

BD FACSDiscover™ S8 Cell Sorter

Quick Reference Guide

This guide contains instructions for using the BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology. See the user's guide for additional information.

Workflow overview

The following figure shows a typical daily workflow when using the BD FACSDiscover™ S8 Cell Sorter.

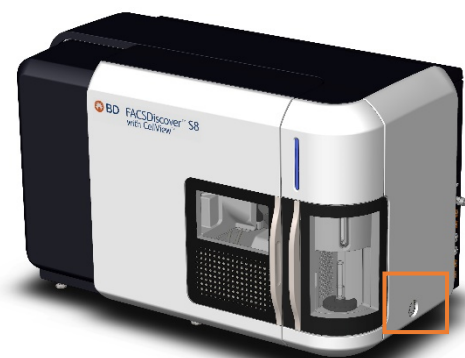


Before you begin

- Verify the sheath tank is full, and the waste tank is empty.
- Prepare the BD FACSDiscover™ Setup Beads, BD FACS™ Accudrop Beads and BD CellView™ Calibration Beads according to the package insert.
- Prepare the single-stain controls for your experiment.

Start up system

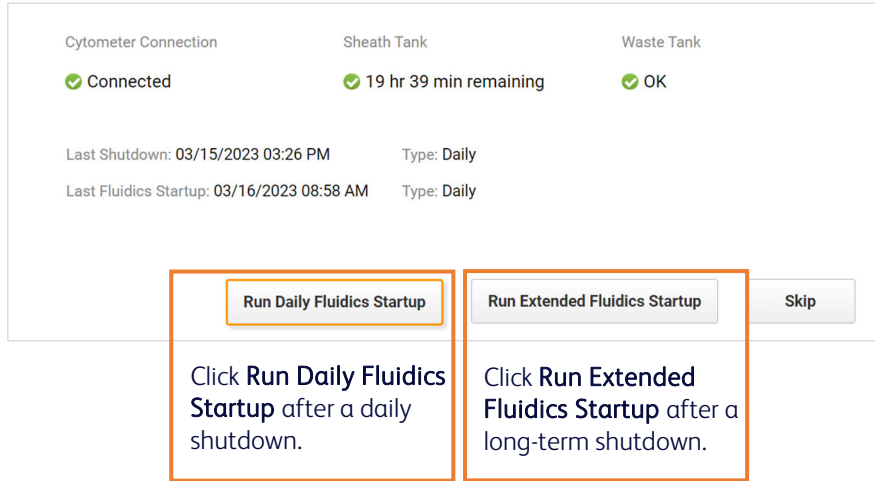
1. Turn on the source of air pressure and verify that the output is 80–95 psi.
2. If you work with a biological safety cabinet (BSC) or an aerosol management system (AMO), ensure it is turned on for 3 minutes before powering on the cytometer.
3. Power on the cytometer.
4. Power on the workstation and log into Microsoft® Windows®.
5. Open BD FACSCorus™ Software.



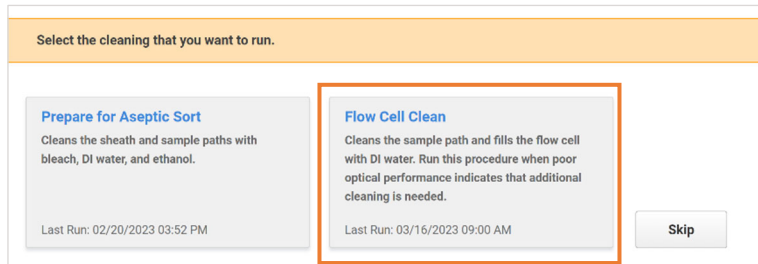
Startup system, continued

- 1 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Setup and QC
- 5 Image Calibration
- 6 Drop Delay

6. Select a startup process and follow the prompts on the screen.

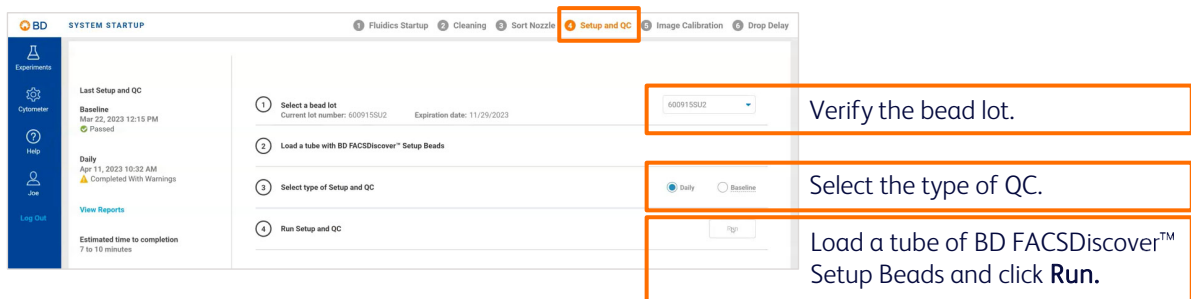


7. Select **Flow Cell Clean** and follow the prompts on the screen.



8. Remove the closed-loop nozzle and insert a sort nozzle.

9. Perform a Setup and QC.



Startup system, continued

10. If needed, perform an Image Calibration.

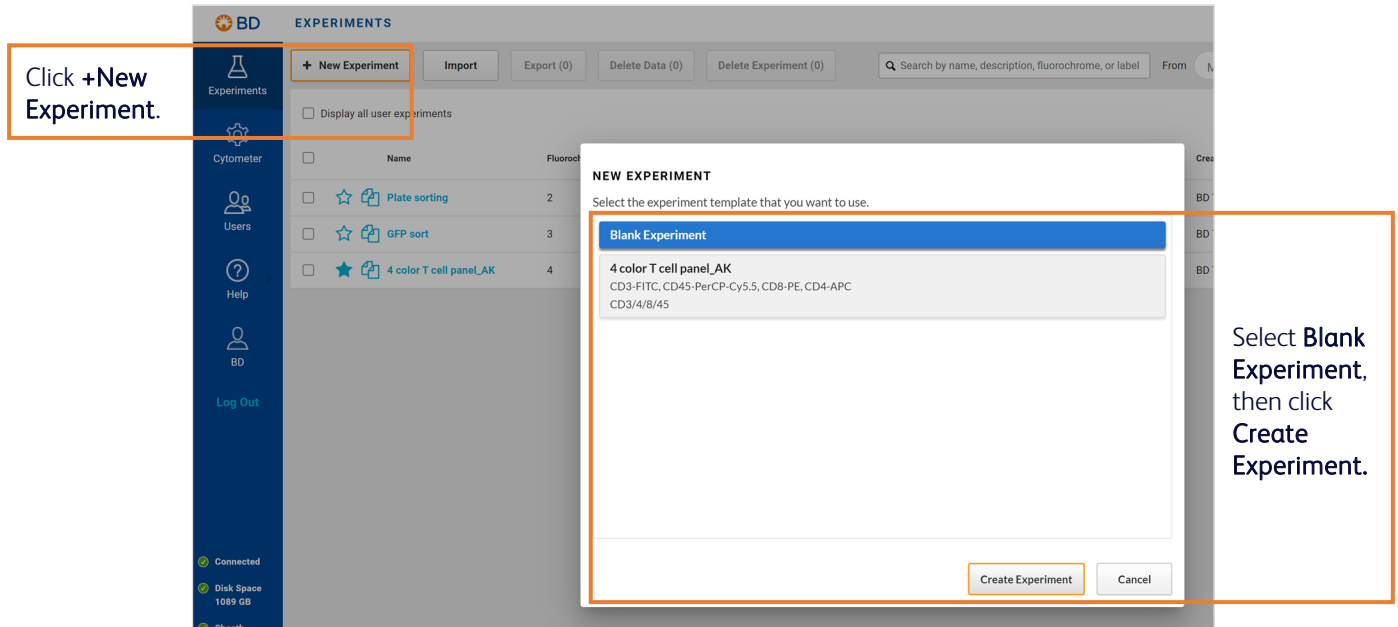
The screenshot shows a software interface with a blue sidebar on the left containing a user profile icon, 'BD', and a 'Log Out' button. The main area displays a 'System Status' window with a list of tasks: 'Nozzle Size: 100 µm', 'Fluidics Startup' (Last Run: 03/20/2023 09:12 AM, Type: Extended), 'Setup and QC' (Last Run: 03/20/2023 02:03 PM, Status: Passed), 'Image Calibration' (Last Run: 03/17/2023 11:44 AM), 'Drop Delay' (Last Run: 03/16/2023 11:14 AM), and 'Configuration: Imaging-3 Blue 16 Violet-20 YellowGreen-12 Red-8 UV-Z2'. A 'Close' button is at the bottom of this window. To the right, a larger panel shows a warning: 'Run Image Calibration bi-weekly or after any change in optical configuration.' Below this, it states 'Last Calibration Run: 03/17/2023 11:44 AM' and 'Status: Passed'. At the bottom of this panel are 'Run Calibration' and 'Skip' buttons. A text box on the right explains: 'Run a tube of BD CellView™ Calibration Beads if the Image Calibration is outdated. Click Skip if the Image Calibration status is green.' A text box on the left says: 'Click System to view the Image Calibration status.'

11. Perform a Drop Delay setup.

The screenshot shows a software interface with a progress bar at the top containing six steps: 1 Fluidics Startup, 2 Cleaning, 3 Sort Nozzle, 4 Setup and QC, 5 Image Calibration, and 6 Drop Delay (highlighted in orange). Below the progress bar, a panel displays a warning: 'Run Drop Delay daily before sorting.' It then shows 'Drop Delay Last Run: 05/30/2023 10:51 AM' and 'Status: Passed'. A text box on the left says: 'Run a tube of BD FACS™ Accudrop Beads.' To the right of this text are 'Run Drop Delay' and 'Skip' buttons.

Set up experiment

1. Create a new experiment on the Experiments page.



The screenshot shows the BD Experiments interface. A callout box on the left points to the '+ New Experiment' button. A modal dialog titled 'NEW EXPERIMENT' is open, showing a list of templates. The 'Blank Experiment' template is selected. A callout box on the right points to the 'Create Experiment' button in the modal.

Click +New Experiment.

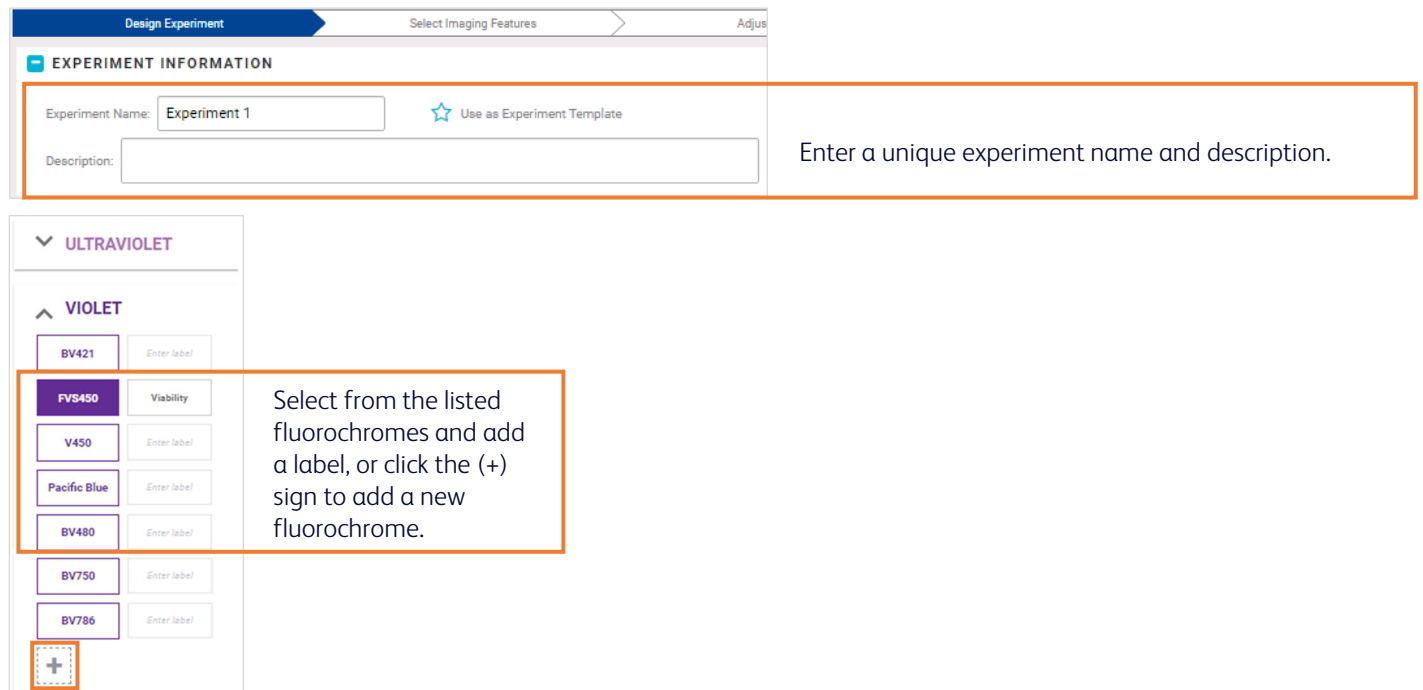
NEW EXPERIMENT
Select the experiment template that you want to use.

- Blank Experiment
- 4 color T cell panel_AK
CD3-FITC, CD45-PerCP-Cy5.5, CD8-PE, CD4-APC
CD3/4/8/45

Create Experiment Cancel

Select Blank Experiment, then click Create Experiment.

2. Enter the experiment information, dyes and labels on the Design Experiment page.



The screenshot shows the 'Design Experiment' page. The 'EXPERIMENT INFORMATION' section has a callout box pointing to the 'Experiment Name' and 'Description' fields. The 'VIOLET' section has a callout box pointing to the list of fluorochromes and the '+' button.

Design Experiment Select Imaging Features Adjust

EXPERIMENT INFORMATION

Experiment Name: Experiment 1 Use as Experiment Template

Description:

Enter a unique experiment name and description.

ULTRAVIOLET

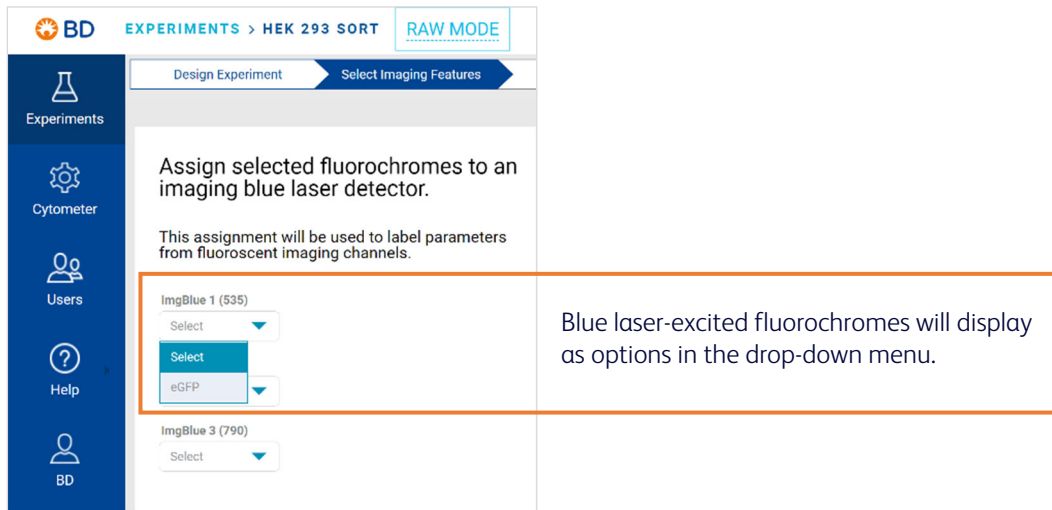
VIOLET

- BV421 Enter label
- FVS450 Viability
- V450 Enter label
- Pacific Blue Enter label
- BV480 Enter label
- BV750 Enter label
- BV786 Enter label
- +

Select from the listed fluorochromes and add a label, or click the (+) sign to add a new fluorochrome.

Set up experiment, continued

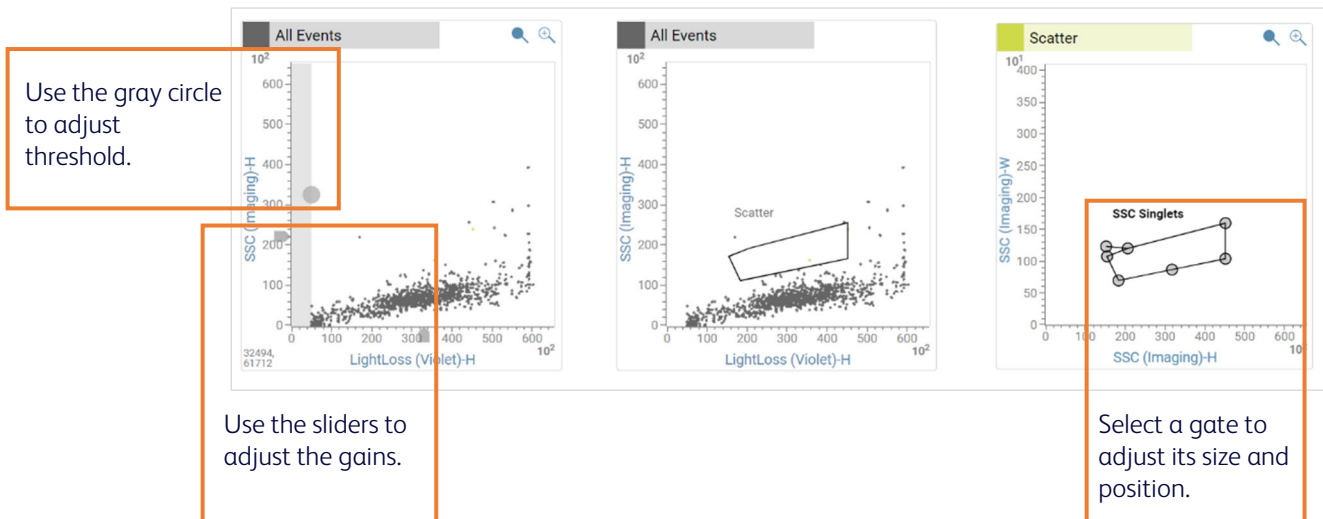
3. Enable fluorescence imaging by assigning dyes to the imaging detectors.



The screenshot shows the 'RAW MODE' interface for 'HEK 293 SORT'. The 'Select Imaging Features' step is active. A sidebar on the left contains navigation icons for Experiments, Cytometer, Users, Help, and BD. The main area displays the instruction: 'Assign selected fluorochromes to an imaging blue laser detector. This assignment will be used to label parameters from fluorescent imaging channels.' Below this, there are three drop-down menus for 'ImgBlue 1 (535)', 'eGFP', and 'ImgBlue 3 (790)'. An orange box highlights the 'eGFP' selection in the first menu, with a text box stating: 'Blue laser-excited fluorochromes will display as options in the drop-down menu.'

Establish settings

1. On the Adjust Gains page, load a tube of brightly stained cells.
2. Adjust the plot zoom, scatter gains, threshold, and gates to encompass cells of interest.



The figure shows three scatter plots illustrating adjustments:

- Plot 1 (All Events):** Shows 'SSC (Imaging)-H' vs 'LightLoss (Violet)-H'. A gray circle on the y-axis is highlighted with a text box: 'Use the gray circle to adjust threshold.'
- Plot 2 (All Events):** Shows 'SSC (Imaging)-H' vs 'LightLoss (Violet)-H'. A rectangular gate is drawn around a cluster of cells, with a text box below: 'Use the sliders to adjust the gains.'
- Plot 3 (Scatter):** Shows 'SSC (Imaging)-W' vs 'SSC (Imaging)-H'. A gate labeled 'SSC Singlets' is drawn around a cluster of cells, with a text box below: 'Select a gate to adjust its size and position.'

Establish settings, continued

- On the Image Wall, adjust the Region of Analysis (ROA).

IMAGE WALL

Adjust Refine

Region of Analysis

Before recording, set the Region of Analysis around the area of interest.

Channel Settings

Adjust the ROA slider until the white area in the images completely encompasses the particles of interest while minimizing background noise.

- Use the spectral plot to adjust the gains, if needed.

SSC Singlets

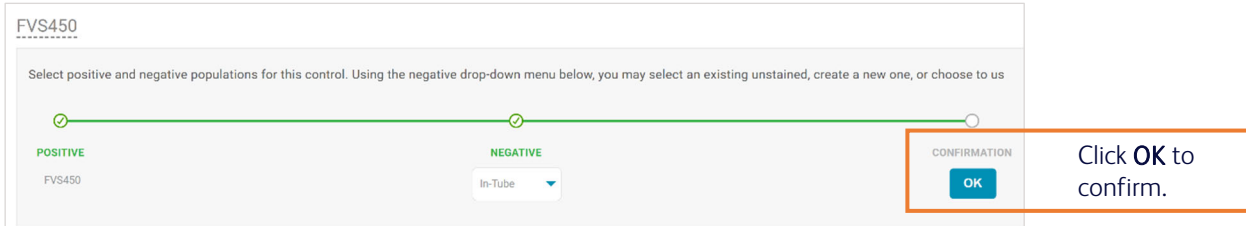
ULTRAVIOLET					VIOLET					BLUE					YELLOW-GREEN					RED					IMAGING		
Adjust All	Gain Value				Adjust All	Gain Value				Adjust All	Gain Value				Adjust All	Gain Value				Adjust All	Gain Value						
+	1 26.55	2 29.44	3 26.10	4 26.07	+	1 26.67	2 26.20	3 27.59	4 27.15	+	1 29.68	2 30.51	3 28.54	4 30.27	+	1 29.22	2 29.00	3 29.41	4 30.42	+	1 29.75	2 29.66	3 29.61	+	1 67.52		
-	5 28.70	6 28.43	7 27.74	8 28.18	-	5 26.88	6 26.67	7 26.59	8 27.84	-	5 30.56	6 29.46	7 30.47	8 28.77	-	5 30.29	6 29.45	7 30.04	8 29.73	-	3 28.85	4 29.12	5 27.31	6 29.08	-	2 75.48	
	9 27.81	10 28.20	11 28.85	12 27.77		9 26.68	10 26.66	11 29.33	12 29.95		9 29.41	10 29.47	11 30.14	12 29.56		9 29.29	10 30.10	11 28.46	12 28.56		7 28.97	8 28.37			3 66.41		
	13 27.90	14 30.79	15 28.58	16 29.23		13 28.80	14 28.63	15 30.64	16 28.21		13 28.90	14 30.07	15 29.39	16 29.99													
	17 29.72	18 28.80	19 28.31	20 29.01		17 28.63	18 28.90	19 29.13	20 29.30																		
	21 29.11	22 28.54																									

Intensity

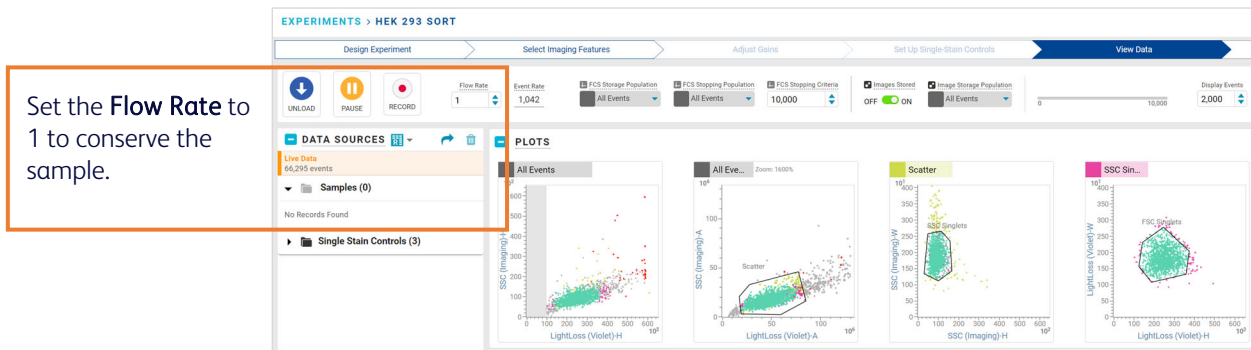
Saturated detectors will be identified with the (!) icon. Lower the gains for these detectors until the icon disappears.

Establish settings, continued

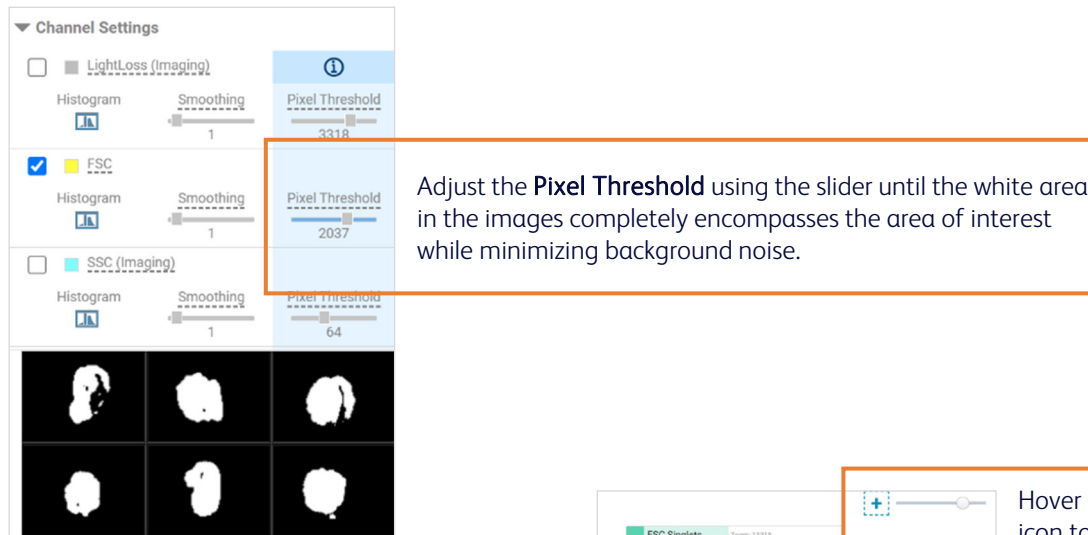
- On the Set Up Single-Stain Controls page, record data for each control tube.
NOTE The Region of Analysis must be appropriately set for the particle type before recording.
- Adjust the gates for each parameter.



- On the View Data page, load a sample tube and adjust scatter and singlet gates to encompass cells of interest.



- Use the image wall to adjust the Region of Analysis and Pixel Threshold for each imaging detector.



- Record a pre-sort data file and then name the file.
- Create new plots and gates to identify populations of interest.



Establish settings, continued

- Use the image wall to adjust the channel settings for each imaging detector.

Channel Settings

← **LightLoss (Imaging)**

Select the color box.

Use the smoothing slider to reduce image blur.

Color

Smoothing: 1

Pixel Threshold: 3003

Histogram

Minimum: 46464

Gamma: 1.00

Maximum: 55202

Adjust the Minimum (red) and the Maximum (blue) bars around the signal peak.

Adjust the Gamma (black circle) as needed.

Sort and analyze

- In the Set Up Sort page, determine the collection setup and the populations in the sample to be sorted.

COLLECTION SETUP

Format: 4-Way Tube

Volume: 5.0 mL

Sort Mode: Purity

Select the format and the volume of the collection device.

Select the **Sort Mode**: Yield, Purity, or Single Cell.

- If you are performing a tube sort:

SORT SETUP

Tube	1	2	3	4
Initial Buffer Volume:	0.50 mL	0.50 mL	0.50 mL	0.00 mL
Number of Events:	50,000	50,000	50,000	781,250
	Max: 703,125 events	Max: 703,125 events	Max: 703,125 events	Max: 781,250 events

Specify the buffer volume and set the target number of events to be sorted for each tube.

Assign a sort population by clicking a tube and selecting the population that you want.

Population Hierarchy:

- All Events
- Saturated
- Unsaturated
- Scatter
- SSC Singlets
- FSC Singlets
- E/RM Singlets
- Viable bright ...
- Punctate
- Intermed
- Diffuse

Assign populations to tubes by selecting the tube, then the population in the hierarchy.

Sort and analyze, continued

b. If you are performing a plate sort:



COLLECTION SETUP

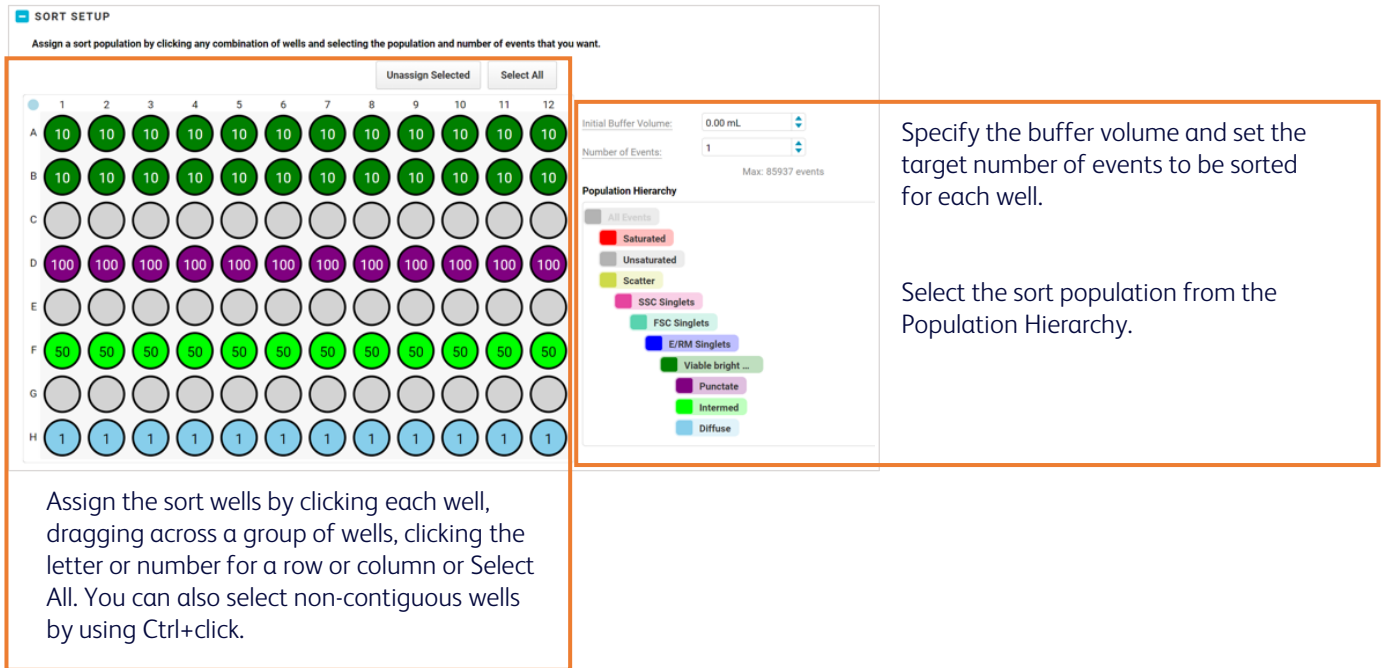
Format: **Plate** Enable Index Sort

BD-Defined Plate: **96 well**

Plate Name: **Default** [Optimize Plate](#)

Sort Mode: **Single Cell**

Select the **BD-Defined Plate** type, **Plate Name**, and **Sort Mode**.



SORT SETUP

Assign a sort population by clicking any combination of wells and selecting the population and number of events that you want.

Unassign Selected Select All

Initial Buffer Volume: 0.00 mL

Number of Events: 1 Max: 85937 events

Population Hierarchy

- All Events
- Saturated
- Unsaturated
- Scatter
 - SSC Singlets
 - FSC Singlets
 - E/RM Singlets
 - Viable bright ...
 - Punctate
 - Intermed
 - Diffuse

Assign the sort wells by clicking each well, dragging across a group of wells, clicking the letter or number for a row or column or Select All. You can also select non-contiguous wells by using Ctrl+click.

Specify the buffer volume and set the target number of events to be sorted for each well.

Select the sort population from the Population Hierarchy.

2. Install the collection device into the sort collection chamber and close the door.
3. On the Sort page, load the sort sample and start the sort.

Sort and analyze, continued

- Monitor the sort as it progresses.

The screenshot displays the Sort software interface with several key sections highlighted by orange boxes:

- Monitor the event rate:** Located in the top left, showing 'Flow Rate' at 1.528 and 'Event Rate' at 2,000.
- Monitor the sort efficiency and sort rate:** A table in the middle left showing population statistics for Punctate, Diffuse, and Intermid.

Population	Punctate	Diffuse	Intermid	0
Target Count:	50000	50000	50000	0
Sort Count:	249	2122	37	0
Sort Rate:	1	29	0	0
Efficiency:	96.14	97.74	100.00	0.00
- Sort Population Plots:** A grid of scatter plots showing various populations like ERM Singlets, Viable bright GFP, All Events, Scatter, SSC Singlets, and FSC Singlets.
- Statistics Table:** A table at the bottom left providing detailed statistics for different populations.

Population	Events	% Parent	% Total	LightLoss (Violet)-A Median	LightLoss (Violet)-A %CV	SSC (Imaging)
All Events	2,000	N/A	100.00 %	47,568,352.00	38.54 %	
Saturated	64	3.20 %	3.20 %	171,424,176.00	63.38 %	
Unsaturated	1,936	96.80 %	96.80 %	47,034,324.00	37.32 %	
Scatter	1,607	80.35 %	80.35 %	45,503,624.00	28.91 %	
SSC Singlets	1,468	91.35 %	73.40 %	44,566,984.00	27.62 %	
FSC Singlets	1,351	92.03 %	67.55 %	43,946,684.00	25.88 %	
ERM Singlets	1,253	92.75 %	62.65 %	43,596,860.00	25.06 %	
Viable bright ...	106	8.46 %	5.30 %	45,674,504.00	28.34 %	

Additional text on the right side of the screenshot says: "Verify that the sort gates are still capturing the appropriate populations throughout the sort."

- When the sort ends or is stopped, name the sort report.
- On the View Data page, analyze the sort purity.

TIP Perform a backflush between tubes to reduce carryover.

The control panel includes a blue 'BACKFLUSH' button and three status indicators: 'Light' (ON), 'Agitation' (OFF), and 'Temperature' (OFF).

Manage data

- On the View Reports page, view and export reports.

The screenshot shows the 'View Reports' page with the following elements:

- Select Sort Report:** A dropdown menu with 'Tube sort' selected. An 'Export Report' button is next to it.
- CYTOMETER SETTINGS:** A section containing 'Gains' and 'Spectral Unmixing: Spillover Values'.
- Export as CSV:** A button located within the 'Spectral Unmixing' section.
- Fluorochromes x Detectors Table:**

Fluorochromes x Detectors	UV1	UV2	UV3	UV4	UV5	UV6	UV7
FVS450	0.413	1.242	4.560	11.143	11.956	6.710	4.972
eGFP	0.044	0.081	0.253	0.300	0.230	0.229	1.269
Autofluoresce...	14.877	24.694	75.605	100.000	92.407	60.288	67.955

Manage data, continued

2. On the Experiments page, export experiments, FCS files, images, statistics, and index sort data.
3. Delete unneeded experiments.

The screenshot displays the BD Experiments web interface. On the left sidebar, the 'Users' icon is highlighted with an orange box and the text 'Select an experiment.' In the main header area, the buttons 'Export (8)', 'Delete Data (4)', and 'Delete Experiment (1)' are grouped together in an orange box with the text 'Export Data, Delete Data, or Delete Experiment.' Below the header, the 'Export (8)' dialog is open, showing the export location as 'C:\Users\BDAdmin\Desktop', an estimated size of 9.26 GB, and 294.3 GB of available disk space. The dialog lists selected items, including 'HEK 293 sort (8)' and 'Single-Stain Controls (3)', with a detailed table of samples.

Name	Events	Created By	Size	FCS	Statistics	Images	Status	Export Completed
<input checked="" type="checkbox"/> HEK 293 sort sample	100,000	BD Training	9 GB	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
<input checked="" type="checkbox"/> Diffuse post-sort	255	BD Training	25 MB	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
<input checked="" type="checkbox"/> Intermed post-sort	9	BD Training	4 MB	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
<input checked="" type="checkbox"/> Punctate post-sort	25	BD Training	5 MB	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		

Shut down system

1. Clean the sample line.
2. On the Cytometer page, perform either a daily or long-term shutdown.

The screenshot shows the 'CYTOMETER' interface with the 'STARTUP / SHUTDOWN' section expanded. Three panels are visible: 'System Startup', 'Daily Shutdown', and 'Long-Term Shutdown'. The 'Long-Term Shutdown' panel is highlighted with an orange box and includes a text box with the instruction: 'Perform a Long-Term Shutdown if the instrument is idle for over two days. Otherwise, perform a Daily Shutdown.'

3. Power off the cytometer and workstation.

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