Trends in Immunology*. 2017;38(2):140–149. doi:10.1016/j.it.2016.12.001

45. Slovin S, Carissimo A, Panariello F, Grimaldi A, Bouché V, Gambardella G, Cacchiarelli D. Single-Cell RNA sequencing Analysis: A Step-by-Step Overview. *Methods in Molecular Biology*. January 2021:343–365. doi:10.1007/978-1-0716-1307-8_19

46. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*. 2018;36(5):411–420. doi:10.1038/nbt.4096

47. Wolf FA, Angerer P, Theis FJ. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biology*. 2018;19(1). doi:10.1186/s13059-017-1382-0

48. Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, Lennon NJ, Livak KJ, Mikkelsen TS, Rinn JL. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nature Biotechnology*. 2014;32(4):381–386. doi:10.1038/nbt.2859

49. Cao J, Spielmann M, Qiu X. The single-cell transcriptional landscape of mammalian organogenesis. *Nature*. 2019;566(7745):496–502. doi:10.1038/s41586-019-0969-x

50. Gambardella G, Di Bernardo D. A tool for visualization and analysis of Single-Cell RNA-Seq data based on text mining. *Frontiers in Genetics*. 2019;10. doi:10.3389/fgene.2019.00734

51. Github [Internet]. scRNA-seq chapter [Accessed July 31, 2025]. Available from: https://github.com/gambalab/scRNAseq_chapter

52. Goetz JJ, Trimarchi JM. Transcriptome sequencing of single cells with Smart-Seq. *Nature Biotechnology*. 2012;30(8):763–765. doi:10.1038/nbt.2325

53. Picelli S, Björklund ÅK, Faridani OR, Sagasser S, Winberg G, Sandberg R. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nature Methods*. 2013;10(11):1096–1098. doi:10.1038/nmeth.2639

54. Sheng K, Cao W, Niu Y, Deng Q, Zong C. Effective detection of variation in single-cell transcriptomes using MATQ-seq. *Nature Methods*. 2017;14(3):267–270. doi:10.1038/nmeth.4145

55. Hashimshony T, Wagner F, Sher N, Yanai I. CEL-SEQ: Single-Cell RNA-SeQ by multiplexed linear amplification. *Cell Reports*. 2012;2(3):666–673. doi:10.1016/j.celrep.2012.08.003

56. Jaitin DA, Kenigsberg E, Keren-Shaul H. Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types. *Science*. 2014;343(6172):776–779. doi:10.1126/science.1247651

57. Xin Y, Kim J, Ni M, et al. Use of the Fluidigm C1 platform for RNA sequencing of single mouse pancreatic islet cells. *Proceedings of the National Academy of Sciences*. 2016;113(12):3293–3298. doi:10.1073/pnas.1602306113

58. Gierahn TM, Wadsworth MH, Hughes TK, Bryson BD, Butler A, Satija R, Fortune S, Love JC, Shalek AK. Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nature Methods*. 2017;14(4):395–398. doi:10.1038/nmeth.4179

59. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, Peshkin L, Weitz DA, Kirschner MW. Droplet barcoding for Single-Cell transcriptomics applied to embryonic stem cells. *Cell*. 2015;161(5):1187–1201. doi:10.1016/j.cell.2015.04.044

60. Zheng GXY, Terry JM, Belgrader P. Massively parallel digital transcriptional profiling of single cells. *Nature Communications*. 2017;8(1). doi:10.1038/ncomms14049

61. Liu C, Wu T, Fan F, et al. A portable and cost-effective microfluidic system for massively parallel single-cell transcriptome profiling. *bioRxiv (Cold Spring Harbor Laboratory)*. October 2019. doi:10.1101/818450

62. Takara Bio [Internet]. SMART-Seq mRNA Single Cell LP and SMART-Seq mRNA Single Cell [Accessed July 31, 2025]. Available from: https://www.takarabio.com/products/next-generation-sequencing/rna-seq/mrna-seq/full-length-mrna-seq/smart-seq-mrna-single-cell-lp-and-smart-seq-mrna-single-cell

63. BD Bioscience [Internet]. BD Rhapsody [Accessed July 31, 2025]. Available from: https://www.bdbiosciences.com/en-us/products/instruments/single-cell-multiomics-systems/rhapsody

64. Scale Bioscience [Internet]. QuantumScale Single Cell RNA [Accessed July 31, 2025]. Available from: https://scale.bio/single-cell-rna-sequencing-kit/

65. Singleron [Internet]. Single Cell RNA Sequencing [Accessed July 31, 2025]. Available from: https://singleron.bio/single-cell-sequencing-solutions/

66. 10X Genomics [Internet]. Chromium Single Cell [Accessed July 31, 2025]. Available from: https://www.10xgenomics.com/platforms/chromium

67. BGI/MGI [Internet]. Single Cell RNA-Seq [Accessed July 31, 2025]. Available from: https://www.bgi.com/us/high-throughput-single-cell-rna-seq

68. BIO-RAD [Internet]. ddSEQ Single-Cell 3' RNA-Seq & Omnition Analysis Software [Accessed July 31, 2025]. Available from: https://www.bio-rad.com/ru-ru/product/ddseq-single-cell-3-rna-seq-kit-omnition-analysis-software? ID=b4738e36–858d-e6a0–61d9–8d96d3d521b8

69. Gao C, Zhang M, Chen L. The comparison of two single-cell sequencing platforms: BD Rhapsody and 10X Genomics Chromium. *Current Genomics*. 2020;21(8):602–609. doi:10.2174/1389202921999200625220812

70. Lei Y, Tang R, Xu J, Wang W, Zhang B, Liu J, Yu X, Shi S. Applications of single-cell sequencing in cancer research: progress and perspectives. *Journal of Hematology & Oncology*. 2021;14(1). doi:10.1186/s13045-021-01105-2

71. Wu J, Xiao Y, Sun J. A single-cell survey of cellular hierarchy in acute myeloid leukemia. *Journal of Hematology & Oncology*. 2020;13(1). doi:10.1186/s13045-020-00941-y

72. Rozenblatt-Rosen O, Regev A, Oberdoerffer P, et al. The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution. *Cell*. 2020;181(2):236–249. doi:10.1016/j.cell.2020.03.053

73. Chen Z, Zhou L, Liu L, Hou Y, Xiong M, Yang Y, Hu J, Chen K. Single-cell RNA sequencing highlights the role of inflammatory cancer-associated fibroblasts in bladder urothelial carcinoma. *Nature Communications*. 2020;11(1). doi:10.1038/s41467-020-18916-5

74. Sinjab A, Han G, Treekitkarnmongkol W. Resolving the spatial and cellular architecture of lung adenocarcinoma by multiregion Single-Cell sequencing. *Cancer Discovery*. 2021;11(10):2506–2523. doi:10.1158/2159-8290.cd-20-1285

75. Pal B, Chen Y, Vaillant F. A single cell RNA expression atlas of normal, preneoplastic and tumorigenic states in the human breast. *The EMBO Journal*. 2021;40(11). doi:10.15252/embj.2020107333

76. Wagner J, Rapsomaniki MA, Chevrier S. A Single-Cell atlas of the tumor and immune ecosystem of human breast cancer. *Cell*. 2019;177(5):1330–1345.e18. doi:10.1016/j.cell.2019.03.005

77. Hu Q, Hong Y, Qi P, et al. Atlas of breast cancer infiltrated B-lymphocytes revealed by paired single-cell RNA-sequencing and antigen receptor profiling. *Nature Communications*. 2021;12(1). doi:10.1038/s41467-021-22300-2

78. Mathewson ND, Ashenberg O, Tirosh I. Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. *Cell*. 2021;184(5):1281–1298.e26. doi:10.1016/j.cell.2021.01.022

79. Zheng Y, Chen Z, Han Y, et al. Immune suppressive landscape in the human esophageal squamous cell carcinoma microenvironment. *Nature Communications*. 2020;11(1). doi:10.1038/s41467-020-20019-0

80. Yuan H, Yan M, Zhang G. CancerSEA: a cancer single-cell state atlas. *Nucleic Acids Research*. 2018;47(D1): D900-D908. doi:10.1093/nar/gky939

81. Pritykin Y, Van Der Veeken J, Pine AR, Zhong Y, Sahin M, Mazutis L, Pe'er D, Rudensky AY, Leslie CS. A unified atlas of CD8 T cell dysfunctional states in cancer and infection. *Molecular Cell*. 2021;81(11):2477–2493.e10. doi:10.1016/j.molcel.2021.03.045

82. Cheng S, Li Z, Gao R. A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell*. 2021;184(3):792–809.e23. doi:10.1016/j.cell.2021.01.010

83. Marcu A, Bichmann L, Kuchenbecker L, et al. HLA Ligand Atlas: a benign reference of HLA-presented peptides to improve T-cell-based cancer immunotherapy. *Journal for ImmunoTherapy of Cancer*. 2021;9(4): e002071. doi:10.1136/jitc-2020–002071

84. Buechler MB, Pradhan RN, Krishnamurty AT. Cross-tissue organization of the fibroblast lineage. *Nature*. 2021;593(7860):575–579. doi:10.1038/s41586-021-03549-5

85. Liudahl SM, Betts CB, Sivagnanam S, et al. Leukocyte Heterogeneity in Pancreatic Ductal Adenocarcinoma: Phenotypic and Spatial Features Associated with Clinical Outcome. *Cancer Discovery*. 2021;11(8):2014–2031. doi:10.1158/2159-8290.cd-20-0841

86. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. *Experimental Biology and Medicine*. 1941;47(2):200–202. doi:10.3181/00379727-47-13084p

87. Gall JG, Pardue ML. Formation and detection of rna-dna hybrid molecules in cytological preparations. *Proceedings of the National Academy of Sciences*. 1969;63(2):378–383. doi:10.1073/pnas.63.2.378

88. Geng S, Wang J, Zhang X, et al. Single-cell RNA sequencing reveals chemokine self-feeding of myeloma cells promotes extramedullary metastasis. *FEBS Letters*. 2019;594(3):452–465. doi:10.1002/1873-3468.13623

89. Jackson HW, Fischer JR, Zanotelli VRT, et al. The single-cell pathology landscape of breast cancer. *Nature*. 2020;578(7796):615–620. doi:10.1038/s41586-019-1876-x

90. Moncada R, Barkley D, Wagner F, Chiodin M, Devlin JC, Baron M, Hajdu CH, Simeone DM, Yanai I. Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. *Nature Biotechnology*. 2020;38(3):333–342. doi:10.1038/s41587-019-0392-8

91. Ståhl PL, Salmén F, Vickovic S. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science*. 2016;353(6294):78–82. doi:10.1126/science.aaf2403

92. Li J, Wang R, Zhou X, et al. Genomic and transcriptomic profiling of carcinogenesis in patients with familial adenomatous polyposis. *Gut*. 2019;69(7):1283–1293. doi:10.1136/gutjnl-2019-319438

93. Praktiknjo SD, Obermayer B, Zhu Q, Fang L, Liu H, Quinn H, Stoeckius M, Kocks C, Birchmeier W, Rajewsky N. Tracing tumorigenesis in a solid tumor model at single-cell resolution. *Nature Communications*. 2020;11(1). doi:10.1038/s41467-020-14777-0

94. Chen YC, Sahoo S, Brien R. Single-cell RNA-sequencing of migratory breast cancer cells: discovering genes associated with cancer metastasis. *The Analyst*. 2019;144(24):7296–7309. doi:10.1039/c9an01358j

95. Wouters J, Kalender-Atak Z, Minnoye L. Robust gene expression programs underlie recurrent cell states and phenotype switching in melanoma. *Nature Cell Biology*. 2020;22(8):986–998. doi:10.1038/s41556-020-0547-3

96. Cook DP, Vanderhyden BC. Ovarian cancer and the evolution of subtype classifications using transcriptional profiling†. *Biology of Reproduction*. 2019;101(3):645–658. doi:10.1093/biolre/ioz099

97. Liu Y, Ye G, Huang L, Zhang C, Sheng Y, Wu B, Han L, Wu C, Dong B, Qi Y. Single-cell transcriptome analysis demonstrates inter-patient and intra-tumor heterogeneity in primary and metastatic lung adenocarcinoma. *Aging*. 2020;12(21):21559–21581. doi:10.18632/aging.103945

98. Zhang M, Hu S, Min M, Ni Y, Lu Z, Sun X, Wu J, Liu B, Ying X, Liu Y. Dissecting transcriptional heterogeneity in primary gastric adenocarcinoma by single cell RNA sequencing. *Gut*. 2020;70(3):464–475. doi:10.1136/gutjnl-2019-320368

99. Peng J, Sun BF, Chen CY, et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. *Cell Research*. 2019;29(9):725–738. doi:10.1038/s41422-019-0195-y

100. Kim N, Kim HK, Lee K. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nature Communications*. 2020;11(1). doi:10.1038/s41467-020-16164-1

101. Kim S, Kim C. Transcriptomic Analysis of Cellular Senescence: One Step Closer to Senescence Atlas. *Molecules and Cells*. 2021;44(3):136–145. doi:10.14348/molcells.2021.2239

102. Hernandez-Segura A, De Jong TV, Melov S, Guryev V, Campisi J, Demaria M. Unmasking transcriptional heterogeneity in senescent cells. *Current Biology*. 2017;27(17):2652–2660.e4. doi:10.1016/j.cub.2017.07.033

103. Casella G, Munk R, Kim KM, Piao Y, De S, Abdelmohsen K, Gorospe M. Transcriptome signature of cellular senescence. *Nucleic Acids Research*. 2019;47(14):7294–7305. doi:10.1093/nar/gkz555

104. Zhou Q, Wan Q, Jiang Y, Liu J, Qiang L, Sun L. A Landscape of Murine Long Non-Coding RNAs Reveals the Leading Transcriptome Alterations in Adipose Tissue during Aging. *Cell Reports*. 2020;31(8):107694. doi:10.1016/j.celrep.2020.107694

105. Ashapkin VV, Kutueva LI, Vanyushin BF. Aging as an epigenetic phenomenon. *Current Genomics*. 2017;18(5). doi:10.2174/1389202918666170412112130

106. Biran A, Zada L, Karam PA, Vadai E, Roitman L, Ovadya Y, Porat Z, Krizhanovsky V. Quantitative identification of senescent cells in aging and disease. *Aging Cell*. 2017;16(4):661–671. doi:10.1111/acel.12592

107. Lafzi A, Moutinho C, Picelli S, Heyn H. Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies. *Nature Protocols*. 2018;13(12):2742–2757. doi:10.1038/s41596-018-0073-y

108. Almanzar N, Antony J, Baghel AS. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature*. 2020;583(7817):590–595. doi:10.1038/s41586-020-2496-1

109. Kimmel JC, Penland L, Rubinstein ND, Hendrickson DG, Kelley DR, Rosenthal AZ. Murine single-cell RNA-seq reveals cell-identity- and tissue-specific trajectories of aging. *Genome Research*. 2019;29(12):2088–2103. doi:10.1101/gr.253880.119

110. Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, Kim SK, Quake SR. Single-Cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. *Cell*. 2017;171(2):321–330.e14. doi:10.1016/j.cell.2017.09.004

111. Hammond TR, Dufort C, Dissing-Olesen L, et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity*. 2018;50(1):253–271.e6. doi:10.1016/j.immuni.2018.11.004

112. Yi W, Lu Y, Zhong S. A single-cell transcriptome atlas of the aging human and macaque retina. *bioRxiv (Cold Spring Harbor Laboratory)*. July 2020. doi:10.1101/2020.07.17.207977

113. Zheng Y, Liu X, Le W. A human circulating immune cell landscape in aging and COVID-19. *Protein & Cell*. 2020;11(10):740–770. doi:10.1007/s13238-020-00762-2

114. De Micheli AJ, Spector JA, Elemento O, Cosgrove BD. A reference single-cell transcriptomic atlas of human skeletal muscle tissue reveals bifurcated muscle stem cell populations. *Skeletal Muscle*. 2020;10(1). doi:10.1186/s13395-020-00236-3

115. Zou Z, Long X, Zhao Q. A Single-Cell Transcriptomic atlas of Human skin aging. *Developmental Cell*. 2020;56(3):383–397.e8. doi:10.1016/j.devcel.2020.11.002

116. Reyfman PA, Walter JM, Joshi N. Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis. *American Journal of Respiratory and Critical Care Medicine*. 2018;199(12):1517–1536. doi:10.1164/rccm.201712-2410oc

117. Roy AL, Sierra F, Howcroft K, Singer DS, Sharpless N, Hodes RJ, Wilder EL, Anderson JM. A blueprint for characterizing senescence. *Cell*. 2020;183(5):1143–1146. doi:10.1016/j.cell.2020.10.032

118. Li Y, Ma C, Liao S. Combined proteomics and single cell RNA-sequencing analysis to identify biomarkers of disease diagnosis and disease exacerbation for systemic lupus erythematosus. *Frontiers in Immunology*. 2022;13. doi:10.3389/fimmu.2022.969509

119. Wang H, Peng C, Hu G, Chen W, Hu Y, Pi H. Integrated single-cell RNA-seq and bulk RNA-seq identify diagnostic biomarkers for postmenopausal osteoporosis. *Current Medicinal Chemistry*. 2024;31. doi:10.2174/0109298673343344240930054414

120. Li J, Zhou M, Feng JQ, Hong SM, Yang SY, Zhi LX, Lin WY, Zhu C, Yu YT, Lu LJ. Bulk RNA sequencing with integrated Single-Cell RNA sequencing identifies BCL2A1 as a potential diagnostic and prognostic biomarker for sepsis. *Frontiers in Public Health*. 2022;10. doi:10.3389/fpubh.2022.937303

121. Yang L, Zhang X, Hou Q, Huang M, Zhang H, Jiang Z, Yue J, Wu S. Single-cell RNA-seq of esophageal squamous cell carcinoma cell line with fractionated irradiation reveals radioresistant gene expression patterns. *BMC Genomics*. 2019;20(1). doi:10.1186/s12864-019-5970-0

122. Srivatsan SR, McFaline-Figueroa JL, Ramani V. Massively multiplex chemical transcriptomics at single-cell resolution. *Science*. 2019;367(6473):45–51. doi:10.1126/science.aax6234

Секвенирование РНК единичных клеток: современные подходы и достижения

А.Е. Гусев, П.В. Чернов, Н.А. Дмитриев, И.А. Кофиади ✉

Государственный научный центр «Институт иммунологии» Федерального медико-биологического агентства,
г. Москва, Российская Федерация
✉ kofiadi@mail.ru

Аннотация. Актуальность. Метод секвенирования РНК единичных клеток (scRNA-seq) является современным подходом к изучению разнообразия и неоднородности транскриптов РНК в отдельных клетках, а также к выявлению состава различных типов клеток и функций в организмах, органах и тканях. Основанный на методе NGS (секвенирование нового поколения) метод scRNA-seq предоставляет огромный объем информации при глубоком клеточном разрешении в различных областях, позволяя делать новые открытия в понимании состава и паттернов взаимодействия отдельных типов клеток в организме человека, модельных животных и растений. Несмотря на активное развитие, оптимизацию и автоматизацию в течение последних 15 лет по всему миру, в нашей стране метод scRNA-seq является относительно новым и применяется сравнительно недавно. Задача освоения и успешного внедрения в практику данного метода актуальна и критична — метод является мощным инструментом для глубокого анализа и диагностики, о чем свидетельствуют результаты исследований, в которых он применялся. В обзоре представлены основные принципы и шаги реализации метода scRNA-seq как в разрезе технической реализации и пробоподготовки в виде надстройки над классическим методом NGS, так и в усложнении и расширении процесса обработки полученных данных, применения новых алгоритмов и баз данных. Рассмотрены уже имеющиеся на рынке коммерчески доступные технологий scRNA-seq и технологии, описанные в научных источниках, послужившие им в качестве прототипов и альтернатив. Представлены примеры и результаты использования таких технологий в различных областях науки и медицины таких как онкология, сенесценция, диагностика и клинические исследования. Выводы. Разработка и успешное применение метода scRNA-seq в научной и клинической практике станет залогом широкого спектра будущих открытий и основой персонализированной диагностики и здравоохранения.

Ключевые слова: секвенирование, РНК, секвенирование нового поколения (NGS), секвенирование единичных клеток (sc-seq, scRNA-seq), клеточная гетерогенность

Информация о финансировании. Работа выполнена при поддержке Министерства науки и высшего образования Российской Федерации в рамках Федеральной научно-технической программы развития генетических технологий на 2019–2030 годы (соглашение № 15.ЦГИМУ.25.3.3).

Вклад авторов. Сбор и анализ литературы, написание статьи — Гусев А.Е.; анализ литературы, написание статьи — Чернов П.В.; анализ литературы, редактирование статьи — Дмитриев Н.А.; анализ литературы, написание статьи, научное редактирование — Кофиади И.А. Все авторы внесли существенный вклад в подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Информация о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов.
Этическое утверждение — неприменимо.

Благодарности — неприменимо.

Информированное согласие на публикацию — неприменимо.

Поступила 27.08.2025. Принята 25.09.2025.

Для цитирования: *Gusev A.E., Chernov P.V., Dmitriev N.A., Kofiadi, I.A.* Single cell RNA sequencing: modern approaches and achievements // Вестник Российского университета дружбы народов. Серия: Медицина. 2025. Т. 29. № 4. С. 421–435. doi: 10.22363/2313-0245-2025-29-4-421-435. EDN: AAFUKT

Corresponding author: Ilya Andreevich Kofiadi — Corresponding Member of RAS, Dr. Sci., Head of Lab. of Molecular Immunogenetics of NRC Institute of Immunology, FMBA of Russia, 115522, Kashirskoe hw., 24, Moscow, Russian Federation; Prof. of the Immunology Chair, MBF of N.I. Pirogov RNRMU of the MOH of Russia, 117513, Ostrovityanova st., 1, Moscow, Russia Federation. E-mail: kofiadi@mail.ru
Gusev A.E. ORCID 0009-0002-5810-098X
Chernov P.V. ORCID 0009-0005-0642-8723
Dmitriev N.A. ORCID 0009-0002-8381-8512
Kofiadi I.A. ORCID 0000-0001-9280-8282

Ответственный за переписку: Кофиади Илья Андреевич — член-корреспондент РАН, д-р биол. наук, руководитель Референс-центра ФГБУ “ГНЦ Институт иммунологии” ФМБА России, 115522, Российская Федерация, г. Москва, Каширское шоссе, д. 24.; проф. каф. иммунологии МБФ, ФГАОУ ВО РНИМУ им. Н.И. Пирогова Минздрава России (Пироговский Университет), 117513, Российская Федерация, г. Москва,. ул. Островитянова, д. 1. E-mail: kofiadi@mail.ru
Гусев А.Е. SPIN 4385-0589, ORCID 0009-0002-5810-098X
Чернов П.В. ORCID 0009-0005-0642-8723
Дмитриев Н.А. ORCID 0009-0002-8381-8512
Кофиади И.А. SPIN 5730-0925, ORCID 0000-0001-9280-8282