

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.

The screenshot shows the Fluidics Startup screen with the following status:

- Cytometer Connection: **Connected**
- Sheath Tank: **19 hr 39 min remaining**
- Waste Tank: **OK**
- Last Shutdown: 03/15/2023 03:26 PM, Type: Daily
- Last Fluidics Startup: 03/16/2023 08:58 AM, Type: Daily

At the bottom, there are three buttons: **Run Daily Fluidics Startup**, **Run Extended Fluidics Startup**, and **Skip**. Two callout boxes provide instructions:

- Click **Run Daily Fluidics Startup** after a daily shutdown.
- Click **Run Extended Fluidics Startup** after a long-term shutdown.

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

The screenshot shows a screen titled "Select the cleaning that you want to run." with two options:

- Prepare for Aseptic Sort**: Cleans the sheath and sample paths with bleach, DI water, and ethanol. Last Run: 02/20/2023 03:52 PM.
- Flow Cell Clean**: Cleans the sample path and fills the flow cell with DI water. Run this procedure when poor optical performance indicates that additional cleaning is needed. Last Run: 03/16/2023 09:00 AM.

A **Skip** button is located at the bottom right.

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

9. Perform a Setup and QC.

The screenshot shows the "Setup and QC" screen with the following steps:

- 1 Select a bead lot: Current lot number: 600915BL02, Expiration date: 11/29/2023. **Callout:** Verify the bead lot.
- 2 Load a tube with BD FACSDiscover™ Setup Beads
- 3 Select type of Setup and QC: **Callout:** Select the type of QC. (Options: Daily, Baseline)
- 4 Run Setup and QC: **Callout:** Load a tube of BD FACSDiscover™ Setup Beads and click **Run**.

The left sidebar shows the "SYSTEM STARTUP" progress bar with steps 1 through 6, and the "4 Setup and QC" step is currently active.

Startup system, continued

10. If needed, perform an Image Calibration.

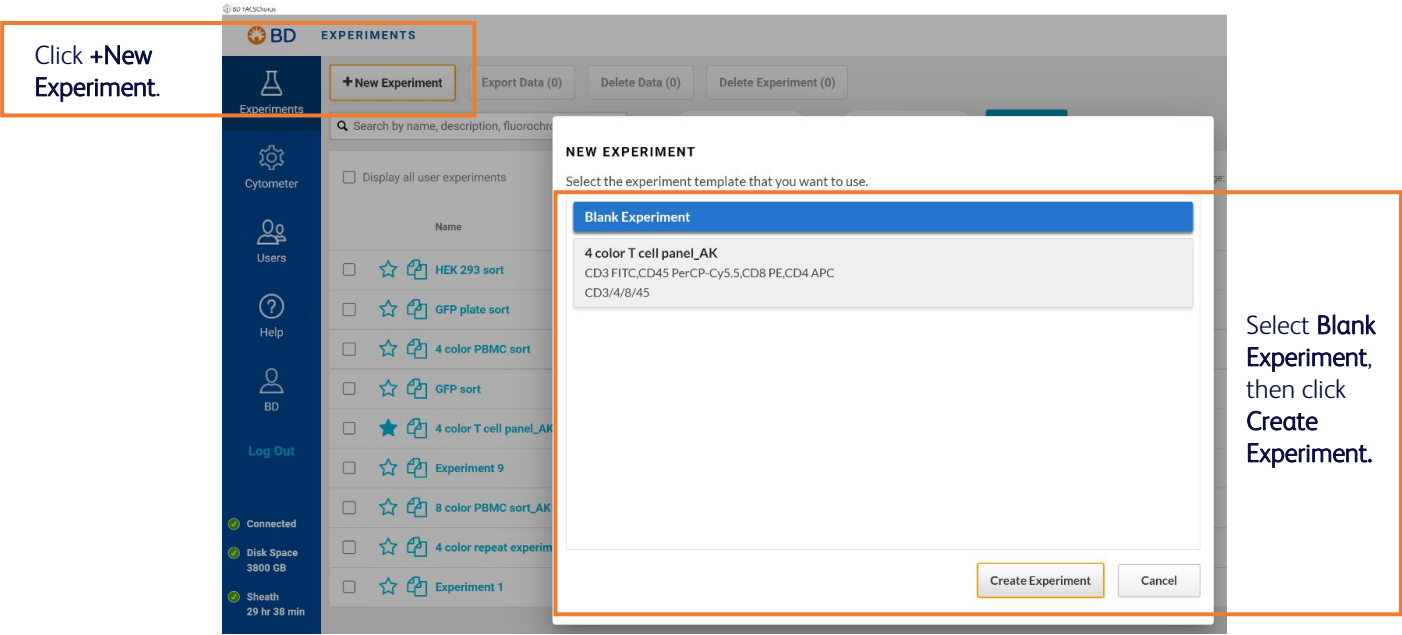
The screenshot shows the BD system startup interface. On the left is a blue sidebar with a user profile icon (BD), a 'Log Out' button, and a list of system components: Connected, Disk Space (3800 GB), Sheath (19 hr 8 min), Waste, and System (highlighted with a right arrow). The main area displays the 'System Status' window, which lists several components with green checkmarks: 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-22). A 'Close' button is at the bottom right of the System Status window. To the right of the System Status window is a larger white panel with an orange warning banner at the top that reads 'Run Image Calibration bi-weekly or after any change in optical configuration.' Below the banner, it shows 'Last Calibration Run: 03/17/2023 11:44 AM' and 'Status: Passed'. At the bottom of this panel are two buttons: 'Run Calibration' and 'Skip'. A text box on the right side of the panel says '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 side of the panel says 'Click System to view the Image Calibration status.'

11. Perform a Drop Delay setup.

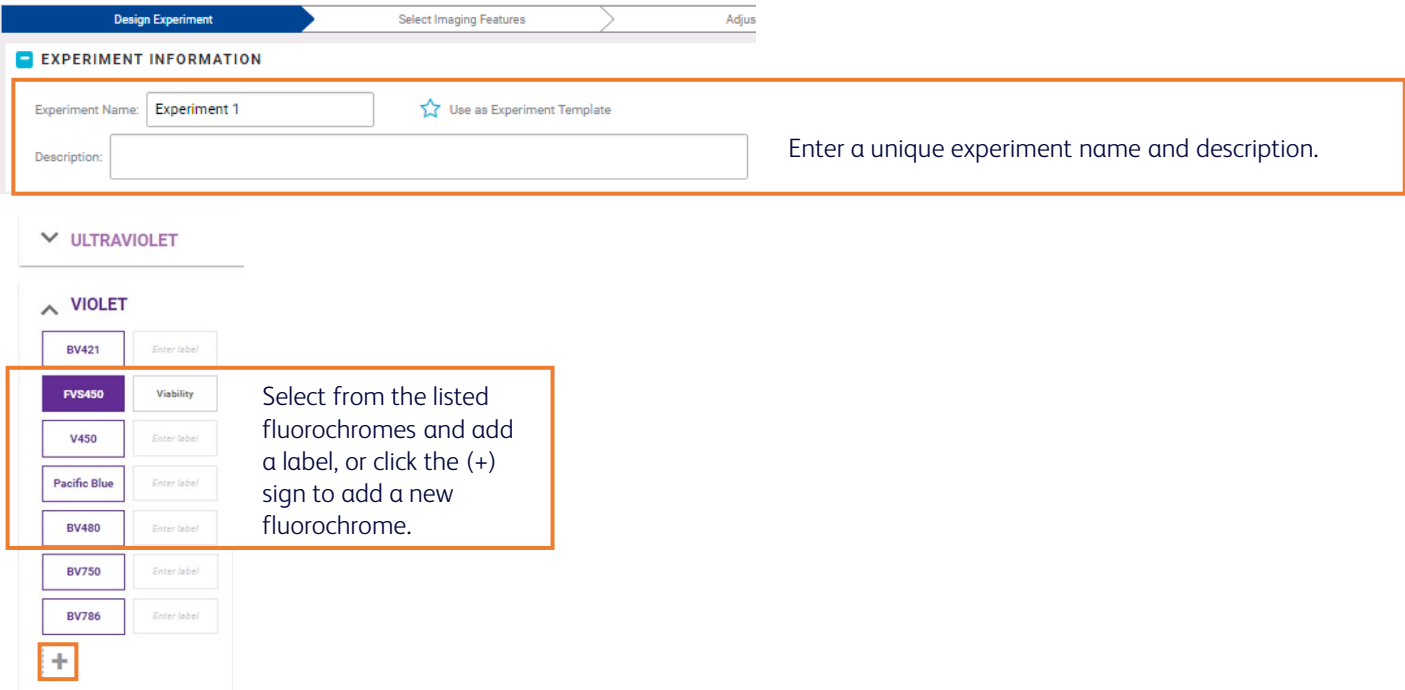
The screenshot shows the BD system startup interface with a progress bar at the top indicating the sequence of steps: 1 Fluidics Startup, 2 Cleaning, 3 Sort Nozzle, 4 Setup and QC, 5 Image Calibration, and 6 Drop Delay (highlighted in orange). The main area displays a white panel with an orange warning banner at the top that reads 'Run Drop Delay daily before sorting.' Below the banner, it shows 'Drop Delay Last Run: 05/30/2023 10:51 AM' and 'Status: Passed'. At the bottom of this panel are two buttons: 'Run Drop Delay' (highlighted with a green border) and 'Skip'. A text box on the left side of the panel says 'Run a tube of BD FACS™ Accudrop Beads.'

Set up experiment

- 1. Create a new experiment on the Experiments page.



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



Set up experiment, continued

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

BD EXPERIMENTS > HEK 293 SORT RAW MODE

Design Experiment Select Imaging Features

Assign selected fluorochromes to an imaging blue laser detector.

This assignment will be used to label parameters from fluorescent imaging channels.

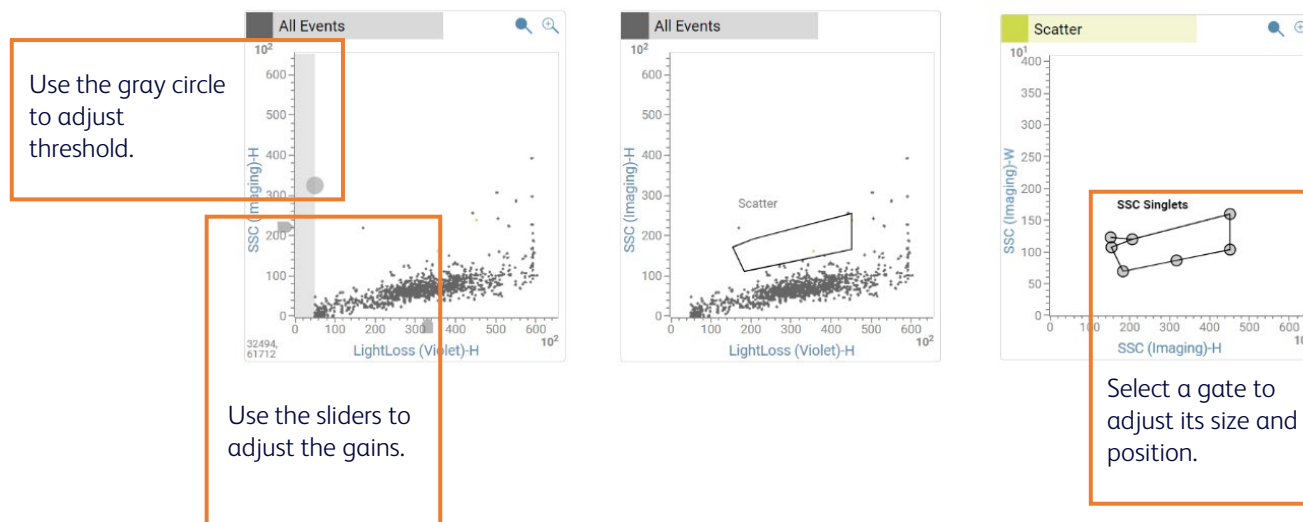
ImgBlue 1 (535)
Select
eGFP
Select

ImgBlue 3 (790)
Select

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 scatter gains, threshold, and gates to encompass cells of interest.



Establish settings, continued

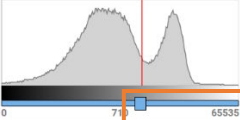
- 3. On the Image Wall, adjust the Region of Analysis (ROA).

IMAGE WALL

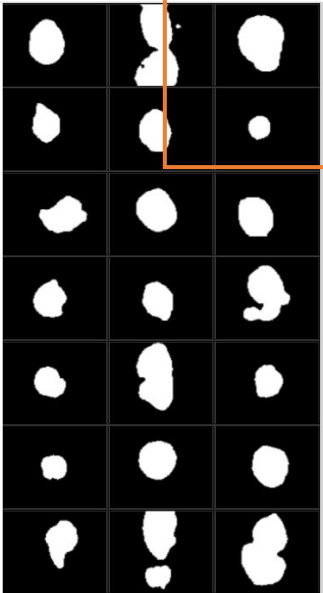
AdjustRefine

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.

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

SSC Singlets

ULTRAVIOLET

VIOLET

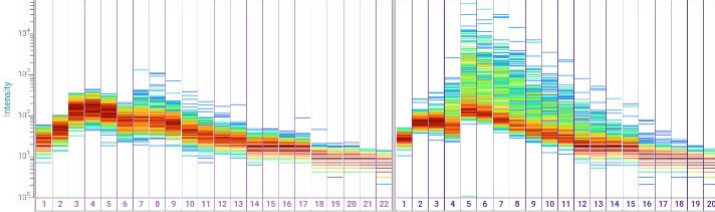
BLUE

YELLOW-GREEN

RED

IMAGING

Adjust All	Gain Values	Adjust All	Gain Values	Adjust All	Gain Values	Adjust All	Gain Values	Adjust All	Gain Values	Adjust All	Gain Values
+	1 26.55 2 29.44 3 26.10 4 26.07	+	1 20.67 2 26.20 3 27.59 4 27.15	+	1 20.08 2 30.51 3 20.34 4 30.27	+	1 28.22 2 28.60 3 29.41 4 30.42	+	1 28.75 2 29.05	+	1 67.52
-	5 28.70 6 28.13 7 27.74 8 28.18	-	5 26.88 6 26.67 7 26.62 8 27.84	-	5 30.56 6 29.46 7 30.37 8 29.77	-	5 30.75 6 29.45 7 30.04 8 29.70	-	3 28.85 4 29.12	-	2 73.45
	9 27.81 10 28.00 11 28.65 12 27.77		9 26.68 10 26.66 11 29.50 12 29.10		9 29.41 10 29.47 11 30.14 12 29.55		9 29.75 10 30.10 11 28.46 12 28.55		5 27.81 6 29.08		3 65.41
	13 27.90 14 30.79 15 28.59 16 29.23		13 20.80 14 20.63 15 30.64 16 20.21		13 20.95 14 30.07 15 29.39 16 29.99				7 28.97 8 28.37		
	17 29.72 18 28.80 19 28.31 20 28.01		17 20.62 18 26.90 19 28.13 20 29.30								
	21 29.11 22 28.54										

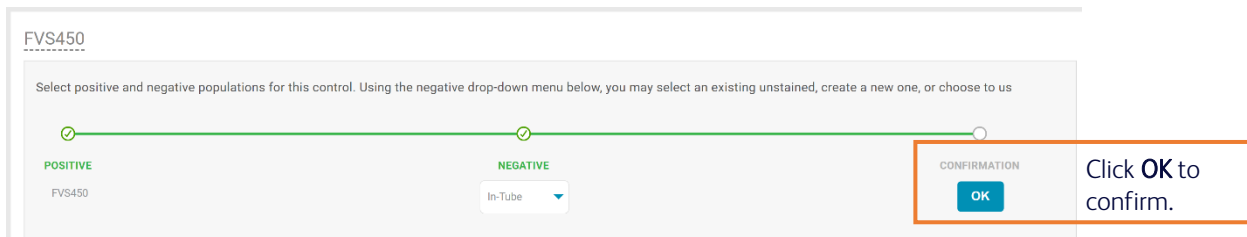




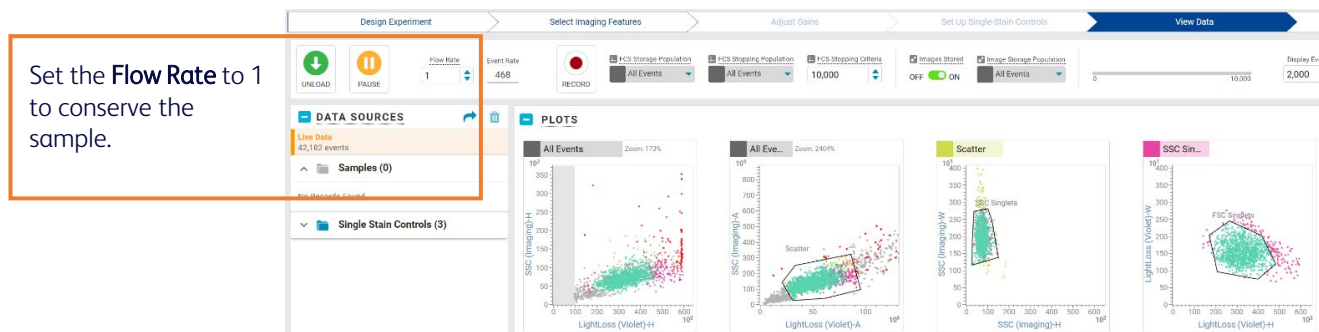
Saturated detectors will be identified with the (!) icon. Lower the gains for these detectors until the icon disappears.

Establish settings, continued

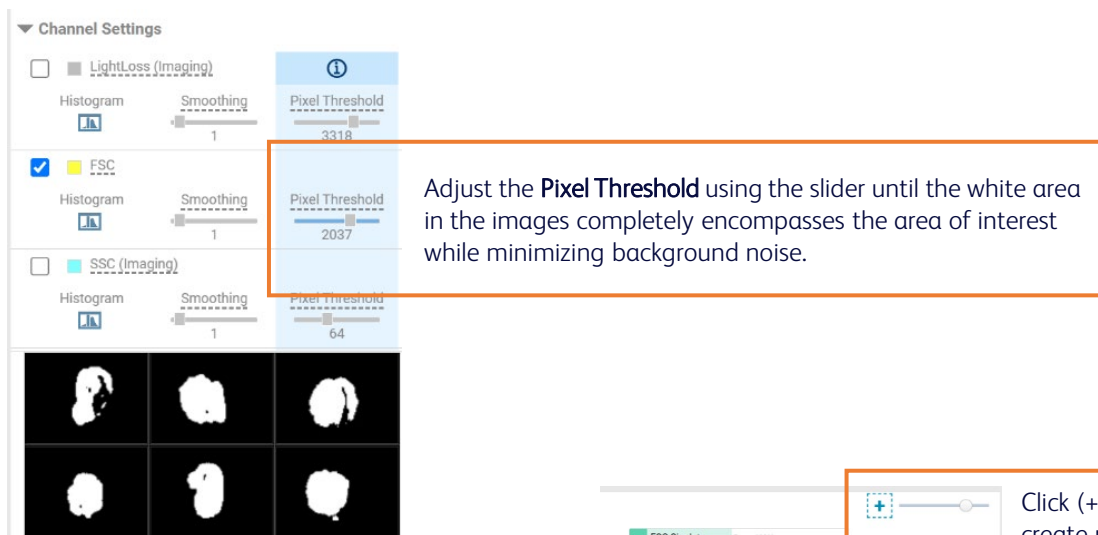
- On the Set Up Single-Stain Controls page, record data for each control tube.
NOTE The Region of Analysis must be appropriately set for the particle type before recording.
- Adjust the gates for each parameter.



- 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.

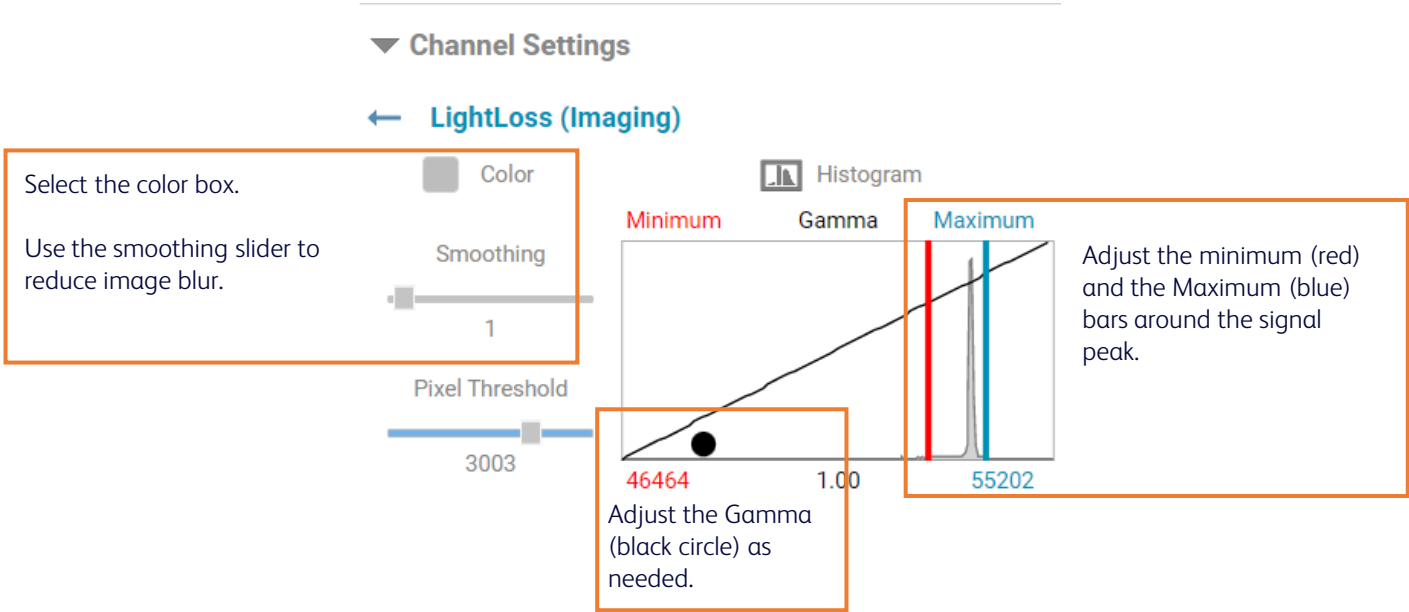


- Record a pre-sort data file and then name the file.
- Create new plots and gates to identify populations of interest.



Establish settings, continued

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

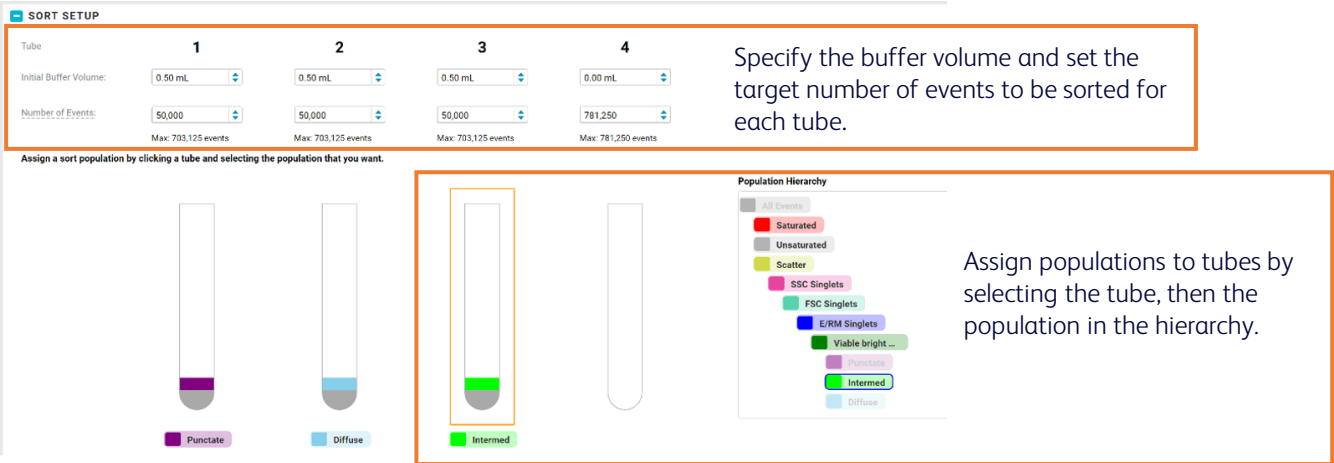


Sort and analyze

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



a. If you are performing a tube sort:



Sort and analyze, continued

- b. If you are performing a plate sort:

COLLECTION SETUP

Format: ☒ Enable Index Sort

BD-Defined Plate: [Optimize Plate](#)

Plate Name:

Sort Mode:

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	10	10	10	10	10	10	10	10	10	10	10
B	10	10	10	10	10	10	10	10	10	10	10	10
C												
D	100	100	100	100	100	100	100	100	100	100	100	100
E												
F	50	50	50	50	50	50	50	50	50	50	50	50
G												
H	1	1	1	1	1	1	1	1	1	1	1	1

Initial Buffer Volume: Max: 85937 events

Number of 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

4. Monitor the sort as it progresses.

Monitor the event rate.

Monitor the sort efficiency and sort rate.

Sort Status - SORTING...

Population	Punctate	Diffuse	Intermed	
Target Count:	50000	50000	50000	0
Sort Count:	3360	5334	1013	0
Sort Rate:	26	19	4	0
Efficiency:	98.29	98.69	98.73	0.00

Sort Population Plots

CHS Singlets

Violet Singlet LP

All Events

Scatter

SSC Singlets

FSC Singlets

Additional Plots

All Events

Statistics

Population	Events	% Parent	% Total	LightLoss (Violet) A Median	LightLoss (Violet) A %CV	SSC (Imaging)
All Events	2,000	N/A	100.00 %	43,495,000.00	41.83 %	
Saturated	111	5.55 %	5.55 %	119,012,332.00	95.86 %	
Unsaturated	1,889	94.45 %	94.45 %	47,382,608.00	40.17 %	
Scatter	1,992	79.60 %	79.60 %	50,175,496.00	31.21 %	
SSC Singlets	1,458	91.50 %	72.90 %	43,566,748.00	30.54 %	
FSC Singlets	1,307	89.84 %	65.35 %	47,527,144.00	27.70 %	
Valid Singlets	1,704	85.17 %	60.20 %	45,566,937.00	27.08 %	

Verify that the sort gates are still capturing the appropriate populations throughout the sort.

5. When the sort ends or is stopped, name the sort report.

6. On the View Data page, analyze the sort purity.

TIP Perform a backflush between tubes to reduce carryover.

BACKFLUSH

Light

Agitation

Temperature

Manage data

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

Design Experiment > Select Imaging Features > Adjust Gains > Set Up Single-Stain Controls > View Data > Set Up Sort > Sort > View Reports

Select Sort Report: Tube sort

Export Report

Tube sort

HEK 293 sort

CYTOMETER INFO

CYTOMETER SETTINGS

Gains

Threshold: LightLoss (Violet) @ 9788

Detector	Gains
SSC (Imaging)	40.17
FSC	18.44
LightLoss (Imagin...	10.12
LightLoss (Violet)	12.86

Spectral Unmixing: Spillover Values

Export as CSV

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

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

Export Data, Delete Data, or Delete Experiment.

Select an experiment.

	Experiment	Created By	Events	FCS	Images	CSV	Status
☒	HEK 293 sort	BD Bio	667	☒	☒		Export Completed
☒	Diffuse post-sort	HEK 293 sort	2,000	☒	☒		
☒	Punctate post-sort	HEK 293 sort	2,000	☒	☒		
☒	More events	HEK 293 sort	10,000	☒	☒		
☒	HEK 293 sort sample	HEK 293 sort	10,000	☒	☒		
☒	Autofluorescence_001	HEK 293 sort	5,000	☒	☒		
☒	eGFP_001	HEK 293 sort	5,000	☒	☒		
☒	FVS450_001	HEK 293 sort	5,000	☒	☒		

Shut down system

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

CYTOMETER

STARTUP / SHUTDOWN

System Startup
Prepares the cytometer for sorting by performing fluidics startup, detector setup and QC, image calibration and setting the drop delay.
Setup and QC Last Run: 03/20/2023 09:40 AM
Image Calibration Last Run: 03/17/2023 11:44 AM
Drop Delay Last Run: 03/20/2023 09:46 AM

Daily Shutdown
Cleans the sample path and fills the flow cell with BD Detergent Solution in preparation for shutdown.
Last Run: 03/20/2023 04:37 PM

Long-Term Shutdown
Removes sheath fluid from the lines, fills the lines with 70% ethanol, and drains the flow cell. Run this procedure when the cytometer will not be used for more than two days.
Last Run: 03/17/2023 04:07 PM

Perform a Long-Term Shutdown if the instrument is idle for over two days. Otherwise, perform a Daily Shutdown.

- Power off the cytometer and workstation.

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