

## Workflow Overview

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




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

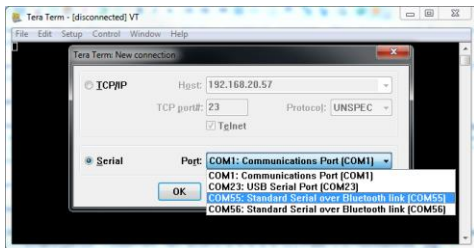



## Starting Up the System

Flow Core staff responsibility – Mon-Fri

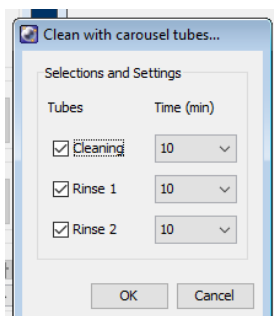
First user of the day responsibility – weekends/holidays


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 (1<sup>st</sup> position), bleach (2<sup>nd</sup> position) and shut down solution (3<sup>rd</sup> position).
8. Once fluidics startup is finished, insert carousel and go to Carousel → Clean - Select 10min for each tube.



 BD FACSDiva Software - Administrator (Canto RUO Special Order 10-color (5B-3R-2V))

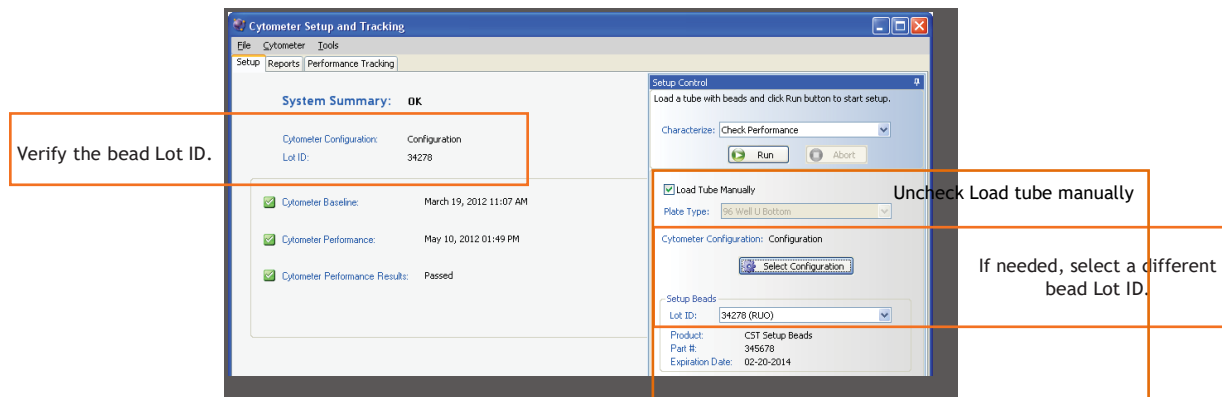
File Edit View Experiment Populations Worksheet **Cytometer** **Carousel** HTS Help

## Checking Cytometer Performance

Flow Core staff responsibility – Mon-Fri

First user of the day responsibility – weekends/holidays

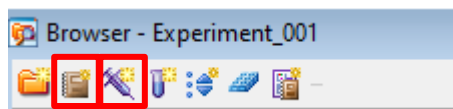
1. Select Cytometer > CST.



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).

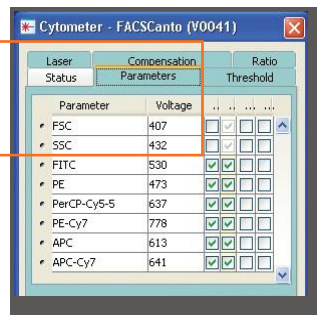


2. Create a new Specimen (syringe icon), click on the plus sign (+) to expand the specimen and place the acquisition pointer on Tube\_001.

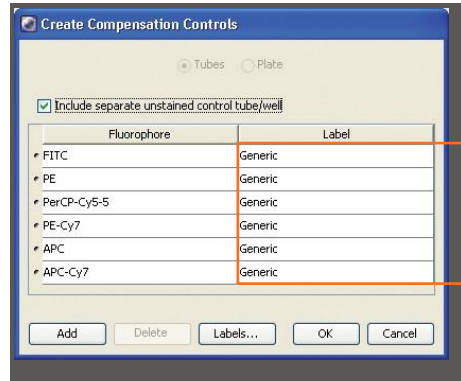


3. Click on the Parameters tab in the Cytometer window. Delete all the fluorochromes and add the ones you will be working with. Do not delete FSC and SSC.

Verify that the FSC and SSC are appropriate.

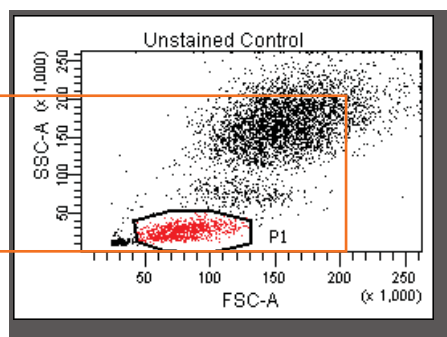


4. Select the H and W checkboxes in the Parameters tab to select height and width for FSC and SSC.
5. If you need Compensation select: Experiment → Compensation Setup → Create Compensation Controls.

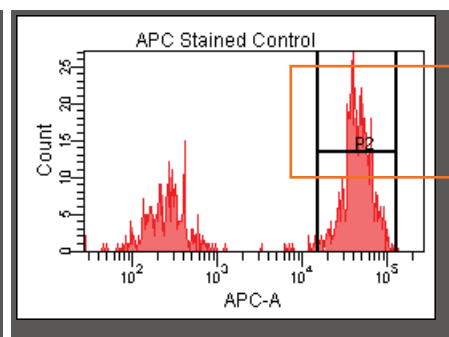


Check your fluorochromes and click OK

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.



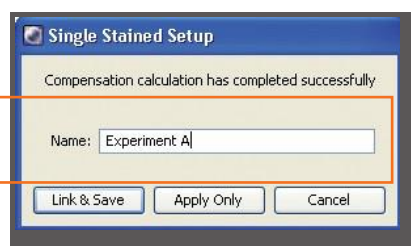
Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.



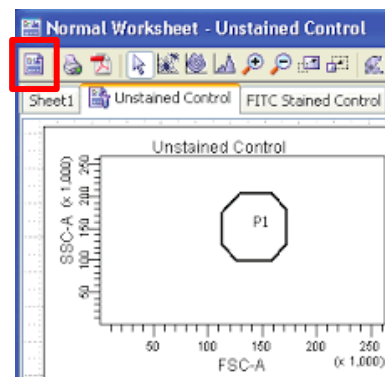
Adjust the P2 gates to fit the positive populations.

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

Rename the compensation setup.

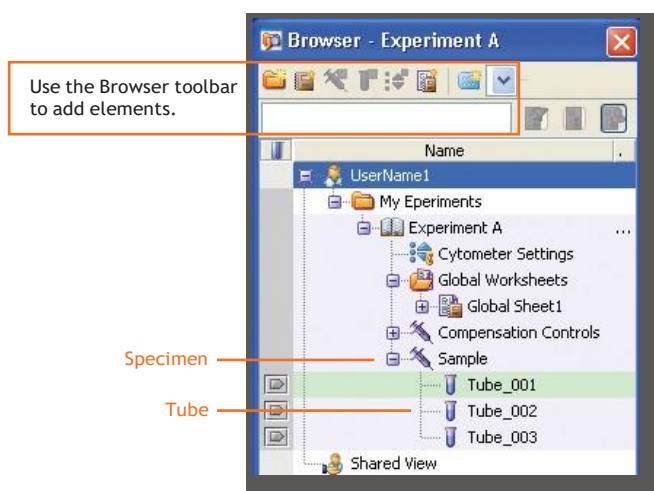


11. Switch back to the Global Worksheet clicking on this icon (  ).

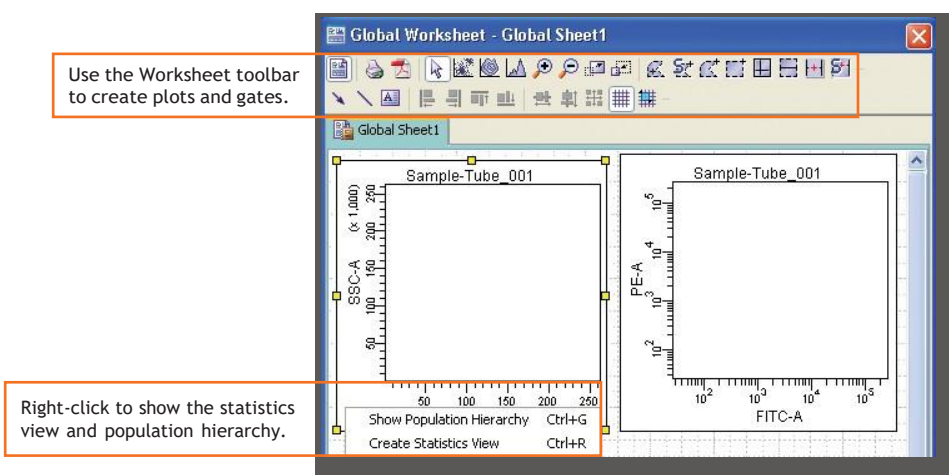


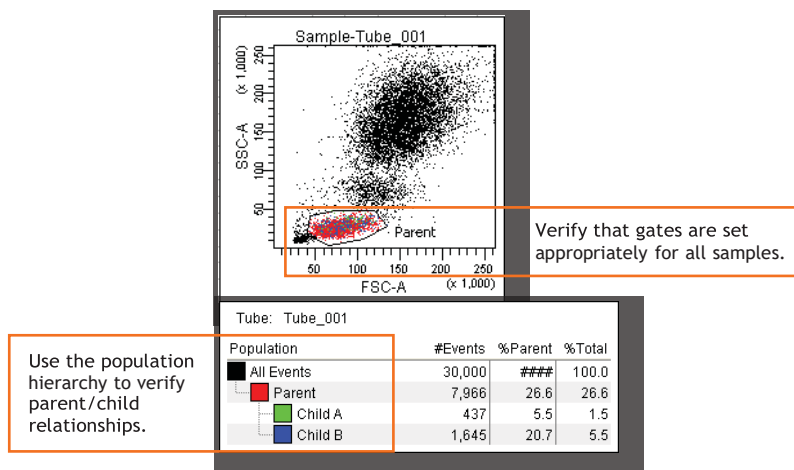
## Recording Specimen Data

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).

Specify reagent labels, keywords, and acquisition criteria as needed.

Labels Keywords Acquisition

Quick Entry  
Label CD3

Name	Label	Label	Label	Label	Label	Label
Sample						
Tube_001	FITC CD3	PE	PerCP-Cy5-5	PE-Cy7	APC	APC-Cy7
Tube_002	FITC	PE	PerCP-Cy5-5	PE-Cy7	APC	APC-Cy7
Tube_003	FITC	PE	PerCP-Cy5-5	PE-Cy7	APC	APC-Cy7

Labels  
Name  
List by user  
● UserName1  
● BD Defined  
Add to List Delete from List  
Assign or Remove Labels  
Assign Remove  
OK Cancel

### 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.

Select the carousel ID number

Choose the mix settings

Carousel Setup

Assignment

Assign a carousel number for each specimen. To create multiple carousels, select a row, click 'New Carousel', and select the carousel number from the drop-down list. To combine carousels, change the carousel number to 'None', select the break line, and click 'Delete Carousel Break'.

Specimen	# of Tubes	Location	Status	Carousel ID	Export Folder
1 Sample	14	1 - 14		None	Undefined
2 Compensation Co...	9	15 - 23		None	Undefined
3 FMO	4	24 - 27		None	Undefined

New Carousel Delete Carousel Break Print

Run Settings  
Recording Delay time  
Seconds 3  
Mix Settings  
☐ Start of carousel mix  
☐ Interim mix: After every 4 tubes  
Tube Pressurization Error Handling (Current Run)  
☐ Show error and wait  
☒ Skip to next tube  
☐ Skip to next specimen  
OK Cancel

Acquisition Dashboard

Current Activity  
Active Tube/Well 1 Threshold Rate 0 evt/s Stopping Gate Events 0 evt Elapsed Time 00:00:00

Basic Controls  
Next Tube Remove Tube Acquire Data Record Data Restart

Carousel Controls  
Run Carousel Run Single Tube Mix Skip Rerun Pause

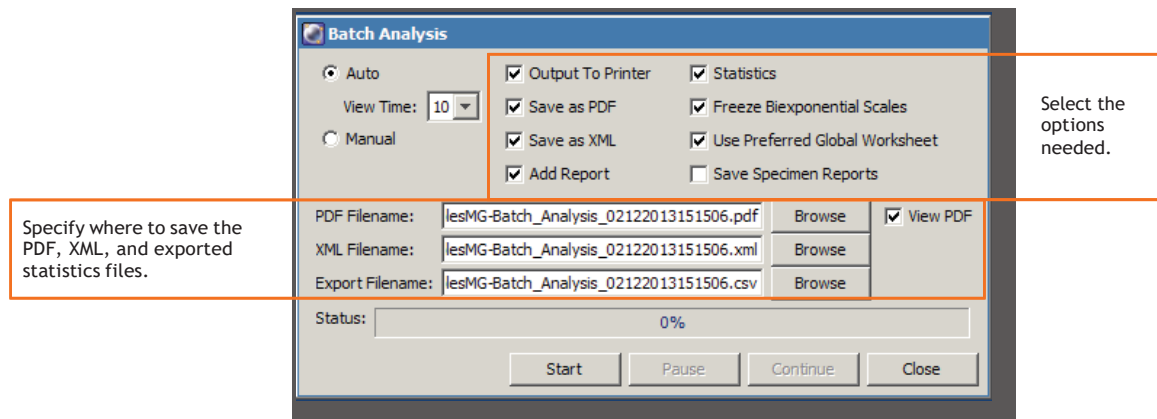
Acquisition Setup  
Stopping Gate: All Events Events To Record: 500000 evt Stopping Time (sec): 0  
Storage Gate: All Events Events To Display: 100000 evt Flow Rate: High

Acquisition Status  
Processed Events: Electronic Abort Rate:  
Threshold Count: Electronic Abort Count:

## 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.