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Scientific Research and Application of Flow Cytometry and Single Cell Analysis

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Abstract: *It is widely used in the clinical practice of small-scale biological separation (fctomet) and other biological separation methods, such as single row flow (FCM). Traditional research is carried out at the multi-cell level, which will lose a lot of heterogeneous information. Single cell technology can reveal the heterogeneity between cells and further explore the essence and law of life activities. It is widely used in the research of tumor cells, tumor networks and tumor stem cells. At the same time, it can help people better understand their occurrence, development and metastasis. Single cell technology has developed rapidly and made many innovative progress in the field of biology. Fully understanding the current development trend of flow cytometry and single cell technology can promote the development of immunology, developmental biology, neurobiology, cancer and other fields.*

Keywords: *Flow Cytometry, Single Cell Analysis Technology, Combined Application*

1. Introduction

Cells in the same tissue are generally considered to be the basic units with the same state. The results obtained by previous technical means are often the average response in the group. With the development of precision medicine and the progress of sequencing technology, people are more and more aware of the huge heterogeneity between cells. In recent years, the research of high-throughput cell sequencing technology has been widely used. The combined application of flow cytometry and single cell analysis technology can better select specific cell communities and analyze individual differences. In 2013, the journal Nature called "single cell sequencing" the annual technology, and in 2019, "single cell technology" became the annual technology again, providing a more reliable scientific basis for the research, development and treatment of severe etiology. It can be seen that the combination of flow cytometry and single cell technology has gradually become the main direction of future development in the field of life science, Broad application prospects.

2. Analysis on the Development Trend of Single Cell Technology

China has paid enough attention to the development of single cell technology, as shown in Figure 1 and table 1. The special plan for Biotechnology Innovation of the 13th five year plan of the Ministry of science and technology lists single cell technology as a key technology, It is suggested to "develop single-cell isolation, genome amplification, transcriptome amplification and single-cell genome analysis technologies, focus on breaking through several cutting-edge key technologies, deploy subversive biotechnology with great impact and can change the competitive pattern of science and technology, economy and society, and establish the core technology and equipment system of China's new generation biochemical technology and industry, "In recent years, single-cell technology has developed rapidly in China, and Chinese scientists have published many single-cell omics research results in the world's top journals [1]. In 2009, Professor Tang Fuzhuo published the world's first article on single-cell mRNA sequencing during his postdoctoral period (naturemethods, 2009) , the leap from 0 to 1 has been realized, and the era of single-cell transcriptome sequencing has been opened. In 2018, Professor Guo Guoji's team of Zhejiang University independently developed a series of national microwell SEQ high-throughput single cell analysis platforms and high-throughput single cell sequencing platforms, systematically analyzed the transcriptome of more than 400000 cells from nearly 50 mouse organs and tissues, and completed the first mammalian single cell atlas [2]. In 2021, Guo Guoji's team published an article entitled high throughput microwell SEQ 2.0 profiles massively

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multiplexed chemical sequencing in cell discovery magazine, showing a high-throughput single-cell sequencing platform that can be used for high-throughput screening, microwell SEQ 2.0.

Figure 1: Annual change trend of single cell technology documents (2009-2019) unit: Articles

At present, conventional high-throughput screening methods focus on the overall phenotype of cells, including transcriptome, metabolome, fluorescence signal, morphology and survival rate at the population level. These indicators can not reflect the subtle and heterogeneous changes between cells. In recent years, high-throughput single-cell sequencing technology has been applied to high-throughput screening (srivatsan, mcfaline Figueroa et al. 2020). These research methods will have a far-reaching impact on the recognition of human normal cell state and disease cell state. In addition to detecting single-cell genes, Chinese scientists have also made a breakthrough in detecting single-cell low copy number proteins. Professor Liu Zhen's team has creatively developed a plasmon sandwich assay (PISA) by combining immune recognition with plasmon Raman detection technology, and successfully realized the analysis of a variety of low copy number proteins in a single living cell and living animals (angeliwandte Chemie International Edition, 2016, 55, 13215)

Table 1: Analysis of the top 10 countries / regions in the number of single cell technology patents (2009-2019)

ranking	country	Number of patents / item	total cites
1	China	3007	7778
2	U.S.A	1097	15119
3	European Patent Office	196	1197
4	Japan	162	475
5	the republic of korea	126	138
6	World Intellectual Property Organization	50	436
7	Germany	45	64
8	britain	37	156
9	Israel	28	135
10	Canada	22	223

Single cell analysis technology is also involved in the study of heterogeneity between various cytokines. When cytokines and their receptors gather, they start complex intracellular molecular interactions and eventually lead to changes in cell gene transcription. This will help to clarify the immune regulation mechanism at the molecular level and contribute to the prevention, diagnosis and treatment of diseases. In particular, recombinant cytokines produced by genetic engineering technology have been used to treat tumors, infections, inflammation, hematopoietic dysfunction and so on.. In recent years, cytokines have been widely used in the research of leukemia treatment, which provides guidance for the individualized treatment of leukemia. The application and characteristics of cytokines in leukemia treatment are shown in Table 2 [3-4].

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Table 2: Application and characteristics of CyTOF in treatment of leukemia

Research applications	Characteristics
Study the mechanism of drug action	Measure the content of metal elements in single cell, the phase changes of cell cycle and the level of phosphorylation of signal pathway
Identify potential targets	Analyze complex cell phenotypic groups, detect heterogeneous cell groups in signal pathways, and then find new therapeutic targets
Evaluate the effect of treatment	Detect abnormal signals and cell cycle effects and provide a real-time signal transduction map of leukemic cells after treatment
Elucidate the mechanism of recurrence and drug resistance	The changes of additional leukemic cells phenotype, signal pathway and metabolic status were found, the characteristics of LSC survived after treatment were described

3. Analysis on the Development Trend of Flow Cytometry

In 1934, a. mordawan first reported the automatic cell counting method of passing suspended red blood cells through a glass capillary placed on the microscope stage. In 1956, W.H. Kurt introduced a device for counting cells and measuring cell volume by using the change of resistance (called Kurt resistance) when cells pass through two small holes (75 ~ 100 microns) in conductive solution. In 1965, L.A. kamensky made a multi parameter flow cytometer, which can measure cell size and nucleic acid content. In the same year, M.J. fullwheeler made a cell sorter. In 1969, van Dilla et al. Established a flow cytometer with liquid flow, illumination optical axis and detector axis orthogonal to each other by using argon ion laser and layered shell flow technology. Later, the cell sorting meter improved by H.R. Hewlett and others can make the cells in the flowing liquid spray into the air for measurement. However, in the above systems, the laser beam used and the limiting light bar set in the detection direction of collecting fluorescence are larger than the cells in the liquid flow, so it can not provide information about cell morphology, so it is called zero resolution system. Subsequently, L.L. Willis and S.F. patten developed a low-resolution slit scanning technique to measure nuclear fluorescence, cell and nuclear size. In 1969, W. GERD and W. Dietrich described a flow cytometer using mercury lamp as light source, which can stimulate cells flowing parallel to the optical axis in the flow chamber under falling light illumination. In the 21st century, flow cytometry has been gradually improved and has become a very important tool in the field of cytological analysis. Flow cytometry uses high-energy laser to irradiate dye stained monocytes or microspheres to measure the scattered light and fluorescence intensity. Colored cells or microspheres are suspended on the focus of a series of high-intensity light sources. When each cell or microsphere passes through the focus, it will emit scattered or fluorescent beams; They are collected in photodetectors (photomultiplier tubes or solid-state devices) through optical filters and mirror systems. The photoelectric detector converts the scattered light into electrical signal quantitatively, then digitizes the scattered light into integer through digital converter, and then stores, displays and analyzes the data electronically; Flow cytometry is a modern analytical technology, which can qualitatively or quantitatively detect the physical, physiological, biochemical, immunological, genetic and other characteristics of cells. Molecular biology and the functional state of cells or microspheres integrate electronics, computer technology, laser technology and fluid theory [5].

Flow cytometry is a cell screening technology developed in recent years. According to its application, it can be divided into flow cytometer and flow cytometer [6]. The technical principle of flow cytometry is to combine the antigen or anti antigen labeled microspheres with the second fluorescent labeled antibody microspheres. The number of antigen or antibody molecules to be detected has a linear relationship with the fluorescence intensity. It is understood that flow cytometry has been introduced into China hundreds of laboratories, flow cytometry has become an optional field of life and an important research tool for routine screening and testing, and in some leukemia diagnosis, AIDS monitoring and other clinical applications are also more and more widely. Tsinghua University AIDS Research Center believes that flow cytometry is an irreplaceable tool in AIDS surveillance. Doctors can accurately determine the absolute value of CD4 and lymphocyte to judge the course and therapeutic effect of AIDS, which will have wide practical significance for the control and treatment of AIDS [7].

4. Correlation Analysis between Flow Cytometry and Single Cell Analysis

In recent years, researchers have combined flow cytometry with mass spectrometry to develop a

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flow cytometry that can simultaneously analyze more than 40 cell parameters at the single cell level. This improves the ability of flow cytometry to evaluate complex cellular systems and processes. The Journal of cell published the Journal of flow cytometry: single cell, multi-function, which introduced the current situation, related instruments, main applications, data analysis methods and development prospects of mobile cell mass spectrometry. The development of life science and technology has two directions: one is to increase the number of cell characteristic analysis and analyze different cell characteristics at the same time; The second is to improve the analysis resolution and observation accuracy. In recent decades, the development of flow cytometry has met these two requirements by accurately analyzing the various characteristics of a single cell and helping scientists understand the molecular mechanisms in complex or multi-stage cell systems [8-9].

Flow cytometry is shown in Figure 2, which is composed of analysis system, electronic system, optical system and liquid flow system. It can only detect suspended particles or single cell signals. After fluorescent staining of particles or cells to be tested, prepare suspended samples. The samples to be tested are pressed into the flow chamber under the action of air pressure. No particle buffer or cells pop out of the sheath under high pressure to form a circular flow [11]. Wrap the outer membrane, arrange the cells to be tested in turn, and then generate fluorescent signals through light excitation through the detection area of flow cytometry. Flow cytometry uses laser as laser light source to focus the reconstructed beam to realize the vertical irradiation of sample flow. Under laser irradiation, fluorescent cells produce fluorescence and light scattering. Light scattering signal Figure 2 flow cytometry configuration

Probe forward at a small angle. This is a kind of forward scattering, which can reflect the size of the unit. The intensity of fluorescence signal can reflect the intensity of cell membrane surface antigen. After receiving the photomultiplier tube, the electrical signal is converted into an electrical signal. The mode converter is then used to convert continuous electrical signals into digital signals that can be recognized by the computer. The computer calculates, processes and collects various measurement signals, displays the results on the computer screen, prints or stores them on the hard disk for in-depth analysis [10-12].

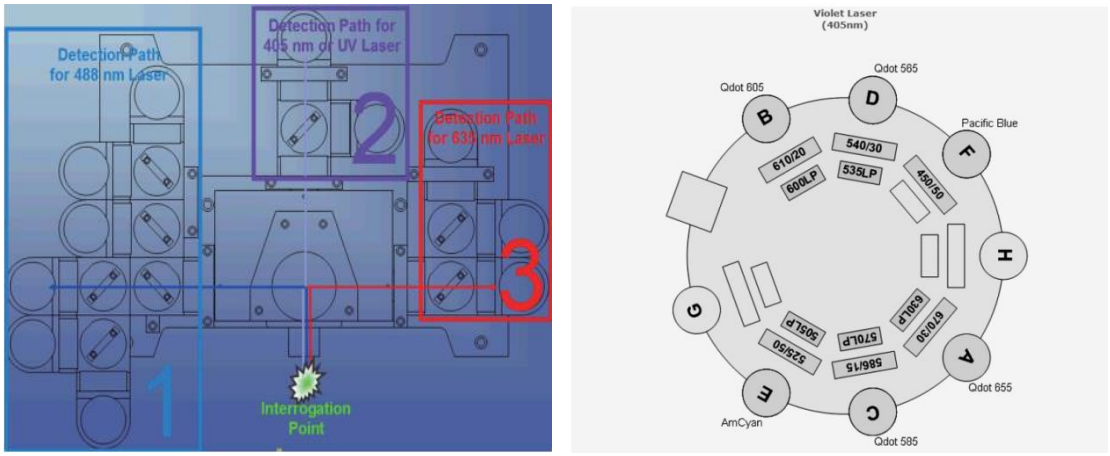

Figure 2: Configuration of flow cytometry

Due to the diversity or heterogeneity of environment or cells, single cell or single molecule methods usually appear in vivo and are difficult to carry out extensive laboratory analysis [13-14]. It can be said that with the development of modern biology, the word "average" can no longer meet our needs. Taking single-cell sequencing as the development direction of current cell biology and molecular biology, combined with multi omics related research such as genomics, transcriptome, translatome, proteomics and metabolomics, it is necessary to develop more sensitive single-cell separation technology. Therefore, the combination of flow cytometry and single cell technology is the general trend. When the two technologies continue to develop in their own depth, the combination of the two becomes logical. As now, flow cytometry combined with mass spectrometry technology inherits the high-speed analysis characteristics of traditional high-resolution mass spectrometry and flow cytometry, which can better serve the separation and identification of cells. At the same time, it also shines in the field of single-cell metabolome and spatial transcriptome. It is a new development direction of flow cytometry technology [15-16].

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5. Conclusion

The greatest advantage of flow cytometry is multicolor and multi parameter detection. It can detect proliferation and other parameters at the same time. It can also detect the proportion of specific cell populations and the characteristics of single cells in mixed samples. Different laser detection channels are used according to different coloring principles. The experimenter can choose according to the laser structure and specific experimental scheme of flow cytometry. With the development of single cell detection technology, it is an inevitable trend to obtain more biological information from the same sample. Combined with fluorescence detection, mass spectrometry and high sensitivity testing methods, it will provide high flow real-time analysis and monitoring of various metabolites in different types of cells in the future. Flow cytometry combined with single cell analysis will become a new and powerful practical technology for the high-throughput development of life science.

References

[1] Meng Qingnan, Li Xiaoying, Xiong Xuelian Single cell transcriptome technology and its application in adipose tissue research . International Journal of Endocrinology and metabolism, 2022,42 (01): 27-31

[2] Peng Juqin, Ren Junguo, Gao Zhuye Application of single cell transcriptome sequencing technology in the study of cardiac protective mechanism of traditional Chinese medicine after myocardial infarction Chinese Journal of experimental prescriptions, 2022,28 (05): 242-249

[3] Zhao Lei Transcriptome and epigenetic analysis of circulating tumor cells based on single cell technology . East China Normal University, 2021

[4] Xiong Heli, Sha Xi, Liu Shaona, Xiang Decai, Zhang Bin, Zhao Zhiyong Application of single cell transcriptome sequencing technology in animals [J / OL] Biotechnology Bulletin: 1-8 [2022-03-07].

[5] Cai Lin, Gong Qiuyuan, Wang Wei, Tian Hengli The latest progress of single cell sequencing analysis technology in the study of phenotypic heterogeneity of microglia . Chinese Journal of Neurosurgery, 2021,7 (03): 156-160

[6] Xiao Lei Xin Hongyi, a long-term teaching assistant professor of Shanghai Jiaotong University: the integration of information science and biomedicine promotes the application of single cell testing technology . China Hi tech, 2021 (11): 19-20

[7] Feng Chao Study on microenvironment heterogeneity of liver cancer and susceptibility of gastric tissue to new crown based on single cell transcriptome . Academy of Military Sciences, 2021

[8] Liu Jian Study on heterogeneity and related genes of cervical malignant tumors based on single cell transcriptome sequencing . Jilin University, 2021

[9] Wu Zihan Application of imaging flow cytometry in algae research . Shenzhen University, 2019

[10] Liu Dejing Screening, optimization and preliminary application of nucleic acid aptamers for marine pathogen Vibrio vulnificus . Second Military Medical University, 2017

[11] Sun Shaojun Study on the diagnostic value of urine dry chemical analysis and flow cytometry in urinary tract infection . Suzhou University, 2016

[12] Kang Zhihong Application of flow cytometry in clinical examination . Seeking medical advice (second half of the month), 2012,10 (11): 861-862

[13] Liu Guangda A new in vivo flow cytometry method for real-time monitoring of tumor cells in circulatory system . Fudan University, 2011

[14] Sha Wei, Sha Li, Xia Yiping, Guo Weina On flow cytometry in clinical medical examination . Chinese and foreign medical, 2011,30 (12): 186

[15] Li Jun, Hao Hongjun, Gao Feng, Huang Yining Clinical application prospect of flow cytometry in detecting T lymphocyte subsets in cerebrospinal fluid . Chinese Journal of neuroimmunology and neurology, 2011,18 (02): 129-131

[16] Julie Flow cytometry promotes the rapid development of life science. Science and technology daily, 2010-01-28 (010)