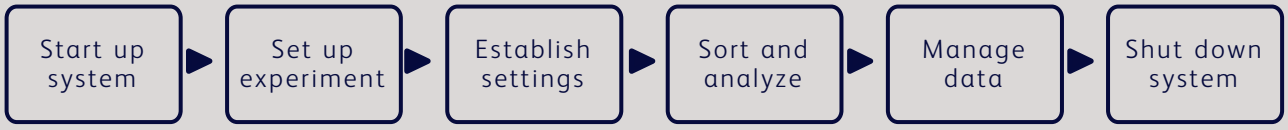
# BD FACSDiscover™ S8 Cell Sorter Quick Reference Guide

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.

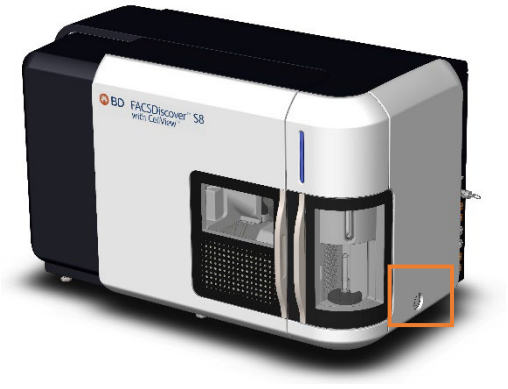



### Before you begin

- 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.

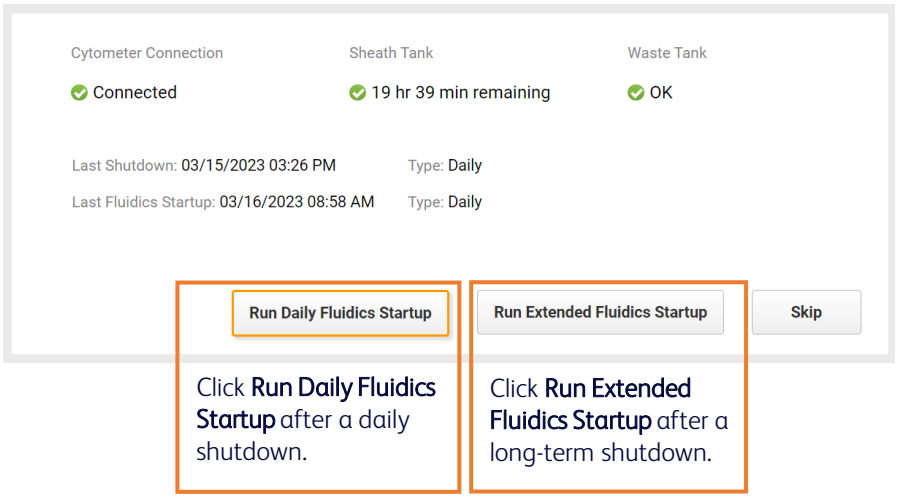
### Start up system

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.

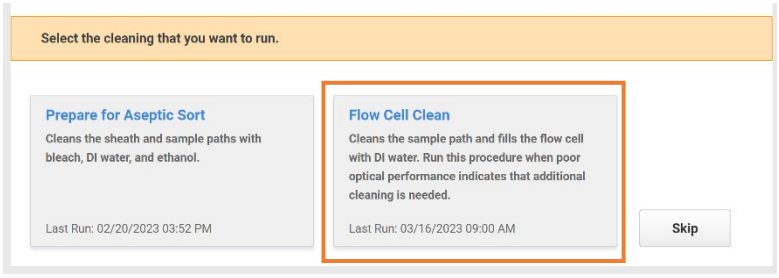



## Startup system, continued


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

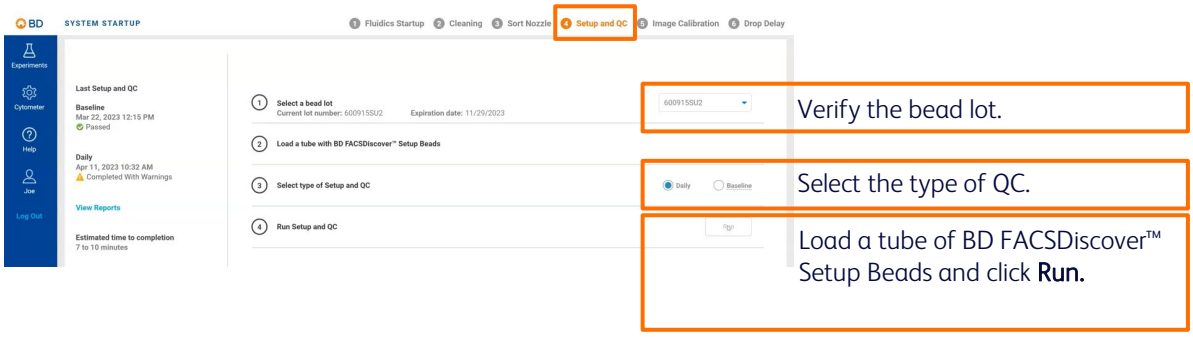



7. Select **Flow Cell Clean** and follow the prompts on the screen.



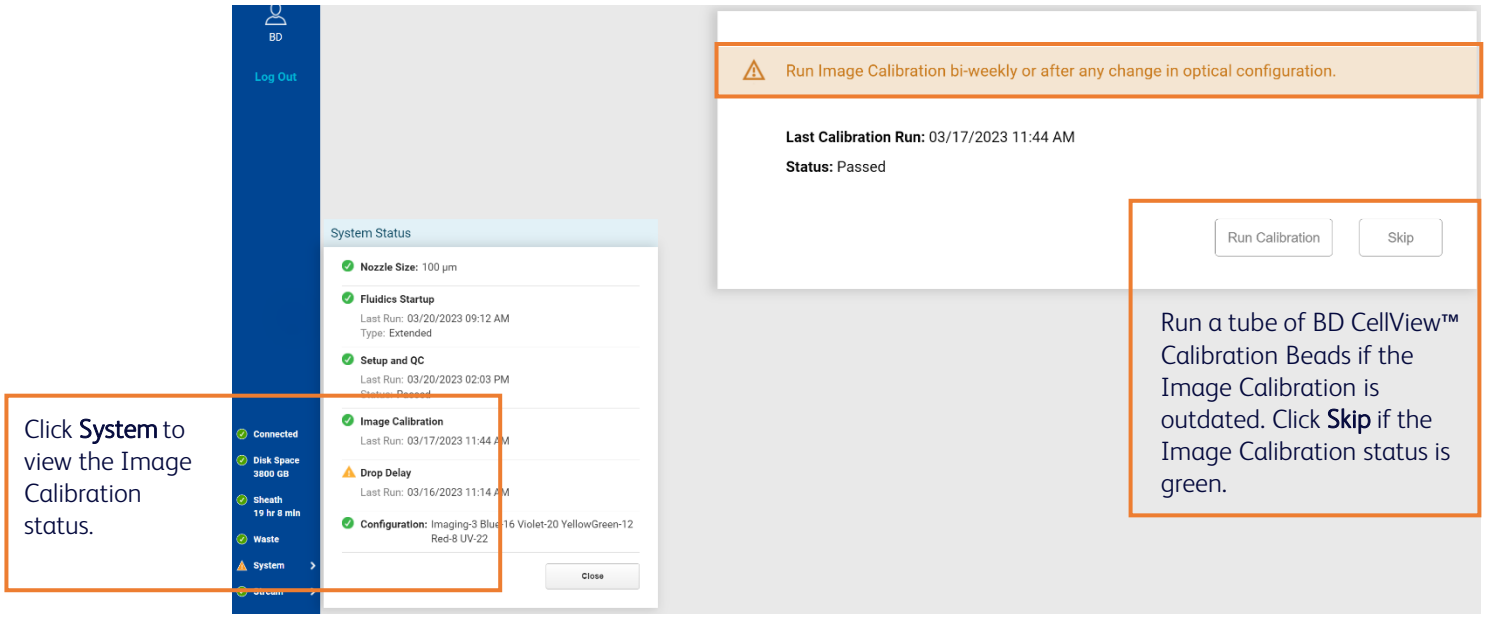

8. Remove the closed-loop nozzle and insert a sort nozzle.

9. Perform a Setup and QC.

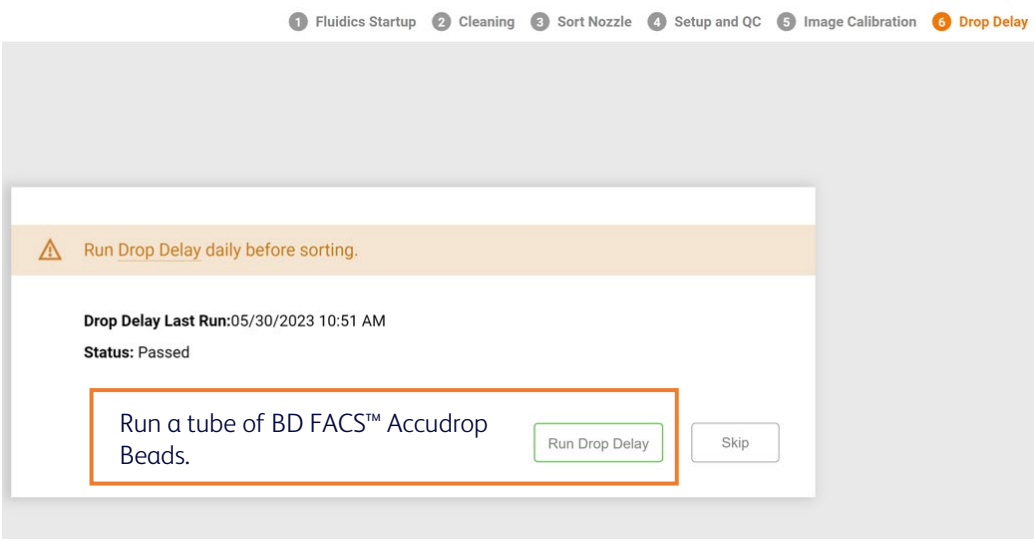


## Startup system, continued

10. If needed, perform an Image Calibration.

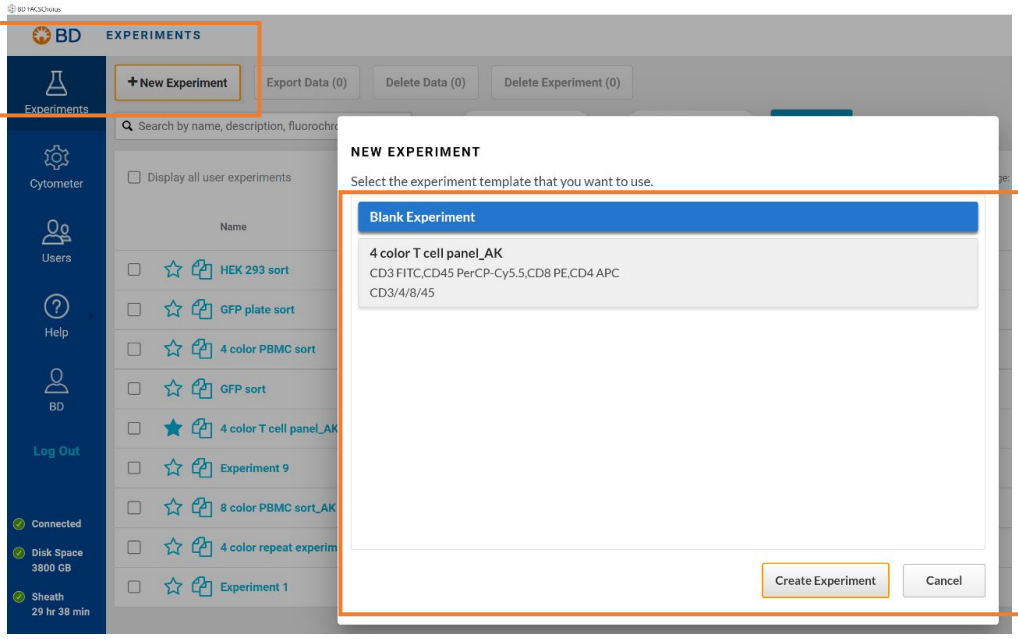



11. Perform a Drop Delay setup.

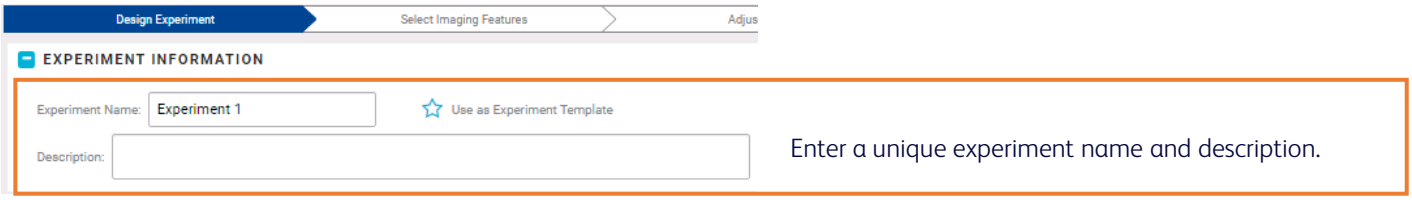


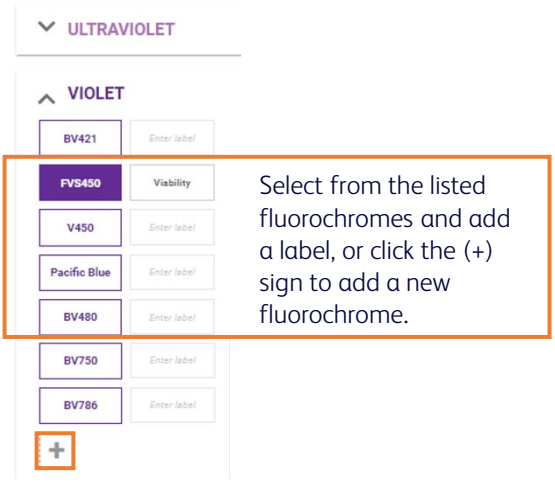
## Set up experiment

1. Create a new experiment on the Experiments page.



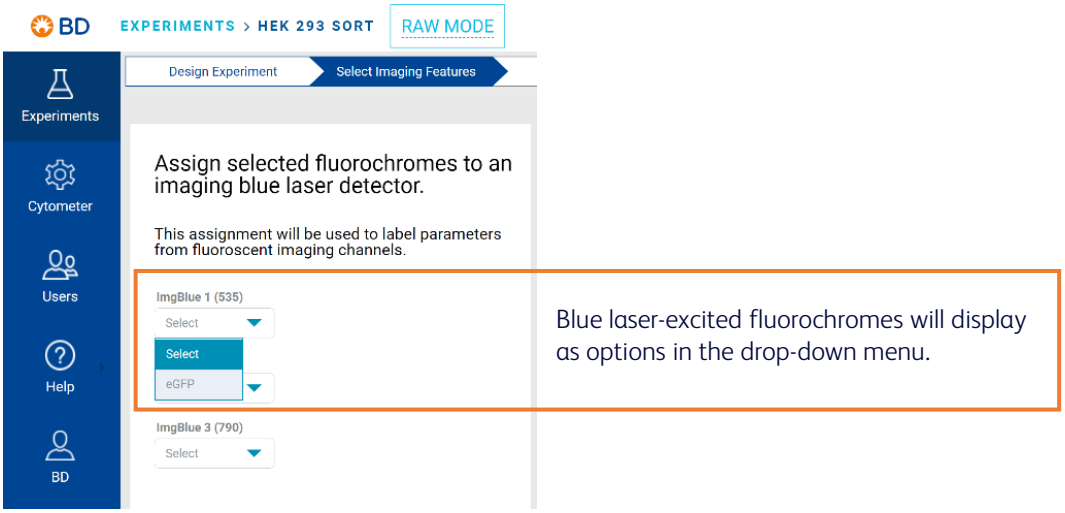

2. Enter the experiment information, dyes and labels on the Design Experiment page.



## Set up experiment, continued

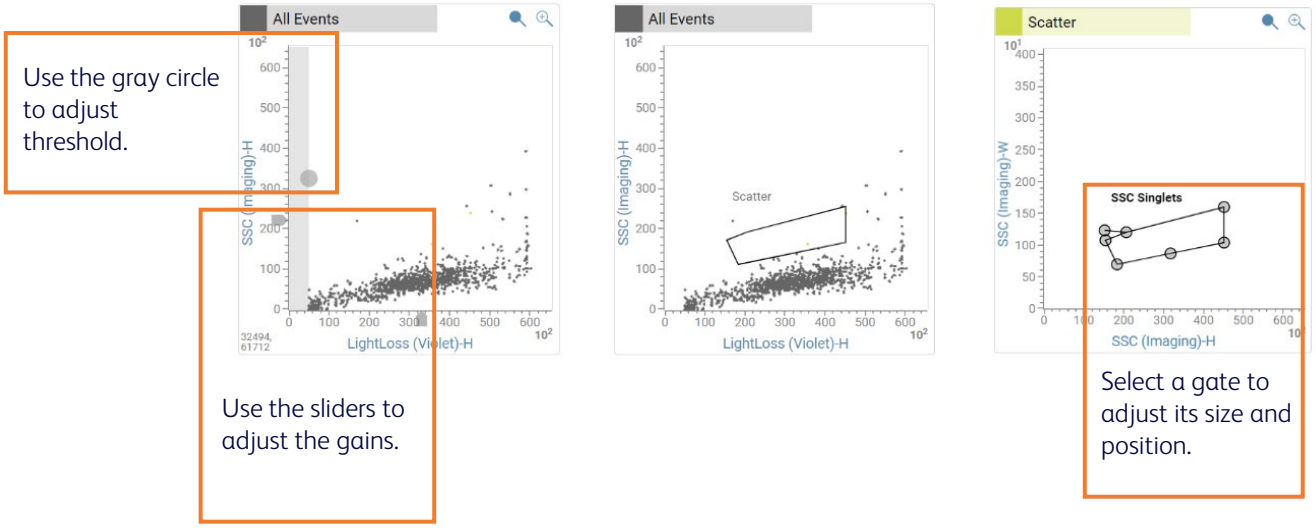
3. Enable fluorescence imaging by assigning dyes to the imaging detectors.




## Establish settings

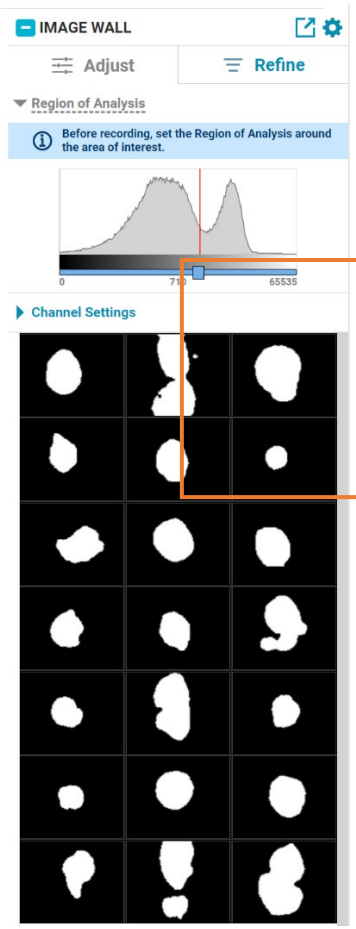
1. On the Adjust Gains page, load a tube of brightly stained cells.

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



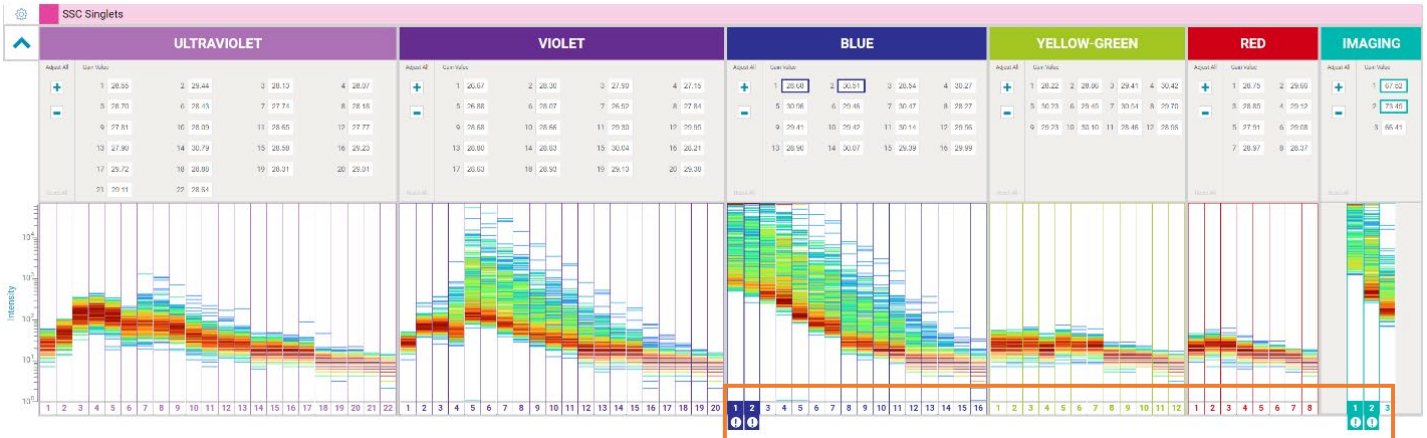
## Establish settings, continued

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




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.



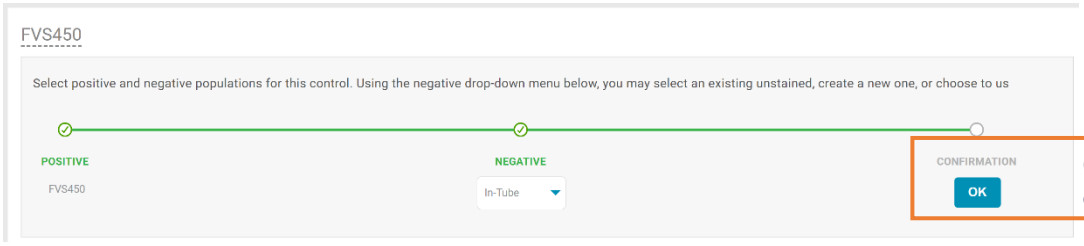

Saturated detectors will be identified with the ( ! ) icon. Lower the gains for these detectors until the icon disappears.

## 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.

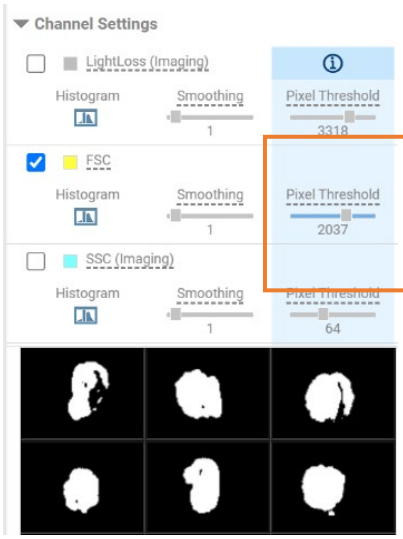



Click **OK** to confirm.

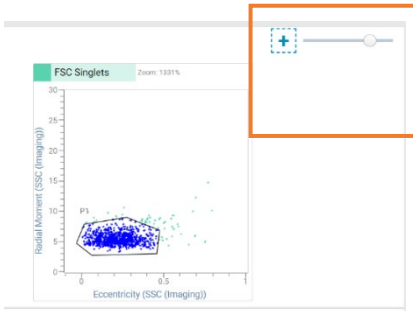
7. On the View Data page, load a sample tube and adjust scatter and singlet gates to encompass cells of interest.

Set the **Flow Rate** to 1 to conserve the sample.


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




Adjust the **Pixel Threshold** using the slider until the white area in the images completely encompasses the area of interest while minimizing background noise.



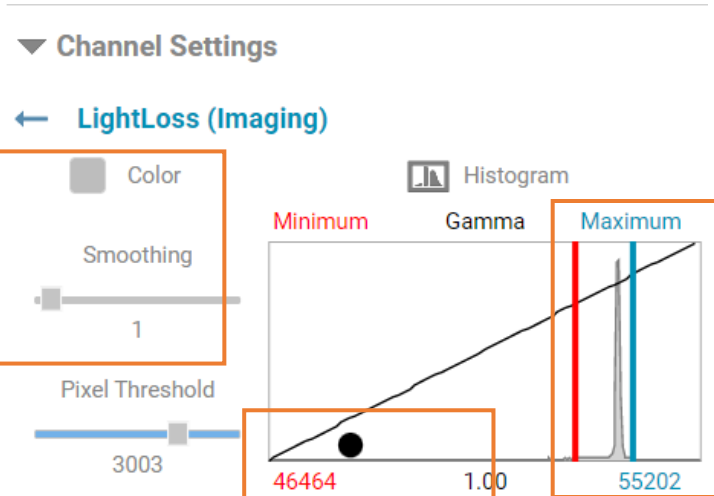

Click (+) icon to create new plots.

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.




Select the color box.

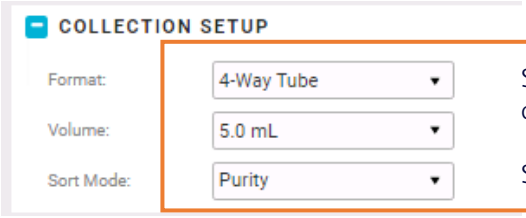
Use the smoothing slider to reduce image blur.

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.

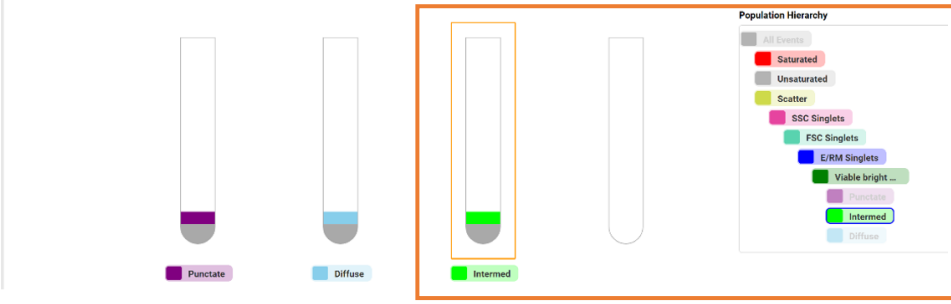



Select the format and the volume of the collection device.

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

a. If you are performing a tube sort:


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

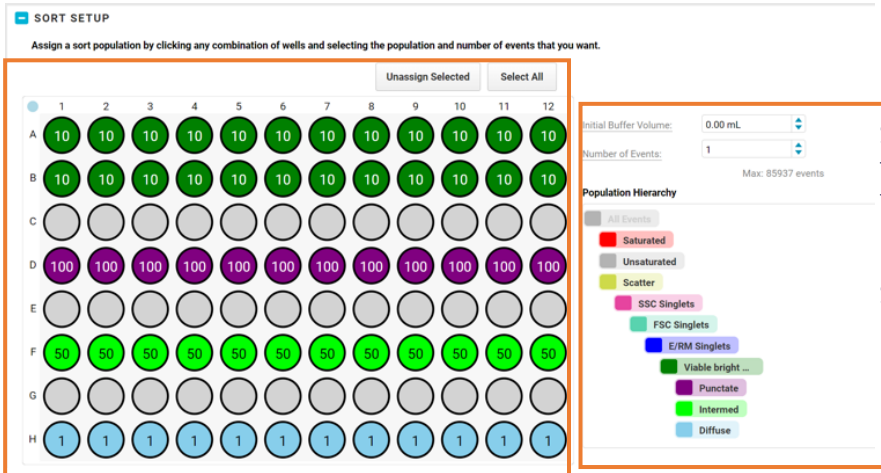



Assign populations to tubes by selecting the tube, then the population in the hierarchy.

## Sort and analyze, continued

b. If you are performing a plate sort:


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




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.

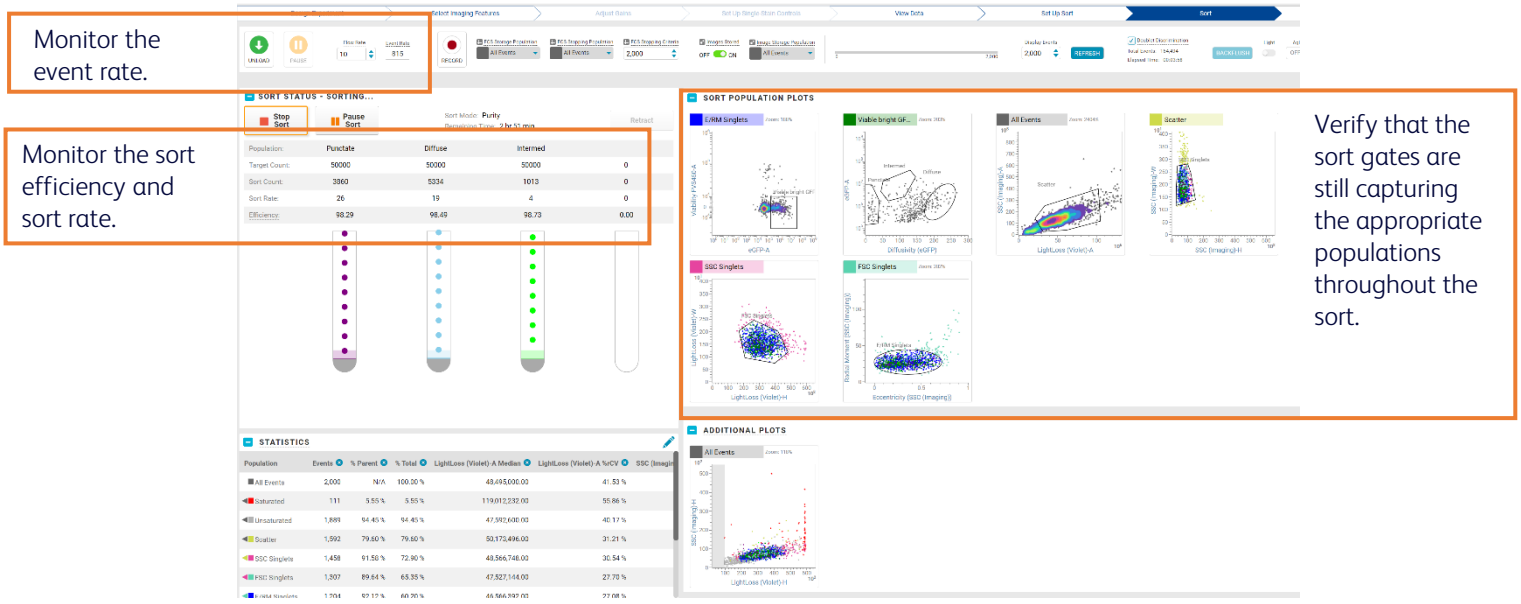
Select the sort population from the Population Hierarchy.

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

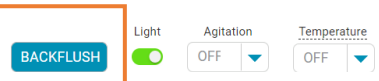
4. Monitor the sort as it progresses.




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

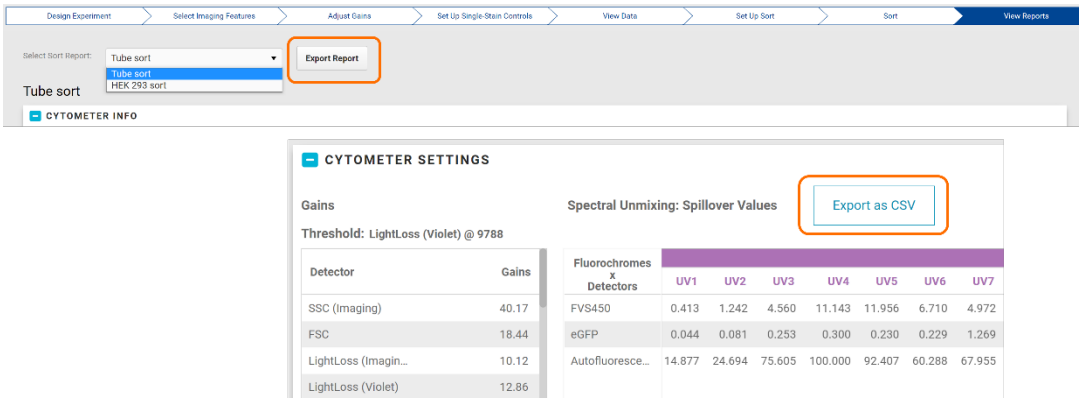
6. On the View Data page, analyze the sort purity.

**TIP** Perform a backflush between tubes to reduce carryover.




## Manage data

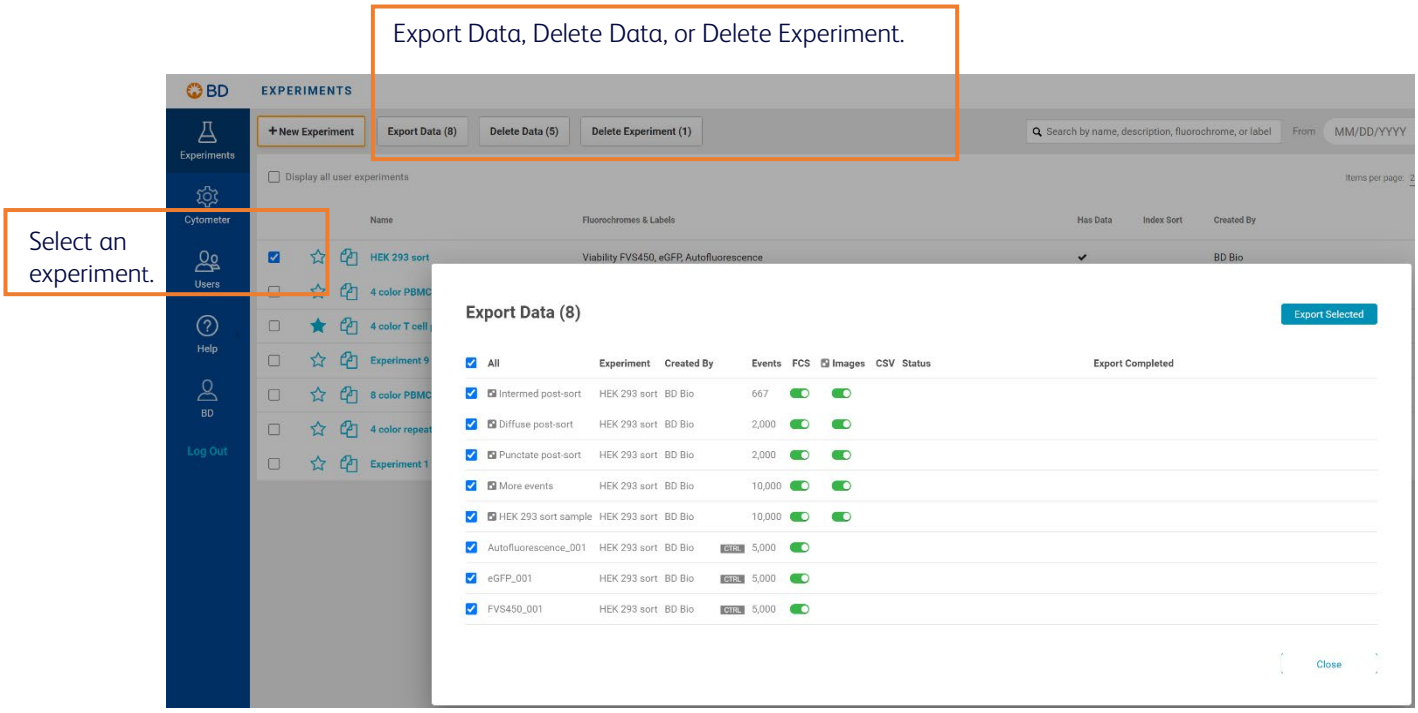
1. On the View Reports page, view and export reports.



## Manage data, continued

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

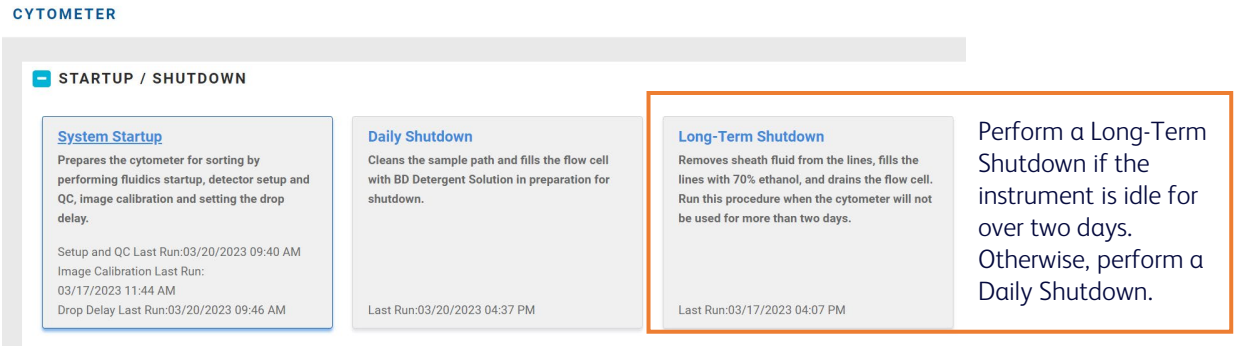
3. Delete unneeded experiments.




## Shut down system

1. Clean the sample line.

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




3. Power off the cytometer and workstation.

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