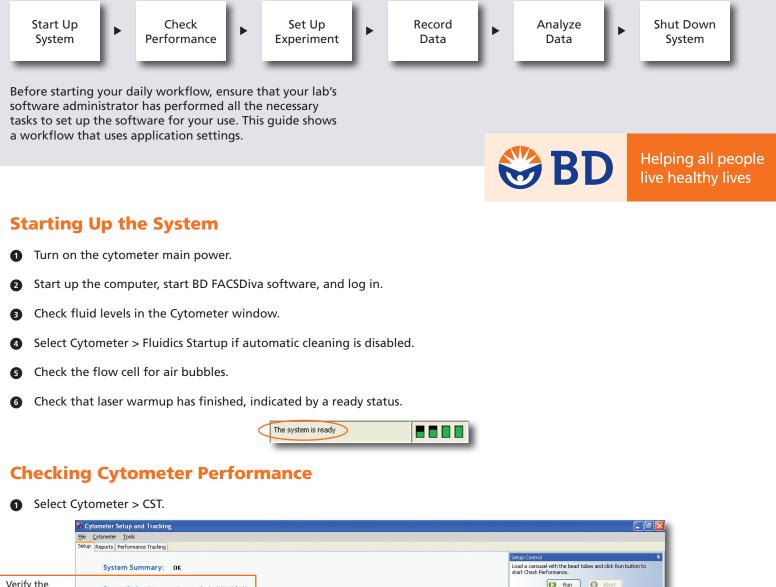
BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems with the Loader Option

This guide contains instructions for using BD FACSDiva[™] software version 8.0 and later with BD FACSCanto[™] and BD FACSCanto[™] II systems equipped with the BD FACS[™] Loader (Loader) option.

Workflow Overview

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



| | System Summary: OK | | Load a carousel with the bead tubes and click Run button to start Check Performance. | |
|---|--|--------------------|---|-------------------|
| Verify the Cytometer Configuration and bead Lot ID. | Cytometer Configuration: 2-laser, 6-color (4-2) (BD default) | | C Run Abort | |
| | Lot ID: 68341 | Load Tube Manually | Clear the checkbox. | |
| | Stormeter Baseline: May 23, 2007 12:27 PM | | Cytometer Configuration 2-laser, 6-color (4-2) (BD default) | |
| | | | Select Configuration | If needed, select |
| | Cytometer Performance: July 13, 2007 02:48 PM | | | a different |
| | | | Setup Beads | configuration or |
| | Sytometer Performance Results: Passed | | Lot ID: 34278 (RUO) | bead lot ID. |
| | | | Product: CST Setup Beads Part #: 345678 | |
| | | | Expiration Date: 02-20-2014 | |
| | | | | |

- Place a tube of BD FACSDiva™ CS&T research beads in position 1 on a carousel and run the beads.
- **3** View the Cytometer Performance Report.
- Close the Cytometer Setup and Tracking window.

Setting Up the Experiment

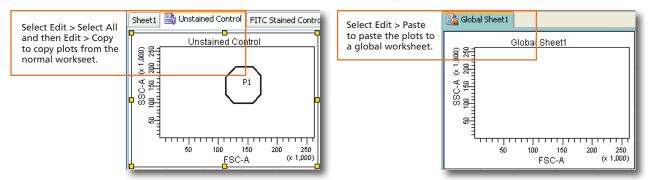
- Select Edit > User Preferences and verify that selected preferences are appropriate.
- 2 Create an experiment in the Browser.
- B Right-click Settings in the Browser. Select Application Settings > Apply.

| | Application Settings | | | Cytometer Settings Mismatch | | |
|--------------------------------|--|---------------------------------|--|--|---|-----------------------------|
| | Cytometer Configuration: 2-laser, 7-color (5-2) (BD default) | | ~ | The application settings to be applied do not match the selected cytometer settings. The following parameters are not in the cytometer settings to be applied: PE-Cy7-A. | | |
| Select an application setting. | Name A Application A Application B | Owner UserName1 UserName1 | Date Created 07/16/07 11:39:58 A 07/16/07 11:40:06 A | | Click Apply to apply PMT Volkage and Threshold values only for matching parameters. | |
| | View | | Apply Cance | | () () () () () () () () () () | ick Overwrite necessary. |

Select Experiment > Compensation Setup > Create Compensation Controls.

| Create Compensation Contro | ls | |
|-----------------------------------|-------------|-----------------------|
| () Tubes | | |
| Include separate unstained contro | l tube/well | |
| Fluorophore | Label | |
| • FITC | Generic | |
| • PE | Generic | |
| PerCP-Cy5-5 | Generic | Create label-specific |
| • PE-Cy7 | Generic | controls as needed. |
| # APC | Generic | |
| # APC-Cy7 | Generic | |
| Add Delete La | | |

6 Copy and paste plots from the Unstained Control normal worksheet to a global worksheet.



6 Specify carousel setup settings.

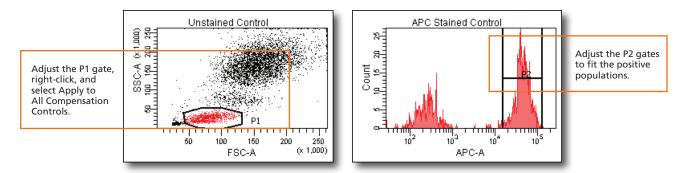
| 2 | Carousel Setup | | | | | | | |
|---|---|------------|---------------------------------------|---|--------|---------------|------------------------|--------------------------|
| | Assignment Assign a carousel number a row, click 'New Carouse To combine carousels, ch and click 'Delete Carousel | | | | | | | |
| | Specimen | # of Tubes | Location | Status | Carous | el ID | Export Folder | |
| | Compensation Co 7 1 - 7 | | | None 1 2 3 4 5 6 7 | × < | ፼ D√(BDExport | Specify a carousel ID. | |
| | New Carousel Delete Carousel Break Print | | | | | | | |
| | Run Settings Recording Delay time Seconds 3 | 10000 | itings rt of carous erim mix: 4 | | ary 4 | ~ | tubes (BD Default) | Specify run settings. |

2

- Verify that the current tube pointer is set to the Unstained Control tube and that a global worksheet is displayed. 9
- Place compensation control tubes in the carousel in the same order as listed in the Browser and install the carousel on 8 the Loader.

🐌 Run Single Tube

- Verify that the cytometer is configured for automatic loading and click 9 🛾 Single Tube Loader lect a tube to load from the llable options highlighted in blue, and Select a tube to load. Carousel 00000 OK Cancel ₩ Cytometer - FACSCanto (V0041) X Laser Compensation Ratio Verify that the Status Threshold Parameters FSC, SSC, and Parameter Voltage threshold settings FSC 407 are appropriate. SSC 432 FITC 530 PE 473 PerCP-Cy5-5 637 PE-Cy7 778 e APC 613 APC-Cy7 641 🕼 Run Carousel 🏠 Unload Click and then click 10
 - View recorded data in the normal worksheets and gate the positive populations. A

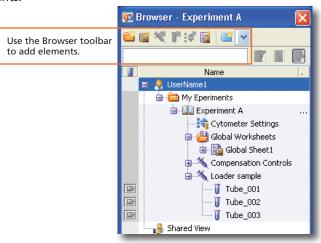


Select Experiment > Compensation Setup > Calculate Compensation. 12

| | Single Stained Setup | | | | | |
|--------------------------------|---|--|--|--|--|--|
| | Compensation calculation has completed successfully | | | | | |
| Rename the compensation setup. | Name: Experiment A | | | | | |
| | Link & Save Apply Only Cancel | | | | | |

Recording Specimen Data

1 Create Browser elements.



2 Specify carousel setup settings.

| 🖉 Carousel Setup | |
|--|--|
| Assignment Assign a carousel number for each specimen. To create multiple carousels, select a row, click News Carousel, and select the carousel number from the drog-down list. To combine carousels, change the carousel number to 'None', select the break line, and click 'Delec Carousel Break'. | |
| Spectrem # of Tubes Location Status Caroused ID Expont Folder 1 Compression Co, 7 1 - 7 4 [2] Undefined 2 Spectrem[001 3 1 = 3 1 = 0 1 = | Specify a carousel ID. |
| New Carousel Delete Carousel Break Print | |
| Run Settings Recording Delay time Mix Settings Seconds | Specify run settings and tube pressurization error handling settings. |
| Skip to next specimen | |

3 Make entries in the Experiment Layout.

| Specify reagent labels, | k Kerwords Acquestion Udd. Entry Udd. Entry None CD3 | Label | Label | Label | Label | Label | Label | Labels Name |
|-------------------------|---|-------------|-------|-------------|--------|-------|---------|------------------------------|
| • | III Experiment A | | Label | Label | Label | Label | Label | |
| • | Compensation Controls | | | | | | | UserName1 |
| | | | | | | | | 🖻 🐣 BD Defined |
| • | Unstained Control | | | | | | | |
| | | FITC | PE | PerCP-CyS-5 | PE-Cy7 | APC | APC-Cy7 | |
| | FITC Stained Control | FITC | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | |
| | PE Stained Control De PE Stained Control De PerCP-CyS-5 Stained Control De PerCP7 Stained Control | | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | |
| | | | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | |
| | | | PE | PerCP-CyS-5 | PE-Cy7 | APC | APC-Cy7 | - |
| | | FITC | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | - |
| | APC-Cy7 Stained Control | FITC | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | - |
| | Loader 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 | Add to List Delete from List |
| | Tube_003 | FITC | PE | PerCP-Cy5-5 | PE-Cy7 | APC | APC-Cy7 | Assign or Remove Labels |

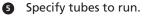
🚱 Run Carousel

4

Install the carousel on the Loader and click

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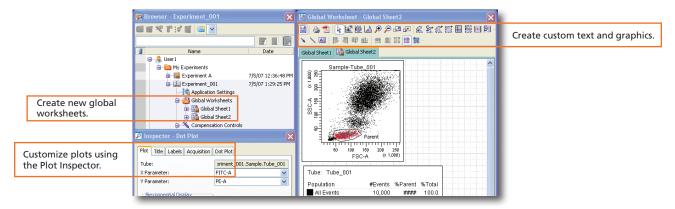
on the Acquisition Dashboard.



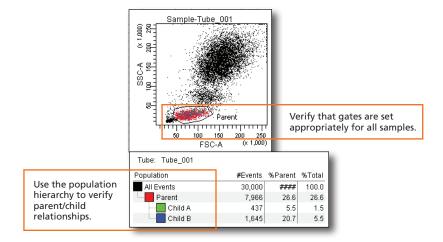
| | 💽 Carousel Rerun | | | | | |
|---------------------------|--|--|--|--|--|--|
| | FCS File/Data Warning, The process of re-running Specimen(s) or Tube(s) deletes the existing FSC files and data before acquisition begins. Stopping the run will not restore these deleted files or data. | | | | | |
| | Select Specimen(s) or Tube(s) to rerun: | | | | | |
| | Compensation Controls | | | | | |
| | Unstained Control | | | | | |
| | FITC Stained Control | | | | | |
| | 🔲 🌆 PE Stained Control | | | | | |
| | PerCP-Cy5-5 Stained Control | | | | | |
| | PE-Cy7 Stained Control | | | | | |
| | APC Stained Control | | | | | |
| | APC-Cy7 Stained Control | | | | | |
| | 🗹 🌂 Loader sample | | | | | |
| Check at the specimen | 🗌 🗹 🥡 Tube_001 | | | | | |
| level to select all tubes | 🗹 🦉 Tube_002 | | | | | |
| under the specimen. | V 😈 Tube_003 | | | | | |
| | Check All Check Remaining Check All | | | | | |
| | OK Cancel | | | | | |

Analyzing Data

1 Create plots, gates, and statistics needed for analysis.



2 Perform quality control of the analysis.



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3 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 elements.
- Right-click a specimen or experiment and select Batch Analysis (using a global worksheet).

| | Batch Analysis | | | | | | |
|----------------------------|---|--|-------------------------------|---------------|---|----------------------------------|--|
| | Auto | Output To Printer Statistic | | Statistics | | | |
| | View Time: 10 💌 | 🔽 Save as PDF | 🔽 Freeze Biexponential Scales | | | Select the options needed. | |
| | C Manual | ▼ Save as XML ▼ Use Preferred Global Worksheet | | Vorksheet | | | |
| | | Add Report | 🔲 Save Sp | ecimen Report | s | | |
| Specify where to save | PDF Filename: lesMG-Batch_Analysis_02122013151506.pdf Browse 🔽 View PDF | | | | | | |
| the PDF, XML, and | XML Filename: lesMG | -Batch_Analysis_02122013 | Browse | | | | |
| exported statistics files. | Export Filename: lesMG | | | | | | |
| | Status: | | | | | | |
| | Start Pause Continue Close | | | | | | |

Shutting Down the System

• Select Carousel > Clean.

| Clean with carousel tubes | | | | | | | |
|---------------------------|------------|---------------------------------|--|--|--|--|--|
| Selections and Se | | | | | | | |
| Tubes | Time (min) | | | | | | |
| 🔽 Cleaning | 5 🖌 | | | | | | |
| Rinse 1 | 5 💌 | Select cleaning tubes and time. | | | | | |
| Rinse 2 | 5 💌 | | | | | | |
| | | | | | | | |
| ОК | Cancel | | | | | | |

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