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.

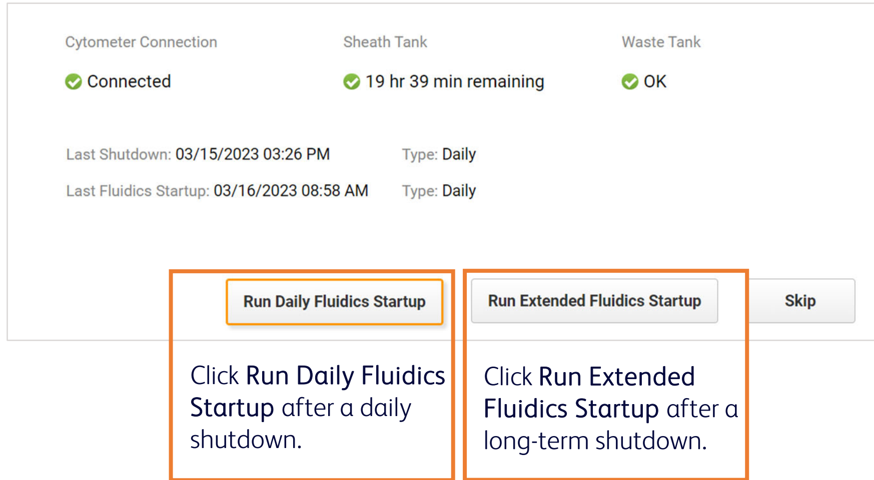
Start up system

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 FACSChorus™ Software.

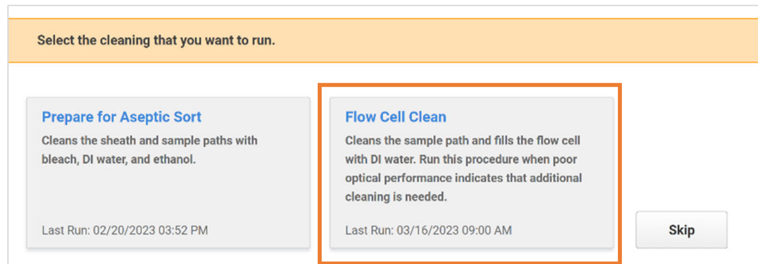
Startup system, continued

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.

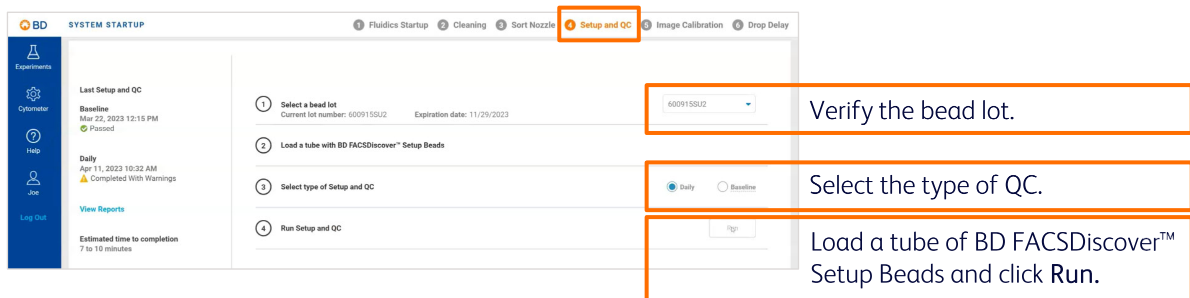


7. Select **Flow Cell Clean** and follow the prompts on the screen.



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.

Run Image Calibration bi-weekly or after any change in optical configuration.

Last Calibration Run: 03/17/2023 11:44 AM

Status: Passed

Run Calibration Skip

System Status

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

Image Calibration
Last Run: 03/17/2023 11:44 AM

Drop Delay
Last Run: 03/16/2023 11:14 AM

Configuration: Imaging-3 Blue-16 Violet-20 YellowGreen-12 Red-8 UV-22

Close

Connected
Disk Space 3800 GB
Sheath 19 hr 8 min
Waste
System

Click **System** to view the Image Calibration status.

Run a tube of BD CellView™ Calibration Beads if the Image Calibration is outdated. Click **Skip** if the Image Calibration status is green.

11. Perform a Drop Delay setup.

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

Run Drop Delay daily before sorting.

Drop Delay Last Run: 05/30/2023 10:51 AM

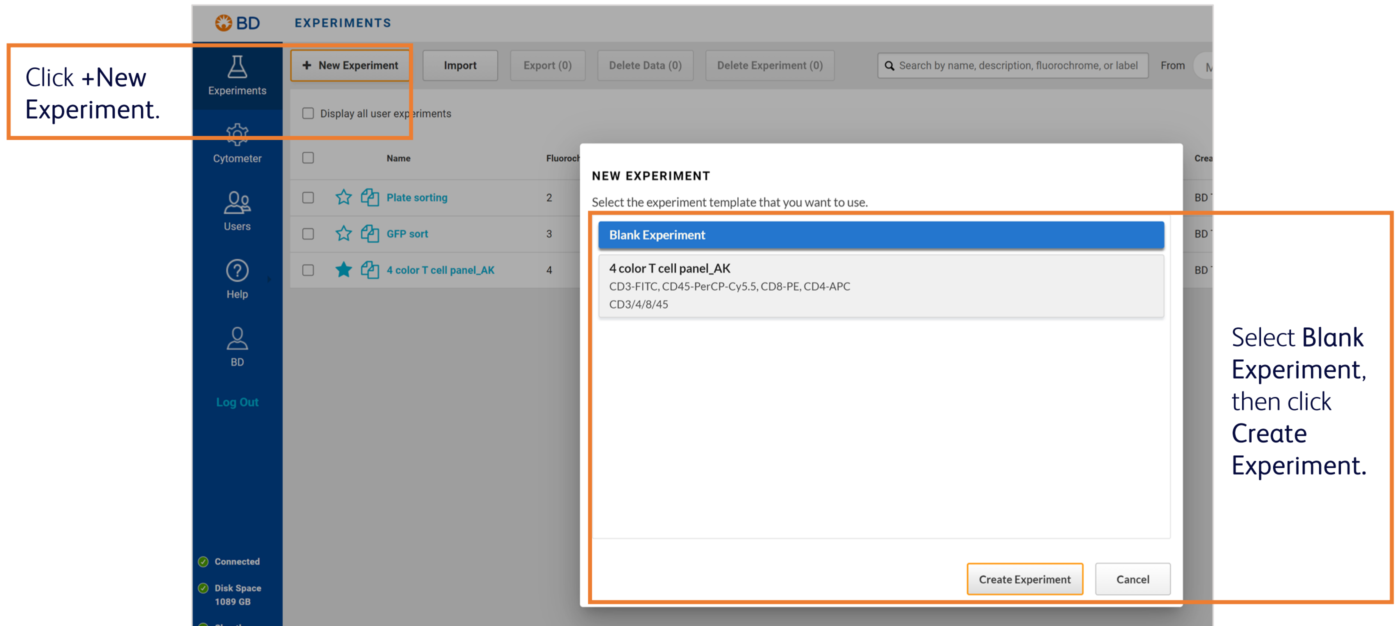
Status: Passed

Run a tube of BD FACS™ Accudrop Beads.

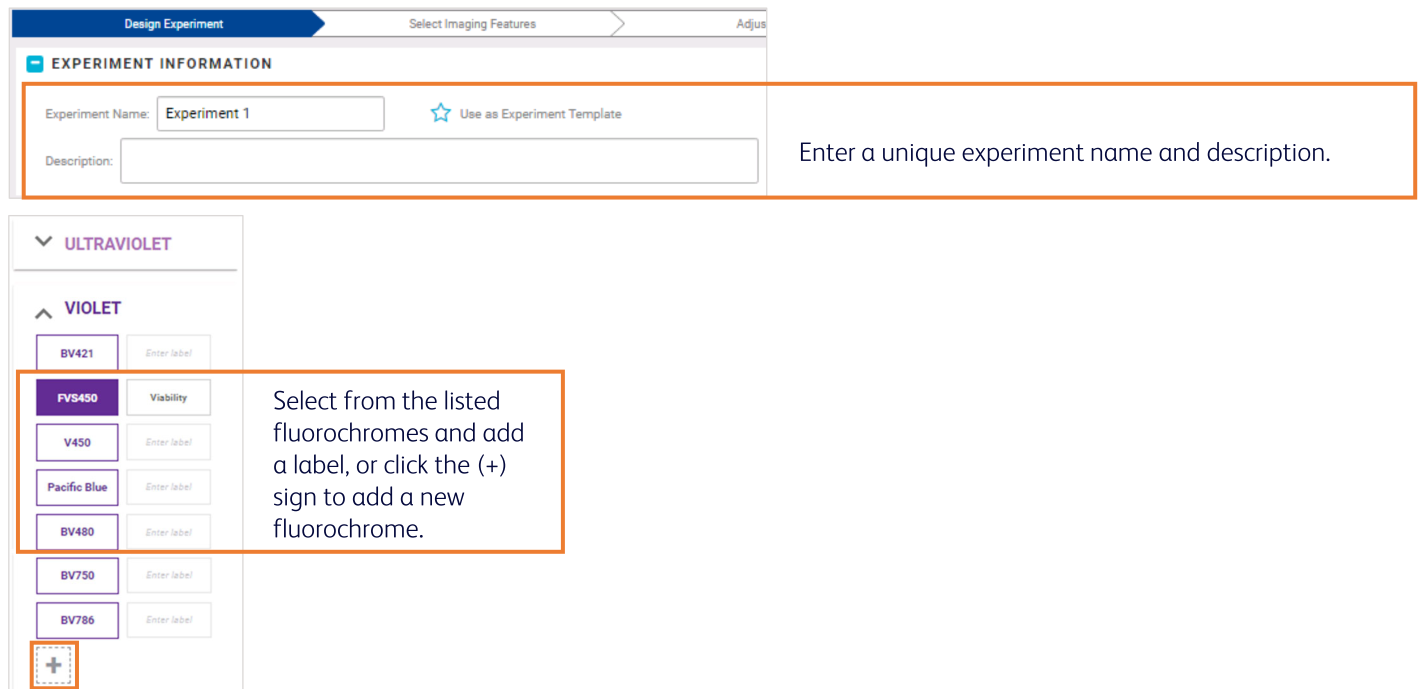
Run Drop Delay Skip

Set up experiment

1. Create a new experiment on the Experiments page.

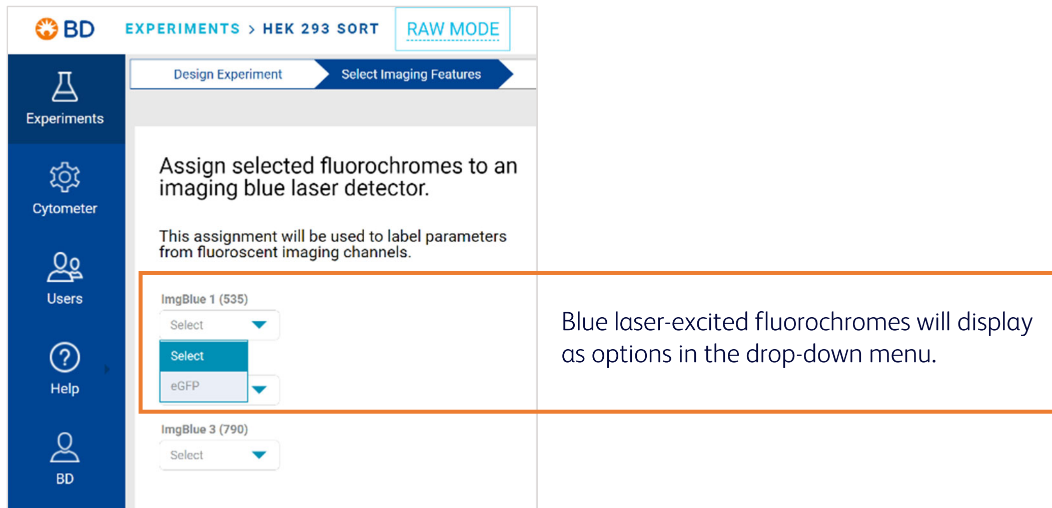


2. Enter the experiment information, dyes and labels on the Design Experiment page.



Set up experiment, continued

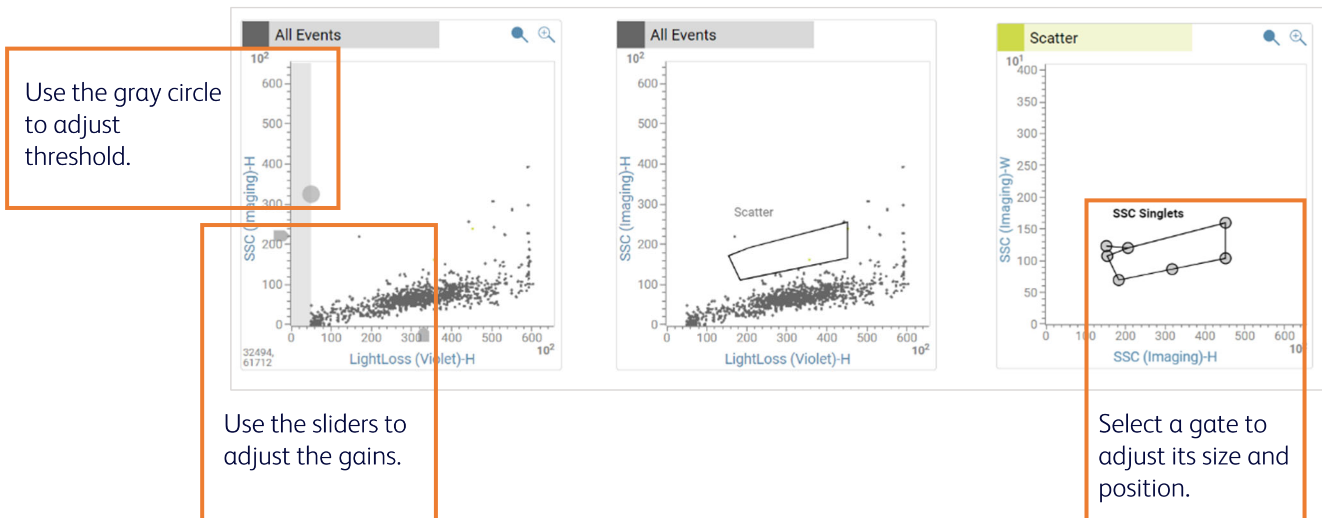
3. Enable fluorescence imaging by assigning dyes to the imaging detectors.



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.



Establish settings, continued

3. 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.

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

SSC Singlets

ULTRAVIOLET | VIOLET | BLUE | YELLOW-GREEN | RED | IMAGING

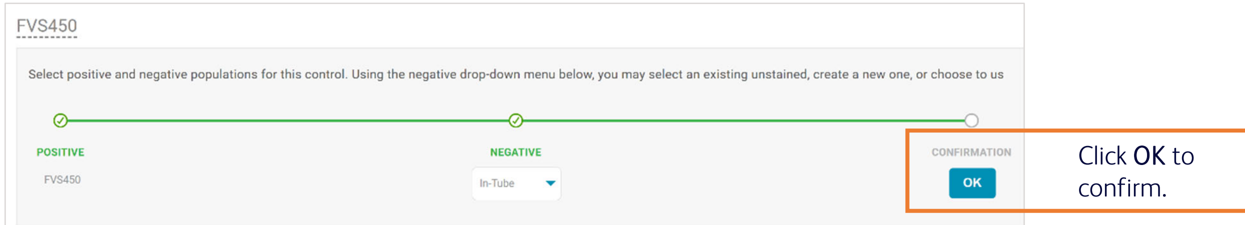
Saturated detectors will be identified with the (!) icon. Lower the gains for these detectors until the icon disappears.

Establish settings, continued

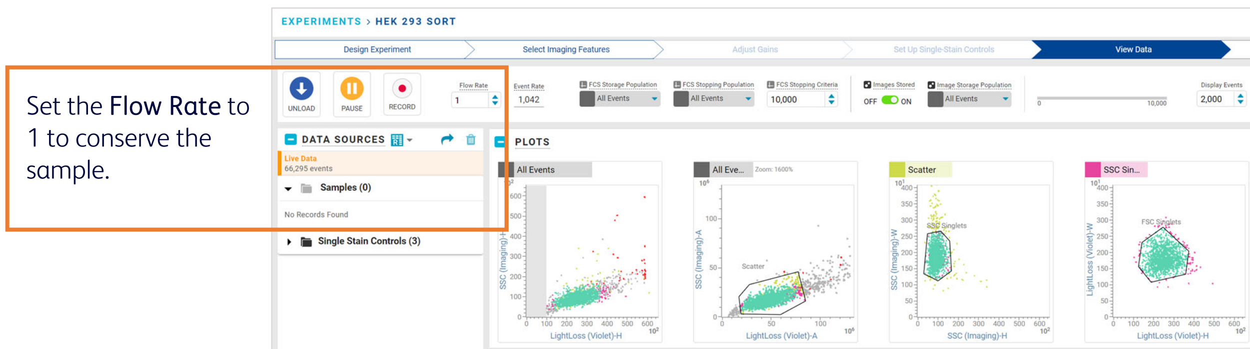
5. On the Set Up Single-Stain Controls page, record data for each control tube.

   NOTE The Region of Analysis must be appropriately set for the particle type before recording.

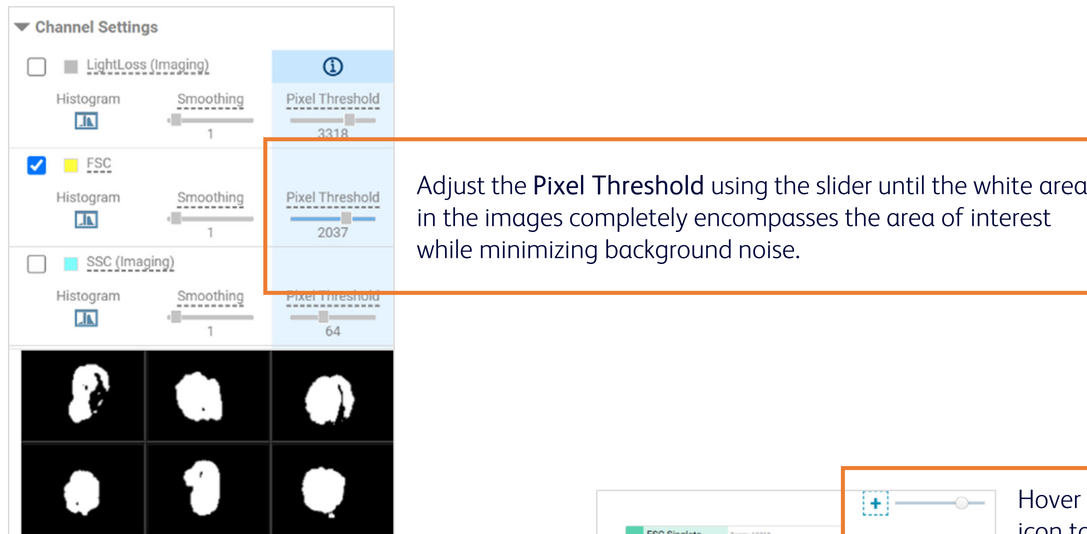
6. Adjust the gates for each parameter.



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



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



9. Record a pre-sort data file and then name the file.

10. 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.

Channel Settings

LightLoss (Imaging)

Color | Histogram

Smoothing 1

Pixel Threshold 3003

Minimum 46464 | Gamma 1.00 | Maximum 55202

Select the color box.

Use the smoothing slider to reduce image blur.

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

Adjust the Gamma (black circle) as needed.

Sort and analyze

1. 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.

a. 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

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

Punctate | Diffuse | Intermed

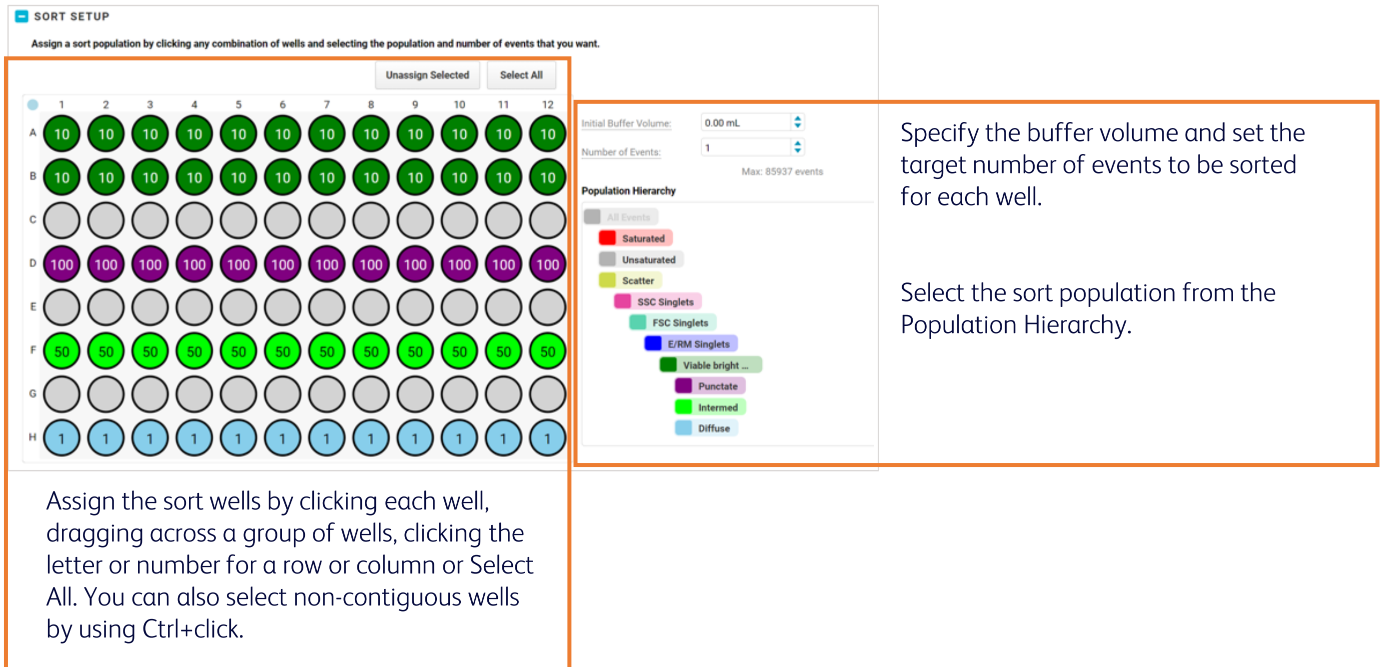
Population Hierarchy: All Events, Saturated, Unsaturated, Scatter, SSC Singlets, FSC Singlets, E/RM Singlets, Viable bright ..., Punctate, Intermed, Diffuse

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

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:



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.

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.

Manage data

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

Manage data, continued

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

3. Delete unneeded experiments.

Export Data, Delete Data, or Delete Experiment.

Select an experiment.

Shut down system

1. Clean the sample line.

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

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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