BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems with Loader Option

This guide contains instructions for using BD FACSDiva™ software version 6 with BD FACSCanto™ and BD FACSCanto II systems equipped with the BD FACS™ Loader option. The workflow shown uses the BD FACS™ Sample Prep Assistant II (SPA II) and BD FACS™ Lyse Wash Assistant (LWA) to prepare lyse/wash samples. The workflow shown also uses application settings in BD FACSDiva software. Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the BD FACS SPA II, LWA, and BD FACSDiva software for your use.

Workflow Overview

The following figure shows the steps for the daily workflow using BD FACSDiva software.

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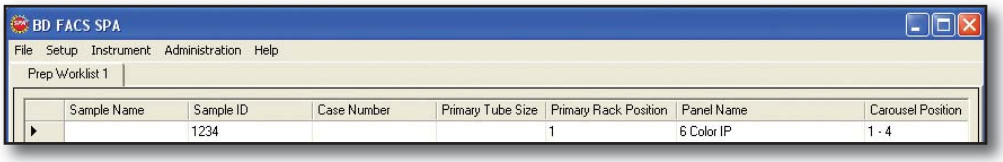
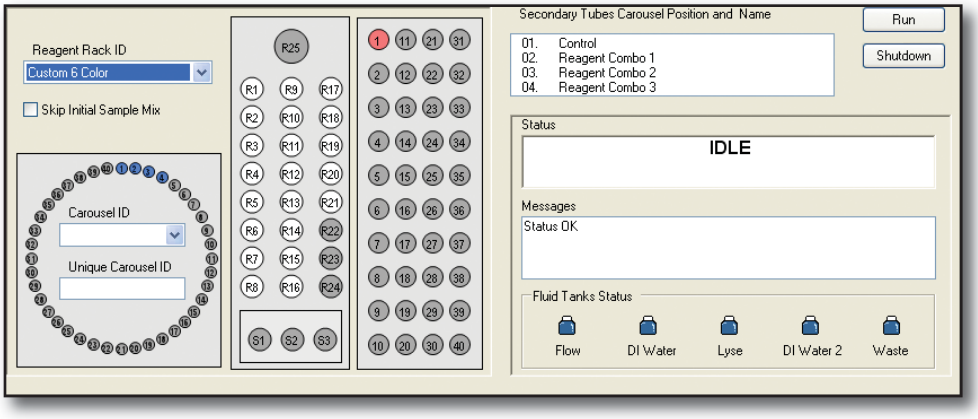
Preparing Samples

1. Perform daily inspection and startup for the SPA II and startup for the LWA.
2. Set up the worklist in the BD FACS SPA software.



3. Load the primary tube rack, secondary tubes into the carousel, and reagents into the reagent rack as specified in the software.



4. Close the safety cover and click Run to process samples on the SPA II.
5. Save and print the SPA II worklist.
6. Transfer the SPA II worklist to a location where it can later be imported into BD FACSDiva software.
7. Transfer the carousel from the SPA II to the LWA.
8. Run the appropriate LWA protocol.
9. Perform daily cleaning for the SPA II and LWA.
10. Shut down the SPA II and LWA.

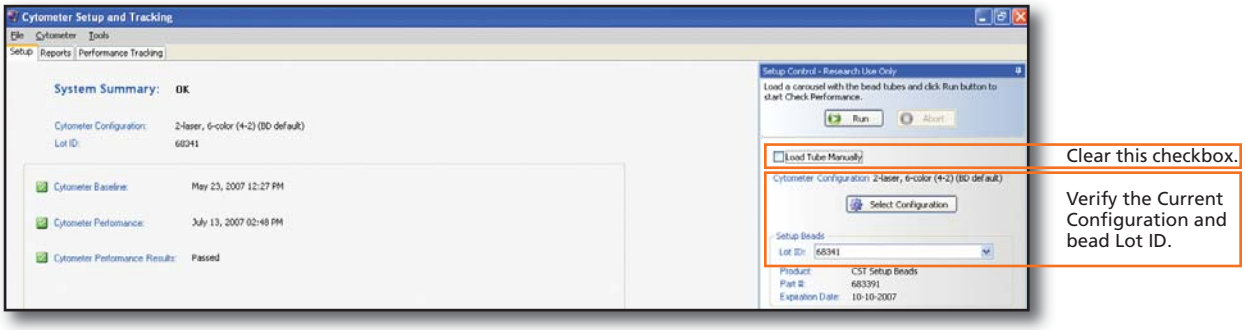
Starting Up the System

1. Turn on the cytometer main power.
2. Start up the computer, start BD FACSDiva software, and log in.
3. Check fluid levels in the Cytometer window.
4. Select Cytometer > Fluidics Startup if automatic cleaning is disabled.
5. Check the flow cell for air bubbles.
6. Check that laser warmup has finished, indicated by a ready status.

TIP Allow the lasers to warm up for 15 to 30 minutes before running samples on the cytometer to ensure laser stability and optimal power.

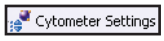
Checking Cytometer Performance

1. Select Cytometer > CST.

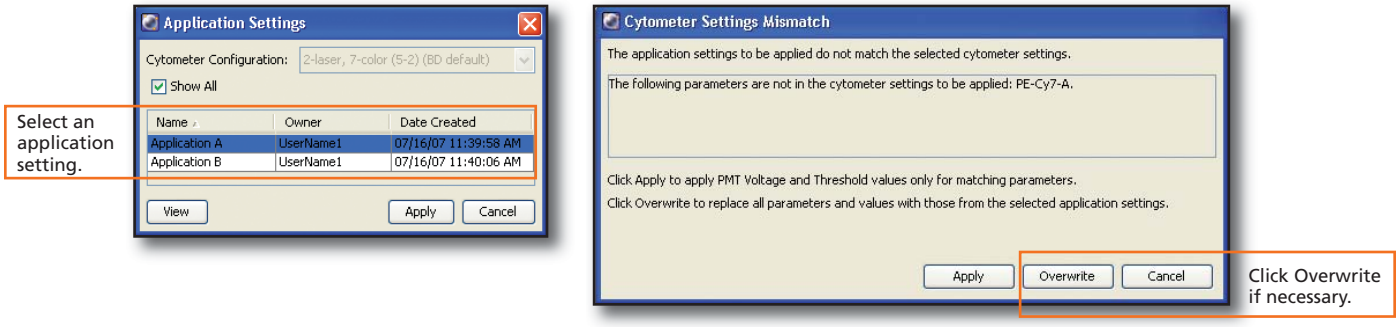


2. Place a tube of the BD™ Cytometer Setup and Tracking beads* in position 1 on a carousel and run the beads.
3. View the Cytometer Performance Report.
4. Close the Cytometer Setup and Tracking window.

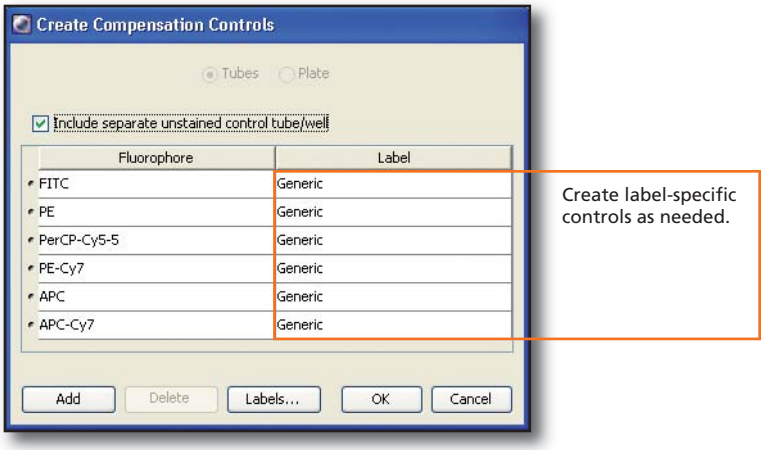
Setting Up the Experiment

1. Select Edit > User Preferences and verify that selected preferences are appropriate.
2. Create an experiment in the Browser.
3. Right-click 

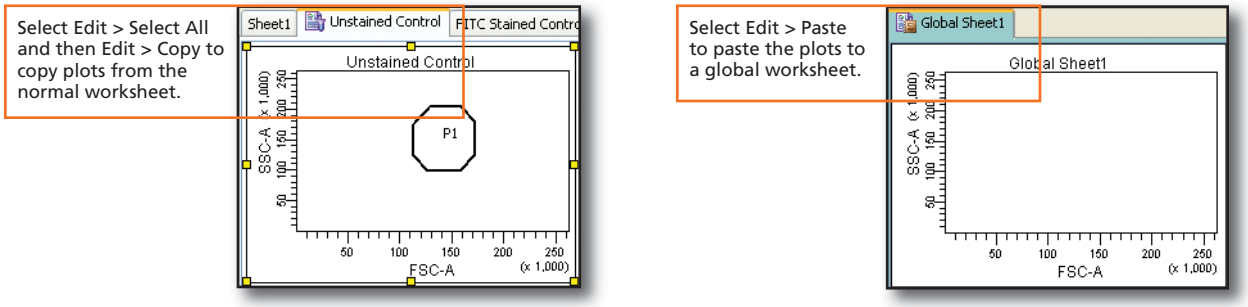
in the Browser. Select Application Settings > Apply.



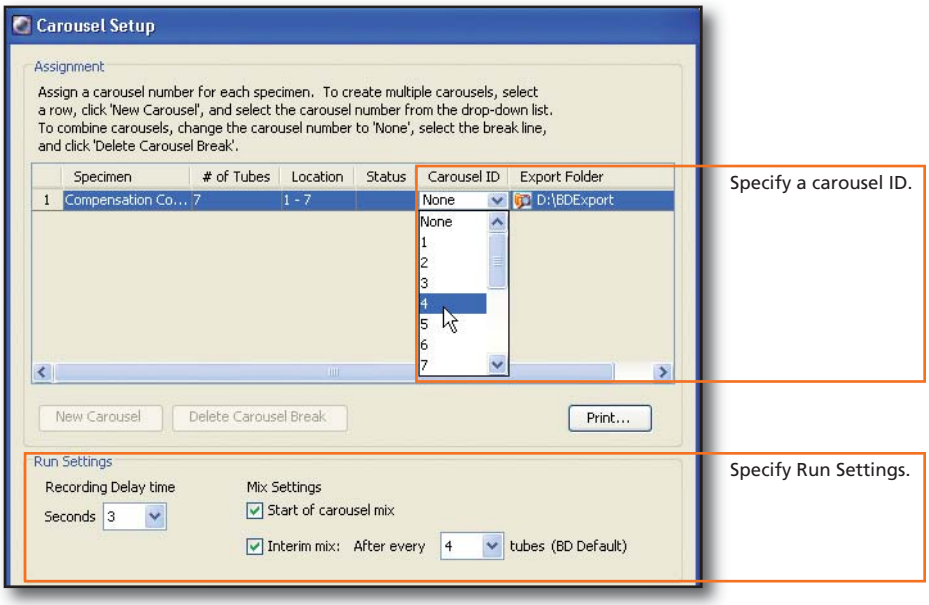
4. Select Experiment > Compensation Setup > Create Compensation Controls.



5. Copy and paste plots from the Unstained Control normal worksheet to a Global Worksheet.



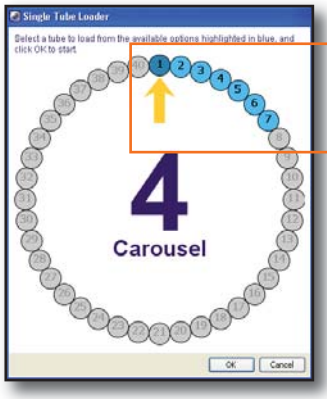
6. Specify Carousel Setup settings.



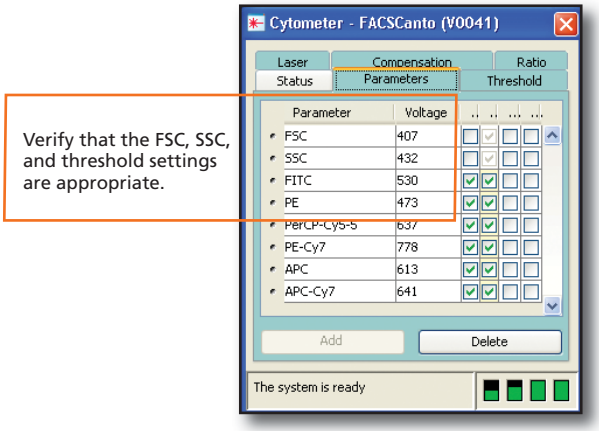
7. Verify that the current tube pointer is set to the Unstained Control tube and a global worksheet is displayed.
8. If using compensation beads, write down the FSC, SSC, and threshold values displayed in the Cytometer window.
9. Place compensation control tubes in the carousel in the same order listed in the Browser, and install the carousel in the Loader.

10. Verify that the cytometer is configured for automatic loading and that settings are appropriate for the compensation controls.

    Application settings are optimized for cellular samples. You might need to adjust settings for compensation controls prior to recording data for controls.



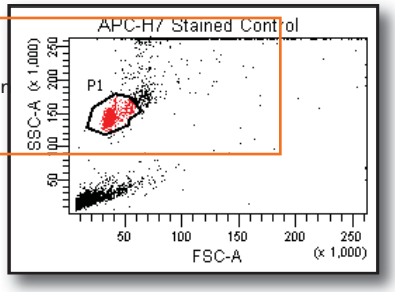
Select a tube to load and click Run Single Tube.

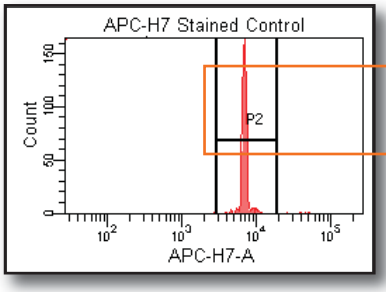


Verify that the FSC, SSC, and threshold settings are appropriate.

11. Click Unload and then click Run Carousel.
12. View the Carousel Report and check for any error messages.
13. View recorded data in the normal worksheets and gate the positive populations.

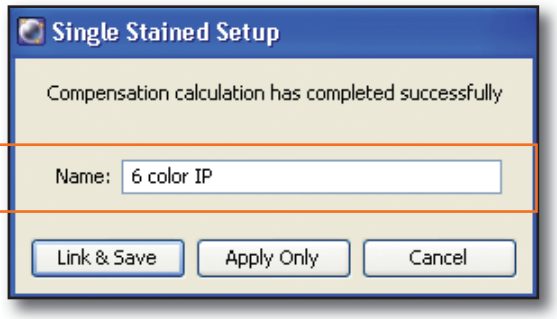
Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.





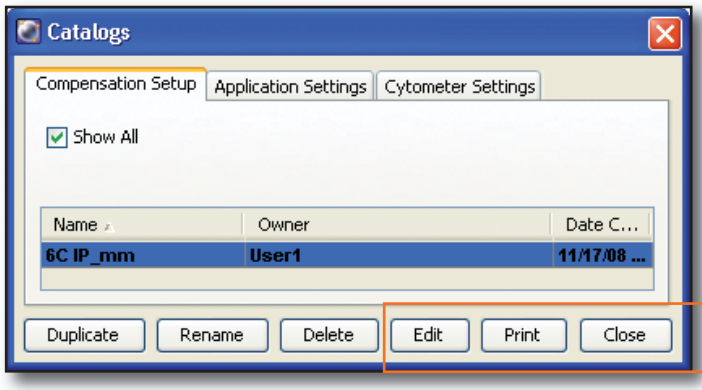
Adjust the P2 gates to fit the positive populations.

14. Select Experiment > Compensation Setup > Calculate Compensation.



Rename the compensation setup.

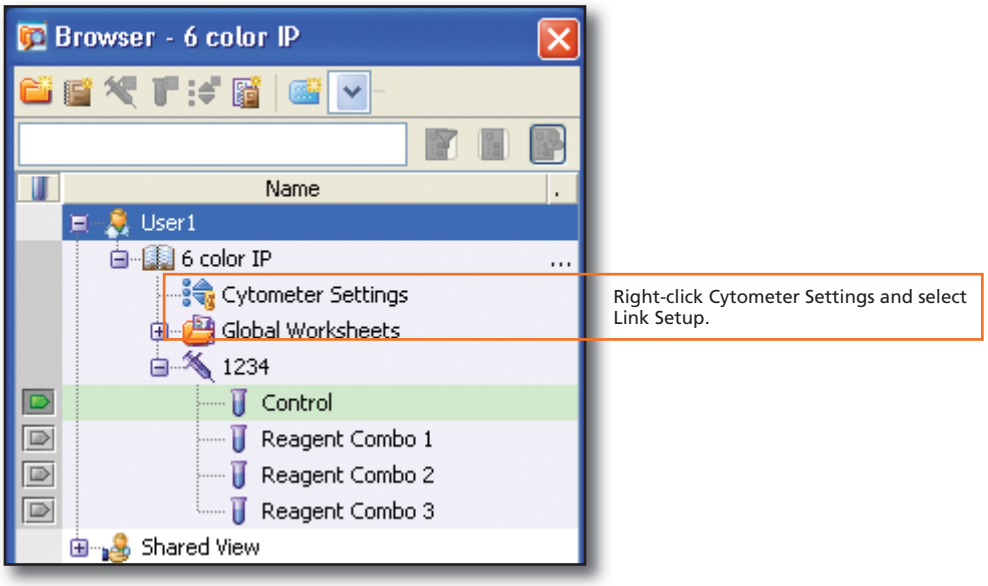
15. If needed, select Cytometer > Catalogs and return the FSC, SSC, and threshold settings to values appropriate for cellular samples.



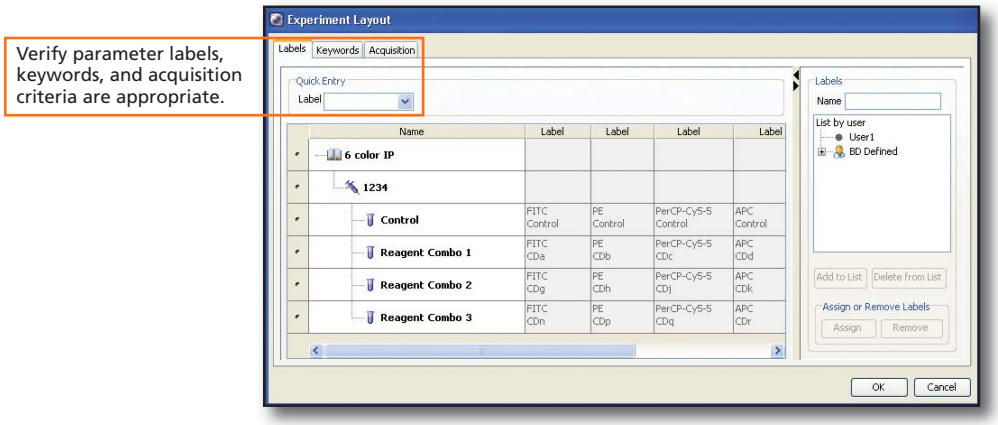
Select the compensation setup and click Edit. Make changes, click Save, and close the window.

Recording Specimen Data

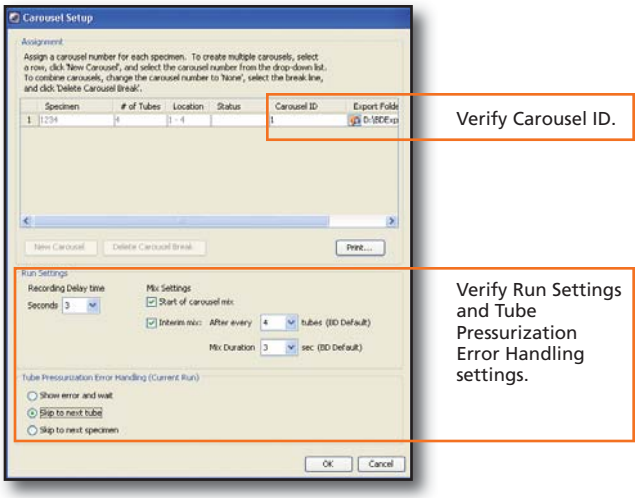
1. Create a new experiment by importing the SPA II worklist.
2. Link to appropriate cytometer settings.



3. Verify that selections and entries in the Experiment Layout are appropriate.



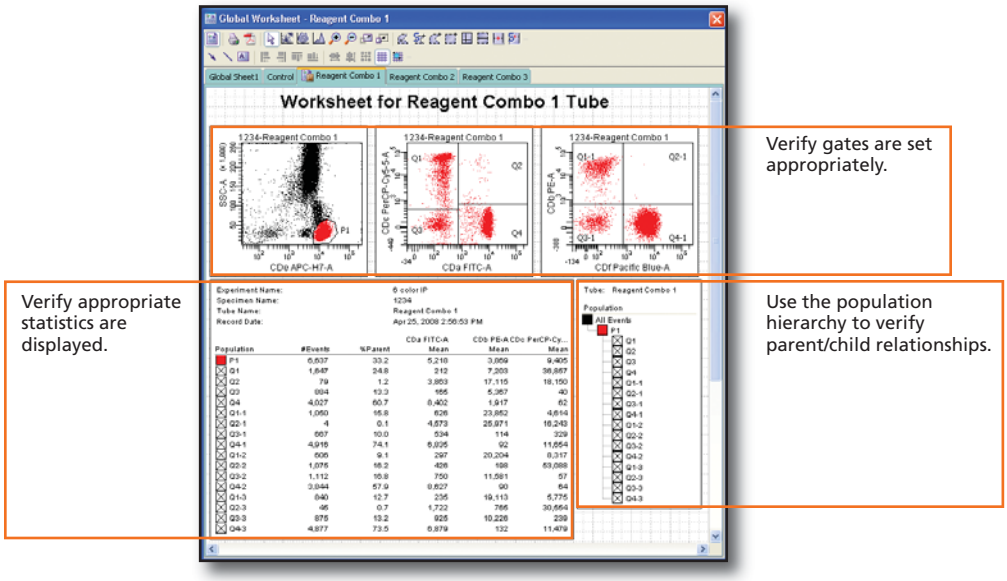
4. Verify Carousel Setup settings.



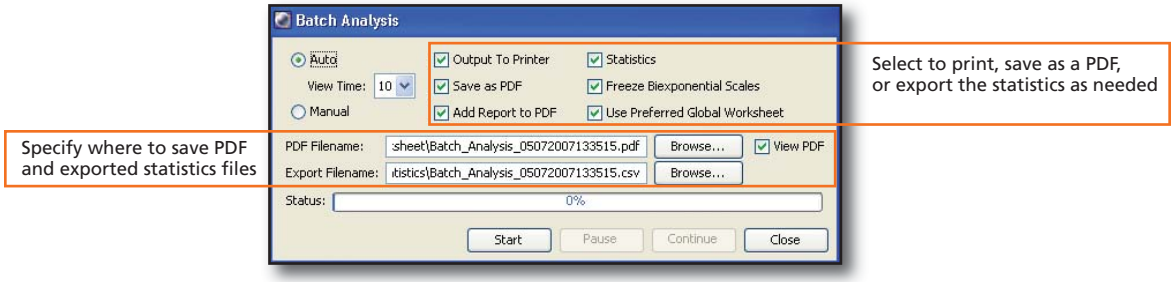
5. Install the carousel on the Loader and click Run Carousel.
6. View the Carousel Report and check for any error messages.

Analyzing Data

1. Verify that plots, gates, and statistics displayed in worksheets are appropriate for analysis of populations of interest.



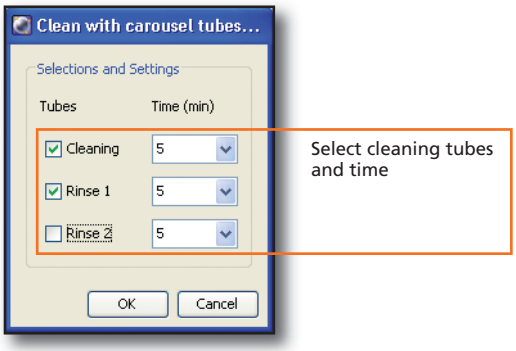
2. Do one of the following to print or export the results.
   - Select File > Print to print the active worksheet.
   - Select File > Export to export selected documents.
   - Right-click a specimen or experiment and select Batch Analysis (using a global worksheet).



3. Review printouts and verify that the analysis is appropriate.

Shutting Down the System

1. Verify that the flow rate in the Acquisition Dashboard is set to Medium or High.
2. Select Carousel > Clean.



3. Install the carousel with the appropriate cleaning tubes and perform the cleaning cycle.
4. Perform a fluidics shutdown.
5. Empty waste and refill fluids if prompted to do so.
6. Turn off the cytometer main power and shut down the computer.