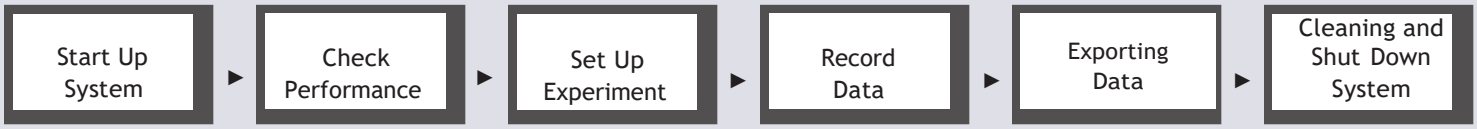
Workflow Overview

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



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

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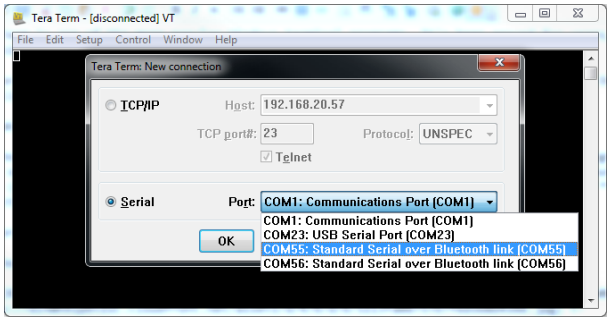
Starting Up the System

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. Click on **Serial,** make sure the port is on **COM1**, and click OK.



4. Turn on the cytometer main power (green button on the side).
5. Start BD FACSDiva software, and log in. 

6. Check if waste tank is empty and there is enough ClearFlow Sheath Fluid with surfactant labeled “Fortessa Only” (if you are emptying the waste, add a layer of bleach to the container).
7. Clean the fluidics: remove the tube with DI water and place the cleaning tube - 10 min for each solution - Contrad, 10% Bleach and DI water. Put the machine on Run mode with high flow rate.

Checking Cytometer Performance

Flow Core staff responsibility – Mon-Fri

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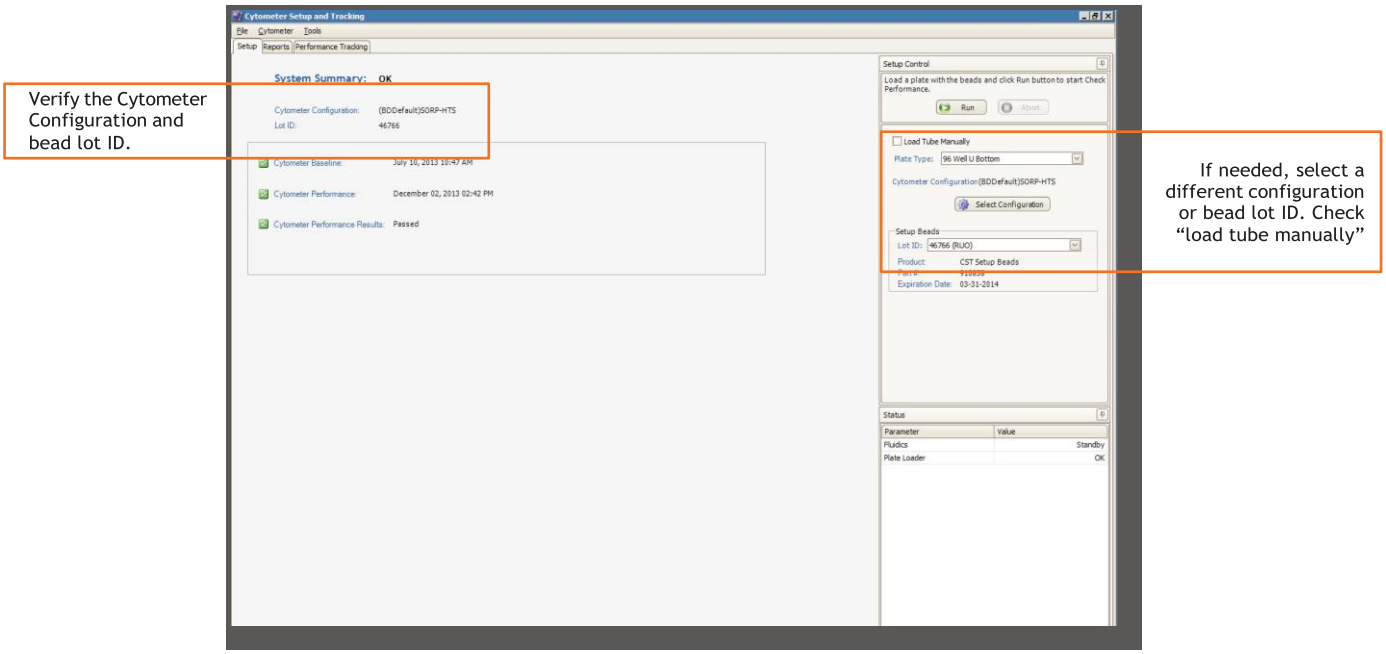
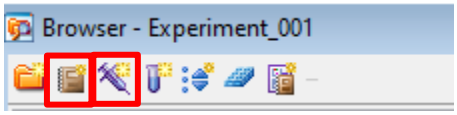


1. Select Cytometer → CST.
2. Put the arm to the side and place the tube in the SIP - two drops of CST + ~500uL of FACS Flow.
3. Run the BD FACSDiva™ CS&T research beads (blue vial) - CST should pass with or without warnings.

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

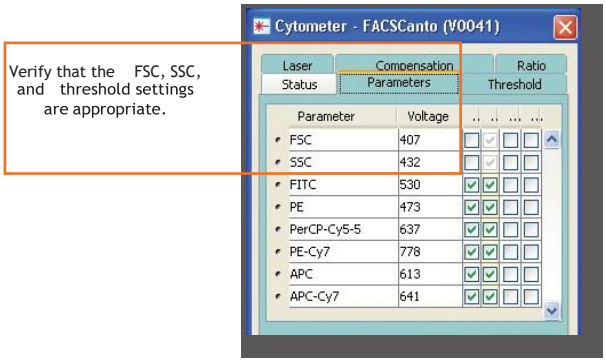
Setting Up the Experiment

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

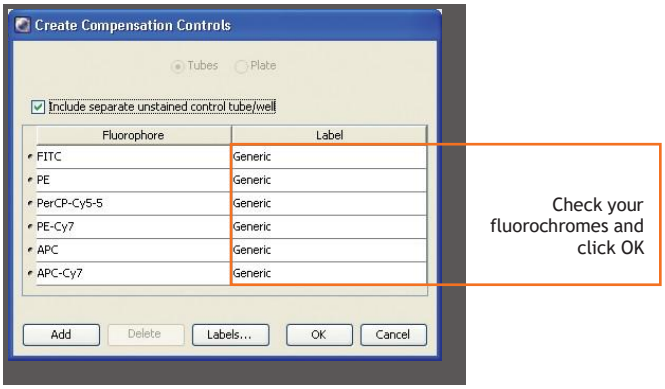


2. Create a new Specimen (syringe icon), click on the plus sign (+) to expand the specimen and place the acquisition pointer on Tube_001.

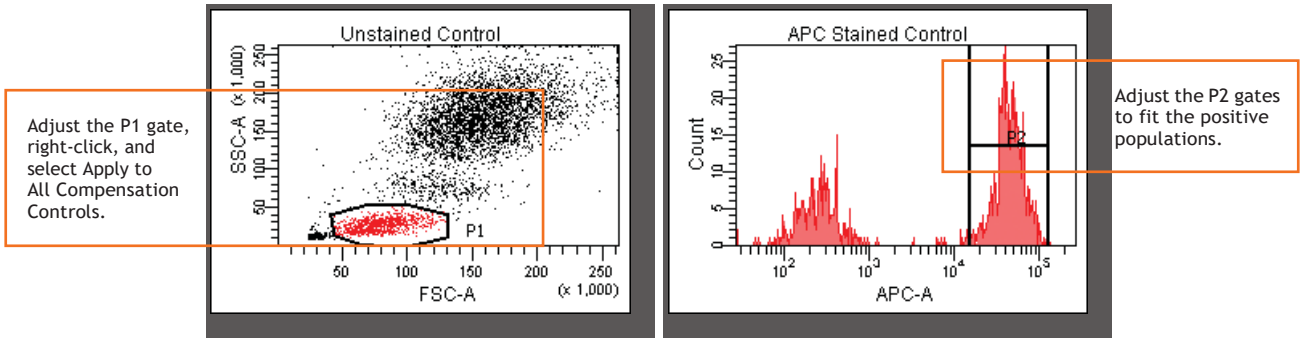
3. Click on the Parameters tab in the Cytometer window. Delete all the fluorochromes and add the ones you will be working with. Do not delete FSC and SSC.
4. Select the H and W checkboxes in the Parameters tab to select height and width for FSC and SSC.



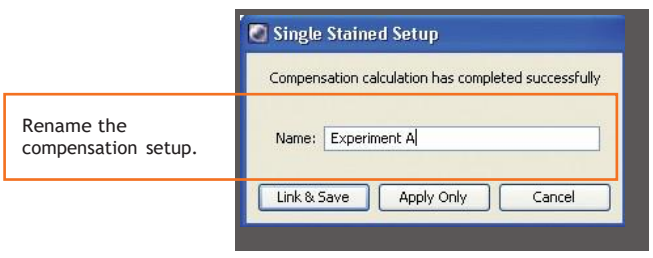
5. If you need Compensation, select: Experiment → Compensation Setup → Create Compensation Controls.

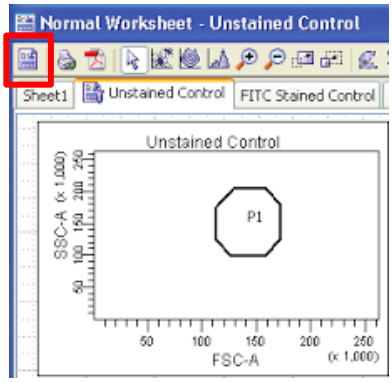


6. A specimen "Compensation Controls" has been created. Expand it and select unstained tube on the pointer; "Normal Worksheets" have been created for each compensation tubes.
7. Install the unstained control tube onto cytometer and click Acquire Data.
8. Adjust the voltages for FSC, SSC and the fluorochromes to put the cells on scale. 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 Next Tube and install your next control tube. Repeat the process for all your control tubes.



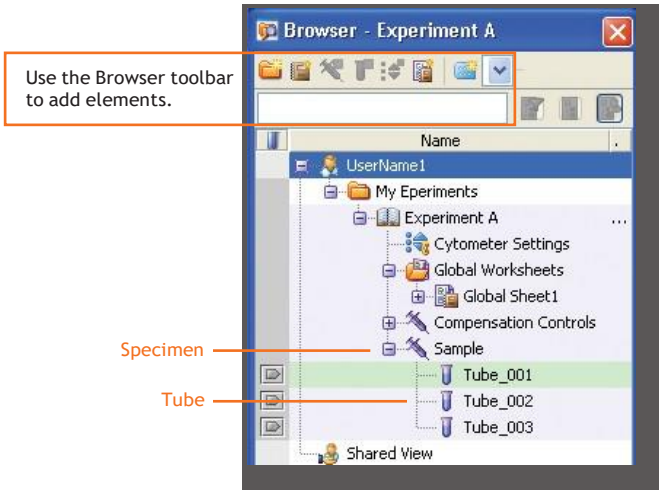
10. Select Experiment → Compensation Setup → Calculate Compensation → Apply Only.
11. Switch back to the Global Worksheet clicking on this icon ( ).



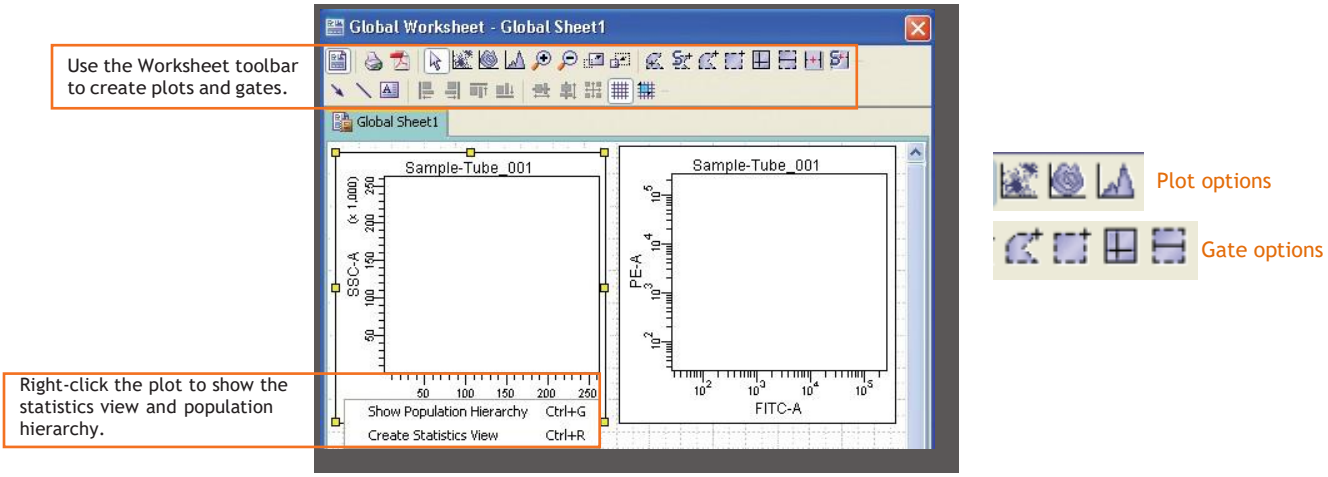


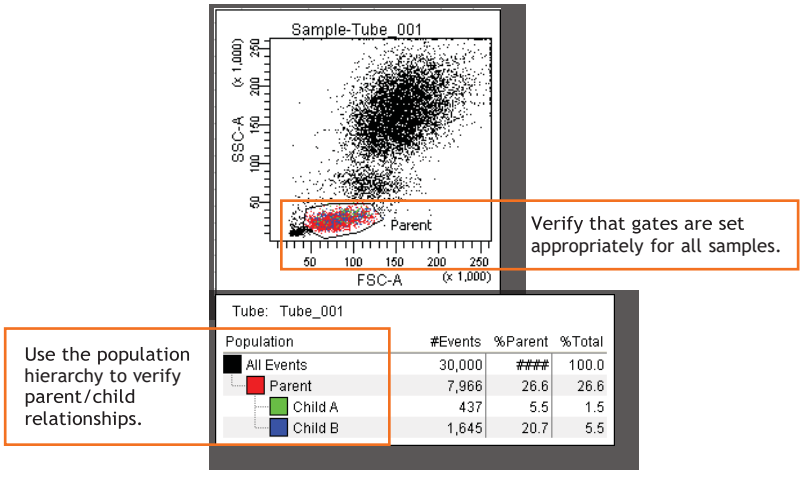
Recording Specimen Data

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

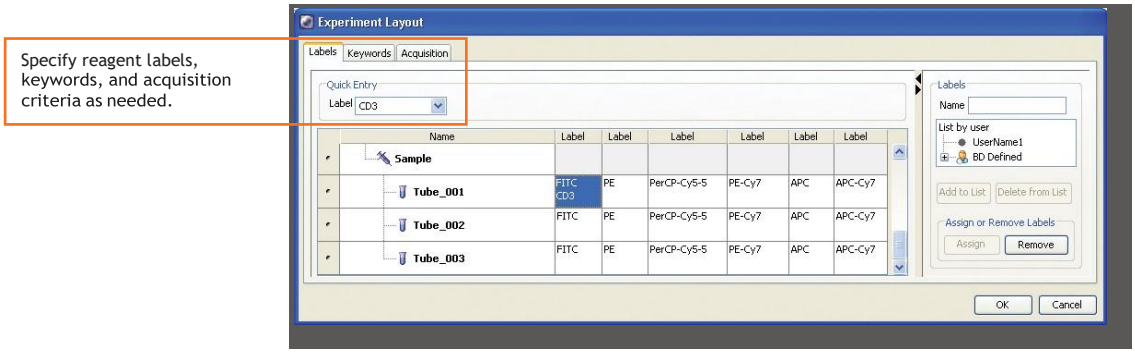


2. Create plots, gates, and statistics according to your experiment.





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

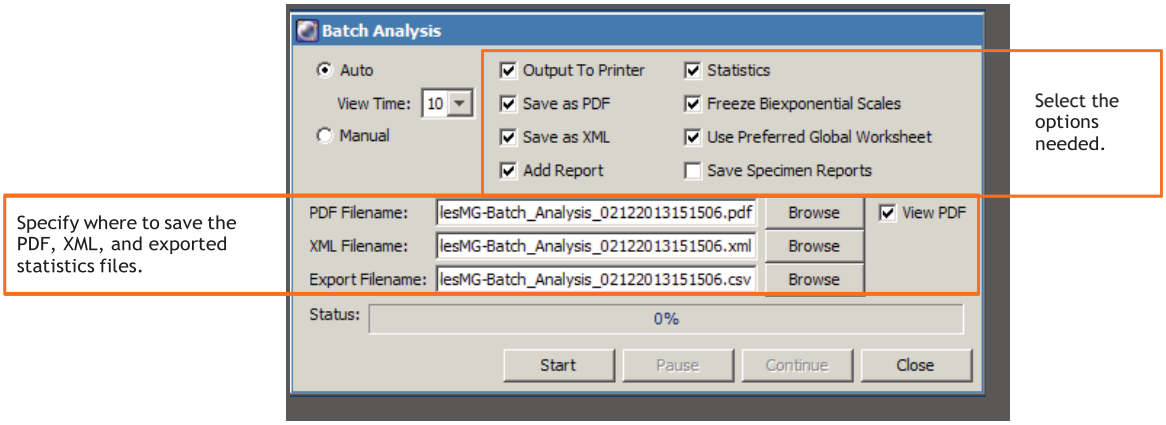


4. Run your samples and "Record Data" on the Acquisition Dashboard.

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. Clean the fluidics: 3 min for each solution - Contrad, 10% Bleach and DI water. Put the machine on Run mode with high flow rate.
2. After the cleaning, leave the machine on Standby and low flow rate with DI water tube on the SIP.

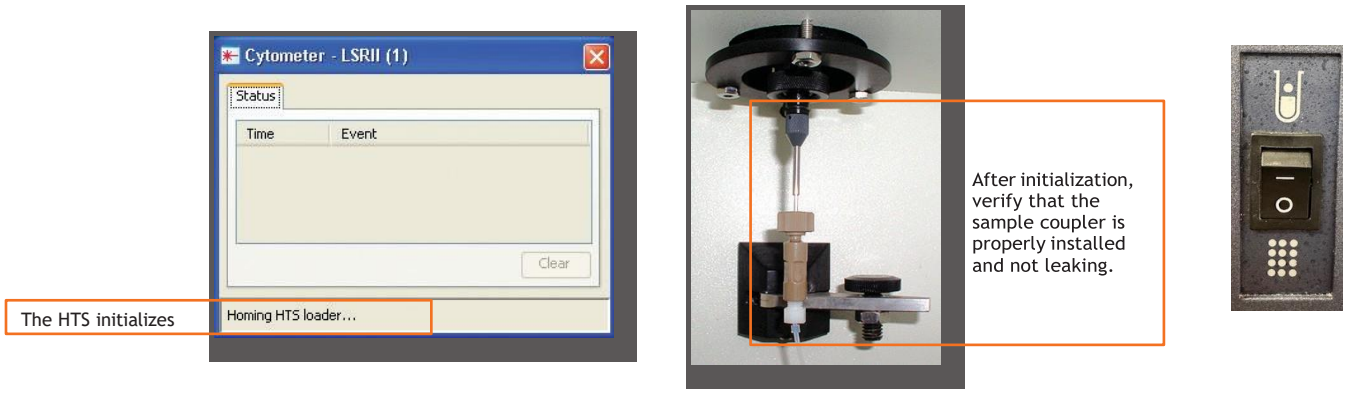
   Do not fill DI water tube more than half way. Water making contact with top of probe will damage the instrument.

If you are the last user of the day:

3. Empty the waste and refill fluids if prompted to do so.
4. Turn off the cytometer main power - green button.

If using the HTS:

1. Turn on the HST (button on the back), switch the machine to “plate mode” and install the sample coupler.



2. Add the plate element to your experiment: 

3. If you need to do **Compensation**, do the following:

   a. Select Compensation wells in the plate window, select the wells where you placed your compensation controls.

   Note: Compensation controls must be arranged horizontally across the plate.

   b. Right-click on your experiment name in the Browser and select: Compensation Controls → Create Compensation Controls.

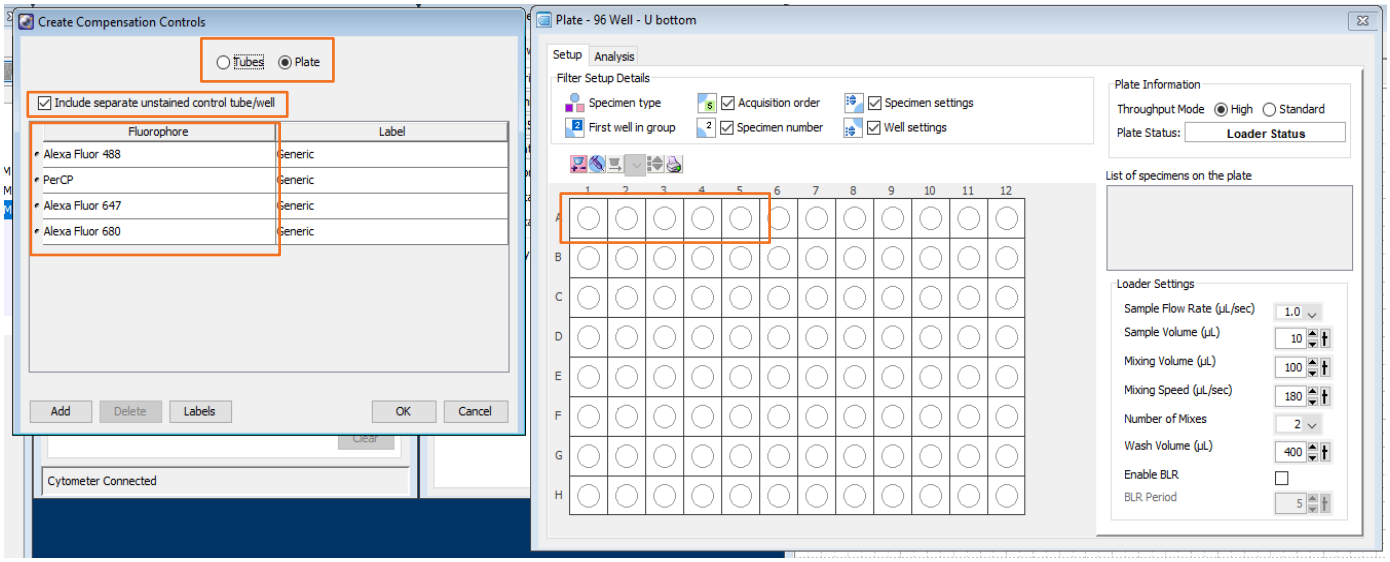
   c. In the "Create Compensation Controls" window:

      i. Ensure the "Plate" option is selected.

      ii. Check the box to include the unstained control, if applicable.

      iii. Adjust the order of fluorochromes to match the layout of your plate by dragging them using the dot on the left of each entry.

      iv. Click OK to save.

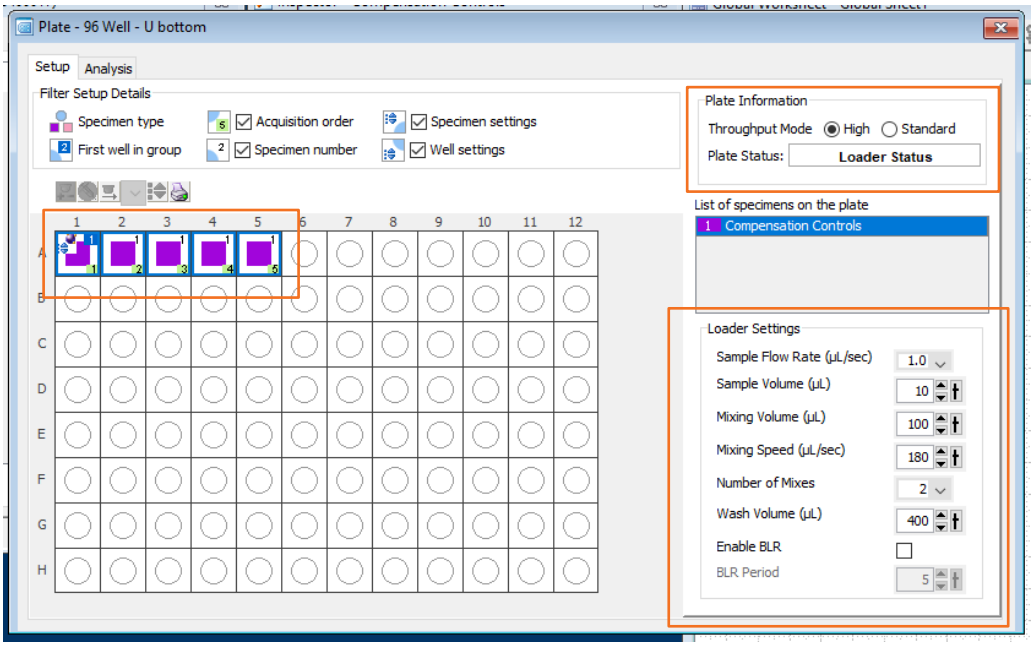


d. In the plate layout view, select all wells used for compensation controls.

e. Change Plate Information to Standard Mode.

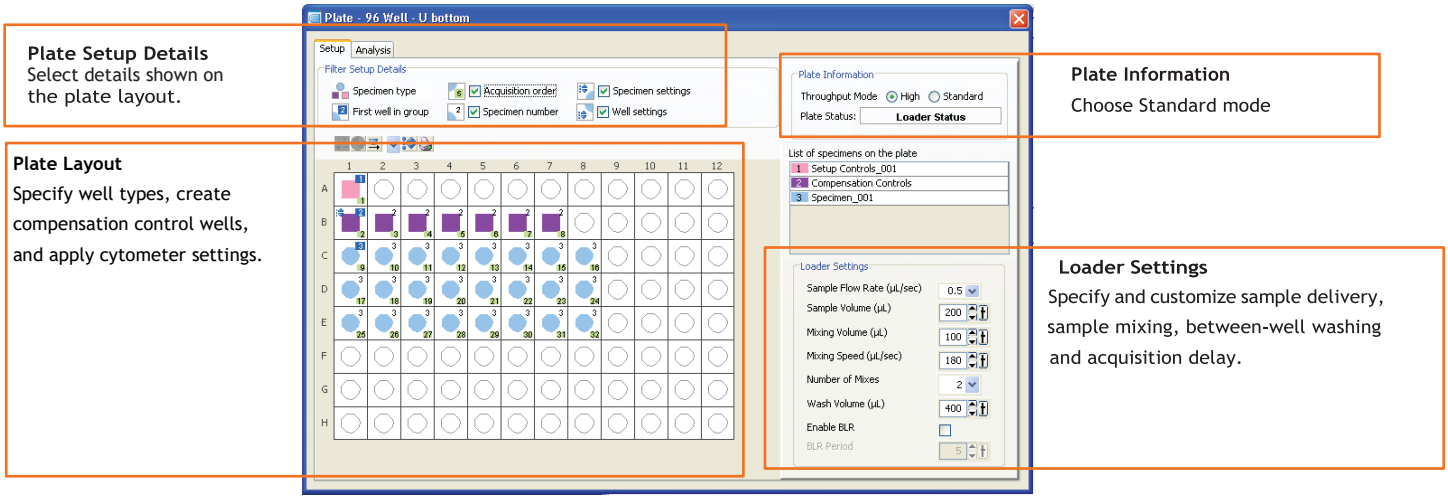
f. Adjust the Loader settings.

g. On the Acquisition Dashboard, click Run Plate to begin acquisition.



h. After the acquisition, adjust all the gates and select Experiment → Compensation Setup → Calculate Compensation → Apply Only.

4. Select the wells and create a Specimen ( ).



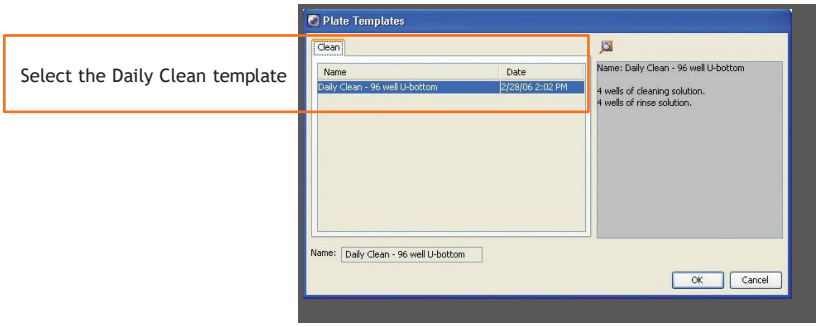
5. Install the prepared plate onto the HTS and place the cytometer in **run mode**.

6. Select the first specimen well and click Run Plate

7. When recording is complete, place the cytometer in standby mode.

CLEANING THE HTS:

1. Select HTS → Clean → DailyClean Template.
2. Install the plate and click OK to begin cleaning: 4 wells with contrad (A1 to A4), 2 with 10% bleach (B1 and B2) and 2 with DI water (B3 and B4).



Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in µL per second) that is delivered to the flow cell. Select a rate between 0.5 and 3.0 in increments of 0.5 µL per second.	The larger the value entered, the shorter the plate running time, but this increases the sample core, causing more variation of data.
Sample Volume	Amount of sample (in µL) aspirated from the well and delivered to the flow cell. Select a volume between 2 and 200 µL.	This value does not include the system default volume or the plate-dependent dead volume. For High Throughput mode, the system aspirates a set amount of 22 µL of sample, but records data for a volume between 2 and 10 µL. For Standard Throughput mode, the system aspirates the sample volume amount plus 20 µL.
Mixing Volume	Amount of sample (in µL) aspirated and dispensed from the well to resuspend the particles.	To avoid introducing bubbles into the fluidics, this value should be half the total well volume.
Mixing Speed	Rate (in µL per second) that the mixing volume sample is aspirated and dispensed.	The faster the rate, the more likely that cell shearing occurs, especially for delicate cells. A faster rate can introduce bubbles in the sample delivered to the cytometer and compromise the separator bubble.
Number of Mixes	The number of times the mixing volume sample is aspirated and dispensed at the mixing speed. Select a number between 0 and 5 mixes.	The larger the number, the longer the plate running time.
Wash Volume	Amount of sheath fluid (in µL) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 µL.	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.
Enable BLR/ BLR Period	Amount of initial data ignored at the start of data recording. Select a value between 5 and 150. The value selected is multiplied by 10 to determine the recording delay in ms.	Enable this feature when you expect a large fluorescence intensity difference between one well and the next. (Feature available on BD LSR II, BD LSRFortessa family, and BD FACSCanto A) This function delays recording for the selected amount of time multiplied by 10 (in millisec-onds). For example, setting the BLR period to 5 delays recording for the first 50 ms.