

BD FACS Vantage SE Flow Cytometer

Introduction and Instrumentation Specifications

Flow cytometry is a powerful tool in modern biology and in recent years has become widely used in all branches of biological science. The applications to which it can be applied have expanded rapidly from cell sorting, to measurement of cell surface and intracellular antigens, and the analysis of DNA.

What makes Flow cytometry such a powerful technique, is its ability to measure several parameters on many thousands of individual cells in a very short period of time, by the measurement of their fluorescence and the way they scatter light. This involves shining a laser on discrete groupings of cells. The light passes through the group (forward scatter) or reflects off it (side scatter). The side scatter and forward scatter are measured by photodetectors. Most of the time, fluorescent material(s), tuned to the wavelength of the laser, is/are selectively bound to the cells. The emitted light is filtered out through the use of wavelength-specific light filters, which transmits certain wavelengths of light and reflects others. The reflected light is turned to an electrical signal by photomultiplier tubes (PMTs). All of this data is sorted out by a computer, and plotted onto the computer screen. With a little practice and knowledge, one can tell different types of cells by the characteristics of the data produced.

The BD FACS Vantage SE housed in the IBB Flow Cytometer and Confocal Microscopy Laboratory can perform up to 5-colour fluorescence analysis as well as cell sorting. It is equipped with 2 water-cooled lasers (Coherent Interprise II and Coherent Innova 70C Spectrum and Dye lasers). The optical block consists of 7 PMT's. The COMPENSATION option allows for compensation of the spectral overlap between similar fluorochrome emissions derived from different lasers, such as observed when using PE-CY5 (ex. 488 nm, em 670 nm) and CY5 or APC (ex. 600 nm, em. 660-675 nm). This allows for greater flexibility in choosing possible combinations of fluorochromes to use and in the number of fluorescence signals that can be resolved.

Primary laser fluorochromes
(excitation at 488 nm)

Second laser fluorochromes
(excitation at 351-364 nm)

Third laser fluorochromes
(excitation at 568-647 nm)

Fluorochrome	Emission	Fluorochrome	Emission	Fluorochrome	Emission
FITC	525nm	Cascade Blue	420 nm	Texas Red	613 nm
PE	575nm	Hoechst	465 nm	CY5	675 nm
PE-TexasRed (Red613)	613nm	DAPI	465 nm	APC	660 nm
GFP	515nm	AMCA	460 nm		
Propidium Iodide	600nm				

It is requested that those wishing to do multicolour analysis check with the Johnafel Crowe prior to the experiment to devise the most appropriate fluorochrome/antibody panel. Data Acquisition on the FACS Vantage SE is achieved using BD CellQuest

software and the analysis is performed using the Win MDI program all available within the core laboratory.

Cell Sorting can also be performed on the FACS Vantage SE. Cells (particles) can be sorted at rates up to 25,000 cells/second maintaining sterility to allow for subsequent culturing of the sorted cells. The FACS Vantage SE uses electrostatic droplet sorting by the generation of droplets from the core stream. This produces a stable line of droplets at 10,000 to 60,000 Hz, depending upon nozzle size. Larger cells use a larger nozzle and lower drop-drive frequencies and so are sorted at lower optimum rates. To prevent distortion of the laminar flow of the sample/sheath fluid and disruption of the stable droplet break-off (clogging of the Nozzle), it is required that ALL samples be filtered prior to sorting. Sterilized nylon mesh (20 - 70 μ M pore size) can be used. The number of cells that can be sorted depends upon factors such as frequency of events to be sorted, total number, required purity and these can be discussed prior to sorting. Sheath fluid used is sterile 0.9% Sodium Chloride or PBS. Sterilization of the cell sorter is performed as required using 10 % bleach, 70 % ethanol and distilled water.

BD FACS Vantage SE Flow Cytometer Operation and Guidelines

Policy

Projects:

All new users should meet with Johnafel Crowe (Office: 404.894-2212, johnafel.crowe@ibb.gatech.edu) to discuss their project **BEFORE** they use the instrument. An abstract or brief description of each user's project involving flow cytometry should be discussed in person or forwarded to johnafel.crowe@ibb.gatech.edu. At that time objectives and goals of the user can be discussed as well as the best means of achieving these objectives. **Important note:** If projects involve potential biohazards, the user must inform Johnafel and supply protocols for decontamination at this time.

Instrument Use:

The cell sorter is a very expensive and sensitive piece of equipment. We expect all users to be properly trained before they use this instrument. This is done after project consultation described above. Visit ibb.gatech.edu/~avesper/confocal/ to schedule a training session. Basic training sessions are administered once a semester or as needed. These sessions usually last about 2 hrs. Basic training session topics include but are not limited to operation of the system, how to acquire, save, transfer data, and shut down the instrument.

User Certification:

Only certified users may operate the instrument unassisted. Certification is attained after a person has attended a Basic Training session and logged 3 sessions with Johnafel.

Who is a certified user?

A certified user is defined as a person who has:

1. Attended a basic training session on the operation of the flow cytometer.
2. Logged **three** sessions with operator assistance.
3. Demonstrated proficiency in shutdown procedures.
4. Agreed to follow all of the guidelines set forth in this document.

Flow Cytometry Sessions:

Each user is required to sign a log before and after each session. Each user is expected to be punctual for their appointments. If you suspect any delays, please contact the core lab. Equipment will be left as clean or cleaner than the user originally encountered it. Users are required to report any instrument problems encountered during each session to the Johnafel Crowe. This action will help to keep the instrument optimized and detecting properly. Each user will create a folder to temporarily store their data. The hard drive connected to the flow cytometer has a limited amount of disk space. **Therefore, users are required to bring blank storage media** (e.g. blank MO disks, ZIP disks) **to each session** or transfer their data to another computer via the GT network **BEFORE** each session is complete. **The core laboratory is not responsible for the backup of user folders.**

Instrument Modifications:

No modifications are to be made to the flow cytometer's optics, fluidics, laser sources, or the attached computer. Only certified Becton Dickinson representatives may make hardware and software modifications to the system.

Reserving Time for Flow Cytometer sessions:

In order to reserve the cell sorter for a specific period of time, each user must: Go to <http://www.ibb.gatech.edu/~avesper/confocal/> and follow the instructions provided. **If you plan to sort cells, you will require operator assistance and MUST start no later than 3:30 P.M.**

IF YOU REQUIRE OPERATOR ASSISTANCE, YOU SHOULD NOTIFY JOHNAFEL AND RESERVE A TIME NO LATER THAN THE DAY BEFORE YOUR APPOINTMENT. In the event of a cancellation or an error when scheduling a time, please notify Johnafel at 404-894-2212; johnafel.crowe@ibb.gatech.edu) as soon as possible and he will make the necessary corrections to the schedule. If the core laboratory has to cancel an appointment, then the user will be notified in a timely manner.

Supplies

The core laboratory is primarily equipped for data acquisition, and on a limited basis, for data analysis. The staff will be available for technical support Monday –Friday between the hours of 9 A.M. and 5 P.M. EST. The core laboratory will provide two types of storage devices: a 650 MB MO drive, a ZIP drive and access to the GT network. We do not supply storage media.

What users should consider purchasing?

Users should purchase the following items prior to their scheduled session:

Falcon sample tubes (Fisher) catalog number 14-959-92B (sterile) or 14-959-6 (nonsterile in bulk)

Nitex 35 micron mesh 3-35/27 (Tetco Inc. (818) 289-9153)

Positive controls, internal standards

Guidelines for samples preparation:

Users must prepare their samples prior to entering the core laboratory. The samples should be free of any clumps or aggregates. **IF YOU ARE NOT SURE, YOU SHOULD FILTER THEM.** The samples should be in some type of buffered saline solution. If the samples are live, they should be on ice. Sample volume, cell size and cell densities are important factors. The minimum volume for running samples is around 0.5 ml considering that you have 0.5 million cells. If possible, your cell concentration should be around 1million cells/ml. Lower cell densities increase the time it takes to run samples. Individual cells should be less than 35 microns in diameter.

Guidelines for data analysis

Most users perform basic data analysis with a program called Win MDI. You can download the program in a PC or Mac format from the Web at <http://facs.scripps.edu/software.html> or (<http://pingu.salk.edu/software.html>).

Safety

Emergency procedures:

In case of an emergency, please contact:

Johnafel Crowe Office: 404-894-2212, Home: 770-210-9145

Steven Woodard Office: 404-894-5891, Cell: 404-725-0023, Home: 770-322-0187

Kay Kinard Office: 404-894-8896, Home: 770-944-0519

In the event we cannot be reached and the matter requires immediate attention, call Georgia Tech Police 404-894-2500. These numbers will be posted near the instrument and on the outside of Room 1326.

Laser Safety

We are operating a type IV laser and there are some precautions. Do not look directly into the beam and do not disable any of the safety features on the flow cytometer. Knock before entering a room with this type of laser. Do not use any type of reflective surfaces when operating this instrument.

Biohazardous Materials:

Please notify Johnafel of any potentially biohazardous samples that are used with the flow cytometer. Any specimen that is either human or primate in nature is considered a potential biohazard. Therefore it is the user's responsibility to perform their data acquisition and analysis in such a way as to not contaminate the instrument.

Furthermore, it is the user's responsibility to provide the facility with decontamination protocols. In the event that the instrument becomes contaminated by the sample or specimen (e.g. blood, sputum or waste products), it is the user's responsibility to notify Johnafel Crowe immediately. All biohazardous waste will be disposed of in red biohazardous bags and placed in a designated waste area. Regular waste pick-ups will be scheduled with the Department of Environmental Health and Safety.

IMMUNOFLUORESCENCE ANALYSIS

DYES and FLUOROCHROMES STAINING PROTOCOLS

Phenotypic analysis of cells using immunofluorescence is the most widely used application of flow cytometry. Antigen expression, both extracellular and intracellular, can be quantitated using fluorescently labelled antibodies or ligands directed towards these biomolecules. Over the past few years, the introduction of novel fluorochromes with unique spectral (excitation and emission) properties has facilitated the use of multicolour immunofluorescence analysis permitting the identification and characterization of specific subpopulations of cells. Furthermore, this has also facilitated the combination of flow cytometric assays for both phenotype and function.

Staining Protocols

There are many protocols for staining cells with monoclonal antibodies for immunofluorescence analysis by flow cytometry. The protocols outlined below are examples and can or may need to be modified for optimal staining of different cell types. Cells can be stained in FACS tubes allowing for larger volumes of wash buffer to be used, which may be important in removal of residual stain.

Directly conjugated antibodies require only single-step incubation and are therefore easier and faster to use. They are also advantageous when performing multicolour immunofluorescence analysis or using several antibodies of the same isotype.

Indirect immunofluorescence staining uses unconjugated primary antibodies, which recognize the epitope/antigen of interest and then a second-step staining with a fluorescently conjugated secondary antibody that will bind to the primary antibody. Care must be taken when using indirect staining with multiple antibodies to avoid cross-reactivity between secondary antibodies and different primary antibodies. It may be beneficial to combine direct and indirect staining techniques with conjugated and unconjugated primary antibodies.

Considerations should also be given to the choice of fluorochrome. Try to match the antibody-fluorochrome combination to the antigen density so use brightest fluorochromes to detect those antigens expressed at the lowest levels.

A. General Immunofluorescence Staining Protocol using Directly Conjugated Antibodies:

1. Prepare single cell suspension and wash in staining buffer (PBS, 2% FCS, 0.1% azide).
2. Centrifuge (300 xg, 5 min, 4oC.), discard supernatant and resuspend to 1 x 10⁷ cells/ml with staining buffer.
3. Aliquot 100 ul of cells into a 12 x 75 mm polypropylene FACS tube.
4. Add 5ul/tube of blocking antibody (e.g. Fc Block).
5. Vortex and incubate for 2 min at room temp.
6. Add monoclonal antibodies, vortex and incubate for 30 min at 4oC (on ice) in the dark.
7. Add 2 ml of staining buffer, vortex and centrifuge (300 xg, 5 min, 4oC).
8. Discard supernatant, resuspend cells in 100 ul staining buffer and add 20ul second antibody.
9. Vortex and incubate for 30 min at 4oC on ice in the dark.
10. Add 2 ml staining buffer, vortex and centrifuge as previous.
11. Discard supernatant.
12. Wash again in 1 ml staining buffer and resuspend in 500 ul staining buffer for FACS analysis.
13. Keep cells on ice prior to analysis.
14. Cells may be centrifuged and fixed in 1 ml of 1% paraformaldehyde (in PBS) at 4oC for analysis next day.

NOTES:

Use buffers without Phenol Red

The blocking antibody step (4 and 5) is optional but should be included if cells express high levels of Fc receptors which will contribute to non-specific binding and background fluorescence.

B. General Immunofluorescence Staining Protocol using Indirectly Conjugated Antibodies:

1. Prepare single cell suspension and wash in staining buffer (PBS, 2% FCS, 0.1% azide).
2. Centrifuge (300 xg, 5 min, 4oC.), discard supernatant and resuspend to 1 x 10⁷ cells/ml with staining buffer.
3. Aliquot 100 ul of cells into a 12 x 75 mm polypropylene FACS tube.
4. Add 5ul/tube of blocking antibody (e.g. Fc Block).
5. Vortex and incubate for 2 min at room temp.
6. Add primary antibody, vortex and incubate for 30 min at 4oC (on ice) in the dark.
7. Add 2 ml of staining buffer, vortex and centrifuge (300 xg, 5 min, 4oC).
8. Discard supernatant, resuspend cells in 100 ul staining buffer and add 20ul second antibody.
9. Vortex and incubate for 30 min at 4oC on ice in the dark.
10. Add 2 ml staining buffer, vortex and centrifuge as previous.
11. Discard supernatant.

12. Wash again in 1 ml staining buffer and resuspend in 500 ul staining buffer for FACS analysis.
13. Add secondary antibody, vortex and incubate for 30 min at 4oC (on ice) in the dark.
14. Wash twice as previous and resuspend in 500 ul FACS buffer.
15. Keep cells on ice prior to analysis.
16. Cells may be centrifuged and fixed in 1 ml of 1% paraformaldehyde (in PBS) at 4oC for analysis next day.