Reference:

BD FACSDiva 6 Online Course

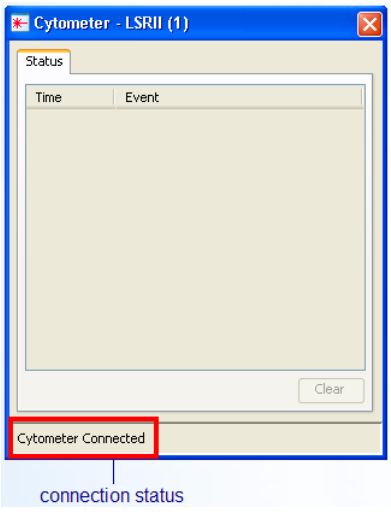
BD Training & eLearning

Startup

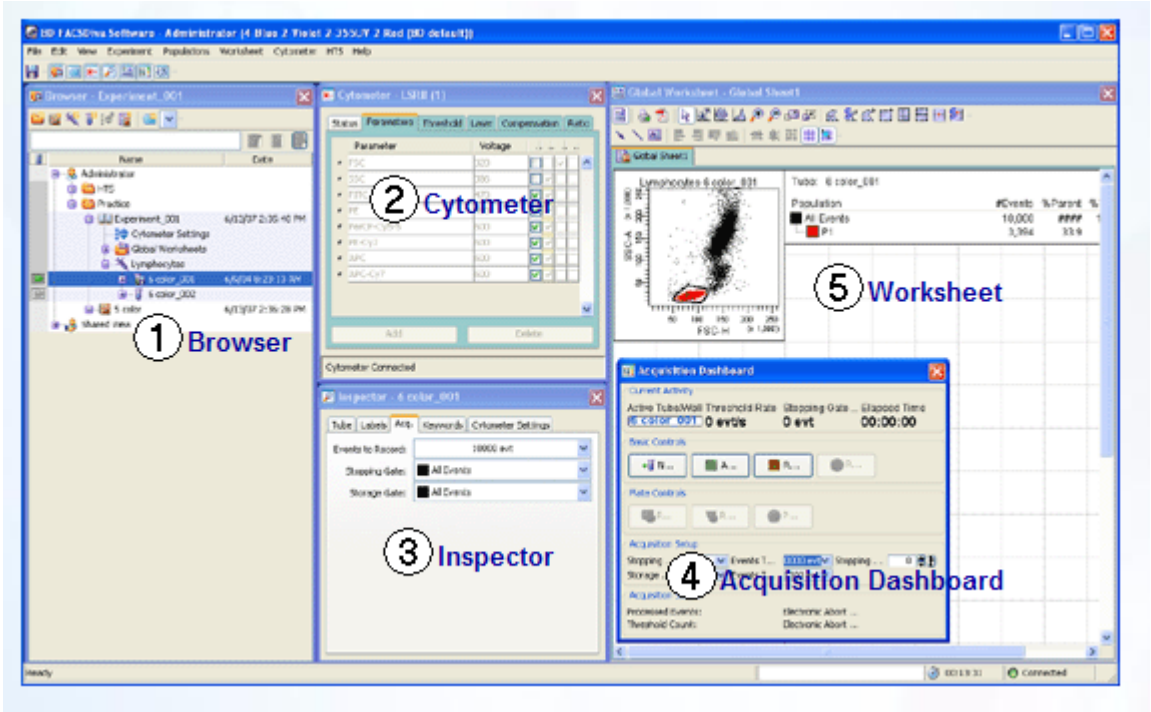
1. Check **Sheath and Waste** containers:
   - Empty Waste container in the sink – Add bleach to the black mark (~10% final concentration).
   - Fill Sheath container with Sheath Solution (1X PBS) – Do not let fluid level go below the black mark.
2. Turn on **FACSFlow Supply System** (Fluidics Cart) – Green button on the right-hand side.
3. Turn on **Cytometer** – Green button on the right-hand side (watch for position of the HTS/tube switch).
4. Check Sheath filter for air, bleed if necessary. Bleed side tubing.
5. Log in to Windows (Onyen + password).
6. Turn on necessary **lasers**:
   - GUI-VFL (green arrow icon) for 592nm laser – Select laser power, set to 100mW, turn 'On' and 'Activate'.
   - BD Coherent Connection 4 for 355, 405, 488, 532, 561, and 633nm lasers – Click 'Laser On'.
7. Launch **BD FACSDiva Software** (Username + password).
8. Check Flow Cell for bubbles. **Prime** if necessary.

Wait for the computer to establish a connection with the cytometer

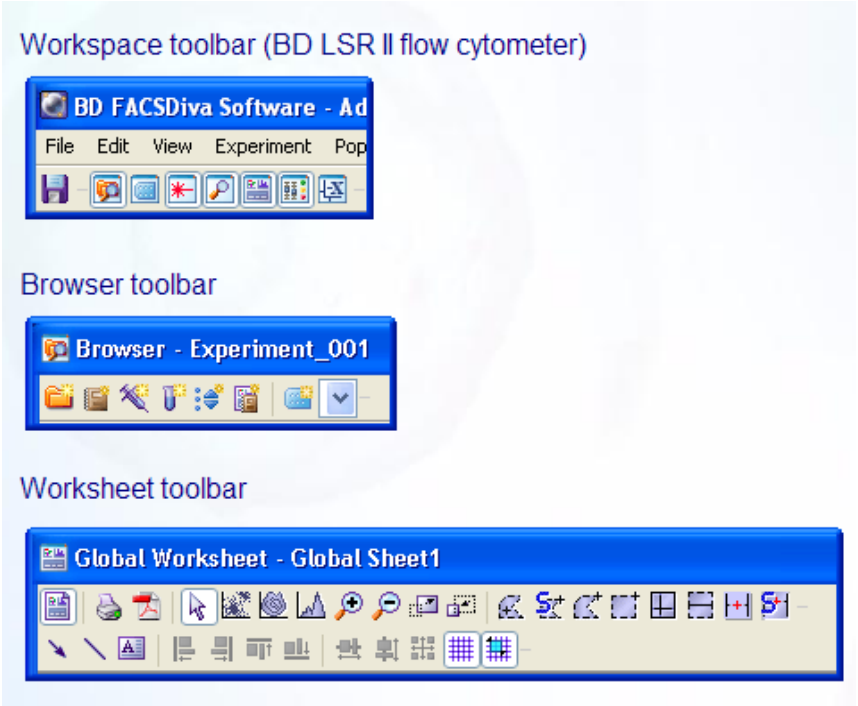
If the connection fails, restart the cytometer (green button) or alternatively power down the cytometer and the computer and then restart them both.



BD FACSDiva Software



Menus Overview

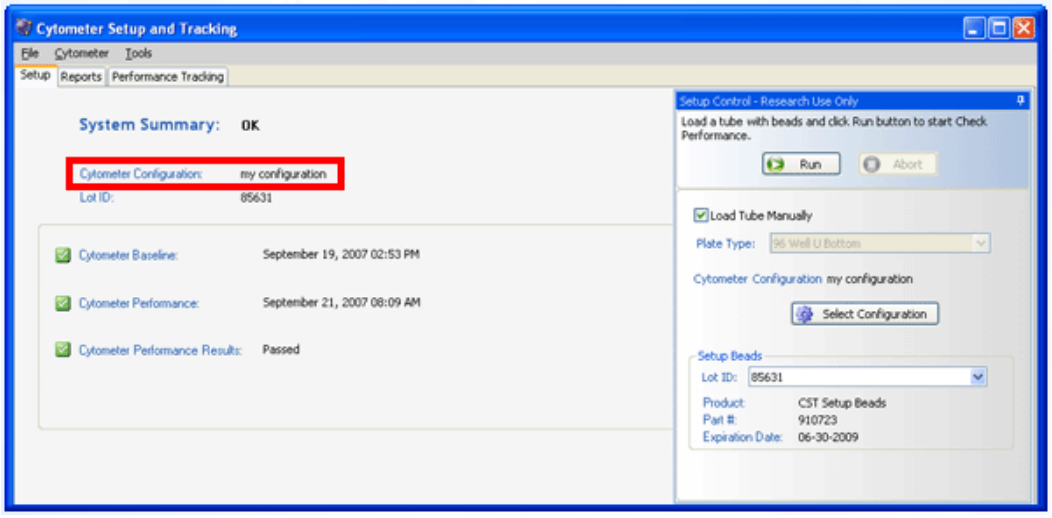


Quality Control – Cytometer Setup & Tracking (CST)

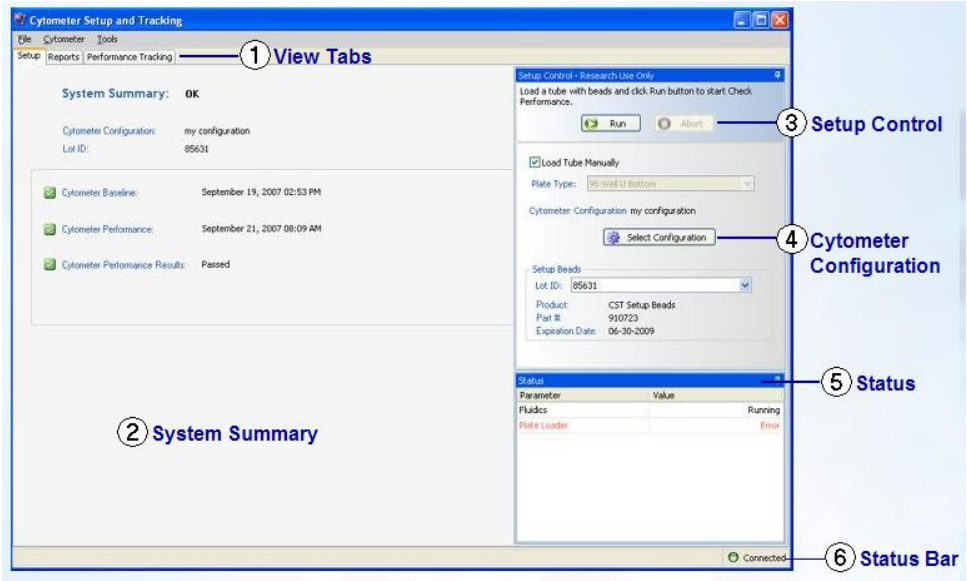
CST beads are used to optimize each PMT. This ensures that the ratio of signal to noise is the highest, and the CVs of the positive peak are the smallest.

Launching the CST Workspace

Select Cytometer Menu > CST



CST Workspace Overview

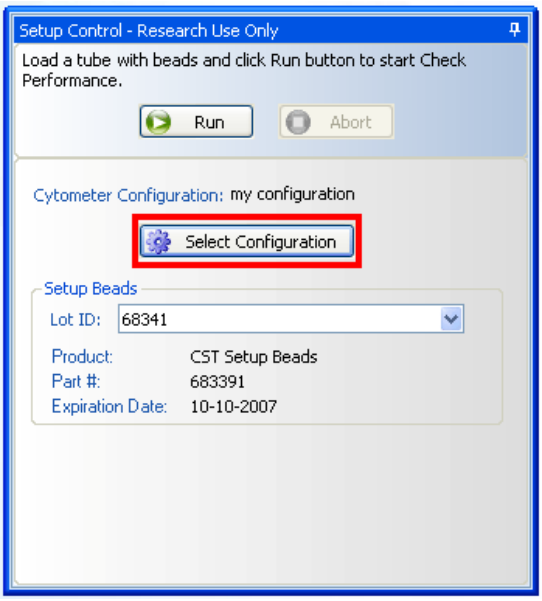


Instrument Configuration

Select the Configuration that matches the optical bench layout (filters & mirror installed on the cytometer).

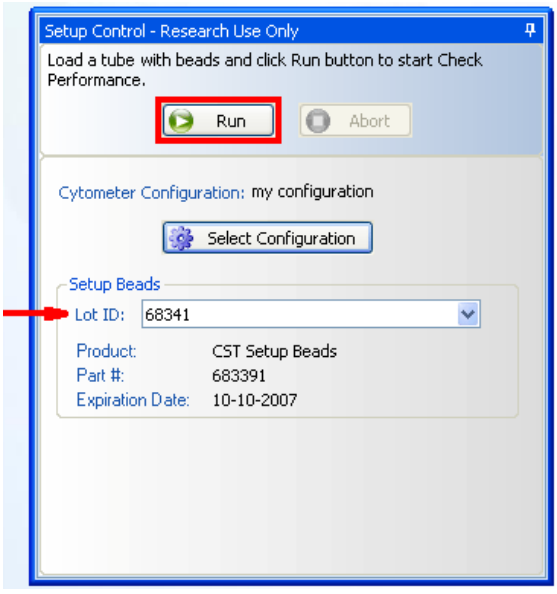
Within the Cytometer Configuration windows, select a configuration from the left-hand side menu and click Set Configuration. Click OK to close the Window. Close CST Workspace.

Note: If necessary change filters & mirror to accommodate your experimental staining panel.



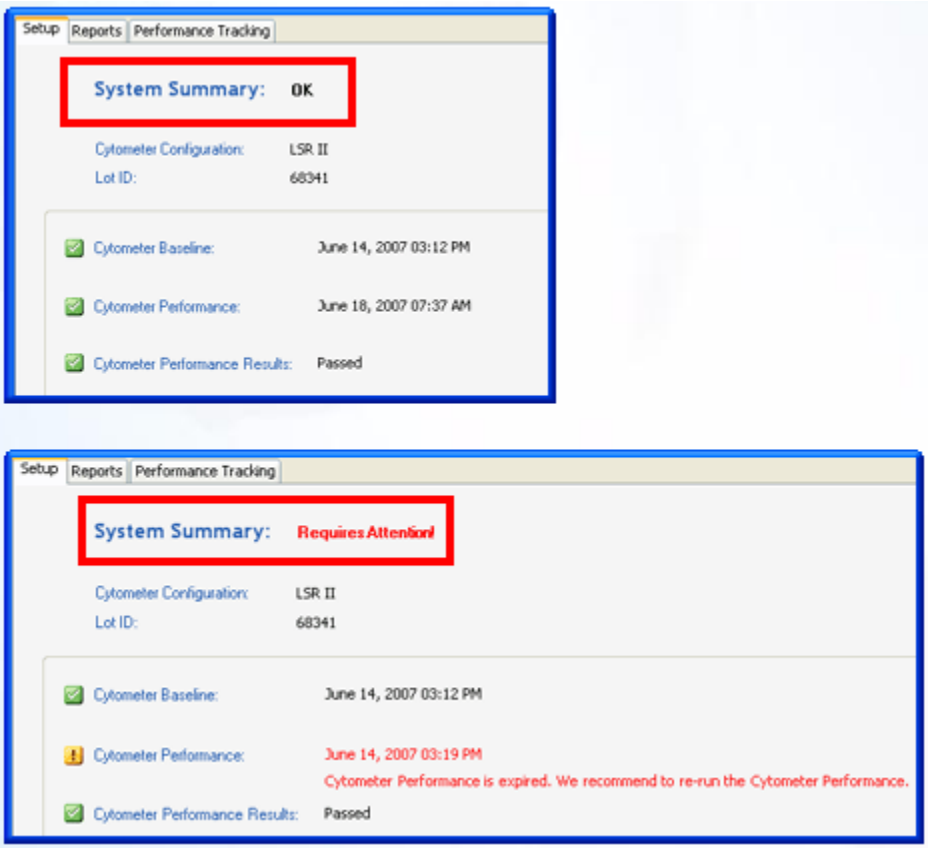
Running CST (Performance Check)

- Prepare the CST beads (1 drop in 350µL of PBS)
- Verify the bead Lot ID is correct
- Select Run mode and Low on the fluidics control panel of the cytometer.
- Click Run in CST Workspace

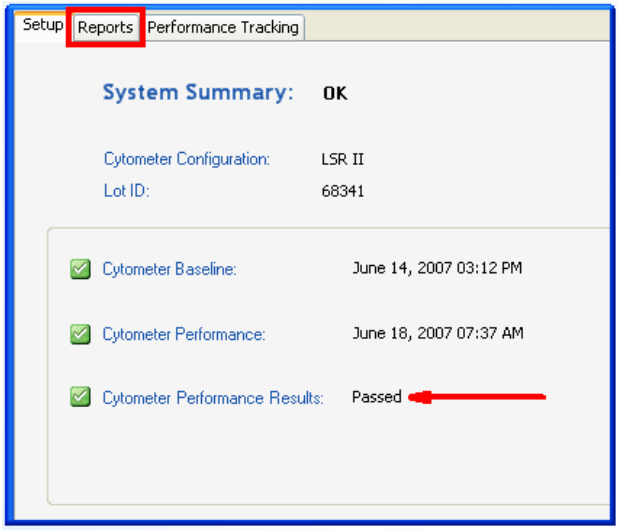


CST Results

Under System Summary, check the system's status.
If the status is 'Requires Attention!', you must run a Performance Check for that configuration.
If the status is 'OK', proceed with setting up your experiment.



Click the **Report** and troubleshoot any warnings or failures.

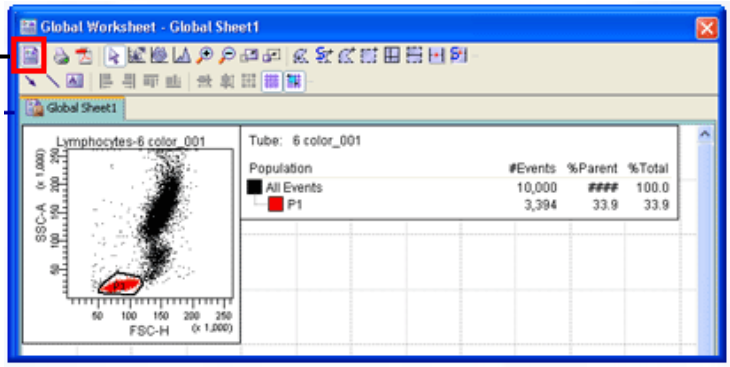


Working with BD FACSDiva Software

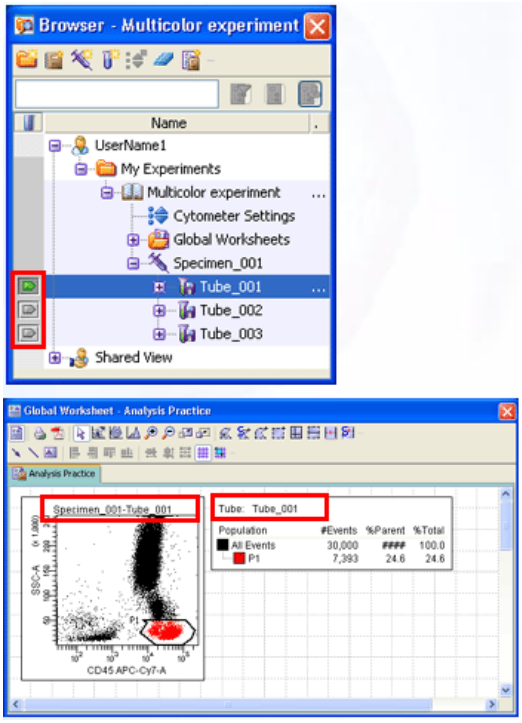
Worksheets are where you create plots, gates, population hierarchies, statistic views, and custom text.
There are 2 types of worksheets between which you can toggle (Worksheet View button):

- ***Global***:
  Displays data from one tube at a time
  Uses the same set of plots/gates to analyze any tube in an experiment
  Used to record data using the Loader or HTS options
  Used to perform batch data analysis
- ***Normal***:
  Used to perform compensation
  Displays data from several tubes simultaneously
  Uses different gating strategies (on the same worksheet) for tubes within an experiment

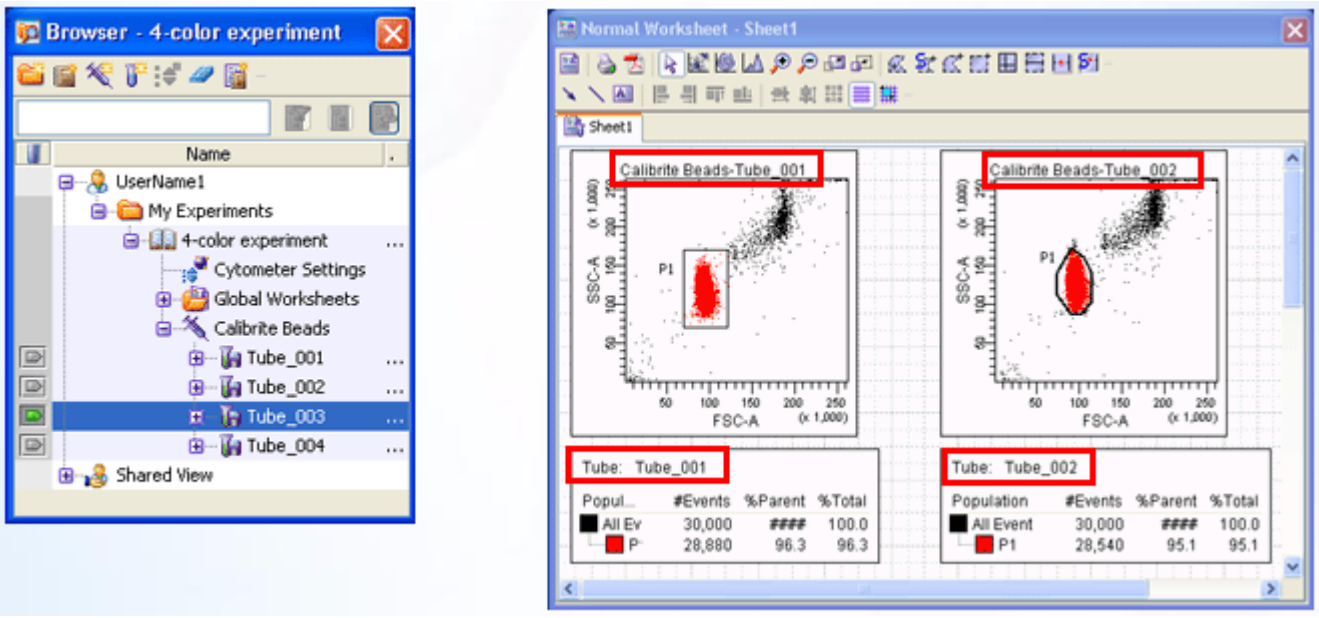
Worksheet View button



Data displayed in a **Global Worksheet** changes when the current tube pointer is moved to another tube.



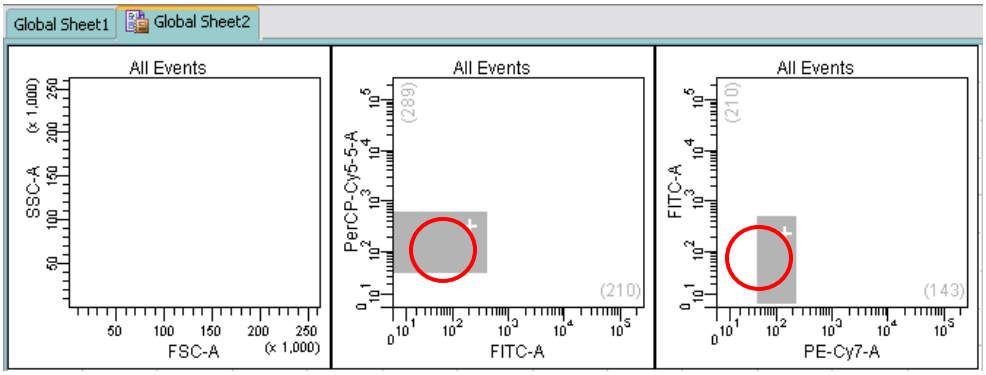
Data displayed in a **Normal Worksheet** is not dependent on where the current tube pointer is set.
For example, the plots in the graphics below display data from tubes 001 and 002.
Additionally, you can use different gates and gating strategies for each tubes. Here, the P1 gates in the plots are different.



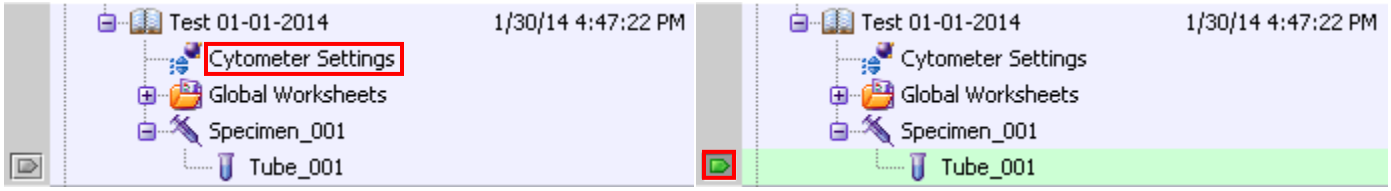
Setting Up Your Experiment

Adjusting Baseline Voltages

- Click 'New Experiment' (2nd icon in the Browser toolbar). ***OR*** Recall a saved Experiment Template (Experiment menu>New Experiment…).
- Rename the Experiment (R-click>Rename).
- Click on Cytometer Settings and delete unused parameters in the Inspector window.
- Check the Height (H), Width (W), or both for FSC, SSC, or both to perform doublet discrimination.
- Check the Log box and the Area (A) box for all fluorescent parameters if running a phenotypic experiment.
- Right-click Cytometer Settings>Application Settings>Create Worksheet. A sheet titled "Global Sheet2" opens.



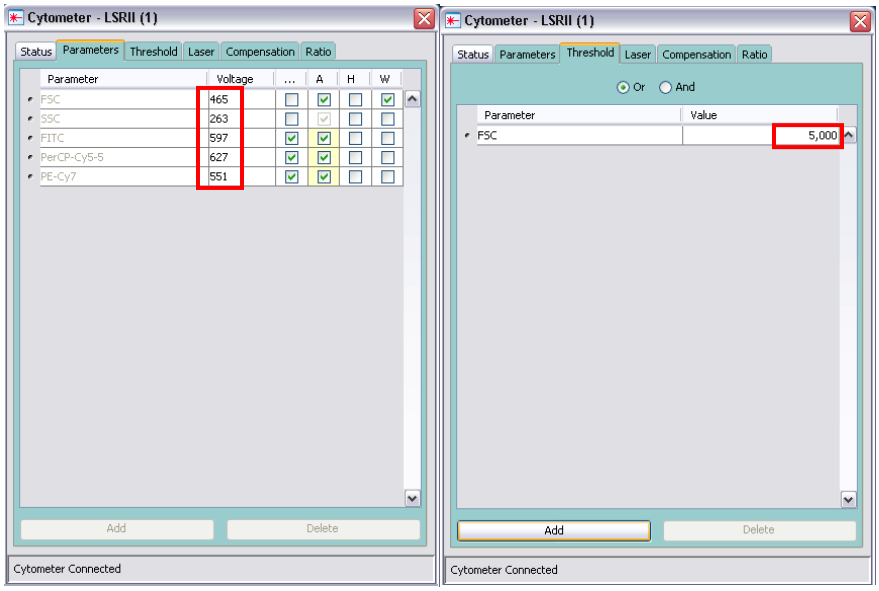
- Click on the Experiment (in Browser) and create a New Specimen (3rd icon in the Browser toolbar) then place the tube pointer on Tube_001.



- The Acquisition Dashboard becomes available.

- **Filter your samples prior to running on the instrument**
- Load your unstained control on the cytometer.
- Click Acquire on the Acquisition Dashboard.

- Adjust FSC-A and SSC-A PMT voltages, as well as the threshold if needed.
- Adjust the voltages of all your fluorescent parameters in order to place the negative population in the grey box, below the white cross hair.



- Run a fully-stained sample to make sure all fluorescent parameters are on scale.
- R-click Cytometer Settings>Application Settings>Save
- To recall Application Settings: R-click Cytometer Settings>Application Settings>Apply and then R-click Application Settings>Apply Current CST Settings.

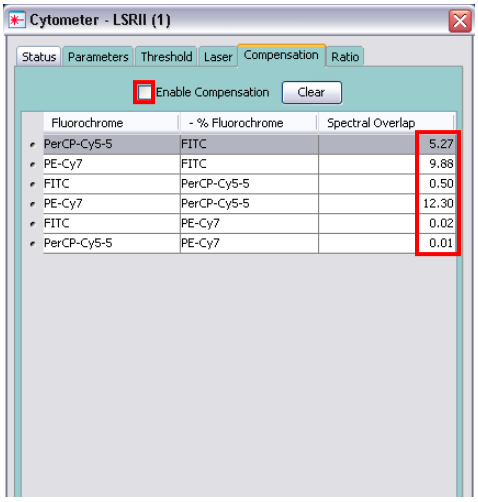
Note: Using Application Settings, synchronizes the voltages of a specific experiment with the voltage fluctuations recorded by CST during the daily Performance Check. Your voltages will be adjusted to reflect day to day changes in the cytometer signal detection.

Note: Alternatively, baseline voltages can be set using an unstained control, adjusting PMT voltage so the population is approximately at 2.5x rSD_{EN} (rSD_{EN} is calculated during CST Baseline determination. See Technical Bulletin "Standardizing Application Setup Across Multiple Flow Cytometers Using BD FACSDiva V6" – March 2012).

Compensation

- Go to Experiment menu>Compensation Setup>Create Compensation Controls.
- Change the Generic Label to the antibody used in your single-stained controls.
- If using an unstained control, check the "Include separate unstained control tube/well" box. If not, you will have to manually create a gate around the negative population of all your single-stained controls.
- Click OK.
- A Specimen called "Compensation Controls" is created. Open it by clicking on the +.
- Place your tube pointer on your first single-stained control or unstained control.
- Load the corresponding sample on the cytometer.
- Click Acquire (adjust P1 if necessary – you can R-click on the P1 to "Apply to All Compensation Controls").
- Click Record (5000 events are automatically recorded).
- Click Next Tube.
- Load then Acquire/Record your next single-stained control.

- Once all have been recorded, review/adjust the placement of the "snap-to gate" P2 for each control.
- Go to Experiment menu> Compensation Setup>Calculate Compensation…
- Select 'Apply Only' and click OK.
- Toggle back to the Global Worksheet (Worksheet View button).
- Review and Enable Compensation



Running Samples

- Place the tube pointer on Tube_001.
- While Tube_001 is selected, go to the Inspector window>label tab and input the names of your antibodies.

Note: If naming multiple tubes simultaneously, use the Experiment Layout (Experiment menu> Experiment Layout…).

- Rename Tube_001.
- Create Plots & Gates in the Global Worksheet.

Note: Apply a gate to a plot by R-clicking a plot and selecting Show Populations>"Name of the Gate" or by using the Inspector window (plot tab).

- Set the 'Events To Record' and the 'Stopping Gate' on the Acquisition Dashboard or in the Experiment Layout (Acquisition tab).

Note: To display the name of the applied gate on the header of a plot: click on a plot, go to the Inspector window (title tab) and check the 'Populations' box.

- R-click on any of your plot and select Show Population hierarchy to visualize simple statistics.

Note: To display data using Biexponential display: click on a plot, go to the Inspector window (plot tab) and check the Biexponential X and Y boxes.

- Load then Acquire/Record your samples (FMOs, biological controls, and experimental samples).

Exporting Data

- Close your experiment (double-click on header)
- R-click Experiment>Export>FCS files… [FCS 3.0]
- Select Location (path)
- R-click Experiment>Export>Experiment Template… (if desired add information pertaining to your experiment)
- **R-click Experiment>Delete**

Shutdown

Be considerate of others, start cleaning ~9 minutes before the end of your scheduled time

1. Install a tube with 3mL of **1% Contrad** with support a**rm to the side** for **1 minute**
2. Put support **arm under** the tube and **RUN on HI** for **2 minutes**
3. Install a tube with 3mL of **FACSClean** with support a**rm to the side** for **1 minute**
4. Put support **arm under** the tube and **RUN on HI** for **2 minutes**
5. Install a tube with 3mL of **diH_2O** with support a**rm to the side** for **1 minute**
6. Put support **arm under** the tube and **RUN on HI** for **2 minutes**
7. Leave a tube with ~**1ml** of diH_2O with support **arm under**
8. Switch cytometer to **STANDBY** mode
9. Log off FACSDiva then Quit
10. Turn off lasers
11. Log out of Windows

Last user turns off cytometer & FACSFlow Supply System

Troubleshooting

- Coherent Connection not visible
  Alt + Space, Maximize
- Coherent Connection not displaying all lasers
  Tools > Serial Settings… > Scan Ports **or** turn the cytometer off then on
- Coherent Connection not displaying the 488 laser
  Open the LSR II lid: if there is blue light, the laser is on.
- UV laser (355nm) not firing up
  Set the laser power to 5mW and turn On, then increase to 100mW
- Cytometer not connected
  Turn the cytometer off then on
- Waste Alarm
  Take the waste lid off and wipe the silver probe dry
- Sample Not Running due to an empty reservoir
  Fill the sheath tank, restart, fill the reservoir (halfway), and bleed the sheath filter & side tubing