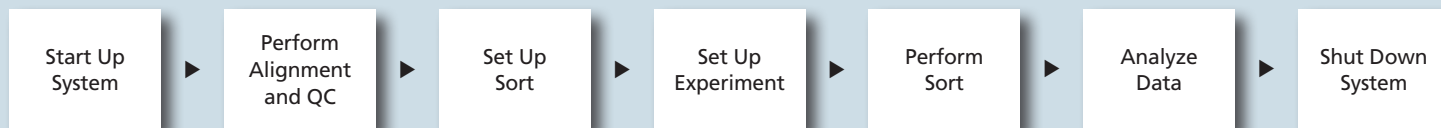


Getting Started Guide for Using BD FACSJazz™ Cell Sorters with BD FACS™ Software Sorter Software

This guide contains instructions for using BD FACS™ Software sorter software v1.X with BD FACSJazz™ cell sorters.

Workflow Overview

The following figure shows the daily workflow steps.



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Start Up the System

- 1 Turn on the cytometer and the computers.
 - a. Turn on the main power.
 - Two connection confirmation beeps sound when the server has connected with the instrument. Three repeating beeps sound if the server is unable to connect to the instrument.
 - Allow 30 minutes for lasers to fully warm up before recording QC data.
 - b. Turn on the BD FACS Software computer, if necessary.
- 2 Start BD FACS Software software.
 - a. Log in to the BD FACS Software computer, if necessary.
 - b. Double-click the BD FACS Software icon on the desktop to start the software.
 - Verify that the BD FACS Software workspace, the SortView pane, and the Pressure Control pane have opened.
 - c. Restore or create a workspace for alignment and QC.
 - A QC workspace contains cytometer settings, sort settings, plots, and gates used for alignment and sort setup.
- 3 Prepare the fluidics.
 - a. Fill the sheath tank and empty the waste tank.
 - Typical starting volume for daily use is 5 liters. This is based on an average usage of 0.5 liter per hour, plus 1 liter additional volume to account for startup rinsing and drop stability. The maximum tank volume is 7 liters.
 - Bleach may be added to the waste tank so that the end-of-day concentration of bleach is 10%. Do not let undiluted bleach sit in the waste tank while the instrument is not in use.
 - b. Reattach the sheath filter, if necessary.
 - c. Turn on the vacuum and air supply sources.
 - d. Turn the AIR switch to on.

Start Up the System (Continued)

- e. Connect the air, vacuum, and fluidic lines to the sheath and waste tank.
- f. Verify that the sheath tank is pressurized to ~27 psi, and the waste tank is between 5" and 15" Hg vacuum.

4 If performing a **dry startup**, clean and attach the nozzle tip.

- a. Sonicate the nozzle tip in a tube of distilled water for one minute.
 - If sonicating the tip in ethanol or detergent, rinse the tip with distilled water before installing on the instrument.
- b. Use a syringe to flush the nozzle tip with filtered deionized water.
- c. Place the flush bucket under the nozzle assembly, if necessary.
- d. Install the nozzle tip onto the nozzle assembly.

4 If performing a **wet startup**, remove the debubble reservoir and leave the flush bucket under the nozzle assembly.

5 Flush the system to remove air from the sheath filter.

- a. Click **Rinse** to begin flushing the system.
- b. Tap the sheath filter gently to dislodge any air bubbles.
- c. Click **Rinse** to stop flushing the system.

6 Remove air from the sample line and nozzle.

- a. Click **Stream** to start the stream.
- b. Click **Backflush** and verify that sheath fluid is dripping from the sample line.
- c. After about 20 seconds, click **Backflush** and then click **Stream** to stop the stream.
- d. Install the debubble reservoir filled with filtered sheath fluid so that the nozzle tip is submerged.
- e. Click **Purge** to remove air from the nozzle.
- f. Click **Pulse** to help dislodge bubbles.
 - If performing the dry startup, allow the initial air bubble from the nozzle to pass the y-fitting before clicking **Pulse**.
- g. (Optional) If bubbles are still present after pulsing:
 - Use the "nozzle prime" technique to dislodge bubbles: While in purge mode, remove the debubble reservoir to introduce a small amount of air into the nozzle. Then, refill the debubble reservoir and re-submerge the nozzle.
 - Or, stop the PURGE and fill the debubble reservoir with 70% ethanol and click **Purge** to fill the nozzle.
Click **Pulse** to help dislodge bubbles. Stop the purge and refill the debubble reservoir with sheath fluid. Purge with sheath fluid to clear away the ethanol.
- h. Click **Purge** once air has been removed from the nozzle.

Start Up the System (Continued)

7 Start the stream.

- a. Install the two-tube sort device with 50-mL conical, and put the tray in the Safe position.
- b. Open the deflection plates before removing the flush bucket to reduce the risk of getting them wet.
 - (Optional) Before removing the flush bucket, remove and clean the deflection plates. Dry the plates thoroughly before replacing.
- c. Click **Stream** to start the stream.
- d. Remove the debubble reservoir.
- e. Remove any excess fluid from the nozzle tip using a tissue or cotton applicator.

Perform Alignment and QC

- 1 Align the stream to the pinholes, the stream drain, and the BD FACST[™] Accudrop laser.
 - a. Open the nozzle access door and close the sort chamber door, if necessary.
 - b. Press Illum to turn on the Accudrop laser.
 - c. Remove the flush bucket from under the nozzle, if necessary.
 - d. Use the three translational (horizontal, vertical and focus) silver knobs on the nozzle stage to align the upper section of the stream to the pinholes.
 - Usually, the vertical-axis knob should not be adjusted for daily use.
 - The stream should be centered on the pinholes with crisp edges on the sides of the stream.
 - The nozzle tip should be about one pinhole distance above the first pinhole.
 - e. Use the two rotational (pitch and roll) black knobs on the nozzle stage to align the lower section of the stream with the stream drain and the Accudrop laser.
 - (Optional) Advanced users can use Test Streams to align the side streams to the Accudrop laser. The nozzle access door must be closed to charge the stream.
 - f. Repeat steps 1d and 1e until the stream is centered and in focus over the pinholes and centered in the stream drain and Accudrop laser beam.
 - g. Close the nozzle access door.

- 2 Optimize the fluorescence and side scatter parameters from the primary laser.
 - a. Open the shutter for the primary laser.
 - b. Verify that the nozzle access door is closed and activate the laser reset sensor.
 - c. Run a sample tube of QC beads and adjust the sample offset to produce a narrow sample core.
 - Verify that the bead flash for the primary laser is aligned with the first pinhole, and adjust if necessary.
 - Verify that the trigger parameter is set to SSC or primary fluorescence.
 - d. While looking at dot plots for primary fluorescence and SSC channels, make small adjustments to the stream alignment to obtain the brightest signal strength and lowest CV.
 - If CVs remain high or signal strength low, lower the Sample Offset and/or make small adjustments to the stream primary laser adjustment knob.

- 3 Optimize forward scatter (standard FSC).
 - a. Examine the FSC signal.
 - With good consistent daily alignment, little or no adjustment is needed.
 - b. If you need to optimize the FSC signal:
 - Lower the Sample Offset, being careful not to collapse the core stream.
 - Make small adjustments using the knobs on the forward scatter stage.

Perform Alignment and QC (Continued)

- 4 Align the remaining lasers, if present.
 - a. Open the appropriate laser shutter.
 - Verify that the laser is aligned with the appropriate pinhole.
 - b. Optimize the laser delay for each laser to achieve the brightest and tightest signal.
 - Typical values for laser delay for the second and third pinhole are 5.2 and 10.4, respectively.
 - Laser delays may be further optimized after laser adjustment in step 4c.
 - c. Make small adjustments using the appropriate laser adjustment knob to obtain the brightest signal strength and lowest CV.

- 5 Enter values in the QC log.
 - a. Adjust the voltages to place the fluorescence and scatter signals at their target values.
 - b. Record the PMT voltage and rCV for each parameter in the QC log.
 - Optionally, QC may be performed by keeping the PMTV constant and recording the median signal strength and rCV for each parameter.
 - c. Record a data file for QC.

Set Up to Sort

- 1 Optimize the frequency.
 - a. Visualize the breakoff using the Drop Camera image.
 - The typical starting value for piezo amplitude is between 4 and 5.
 - If the drop position is off-screen, adjust the **Piezo Amplitude** settings.
 - If the piezo needs to be adjusted dramatically, or the drops are moving, check the sheath tank for leaks and the nozzle for air bubbles.
 - b. Scan for the optimal frequency to find the shortest breakoff.
 - The typical starting value for the drop frequency is 39.00 kHz.
 - c. Adjust the **Piezo Amplitude** to position the breakoff at the drop reference marker.

- 2 Optimize the drop phase.
 - a. Verify that the two-tube holder is installed, with the waste conical in place, on the sort stage.
 - b. Select **2 tube holder – 2 way sort** as the sort device in the **Sort Layout**.
 - c. Click **Safe** in the **Sort Layout** to place the waste conical under the waste drain.
 - d. Close the deflection plates then click **Plates** to charge them, if necessary.
 - e. Click **Test Streams** in the **Sort Settings** pane to initiate stream deflection.
 - Confirm that the nozzle chamber door, the sort chamber door, and the plate access panel are closed.
 - f. Click **Flash Charge** and adjust the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.
 - If you cannot get side streams to deflect without fanning, adjust the frequency and/or clean the deflection plates and repeat.
 - Verify that the breakoff is at the drop reference marker. If it is not, adjust the **Piezo Amplitude** to bring the breakoff to the marker and repeat the Flash Charge.
 - g. Click **Short Flash** and adjust the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.
 - h. Turn off the Short Flash and Flash Charge and adjust the stream focus to eliminate second drop deflection, if necessary.
 - The typical starting value for stream focus is 10 to 12%.
 - i. Turn off the Test Streams.

- 3 Determine the drop delay using BD FACST[™] Accudrop.
 - a. Click **Safe** in the **Sort Layout** pane and verify that the waste flask is under the stream drain.
 - b. Select **Accudrop Setup** as the sort device in the **Sort Layout**.
 - c. Prepare and run a sample tube of BD FACST[™] Accudrop beads and adjust the sample offset to produce an event rate near 2,000 events per second.
 - d. Click **Accudrop** to place the Accudrop optical filter into position.
 - e. Create a gate for the Accudrop beads, if necessary.
 - f. Assign the gate for the Accudrop beads in the **Sort Layout** pane and click **Start** to begin sorting.

Set Up to Sort (Continued)

- g. Adjust the drop delay until the left stream is as bright as possible and the center stream has almost no fluorescence.
- h. Click **Accudrop** to place the neutral density filter into position

4 Align the sort streams to the sort device.

- a. Select the desired sort device in the **Sort Layout**, and install the appropriate sort device on the sort tray.
- b. Adjust the side streams and/or tray position so they are centered in the sort collection tube or well.

Set Up an Experiment (with Compensation)

1 Prepare the workspace.

- a. Open a new workspace or load configuration files.
 - If restoring a workspace, clear the **Fluidics Setup** checkbox to prevent overwriting the optimized daily sort settings.
- b. Verify the user preferences.
- c. Verify the following cytometer settings: trigger detector level and parameter, and the Lin or Log amplifier setting for each parameter, then enter the detector channel labels.
 - It is important to remember that you can sort only what you see, and you can exclude from your sort only what you see.
- d. Create and/or verify worksheet elements such as plots, statistics, and gate hierarchies, if necessary.

2 Adjust PMT detector voltages to optimize for the sample.

- a. Run an unstained sample tube, and verify the scatter detector voltages and trigger level, if necessary.
 - If you cannot determine the scatter voltages, use a single or fully stained control to help identify the population through gating.
- b. Run the single stained control sample tubes or the fully stained sample, and verify the fluorescence detector voltages.
 - PMT voltages for all detectors to be compensated must be adjusted before recording compensation controls. The Autocompensation tool will not process tubes with different detector voltages.

3 Record compensation controls.

- a. Load and run the unstained control sample tube.
- b. Change the save location and file name to reflect the sample type (for example, *unstained control*).
- c. Verify the recording rule settings, including: the event limit, stopping gate, and storage gate.
 - A minimum of 100 events in each gate is necessary per compensation control population to properly calculate compensation.
- d. Record a data file for the unstained control tube.
- e. Repeat steps 3a–3d for each of the single color control tubes.

4 Perform compensation.

- a. Add a new worksheet page.
- b. Create ADC data plots for each of the compensation control data files.
- c. Create Local gates for each compensation control population, and name them accordingly.
- d. Verify that **Cytometer** is the source in the **Compensation** pane.
- e. Select the parameters to be compensated using **Manage Parameters**.

Set Up an Experiment (with Compensation)

- f. Assign the positive and negative compensation control populations to the parameters for autocompensation.
- g. Click **Calculate** to populate the compensation matrix.
- h. (Optional) Select the **Visualize** box in the **Compensation** pane to view compensation applied to the ADC parameters.
 - Compensation visualized using the ADC parameters can be displayed in Logicle scaling, and the compensation values can be adjusted post-recording.

Perform a Sort

1 Create sort gates and worksheet elements.

- a. Load the sort sample and acquire some data.
- b. Create or modify plots, if necessary.
 - Verify that *DSP parameters are used to display compensated parameter data.
- c. Create or modify gates, if necessary.
 - Verify that *DSP parameters are used to define sort gates using compensated parameter data.
- d. Create or modify population hierarchies and/or statistics views, if necessary.
 - Verify the percentages of target populations and plan the sort accordingly.

2 Set up the Sort Layout.

- a. Select a sort device in the **Sort Layout** and install the sort device onto the sort tray, if you have not already done so.
- b. Verify that the side streams are aligned to the sort device, and adjust if necessary.
- c. Assign the sort populations to sort positions.
- d. Assign the sort limit for each sort position.
- e. Select the sort mode to be used during the sort.
- f. Save the **Sort Layout**.

3 Start the sort.

- a. Verify that the droplet position and breakoff either visually or by using the Flash Charge, before starting the sort.
 - If using the Flash Charge, remember to remove the sort device and replace with the waste flask while testing streams.
- b. Mix and or filter the sample, if necessary.
- c. Load and run the sort sample tube.
- d. Adjust the sample offset to achieve the desired acquisition event rate.
 - In general, the higher the event rate the lower the sort efficiency. We recommend that the event rate not exceed 1/4 of the drop frequency.
- e. Click **Sort Ready** in the **Sort Layout** pane to place the sort device in the starting sort position.
- f. Start the sort.

4 Monitor the sort.

- a. Monitor the acquisition event rate and acquisition efficiency.
 - Adjust the sample offset, if necessary.
- b. Monitor the sort rates, sort abort rates, and sort efficiency.
- c. Monitor the sort streams and minimize fanning, if necessary.
 - Mitigate fanning by adjusting the piezo amplitude, event rate, sort mode, or by filtering the sample.
 - Fanning due to cell size and shape may necessitate using a larger nozzle for sorting. Generally, we recommend using a nozzle at least five times larger than the cells in the sample.

Perform a Sort (Continued)

- 5 Complete the sort.
 - a. Verify that the sort has completed, or stop the sort manually.
 - b. Stop and unload the sample.
 - c. Click **Eject** in the **Sort Layout** pane, if necessary, and remove the sort device.
 - d. (Optional) Preview, print, and/or save the sort report.
 - The sort report can be reset by clicking **Reset** in the **Sort Layout** or by changing the sort device.

Analyze Data

1 Record post-sort data files.

- a. Transfer a portion of the sort sample to a 12 x 75-mm polypropylene sample tube.
- b. Backflush the sample line between samples to prevent cross-contamination.
 - If desired, run a clean sample tube of filtered water between samples to check for residual sample in the sample lines.
- c. Run and record your post-sort samples.

2 Verify the analysis.

- a. If you have created an Analysis Template for the experiment, restore the Analysis Template for the post-sort data file. Or, add new worksheet pages and create plots, population hierarchy, and/or statistics view for the post-sort data files.
- b. Create, modify, and/or localize gates for analysis, if necessary.
- c. (Optional) Select the **Visualize** box in the **Compensation** pane to view compensation applied to the ADC parameters.
 - Compensation visualized using the ADC parameters can be displayed in Logicle scaling, and the compensation values can be adjusted post-recording.
 - Compensation applied to the DSP parameters cannot be adjusted after recording.
- d. Check the sort purity using post-sort data files.
 - Sort gates may need to be localized and modified to analyze post-sort purity due to wider distribution of target populations after sorting.
 - If sort purity was lower than expected, modify the sort mode, sort gates, and/or include a doublet discrimination gate, and perform the sort again.

3 Save, print, or export the results.

- a. Save the Workspace.
- b. Save any additional configuration files, if necessary, including Cytometer Settings, Sort Layout, Analysis Template, and Compensation.
- c. Save and/or print the worksheet report as a PDF file.
- d. (Optional) Export data files as CSV files or in BD FACSDiva™ software compatible format.

Shut Down the System

1 Clean the sample line.

- a. Load and run a sample tube of 10% bleach for 5 minutes.
- b. Load and run a sample tube of filtered distilled water for 5 minutes.

2 Perform a **wet shutdown**.

- a. Leave the tube of water on the sample port.
- b. Place the flush bucket under the nozzle.
- c. Fill the debubble reservoir with filtered distilled water and install it on top of the flush bucket.
- d. Verify that the nozzle tip is submerged.
- e. Stop the stream and PURGE for 1 minute to fill the nozzle with water.
- f. Remove the air, vacuum, and fluidic lines from the sheath and waste tanks.
 - DO NOT connect the fluidic and air lines together during a wet shutdown.
- g. Empty the sheath and waste tanks.
 - If bleach was not added now before sampling, bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.

2 Perform a **dry shutdown**.

- a. Remove the tube of water from the sample port.
- b. Place the flush bucket under the nozzle.
- c. Stop the stream, and remove the air, vacuum, and fluidic lines from the sheath and waste tanks.
- d. Empty the sheath tank and fill it with 0.5–1.0 L of distilled water.
- e. Empty the waste tank.
 - Bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.
- f. Reconnect the air, vacuum, and fluidic lines to the sheath and waste tank and verify that the vacuum and pressure have been applied to the tanks.
- g. Bypass the sheath filter.
 - (Optional) Bypass the sheath filter one minute after beginning step 2i so that the sheath filter is stored in water instead of sheath.
- h. Click **Rinse** and then **Backflush** to run water through all the lines.
- i. When air starts to run through the system, click **Rinse** and then **Backflush** to stop the stream.
- j. Remove the air and sheath lines from the sheath tank and connect them to each other.
 - Do not connect the air and sheath lines to each other if the sheath filter is still in place.
- k. Click **Rinse** and **Backflush** to remove any residual water, then click **Rinse** and **Backflush** to stop the flow, then remove the nozzle tip and place it in a storage vessel.
- l. Click **Rinse** and **Backflush** to dry the fluidic lines, and stop fluidics when system is completely dry.

Shut Down the System (Continued)

m. Empty the sheath and waste tanks.

- Bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.

3 Clean the sort chamber.

- a. Verify that the power to the deflection plates is turned off.
- b. Remove the plate access panel and clean the deflection plates.
- c. Clean and decontaminate any spills in the sort chamber or sample port area.
- d. Clean any area of salt buildup and check for leaks.

4 Turn off the power.

- a. Export files to secondary storage device, if needed.
- b. Save the workspace, if needed.
- c. Save Cytometer Settings and Sort Layout, if needed.
- d. In BD FACS Software, select **Cytometer > Shutdown Cytometer**.
 - The Shutdown Cytometer command disconnects and shuts down the server.
 - (Optional): If the instrument needs to be used after Cytometer > Shutdown Cytometer was selected, switch the cytometer power off, then on again to restart and reconnect the cytometer server.
 - Turn the AIR switch to Off.
- e. Turn off the vacuum and the air supply.
- f. Turn off the BD FACS Software computer.
- g. Turn off the cytometer power.