BD Life Sciences FACs Celesta

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Definition and use

Flow cytometry is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles. A sample containing cells or particles is suspended in a fluid and injected into the flow cytometer instrument. The sample is focused to ideally flow one cell at a time through a laser beam and the light scattered is characteristic to the cells and their components. Cells are often labelled with fluorescent markers so that light is first absorbed and then emitted in a band of wavelengths. Tens of thousands of cells can be quickly examined and the data gathered are processed by a computer.

Flow cytometry is routinely used in basic research, clinical practice, and clinical trials. Uses for flow cytometry include: Cell counting, Cell sorting, Determining cell characteristics and function, Detecting microorganisms, Biomarker detection, Protein engineering detection, Diagnosis of health disorders such as blood cancers

A flow cytometry analyser is an instrument that provides quantifiable data from a sample. Other instruments using flow cytometry include cell sorters which physically separate and thereby purify cells of interest based on their optical properties.

System composition

Fluidic system

* Sample inject port (SIP)
* 3 modes. Run (low, medium and high speed)/Standby mode/Prime (purge)

Optics. Generates and collects light

* 16 colours + Forward Scatter (FSC) and Side Scatter (SSC)
* 6 violet (BV421, 510, 605, 711, 786)
* 3 Blue (FITC, BB630, PerCP Cy55)
* 4 Green (PE, PE CF594, PE Cy5, PE Cy7)
* 3 Red (APC, APC R700, APC Cy7)

Electronics. Transforms light into voltage and generates data. FACs Diva Software (files .fcs)

NOTE. The cytometer should be switched on every 1-2 weeks to avoid clogs in the lines, even if we do not need to run any experiment. Simply switching it on (Cytometer and FACs trolley) should be sufficient.

**Start up.**

* Check that we have enough FACs sheath to perform the experiments. In the FACs flow trolley there are two containers (FACs flow on the left and FACs waste on the right). Each of those are connected to a probe that would let us know when the FACs flow solution is low (below 1.5L, recommended to change) or too high (FACs waste).
* Unscrew and screw back the lid of the waste container to release any remaining pressure
* Prime purge in the trolley must be performed every time that the flow or the waste containers are opened or changed
* Switch on FACs Celesta at least 30 min before using it. (keep it on STANDBY mode)
* The order should be as follows. Switch on the FACs fluid trolley (below)-Switch on the cytometer and then the computer/software. It must be done inversely to switch off the system.
* Check that there are no bubbles all over the visible circuit lines of the cytometer or the FACs flow trolley. If so, press prime to purge both in the FACs flow trolley and in the cytometer
* On the right hand side of the cytometer we can find a switch to select tube or plate mode.
* Check that HTS is active on the screen of the cytometer. If not, press “mode” until the HTS appears on the screen and press “up” to switch it on, then press “mode” several times to see the exit screen and confirm with the “up” button
* Set the fine adjustment of the speed to 250 using the up or down button

NOTE. Having HTS on or off will not affect our experiment. Simply keep it on as routine.

* Go to the computer—software DIVA—Administrator—password (no password)
* Prime purge (x2) the cytometer at the beginning of our experiment or if we experience any problem while collecting data. For that, either remove the tube or keep the tube with water and the SIP inserted (we should see some bubbling during the process of prime purging)
* Check that the computer and the cytometer are connected. On the lower right side of the screen it should say “connected”. If not, go to set up—press connect
* DO QUALITY TEST of the cytometer with Cytometer Setup and Tracking beads (CST)

For that, we need CST beads, tubes 12x75mm, FACsflow solution and a vortex

Baseline-----------------------------------------------------------------------------------------------------

Baseline must be done every 6 months or every time we receive a new batch of CST beads

Download data info for the batch of beads we will use in [www.bdbiosciences.com/en-eu/instruments/research-instruments/resaerch-software/flow-cytometry-acquisition/facsdiva-software](http://www.bdbiosciences.com/en-eu/instruments/research-instruments/resaerch-software/flow-cytometry-acquisition/facsdiva-software)

paste the file in D:/BD/FACSDiva/CST/beads lot

Go to software--cytometer—CST—Tool—Beads lots—import—(select beads batch)-press OK

For the baseline, mix up 0.5 mL FACsFlow+3drops of beads

Insert the CST tube—press RUN LOW (Fine adjust 250) in the cytometer—press RUN in the software

When completed, check cytometer baseline report.

We must check that the variance coefficient has a value below 6 in the primary filters. Any altered value will be highlighted in red. NOTE: the 605 filter might show some values highlighted in red, but those are not really a problem as it is sometimes a limitation of the cytometer per se.

Performance check---------------------------------------------------------------------------------

Must be done every 24-48h. Mix in a tube 0.35mL FACsFlow and 1 drop of CST beads---vortex it

As like per the baseline, we must ensure we have the correct data files for the CST batch in use. Go to the software---cytometer—CST---Tools---Beads lot (compare that with the actual batch indicated in the CTS stock solution)

Prepare 0.350mL FACsflow + 1 drop CTS beads

RUN LOW (fine adjustment 250)----go to software RUN

This time the report would analyse the values obtained after the performance check as compared to those of the baseline.

At the end of the process a dialog screen will pop up…”The settings from CTS are different from those on the cytometer. Do you want to use CTS values?”---Press “Use CTS Settings”

After this the cytometer is ready to use.

You are now ready to run an experiment.

Create a new folder with date and name of your experiment

Threshold.

Threshold should be adjusted depending on our interest (200 min, 5000 normal)

COMPENSATION

Compensation is not necessary for single-color experiments. Compensation can be done off-line?

Create compensation controls for multi-colour experiments.

Select Experiment > Compensation Setup > Create Compensation Controls.

Prepare a battery of tubes with a negative control (just to check autofluorescence of your samples) and single labelling for each of the fluorochromes of interest

In the Create Compensation Controls window, ensure that all of your colours are labelled (i.e CD20, CD16…) and that the separate unstained control box is checked. Then select “OK.”

Diva will create a new specimen called “Compensation Controls.” Expand the specimen and make the Unstained Control tube active. Load your unstained specimen on to the instrument and press the RUN button. Then select “Acquire Data” on the Acquisition Dashboard.

Use the unstained specimen. Adjust your FSC and SSC voltages in the Cytometer window to ensure your cells are all on scale. Move the P1 gate so that it contains the majority of your cells

Use the single staining to adjust voltages for other channels of interest

The population of cell with a negative staining should appear on the left part of the histogram (below 10exp3?)

Select Experiment > Compensation Setup > Calculate Compensation.

When the Single Stained Setup box appears, write the name of your cell type/experiment… select “Link Setup & Save” to apply the calculated values to your experiment (overwrite). Load a tube of water on to the instrument and place it in STANDBY

Return to your original sample tube (“Tube\_001”) in the Browser. Return to the blank Global Worksheet by clicking on the Global Worksheet icon in the Worksheet window.

**RUNNING SAMPLES**

Create a FSC-A by SSC-A plot on the global worksheet and create a polygon gate to select your cells.

Create a population hierarchy by right-clicking on any dot plot and selecting “Show Population Hierarchy.” Create other dot plots or histograms as needed for your experiment.

Load your first sample tube onto the instrument and press RUN. Click “Acquire Data”. Adjust your FSC and SSC voltages as needed. Select the number of cells to record in the Stopping Gate settings on the Dashboard and click “Record Data!!.” After recording all samples, place the instrument in STANDBY with a tube of water.

**FINISHING UP**

Clean the instrument before leaving the lab.

Load a tube with 3 mL of 1% Bleach solution (FACSclean solution) and select RUN HIGH. Run the tube with the support arm to the side for 1 minute (liquid will be drained quickly) and then with the support arm under the tube for an additional 5 minutes.

Repeat that with 3 mL FACSrinse (diluted detergent). Run the tube with the support arm to the side for 1 minute and then with the support arm under the tube for an additional 5 minutes.

Repeat that with 3 mL distilled water Run the tube with the support arm to the side for 1 minute and then with the support arm under the tube for an additional 5 minutes.

After cleaning is complete, place the instrument in STANDBY (leave water tube in place) and log off the Diva software. Always leave a tube of water on the instrument when not in use.

NOTE: The cleaning process should be done at the end of our experiments or every 50 samples.

NOTE2: There are certain staining that require an extra step using 3 mL of ETOH 70% (DAPI, Hoeschst, Tiazole orange, acridine orange, Etidium Bromide and Propidium Iodide)

**EXPORTING DATA**

To export your data, right-click on your experiment name in the browser, select Export then either Experiments or FCS Files. The experiment folder will be given the same name as your experiment in the browser, so be sure to name it so you can easily find it again. Names, dates, and brief experiment titles are recommended.

Once your experiment is backed up, delete it from the browser to prevent data corruption or slowing of the computer???