BD FACSCanto II Flow Cytometer
Quick Reference Guide

This guide contains instructions for both BD FACSCanto™ clinical and BD FACSDiva™ software. Follow only those sections that apply to the software you are running. Sections are indicated with the appropriate software icons.

or

BD FACSCanto Software
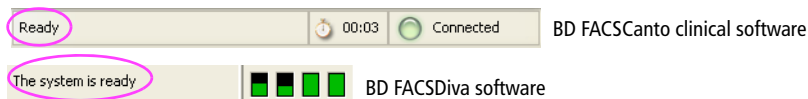
BD FACSDiva Software

Starting Up

1 Turn on the cytometer main power.

2 Start up the computer, launch the software, and log in.

3 Make sure the software is connected to the cytometer. If needed, make the connection manually (choose Cytometer > Connect).

| Ready | 00:03 | Connected | —— BD FACSCanto clinical software |

| Connected | Fluidics Startup done | —— BD FACSDiva software |
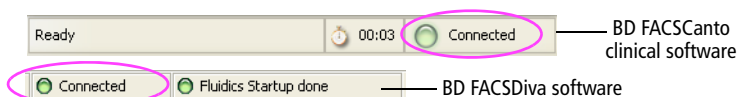
4 Check fluid levels in the Status or Cytometer window.

Empty the waste or replace fluid containers, if needed.

5 If automatic cleaning is disabled, run the fluidics startup procedure.

⚠ To prevent fluid overflow, make sure there is no tube on the sample injection tube (SIT) during fluidics startup.

6 Check the flow cell for air bubbles. If needed, run the de-gas flow cell procedure.

7 Check that laser warmup has finished.

| Ready | 00:03 | Connected | BD FACSCanto clinical software |

| The system is ready | | BD FACSDiva software |

Daily Setup: BD FACSCanto Clinical Software

Set up the cytometer with BD FACSCanto clinical software, even if you will optimize and run samples using BD FACSDiva software.

1 Prepare BD FACS™ 7-color setup beads.

2 Run the cytometer setup procedure for the bead lot you prepared.

Follow the Cytometer Setup Wizard to complete setup. When prompted, install the tube containing the setup beads, or the rack containing the beads tube and optimization samples.

3 If you are running samples manually, unload the tube when prompted.

- Hold the tube while moving the aspirator arm to the left.

- Remove the tube from the SIT.

- Release the aspirator arm and wait for cleaning to finish.

4 (Optional) When setup is complete, view and print the Setup Report.

5 Click [Next >] to optimize the settings, or click [Finish].

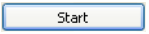**6** Review the Levey-Jennings report for trends in the setup data.

Optimizing Cytometer Settings: BD FACSCanto Clinical Software

Before you record data for a sample, optimize the detector voltages, spectral overlap, and threshold settings for the sample type and fluorochrome to be run.

1 Follow the prompts on the screen to proceed with optimization.

- Click Yes to save results.

- Choose the panel type and the X and Y parameters, and click [Next >].

- If you are using the Loader for optimization, click [Next >].

- Click [Next Tube] at the Cytometer Setup Optimization screen.

- If you are running tubes manually, install the first optimization tube when prompted, and click [OK].

- Click [Start].

2 Optimize settings, if needed.

- To adjust the optimization gate, click [Stop], make the needed adjustments, and then click [Start].

- To adjust detectors, thresholds, or spectral overlap settings, click the corresponding tab and use controls to adjust the settings.

3 If there are more tubes for that panel type, click [Stop], and then [Next Tube].

Otherwise, click [Stop], and then view and print the Setup Report for optimized settings.

4 Click [Save], remove the tube (if prompted), and then click [Finish].

Optimizing Cytometer Settings: BD FACSDiva Software

BD FACSDiva
Software

Before you record data for a sample, optimize the PMT voltages, compensation, and threshold settings for the sample type and fluorochrome.

1 Verify the cytometer configuration.

2 Create an experiment.

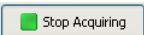**3** Select the experiment-level settings, and change, add, or delete parameters as needed.

4 Apply setup results.

- Right-click the experiment-level settings, and choose Apply from Catalog.

- Select a setup and click Apply.

5 Create compensation controls. Click OK to remove the setup link.

- If needed, edit labels for the compensation controls.

6 Optimize cytometer settings using a mixed sample tube.

- Set the current tube pointer to the unstained control tube, install the tube, and click [Acquire Data].

- Adjust FSC and SSC voltages, and the FSC threshold, if needed.

- On the Unstained Control worksheet, adjust the P1 gate, and apply gate changes to the remaining compensation controls.

- Optimize voltages to place the positive population for each fluorescent parameter on scale.

- Click [Stop Acquiring]. Remove the mixed sample tube.

7 Record data for the unstained control and for each single-stained control.

8 Adjust the gates around the positive populations on the histogram for each stained control tube.

9 Calculate compensation, and name the resulting setup.

Running Samples: BD FACSCanto Clinical Software

BD FACSCanto
Software

1 Prepare a worklist.

2 Install the first sample tube (manual acquisition) or the Loader rack (automated acquisition), and click ▐▶.

3 When asked if you want to save the worklist, click No.

4 (Loader option only) Click Ignore to run tubes manually.

5 (Optional) To optimize settings during initial acquisition, click ▐▇, and then ▐▚.

Adjust settings, and click ▐▶ to resume.

6 For manual acquisition, remove the sample tube when prompted and install the next tube. Repeat until data for all tubes has been recorded.

7 Inspect the lab report for each sample on the worklist.

Double-click the Status field to see a sample's report.

Running Samples: BD FACSDiva Software

BD FACSDiva
Software

1 Create a new specimen and tubes. Rename them appropriately.

2 Create a global worksheet and rename it.

3 Use the Experiment Layout dialog to define tube labels and keywords, and enter events to record.

4 On the global worksheet, create appropriate plots for viewing data during acquisition.

5 Install the first tube onto the cytometer. Acquire data for the corresponding tube in the Browser.

6 While data is being acquired, draw a gate around the population of interest. Set the other plots to show data from this population.

7 Click ■ Record Data . Remove the tube when recording is finished.

8 Install the next tube.

9 Repeat until data has been recorded for all tubes.

Shutting Down: BD FACSCanto Clinical Software

1 Create a worklist with a two-tube panel. Enter *Clean* as the Sample ID.

2 Click ▐▶ ; when asked to save the worklist, click No.

3 (Loader option only) Click [Ignore] to run tubes manually.

4 When prompted, install a tube containing ≤3 mL of BD™ FACSClean solution or 10% bleach.

5 Click [OK]. Wait 5 minutes, and then click [▌🧪] .

6 When prompted, unload the tube.

7 Install a tube with ≤3 mL of deionized water. Repeat steps 5 and 6.

⚠ To prevent fluid overflow, do not leave a tube on the SIT during fluidics shutdown.

8 Choose File > Exit, and select *Run fluidics shutdown and exit*.

9 When fluidics shutdown completes, turn off the power.

Shutting Down: BD FACSDiva Software

1 Install a tube with ≤3 mL of BD FACSClean solution or 10% bleach, and click [Acquire Data] .

2 After 5 minutes, click [Stop Acquiring] and remove the tube.

3 Repeat steps 1 and 2 with DI water.

4 Run fluidics shutdown.

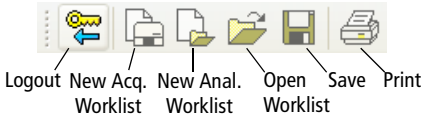⚠ To prevent fluid overflow, do not leave a tube on the SIT during fluidics shutdown.
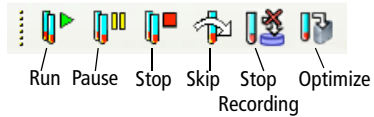
5 Turn off the cytometer main power.

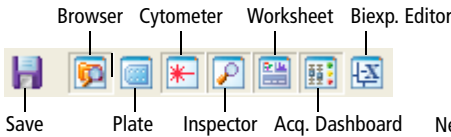# BD FACSCanto Clinical Software Toolbars

Standard toolbar

Logout | New Acq. Worklist | New Anal. Worklist | Open Worklist | Save | Print

Worklist toolbar

Run | Pause | Stop | Skip | Stop Recording | Optimize

BD FACSDiva Software Toolbars

Workspace toolbar

Browser | Cytometer | Worksheet | Biexp. Editor

Save | Plate | Inspector | Acq. Dashboard

Browser toolbar

New Expt | New Tube | New Global Worksheet | Number of Wells

New Folder | New Specimen | New Cytom Settings | New Plate

Worksheet toolbar

Worksheets View | Save as PDF | Dot Plot | Histogram | Zoom Out | Decrease Plot Size | Snap-To Gate | Rectangle Gate | Interval Gate | Snap-To Interval Gate

Print | Select | Contour Plot | Zoom In | Increase Plot Size | Autopolygon Gate | Polygon Gate | Quadrant Gate | Autointerval Gate

Arrow | Text | Align Right | Align Bottom | Distribute Vertically | Show Grid

Line | Align Left | Align Top | Distribute Horizontally | Make Same Size | Snap-To Grid