Flow Cytometry & FACS
Core Facility

BD FACSCanto^{TM} II – User's Guide

For authorization to use the FACSCanto II cytometer, you have to receive a training and must accept the user rules of the facility!

Cytometer Startup

1. If the cytometer and computer are already on: Launch the Diva-Software, log in and start your measurement.
2. If not: Turn on the cytometer main power (green switch).
3. Start up the computer.
4. Windows log in: BDOperator – Password: Welcome#1
5. Launch the Diva-Software and log in.
6. Wait until the software is connected to the cytometer.
7. If the CS&T Settings mismatch box appears select: Use CS&T settings.
8. Check the fluid levels. If needed, empty the waste and replace empty fluid containers. Then select: Cytometer menu > Cleaning modes > Prime after tank refill
9. Run the fluidics startup: Cytometer menu > Fluidics startup
10. Check the flow cell for air bubbles. If necessary, run the de-gas flow cell procedure: Cytometer menu > Cleaning modes > De-gas flow cell

Data Acquisition

<u>Creating an experiment and worksheet</u>

1. Select your folder in the browser.
2. Create a new experiment or copy (right-click: copy > paste or duplicate without data) or import an old one. Rename it appropriately.
3. Right-click the experiment-level cytometer settings in the browser. Choose: Apply current CST-Settings.
4. Change, add or delete parameters as needed in the inspector window.
5. Create a new specimen (Specimen_001 containing the first tube named Tube_001 by default) and rename it appropriately.
6. On the global worksheet create appropriate plots for viewing data during acquisition. Display population hierarchy and statistics view (right-click within any plot). To edit statistics right-click within the statistics view.

Optimizing instrument settings and acquiring data

1. Set the current tube pointer (green arrow) to the first tube in the browser and install the unstained control tube. Then click acquire data in the acquisition dashboard and choose a flow rate (high, medium, low).
2. Please make sure, that the flow rate does not exceed 10,000 evt/s. Dilute the sample if it is too concentrated.
3. Adjust the FSC and SSC voltages and threshold. The population of interest should be completely visible within the FSC/SSC-Plot. FSC and SSC voltage are displayed in linear mode by default, but may be changed to log in case of a widely scattered population (e.g., for yeast or bacteria).
4. Optimize fluorescent parameter voltages. Voltages are displayed in log by default. The peak of the negative population should be at 10^2.
5. By option you can define a gate around the population of interest in the FSC/SSC plot and show only the data of the P1 population in the other plots (right-click within plot > show population).
6. Enter events to record, stopping gate, stopping time and storage gate in the acquisition dashboard and click record to save the data and settings of the unstained control tube.
7. Remove the unstained control tube.
8. From now on you should not change any voltage settings for the rest of your measurement.
9. Install the first sample tube onto the cytometer and acquire data for the corresponding tube in the browser.
10. Enter events to record.
11. Click record data. Remove the tube when recording is finished.
12. Click next tube, install the next sample and go on with recording.

Compensation

Compensation is necessary only in case of overlapping emission spectra and same excitation laser. You need an unstained control and single stained controls for all fluorochromes used in your experiment.

1. Create a new experiment as described above and rename it appropriately.
2. Go to Experiment menu > Compensation setup > Create compensation controls
3. Set the current tube pointer (green arrow) to the unstained control tube in the browser and install the unstained control tube. Then click acquire data in the acquisition dashboard and choose a flow rate (high, medium, low).
4. Please make sure, that the flow rate does not exceed 10,000 evt/s. Dilute the sample if it is too concentrated.
5. Adjust the FSC and SSC voltages and threshold. The population of interest should be completely visible within the FSC/SSC-Plot. Move the P1 gate around the main population, right-click the gate boarder and select: apply to all compensation controls.
6. Optimize fluorescent parameter voltages. Fluorescence parameter voltages are displayed in log by default. The peak of the negative population should be at 10^2.
7. Click record to save the data and settings of the unstained control tube.
8. Remove the unstained control tube.
9. From now on you should not change any voltage settings for the rest of your measurement.
10. Install the first single stained control tube onto the cytometer and acquire data for the corresponding tube in the browser.
11. Click record data. Remove the tube when recording is finished.
12. Click next tube, install the next single stained control and go on with recording.
13. Go through all single stain worksheets and if needed adjust the P2 gate to include the positive peak completely.
14. Go to Experiment menu > Compensation setup > Calculate compensation
15. Choose: Apply only.

Saving, Exporting and Importing Data

Experiments in the browser are saved to a database automatically. But it is recommended to keep the database size as small as possible!!! Please backup your raw data by exporting as Experiment or FCS file. It is also possible to create a PDF or CSV file. Exported Experiments are compatible with Diva and contain all settings, plots and data. FCS files (Flow Cytometry Standard) are compatible with any analysis program but do not include gates.

1-2 experiments per user can be stored in the browser as a template – please name it appropriately. Any additional experiments are removed from the computer on a regular basis!

You can transfer your data files to your own storage device (virus free!).

There is no data backup done by the facility!

Exporting Experiments or FCS Files
1. Select your experiment. In the File menu select: Export > Experiments or FCS Files
2. Define the storage location.
3. Delete the experiment in the browser.

Importing Experiments
1. In the File menu select: Import > Experiments
2. Select the storage location and import the experiment you need.

Creating PDF and CSV files
- You can create a PDF file from normal worksheet view via the PDF button in the worksheet toolbar.
- To make a batch analysis report of your experiment select the first sample tube in the browser and then click on the experiment name > right-click and choose batch analysis > make sure to select Save as PDF and define location and file name and start the batch analysis.
- To create a CSV file from the statistics table displayed on the worksheet select Statistics in the batch analysis box and define location and file name and start the batch analysis. CSV files can be imported in spreadsheet programs like MS Excel.

Cytometer Shutdown
1. Install a tube with 3 ml FACSClean. Choose: Cytometer menu > Cleaning modes > Clean flow cell (3 x)
2. Install a tube with 3 ml DI Water. Choose: Cytometer menu > Cleaning modes > Clean flow cell (3 x)
3. Remove the tube!
4. If another person has booked within the next 2-3 hours: Log out from Diva-Software and leave on the machine (without fluidics shutdown).
5. If nobody has booked: Run the fluidics shutdown: Cytometer menu > Fluidics shutdown
6. Quit the Diva-software.
7. Shut down the computer.
8. Switch off the cytometer main power.
9. Empty the waste if it is more than half full.
10. Check the fluid levels of the FACSFlow, FACSShutdown-Solution and FACSClean containers and replace if empty.
11. Please update your logged time in the schedule (logged time is used for billing).