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AN OVERVIEW ON FLOWCYTOMETRY

Jyothi P*, Geetha K, Ajitha A, Uma Maheshwara Rao V

Department of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

Flowcytometry is a powerful technology mainly used for investigations in cell biology and isolation of individual cells.It is a laser based biophysical analytical technique used mainly for cell sorting, cell counting, biomarker detection and protein engineering. It is one of the high powerful, sensitive and specific for simultaneous estimation of multiparameters of individual cells in heterogeneous suspension. Flowcytometry can analyze thousands of particles per second. In flowcytometry, analysis is done based on physical interaction of particles with laser light. It is used both for single cell analysis and multi cell analysis. Now a day's flowcytometry has become an essential tool in the clinical field for diagnosis of different types of diseases. Because of this analytical capability, flowcytometry become an integral part in medical research centers .At the present time flowcytometry is available as a new and specialized flowcytometer called High Throughput Pharmacological System(HTPS).It is mainly used for high throughput analysis of 9-10 samples/minute. The flowcytometry is applicable to few branches of sciences both alone and in combination with other methods. It gives new and valuable results in clinical field that could not be obtained by any other analytical methods.

Keywords: Cell sorting, Laser based technique, High Throughput analysis, Single cell analysis.

INTRODUCTION

Flow ctometry means- Flow~ cells in motion, Cyto~cell, Metry~measure, measuring the properties of cells in a fluid stream. Flowcytometry is a laser based biophysical technology employed in cell sorting, cell counting, biomarker detection and protein engineering by suspending the cells in a stream of fluid and passing them by an electronic detector apparatus. The modern flowcytometry begins in late 1940 and 1950 of Wallace coulter's invention of coulter counter. Later in 1970, Mack Fulwyler, Louis Kamentsky, Wolfgang Gohdeand Len Herzenberg's group at Stanford fined a new detection technology. It measures size and granularity by light scatter and fluorescence. This system termed Fluorescence Activated Cell Sorting (FACS).Later it is referred as flowcytometer. Now it becomes the essential part in the medical field. It gives the simultaneous estimation of multiparametric analysis of the single cell. Flowcytometry allows analysis of both physical and chemical analysis of thousands of particles per second. Physical parameters estimated based on their physical interaction of particles with laser light, measured at same wavelength as laser. Flowcytometry measures physical parameters like relative size, relative granularity (or) internal complexity of particles. Chemical parameters measured based on signals from reagent that interacts with a laser light. It measures chemical parameters like relative fluorescence intensity.

Theory Involved

Prepared single cell preparation or a suspension of the cells is necessary for analysis. During the preparation of samples the antigens attached with various immune fluorescent dyes (or) antibodies of interest. The prepared samples are injected in to a flow cytometer; the particles are randomly distributed in three-dimensional space. In this case it is necessary to make them in to a stream of single particle file. For this purpose fluidic systems were used. This process of creation of single particle file by using the fluidic system is called as hydrodynamic focusing. The fluidic system contains the central channel (or) core and

Corresponding Author:- P.Jyothi Email:- Jyothipodachowdary@gmail.com

outer sheath that contain faster flowing fluid. If sheath fluid moves it creates the drag effect on the narrowing central chamber. This alters the velocity of the central fluid whose flow front becomes parabolic with great velocity at it center and zero velocity at the wall. Because of this effect the particles are arranged in a single file. This narrow fluid stream passed through the laser beam one at a time. After focusing with the laser beam some of light absorbed or scattered by the cells. The absorbed light of particular wavelength is re-emitted in the form of fluorescence due to the presence of the naturally fluorescent substances in the cell (or) fluorochrome labeled-antibodies on the surface of the cell or internal cell structure. Usually fluorescein isothiocynate (FITC), phycoerythrin, peridinin chlorophyll protein, allophycocyaninare commonly used fluorochromes in biomedical field. The absorbed or scattered light is detected by detector and amplified. Flowcytometer contains the optical filter. It absorbs the unwanted light permits only the absorbed light of particular wavelength. The data is stored, analyzed and displayed on computer-system. The results represent the quantitative information of every cell analyzed by the system.

Instrumentation

The flow cytometer mainly contains three components: fluidics, optical system and electronics.

1) Fluidic systems make the samples in to a single file of stream and transport to the laser beam of interrogation.
2) Optical system contains both laser beams and optical filters. The laser light illuminates the light to the sample stream. The optical filters absorb the unwanted light and permit the resulting absorbed light singles by the samples to detection system.
3) Electronics converts the absorbed light signals into electric signals by using the computer system.

Fluidics

It is one of the major components in flow cytometer. The main purpose of fluidic system is to change a particles stream from three dimensional to single file in order to transport to the optical system for interrogation. In order to provide the optimal illumination, the stream contains the particles should be positioned in the center of the laser and only one cell should be passed through the light at a given time. For this purpose the sample is placed in the center channel. This is enclosed by the outer sheath that contains fluid. If this sheath fluid movies, it creates a massive drag effect in the central chamber. This changes the velocity of the central fluid. In the center it flows with greater velocity and zero velocity in the walls. This effect creates a single file of particles. It is called as hydrodynamic focusing. The flow characteristics of the central fluid can be calculated by using Reynolds number (Re).

$$Re = pVD/\mu$$

Where D = Tube diameter,

V = mean velocity of fluid,

P = density of fluid, and

μ = viscosity of fluid.

The proper working of fluidic system is important for particles in order to intercept the laser beam. Therefore it is necessary to make ensure the fluidic system should be free from air bubbles, debris. The pressure should be adjusted by using the sample pressure settings. For increasing or decreasing the sample pressure by changing the width of the central core. Flow rate is directly related to the pressure. If pressure increases the flow rate also increases and if pressure decreases the flow also decreases. Usually higher flow rate is used for immunophenotyping measurements and lower flow rate is used for DNA analysis.

Optical Systems

1) The optical system in flow cytometer mainly contains two different types of optics-Excitation optics and Collection optics.
2) Excitation optics contains laser and lenses to shape and focus the laser towards the sample stream.
3) The collection optics contains collection lenses to collect the emitted radiation from particles-laser interaction. It also contains the mirrors and filters to absorb the un-wanted radiations and transfer the emitted radiations to the electronics.
4) In order to provide these entire functions optic bench is designed.

Optical bench

The optical bench in the flow cytometer gives stable surface that contains light source, excitation and collection optics in fixed positions. The beam from light sources is entered in to the excitation optics. In excitation optics the light is shaped and arranged in to an elliptical manner then enters in to the focusing lens. From focusing lens the light focused in to the central core. The particle absorbed the some of light. The absorbed light is emitted in the form of fluoresces. This emitted light is collected by collection lens. Collection lens absorbed the un wanted light and transport the absorbed light of particular wavelength to electronic system.

Laser(Light Amplification by Stimulated Emission of Radiation)

Lasers are most commonly used light sources in flowcytometry. Lasers produce a single wavelength of light at one or more different frequencies. Now a day's all instruments fitted with an Argon laser .It provides the blue light at 488nm.Other than this He-Ne, it gives red light at 633nm and He-Cd-Uv at 325nm also available. In case of bench top flow cytometer, laser is fixed at 488nm and second laser red-diode laser is also available. This is a solid state laser it emits at 635nm.In flow cytometer, the

laser light is focused on the central channel/core. This point must be constant. So to maintain this intercept point the laser head is held in a fixed position. The beam from the laser passes through an optics that make the beam in to elliptical shape. From this optics it enters in to focusing lens. The focusing lens focuses the beam at the point between the laser and central core.

Optical Filter

When laser beam passes through the cell or particle, it emitted the light in the form of FSC, SSC and fluorescence signals. These SSC and fluorescence signals are transport to the photomultiplier tubes and a FSC signals are transport to photodiodes. These signals transport to detector trough mirrors and optical filters. These filters absorb the unwanted light signals and allow only the narrow range of wavelength to the detector. The flow cytometry mainly contains 3 types of filters.

1) Band pass filters (BP): These type of filters used when spectral band of light is close to the emission peak of the fluorescent dye.Eg:530±15,it transmit wavelengths of light that are between 515nm and 545nm.
2) Short pass filters (SP): It transmit wavelength of light equal to or shorter than a specified wavelength.Eg:500SP, it transmit all wavelength less than 500nm.
3) Long pass filters (LP): It transmits the wavelength of light equal or longer than a specified wavelength.Eg.600LP, it transmits all wavelengths more than 600nm.

Electronics

When particles pass through the laser beam in a fluid stream generates the light signal. These light signal transport to the detector by filters. Here the filters absorb un-wanted light and transport only the light signals of particular wavelength. The detector converts the light signal in to electric signals or voltages. The detectors used in flow cytometry are photo detector like photodiode and photo multiplier tube (PMTs). The photodiode detectors are less sensitive to light signals when compared to the photo detectors. There for these type of detectors are used for FSC signals. In case of PMTs are more sensitive for light signals so these are used for SSC and fluorescence.

Photo Multiplier Tube (PMTs)

The particles when exposed to a beam of laser light, it absorb the sum of light and emitted it in the form of light signals or photos. These light signals or photos are strikes to the one side of PMTs then they converted in to proportional number of electrons these are multiplied and creates a greater electrical current. These electrical current converts in to voltage pulse in the amplifiers. Here the amplification done by either linear or logarithmic base. The log amplification can be used for separation of negative from dim positive signals. Whereas, linear amplification used to amplify the scatter and fluorescence parameters.

The voltage pulses are converted in to digital value by using Analog to Digital Converter (ADC).it converts the pulses in to channels. Eg: ADC converts 0-1000mv of pulses in to 0-1000mv of channels. This channel number transferred to the computer system. Finally the light signal displayed in the form of digital values.

Computer/Software

The digital values received by the computers are processed by using software. The software translates the digital values in to histogram or pictures, usually single or dual picture plot. The commonly used software programs include Cell Quest, Flowjo, Win Mind and FC Sexpress. The data file of flow cytometry conforms the standards of FCS(Flow Cytometry Standard).The file structure permits the storage of raw data, information about samples and information about how it was collected. The file structure allows the users to combine other pieces of information to get logical information. The other property of flow-software is ability to give information of interest within one histogram and then to gate or restrict a histogram displaying data for other parameters. When analyzing 2 or more colors of fluorescence the emission from one fluorochrome may interfere with other channel of fluorochrome. This can be avoiding by a process called as compensation.

Types of plots

1. single colour histogram: Fluorescence intensity Vs count.
2. Two colour DOT plot: Fluorescence intensity of parameter 1 Vs fluorescence intensityof parameter 2.
3. Two colour contour plot: Fluoresence intensity of parameter 1 Vsfluorescence intensity of parameter 2. Concentric rings from around populations.The more dense the population,the closer the rings are to each other.
4. Two colour density plot: Fluoresence intensity of parameter1 Vs fluoresence intensity of parameter 2. Areas of higer density will have a different colour then other area.

Working

In flow cytometer,the cells or particles of 0.2 -150 µm size are applicable of analysis.after preparation of samples for analysis,they are placed in the central core of fluidic system because these samples are distribute in a three dimensional pattren.The fluidic system makes the sample preparation in to a singlefile for laser interrogation.The sample file passes through the laser for interrogation.the sample cell or particle absorb the some of the laser light and starts scatering in forward and side direction in the form of FSC and SSC. If any natural or systhetic fluorochrome present in the sample,they emitt the

light in the form of fluoresence.These scattered and fluoresence light enterd in to the filters they absorb the un wanted light from scattered and fluorescense light.They transferd the light singles of particular wavelength to detector, usually PMTs are used.Here the light signals are converted in to the voltage signals these are amplified.these voltage signals are converted in to digital values by ADC(Analoge to Digital converters).The digital values are collected and stored in computer system.The data can be analysed to by using different softwares to get information about the sample cell in the preparation.These flow-software gives the data in the form of histograms or plots.These histograms or plots gives the information of sample cell.

APPLICATIONS

Pharmaceutical Applications

1) Flow cytometry is used for dicovery and identification of molecular hostpot.
2) The auto-fluorescent properties of photosynthetic plankton can be explained by flow
cytometry in order to characterize abundance and complexity structurte.
3) Flow cytometry is used in conjunction with yeast display and bacterial display to identify Cell-Surface-Display-Protein variants with desired proteins.
4) Used in preclinical testing of drugs and chemicals in the pharmaceutical & chemical industries.
5) Unidisperses and poly disperses suspensions characterized by flow cytometry.
6) Used for calculating the liposome size distribution.

Clinical Applications

1) Monitoring AIDS patients**.**
2) Monitoring immune phenotyping Leukemia, Lymphomas.
3) Monitoring organ transplant patients for rejection.
4) DNA-analysis of S-phase fraction of solid tumors.
5) Used for conforming diagnosis of chronic lymphocytic leukemia.
6) Measurement of efficacy of cancer therapy.
7) Platelet function analysis.
8) Cell function analysis.
9) Chromosomes analysis.
10) Protein expression, localization and protein modifications.

Research Applications

1. Auto fluorescent proteins.
2. Antigen or ligand density.
3. Analysis of apoptosis.
4. Measures the enzyme activity.
5. Determination of DNA&RNA content and changes in the cell-cycle.
6. Measures the membrane potential.
7. Analysis of drug uptake& efflux.
8. Detection of phagocytosis.
9. Detention of changes in intracellular P^H, Calcium, Glutathione.

Other Applications

1) Microbiology

Flowcytometry applied in bacteremia and bacteriuria for rapid detection of microorganisms on the bases of its cytometric characteristics. It also used for detection of slow growing microorganisms like mycobacteria and fungi.

2) Serological diagnosis:

Flowcytometry mainly used for diagnosis of acute Hepatitis C Virus (HCV).It is used to measure the number of infected cells.

3) Anti-microbial effects:

Anti-microbial effects of a microbial agents on bacteria an be studied based on membrane potential, cell size and amount of DNA.

4) Urine samples analysis:

Flow cytometry used for analysis of urine samples for detection f urinary tract infections.

RECENT DELEVPMENTS IN FLOW CYTOMETRY

1) Development in light source (Polychromatic flow cytometry)

This is recently developed advanced technology in flow cytometry. This type of modern flow cytometry instrumentation contains multiple lasers and fluorescence detectors**.** For this development, the modern flowcytometry can used in all fields like molecular biology, pathology, immunology, plant biology and marine biology etc. Due to the presence of more number of lasers and detectors these flow cytometry used for multiple antibody labeling and identify more precisely a target population by phenotypic expression of fluorescent markers on the surface or intracellular.

2) MACS Quant analyzer

This is a new seven colour flowcytometry. This instrument contains three lasers and seven fluorescence channels. These instruments collect the sample by capillary action and small amount of sample is sufficient for analysis. The analysis rate up to 10,000 cells per second. This instrument provides the absolute cell counting and sheath fluid consumption also low around 2-10ml per min.

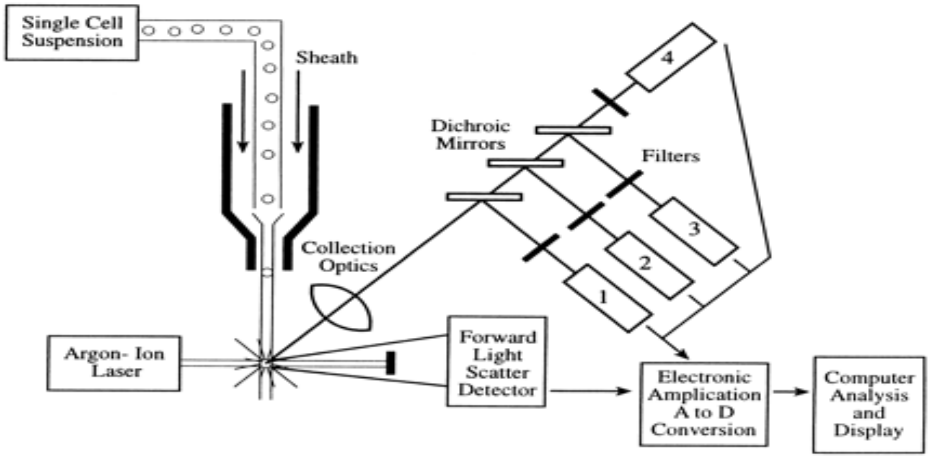
3) Dielectrophorosis flow cytometry

In this type of technology the cell behavior can be developed by micro patterned surfaces, so the cellular properties may be investigated in both single cell and large population. The cell behavior can be changed based on its dielectrophoresis property. Dielectrophorosis manipulation along with flow cytometry gives accurate and high throughput cell sorting. It is used for study of small cell lung cancer.

Limitations

1) Very expensive and sophisticated instrument.
2) Difficult to operate.
3) Need single file particle motion.
4) Tissue architecture is lost.
5) Limited information of intracellular distributions.
6) Special training is necessary for handling.
7) Time consuming process.
8) Special sampling techniques is needed when natural fluorocromes are absent in the cell.

Fig 1. Instrumentation of Flow cytometer



CONCLUSION

Now a day's flow cytometry is a relevant technique used for diagnosis of different diseases so it is used in al clinical fields. Because of this capability, flow cytometry became an integrate part in al medical research centers. It is used in some branches of science field either alone or in combination of other methods. It is safe, rapid, sensitive, specific and user friendly. It appears expensive but because of its capability in drug discovery, diagnostic and therapeutic applications in hematology and immunology it can be afforded. The recent advancements in flow cytometry like mass spectroscopy with single cell flowcytmetry, it is an example of technology merges.at present new and sophisticated flowcytometry avalible called as High Throughput Pharmacological System(HTPS).It is used mainly for high throughput analysis of samples.

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