THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
Popular Article
Sept, 2023; 3(09), 2270-2274

Shining Light on Cellular Magic: The Story of Fluorescence Activated Cell Sorting

Dharamshaw C.A.*, Sylvia Lalhmingmawii, Ishmeet Kumar
Ph.D. Scholar Animal Genetics and Breeding Division
ICAR-National Dairy Research Institute, Karnal, Haryana
https://doi.org/10.5281/zenodo.8346744

Imagine being able to peek into the microscopic world of cells, each of which is an individual world with a variety of distinct features and purposes. Now, envision not only seeing these cells but also having the ability to sort and select them based on their individual characteristics. This remarkable feat is precisely what Fluorescence Activated Cell Sorting (FACS) accomplishes, and it forms the nucleus of our exploration into the captivating world of cellular sorting.

Flow cytometry analyses the cell population according to their physical properties such as size, shape and volume as well as their surface molecules and enables their individual investigation. FACS can be used to sort live cells based on similar principles. It allows to unravel the mysteries hidden within the diverse populations of cells that make up living organisms. Protein specific fluorescent dyes are used to stain the cells for analysis and automatic sorting according to the characteristic wavelength of light scattered by them.

The FACS systems have components to automatically detect, analyze and sort the cell samples loaded in to it.

THE FLUIDIC SYSTEM:

The fluidic system is a crucial part of FACS. It controls the movement of the cell sample suspended in a sheath fluid. This facilitates a smooth laminar flow within the system. Cells are properly lined, spaced and delivered (hydrodynamic focusing - layers of liquid at the outermost edges have zero velocity, while the center runs at a maximum velocity) to the laser for precise analysis and data collection. The fluidics system also helps in transporting sorted cells to their respective collection containers.

THE OPTIC SYSTEM:

They contain the lasers, lenses and filters. for selective emission of light and assimilation of the fluorescence emitted by the tagged cells

i. Laser: Lasers are used for shining beams of light of specific wavelengths uniformly on to the flowing sample within the system. Depending on the dye the cell has been tagged with, they will emit specific colours and allows for differentiating them. Depending on laser wavelength, different dyes are used. E.g., UV light (355nm) - DAPI, brilliant UV: Blue light (488nm) - FITC, Dylight 488.

Lasers may be parallelly arranged so that such that cells only encounter one source of stimulation at a time. While in a colinear arrangement lasers travel along the same optical path causing multiple lasers to simultaneously stimulate the cells.

ii. Filters: After the laser hits the cells, the machine has optics (like lenses) and filters that capture the fluorescent light emitted by the tagged cells. Filters are used to select specific colours of light.

Long pass (LP) filters: Transmission of photons above a specific wavelength. e.g., LP 500 filter. All lights above 500nm will pass.

Short pass (SP) filters: Transmission of photons below a specific wavelength. e.g., SP 500 filter. All lights below 500nm will pass.

Band pass (BP) filters: Transmission of photons within a specific wavelength. e.g., 525/30 BP filter. Light of 30nm below 525nm and 30nm above 525nm will pass

ELECTRONICS SYSTEM:

It is composed of the detectors, amplifiers and the computer. They evaluate the quantity and quality of the light emitted, *i.e.*, the intensity and wavelength (colour). The detectors are used to "see" the fluorescent light using photoelectric effect. The amplifier sends these signals to the computer as proportionally sized voltage pulses. The computer analyses this data to determine which cells are tagged with fluorescent molecules and how bright they glow.

Commonly used detectors are:

Photodiodes: Used for strong signals like forward scatter. When photon hits the photodiodes, it ionizes the atoms of detectors, creating a photocurrent which is directly transferred to the electronic system.

Photomultiplier tubes: Used for detecting small amounts of fluorescence and side scatter. It is sensitive to low levels of signals.

SORTING MECHANISM

If you want to sort cells, FACS machines have a way to do that. Electric charges or air pressure is used to push or divert cells into different containers based on the characteristics identified by the computer.

The entire procedure can be summarized as follows:

1. Preparation of Cell Sample: cell samples like tissues, blood, or cell cultures are ensured to be in a single-cell suspension, meaning they are separated and not clumped together.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published: **11.09.2023**

2. Fluorescent Labelling: Fluorescent molecules called fluorophores or fluorescent tags, are attached to specific parts of the cells. These tags can be designed to bind to particular proteins, DNA, or other cellular components. Each type of tag produces specific fluorescence when illuminated by a laser.
3. Introduction into the FACS Machine: The labelled cell sample is introduced into the FACS machine. The machine fluidic system controls the flow of the cell suspension, ensuring that cells pass in single file through the laser beam for analysis.
4. Laser Illumination: A laser is used to shine a beam of light onto the passing cells. Wavelength of the laser should be corresponding to the fluorescent tags. The resulting fluorescence of the tags are captured by the machine.
5. Optical Detection and Analysis: The emitted light is captured by detectors, which measure the intensity and colour of the fluorescence. This information is then sent to a computer for analysis. By analysing the emitted light, the computer can determine which cells are tagged and how brightly they fluoresce.
6. Sorting Decision: Based on the analysis, the computer makes real-time decisions on whether a particular cell meets the specified criteria for sorting. If so, it sends a signal to the sorting mechanism.
7. Sorting: The sorting mechanism, often using electrical charges or air pressure, guides the cells to different collection containers. This allows researchers to physically separate and collect specific cell populations based on their fluorescent characteristics.
8. Post-Sorting Analysis (Optional): After sorting, researchers may perform additional analyses on the sorted cells to validate their characteristics or perform further experiments

.

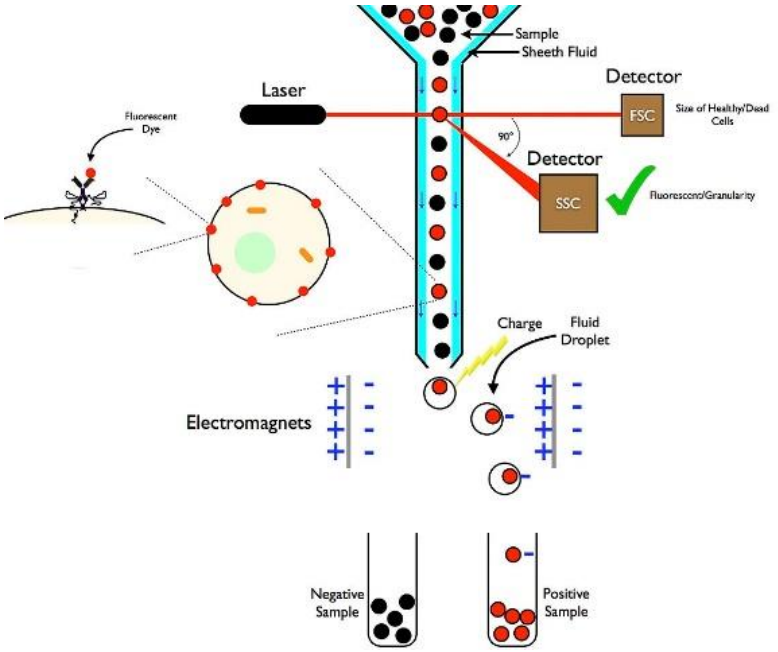

Figure 1: Fluorescence Assisted Cell Sorting (FACS) showing positive cell selection

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published: **11.09.2023**

ANALYSIS

Depending upon the direction, the scattered light is of different types

i. Forward scattered light (FSC): It is the light refracted by a cell in the forward direction. This signal is collected by a detector called the forward scatter channel (FSC), measured and is used to determine particle size. Bigger particles will produce more forward scattered light than smaller ones and vice versa.

ii. Side scattered light (SSC): it is the light refracted by cells and travels in a different direction than its original path. Cells with a low granularity and complexity will produce less SSC, while highly granular cells with a high degree of internal complexity (such as neutrophils) will result in a higher SSC because granules inside the cell will also refract the light.

Table 1: Different lasers and tags used

Laser line	**Wavelength**	**Common fluorophores**
Ultraviolet (UV)	355 nm	DAPI, Hoechst, Brilliant Ultraviolet
Violet	405–407 nm	Pacific Blue, eFluor 450, Pacific Orange, Super Bright 436, Super Bright 600, Brilliant Violet
Blue	488 nm	FITC, Alexa Fluor 488, Dylight 488, PE, PE tandems, PerCP, PerCP tandems, PI, 7AAD, eGFP, YFP
Green	532 nm	PE, PE tandems, Alexa Fluor 532, PI, mCherry, dTomato, RFP
Yellow	561–568 nm	PE, PE tandems, PI, mCherry, dTomato, RFP
Red	633–647 nm	APC, Alexa Fluor 647, Alexa Fluor 700, APC tandems

RESULT PRESENTATION:

The results of a Fluorescence Activated Cell Sorting (FACS) procedure are typically presented in a graphical format on a computer screen. The presentation may include several key elements:

i. Scatter Plots: Scatter plots are commonly used to display FACS data. These plots show two parameters, often forward scatter (FSC) and side scatter (SSC). Each dot on the plot represents a single cell's size and granularity.

ii. Fluorescence Histograms: Histograms display the distribution of fluorescent signals emitted by the cells based on intensity. Peaks in the histogram indicate different populations of cells with varying levels of fluorescence.

iii. Overlay Plots: Overlay plots combine data from multiple fluorescent channels. They allow visualization of relationships between different markers on the same set of cells.

iv. Gating: Gating involves drawing regions or gates on the scatter plots and histograms to select specific populations of cells based on their size, granularity, and fluorescence intensity.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published: **11.09.2023**

v. Back-gating: It is a method of identification of cells to confirm a staining pattern or gating method using different parameters This can be useful if we are unsure of results and need additional information to identify our cells

vi. Statistics and Summary Metrics: The software may provide summary statistics such as the total number of cells analyzed, the number and percentage of cells falling within each gate or population, mean fluorescence intensity and other metrics relevant to the experiment.

vii. Graphical Representation of Sorting Decision: If the FACS machine is used for sorting, the software may display a graphical representation of how cells were sorted into different collection tubes or plates.

APPLICATIONS IN BIOTECHNOLOGY

i. Physiological research: Cell viability is the most widely used parameter for detection. It is possible to discriminate between undamaged, damaged (membrane depolarized) and dead cells.

ii. Cell Line Development: FACS is pivotal for the development and selection of high-producing cell lines and studying the properties and potential therapeutic applications of different types of stem cells. It enables the isolation of cells with desired characteristics, such as higher productivity or specific protein expression levels.

iii. Screening for enzymatic activity: Intracellular enzyme evolution with the aid of FACS can be screened for mutants. An example was demonstrated by the detection of aminoacyl-tRNA synthetase finally leading to the incorporation of unnatural amino acids into proteins

iv. Immunology and Immunotherapy: It plays a critical role in the development of immunotherapies by isolating and characterizing immune cells for therapeutic applications.

v. Cancer Research: It allows for the isolation and analysis of specific cancer cell populations, aiding in the understanding of tumour biology and the development of targeted therapies.

vi. Microbiology and Microbial Ecology: FACS can be utilized to study and sort microbial communities facilitating research in areas like bioremediation and environmental microbiology.

CONCLUSION

Fluorescence Activated Cell Sorting (FACS) stands as a transformative tool in biotechnology. Its ability to precisely analyze and sort cells has wide-ranging applications across various fields. FACS continues to push the boundaries of what is possible in cellular research and plays a nuclear role in advancing biotechnological solutions for human health and beyond.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published: **11.09.2023**