BD FACSDiva Software Quick Reference Guidefor BD FACSCanto

Workflow Overview

The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.

Start Up System ▸ Check Performance ▸ Set Up Experiment ▸ Record Data ▸ Export Data ▸ Cleaning and Shut Down System

To log into the PC: **Username** (BDAdmin) and **Password** (BDIS)

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Starting Up the System

Flow Core staff responsibility – Mon-Fri

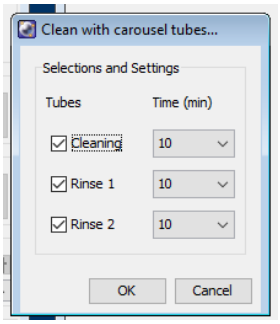
First user of the day responsibility – weekends/holidays

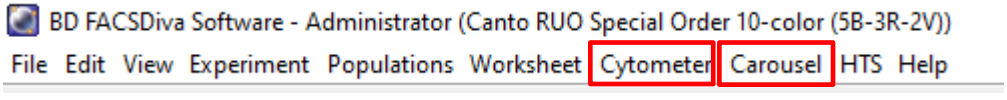
1. Unlock the screen with your PPMS account (UTSW credentials).
2. Launch Tera Term 

3. Turn on the cytometer main power (green button on the side).
4. Click on **Serial,** make sure the port is on **COM1**, and click OK.

5. Start BD FACSDiva software, and log in. 

1. Check if waste tank is empty and there is enough Clear Flow Sheath Fluid (if you are emptying the waste, add a layer of bleach to the container).
6. Select Cytometer > Fluidics Startup (it takes around 7min).
7. Prepare the cleaning carousel: contrad (1st position), bleach (2nd position) and shut down solution (3rd position).
8. Once fluidics startup is finished, insert carousel and go to Carousel → Clean - Select 10min for each tube.



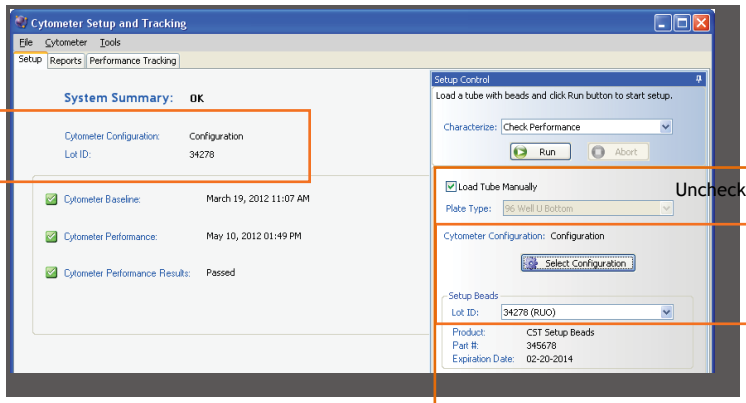


Checking Cytometer Performance

Flow Core staff responsibility – Mon-Fri

First user of the day responsibility – weekends/holidays

1. Select Cytometer > CST.



Verify the bead Lot ID.

Uncheck Load tube manually

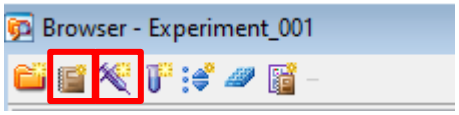
If needed, select a different bead Lot ID.

2. Take out the carousel and replace the tube in position 1 with the CST tube - two drops of CST + ~500uL of FACSFlow.
3. Run the BD FACSDiva™ CS&T research beads (blue vial) - CST should pass with or without warnings.

   Failed CST it not acceptable.
4. Close the Cytometer Setup and Tracking window.

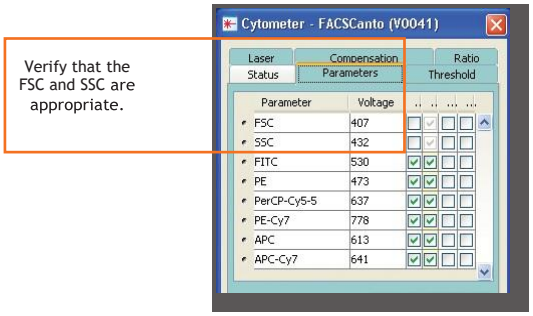
Setting Up the Experiment

1. Create a New Experiment in the Browser (brown notebook icon).

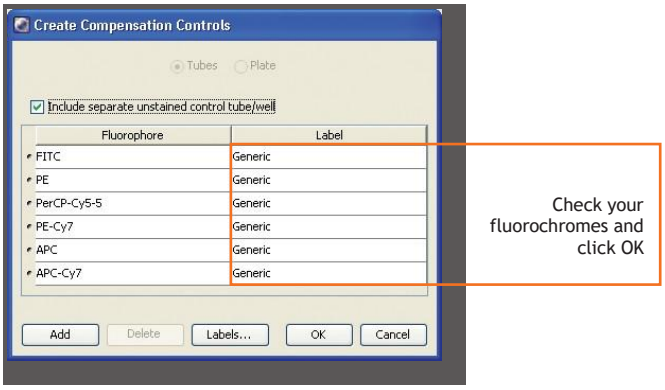


2. Create a new Specimen (syringe icon), click on the plus sign (+) to expand the specimen and place the acquisition pointer on Tube_001.

3. Click on the Parameters tab in the Cytometer window. Delete all the fluorochromes and add the ones you will be working with. Do not delete FSC and SSC.



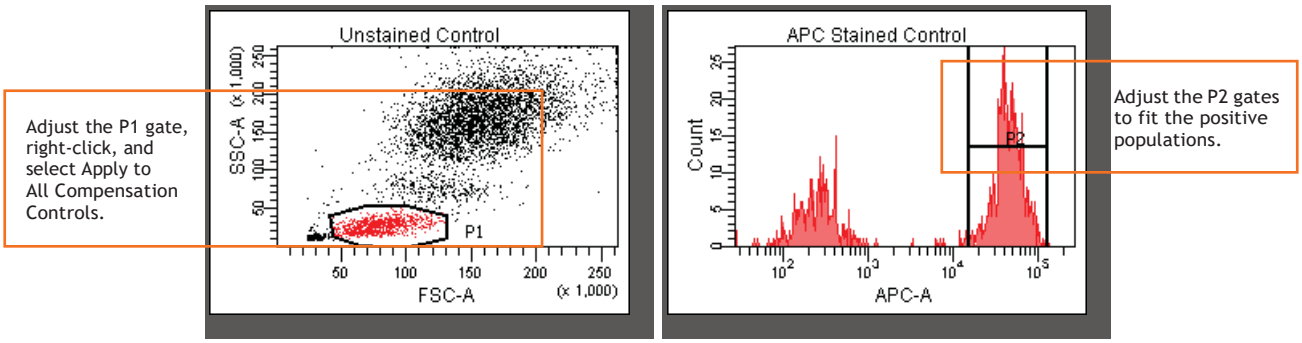
4. Select the H and W checkboxes in the Parameters tab to select height and width for FSC and SSC.
5. If you need Compensation select: Experiment → Compensation Setup → Create Compensation Controls.



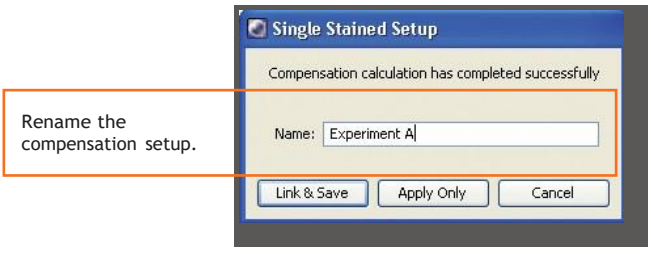
6. A specimen "Compensation Controls" has been created. Expand it and select unstained tube on the pointer; "Normal Worksheets" have been created for each compensation tubes.
7. Place the tubes in the same order as the specimen into the carousel, close the loader and click Run Single Tube on the Acquisition Dashboard. Select the tube on the carousel and click OK.
8. Adjust the voltages for FSC, SSC and the fluorochromes to put the cells on scale. Click on Unload Tube on the Acquisition Dashboard and then Run Single Tube again, repeat the process for all your control tubes.

   * Check if all of your tubes are on scale prior recording data.*

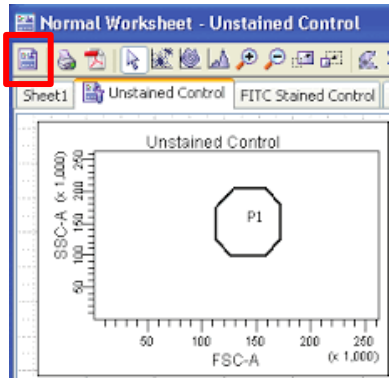
9. Record data for the compensation control tube. After the recording is finished, click on Unload Tube on the Acquisition Dashboard and then Run Single Tube again. Repeat the process for all your control tubes.



10. Select Experiment → Compensation Setup → Calculate Compensation → Apply Only.

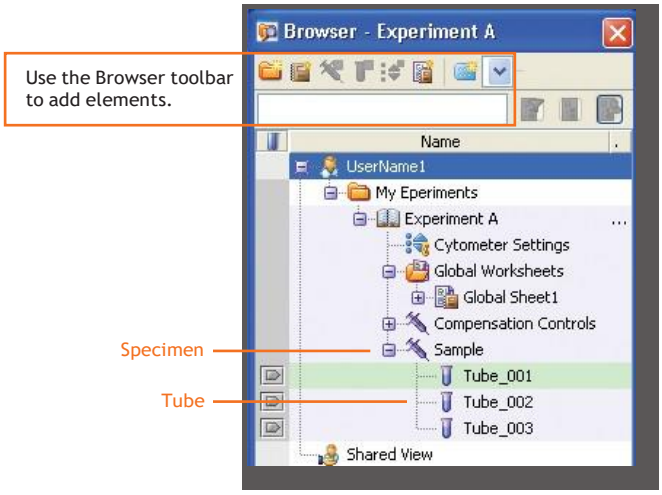


11. Switch back to the Global Worksheet clicking on this icon ( ).

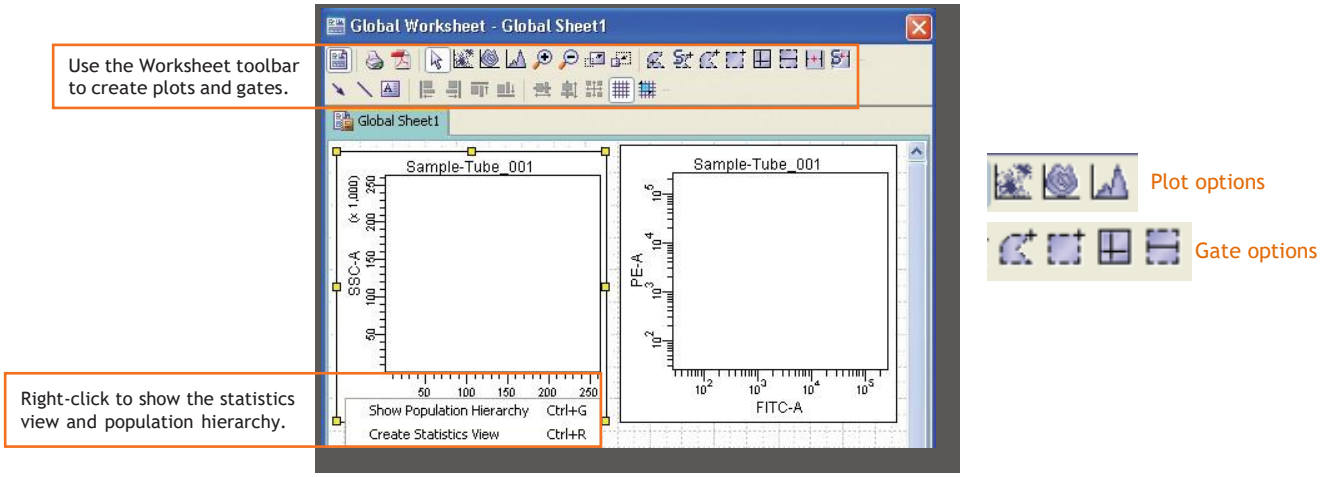


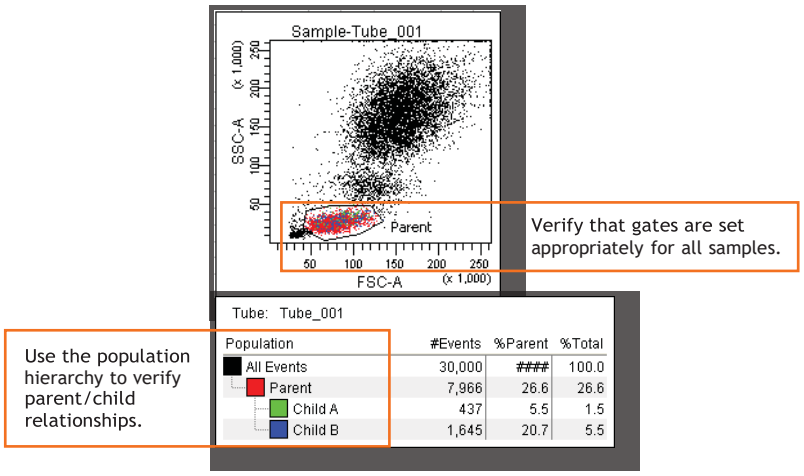
Recording Specimen Data

1. Create Browser elements. To add more samples to your experiment click on the tube icon ( ).

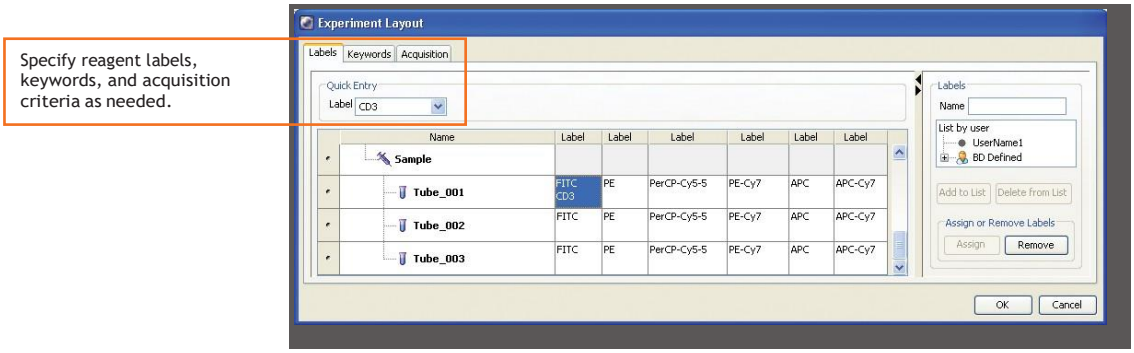


2. Create plots, gates, and statistics needed for recording.

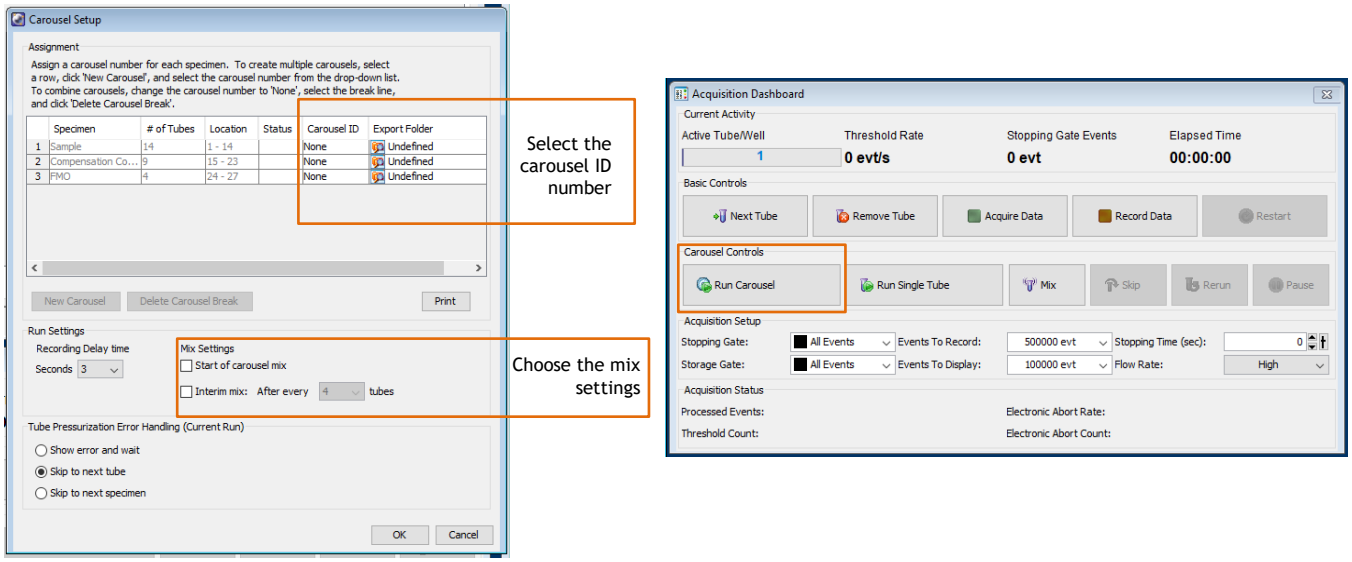




3. Make entries in the Experiment Layout (Experiment → Experiment Layout).



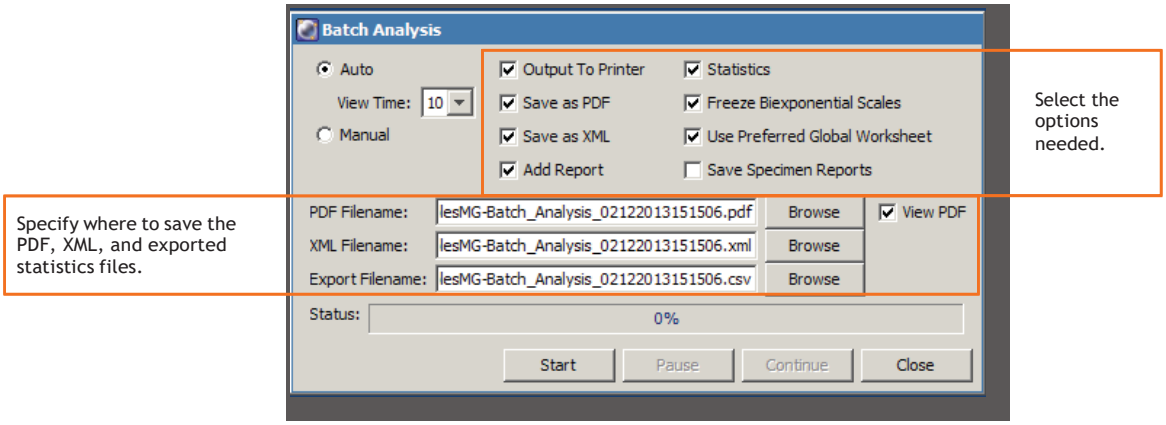
4. To run the samples using the carousel: select Carousel → Carousel Setup → choose the Carousel ID# and mix settings, then click OK. After that, click on Run Carousel on the Acquisition Dashboard.



Exporting Data

1. To print or export the results:

- Right click your Experiment → Export → FCS Files → Do not change the file version, keep FCS3.0 and click OK. (Folder: Documents - Backup - Year - Month - Your folder).
- To create a PDF, right-click a specimen or experiment and select Batch Analysis (using a global worksheet).



Cleaning and Shutting Down the System:

1. Prepare the cleaning carousel and place the tubes on the positions 1, 2 and 3(contrad, bleach and shut down solution) → Carousel → Clean → 3min each tube.

If you are the last user of the day:

2. Perform the cytometer shutdown: Cytometer → Fluidics Shutdown.
3. Empty the waste and refill fluids if prompted to do so.
4. Turn off the cytometer main power.