

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:

- Select a bead lot: Current lot number: 6009158U2 Expiration date: 11/29/2023. A dropdown menu shows "6009158U2". Callout: Verify the bead lot.
- Load a tube with BD FACSDiscover™ Setup Beads. Callout: Select the type of QC.
- Select type of Setup and QC: Radio buttons for "Daily" (selected) and "Baseline".
- Run Setup and QC: A "Run" button is visible. Callout: Load a tube of BD FACSDiscover™ Setup Beads and click **Run**.

On the left, a sidebar shows system status: "Last Setup and QC" (Baseline, Mar 22, 2023 12:15 PM, Passed), "Daily" (Apr 11, 2023 10:32 AM, Completed With Warnings), and "Estimated time to completion: 7 to 10 minutes".

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 components and their 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
Status: Passed
- ✓ 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-Z2

Below the list is a 'Close' button. To the right, a separate panel shows a warning icon and the text: '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.'

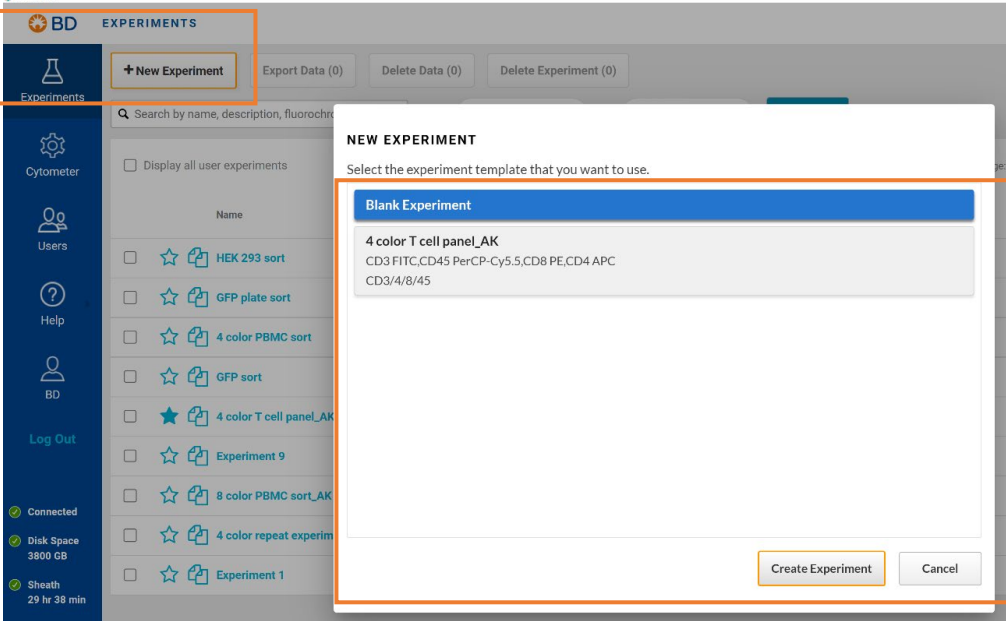
On the left, a text box points to the 'System' menu item in the sidebar: 'Click System to view the Image Calibration status.'

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 icon and the text: 'Run Drop Delay daily before sorting.' Below this, it states 'Drop Delay Last Run: 05/30/2023 10:51 AM' and 'Status: Passed'. At the bottom, a text box explains: 'Run a tube of BD FACS™ Accudrop Beads.' To the right of this text are 'Run Drop Delay' and 'Skip' buttons.

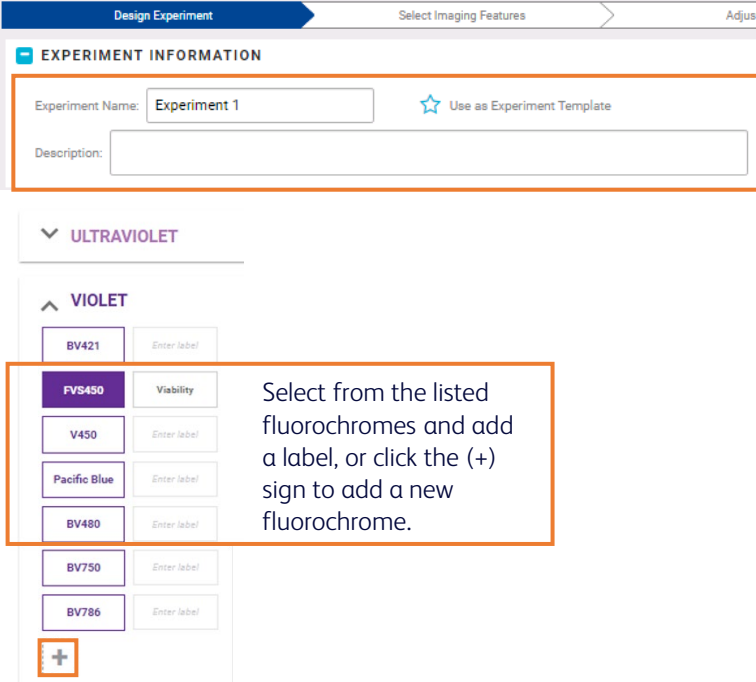
Set up experiment

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 in the top navigation bar. A larger callout box on the right highlights the 'NEW EXPERIMENT' dialog box, which is open. The dialog box prompts the user to 'Select the experiment template that you want to use.' and lists several templates. The 'Blank Experiment' template is selected and highlighted in blue. Below the list, there are 'Create Experiment' and 'Cancel' buttons. A text box on the right side of the dialog box contains the instruction: 'Select Blank Experiment, then click Create Experiment.'

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

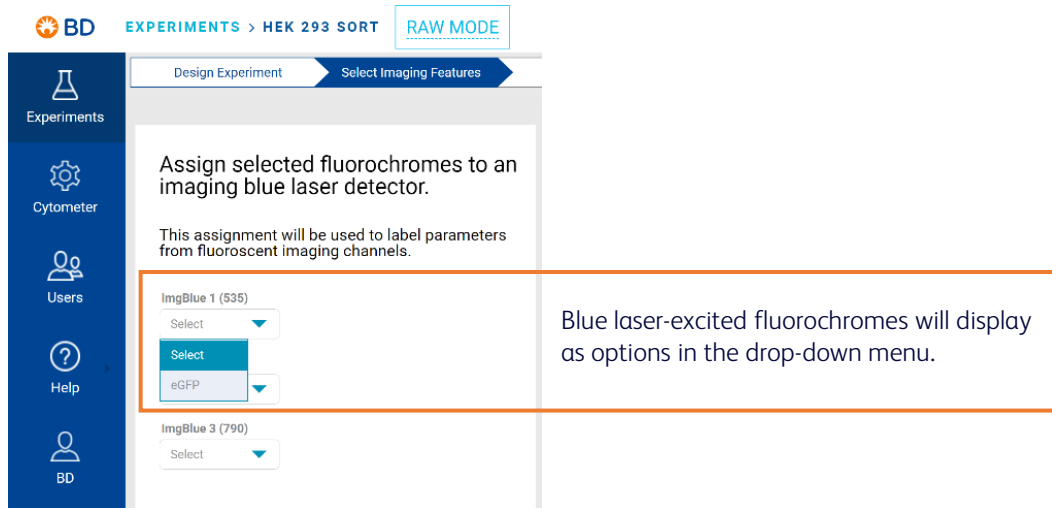


The screenshot shows the 'Design Experiment' page. The 'EXPERIMENT INFORMATION' section is highlighted with a callout box. It contains an 'Experiment Name' field with the value 'Experiment 1' and a 'Description' field. A 'Use as Experiment Template' checkbox is also visible. A text box on the right side of the callout box contains the instruction: 'Enter a unique experiment name and description.'

Below the 'EXPERIMENT INFORMATION' section, the 'VIOLET' section is expanded, showing a list of fluorochromes. A callout box highlights the 'FVS450' fluorochrome, which is selected and highlighted in purple. The 'FVS450' entry includes a 'Viability' label. A text box on the right side of the callout box contains the instruction: 'Select from the listed fluorochromes and add a label, or click the (+) sign to add a new fluorochrome.'

Set up experiment, continued

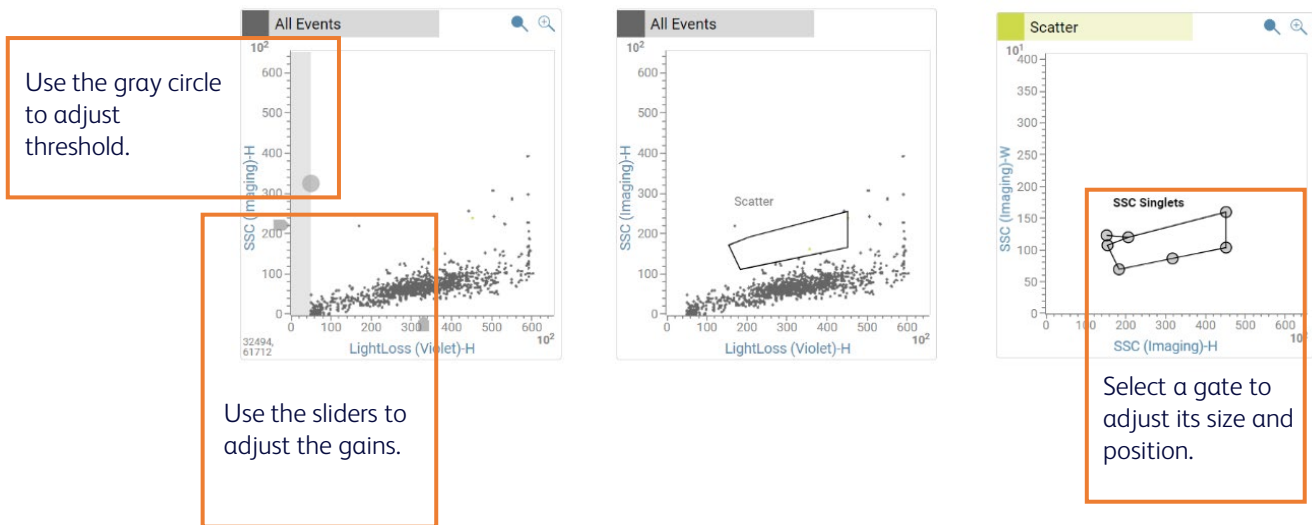
3. Enable fluorescence imaging by assigning dyes to the imaging detectors.



The screenshot shows the 'RAW MODE' interface for 'HEK 293 SORT'. The 'Select Imaging Features' tab is active. 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 two drop-down menus. The first is labeled 'ImgBlue 1 (535)' and has 'eGFP' selected. The second is labeled 'ImgBlue 3 (790)' and has 'Select' chosen. A text box on the right states: 'Blue laser-excited fluorochromes will display as options in the drop-down menu.'

Establish settings

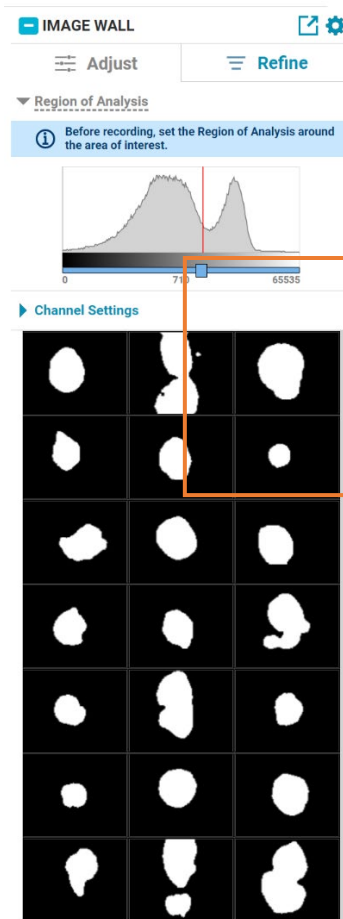
1. On the Adjust Gains page, load a tube of brightly stained cells.
2. Adjust the scatter gains, threshold, and gates to encompass cells of interest.



The first plot, titled 'All Events', shows a scatter plot of 'SSC (Imaging)-H' vs 'LightLoss (Violet)-H'. A gray circle on the y-axis is highlighted with a callout: 'Use the gray circle to adjust threshold.' Below it, a callout says: 'Use the sliders to adjust the gains.' The second plot, also titled 'All Events', shows a rectangular gate drawn around a cluster of cells, with a callout: 'Scatter'. The third plot, titled 'Scatter', shows a gate labeled 'SSC Singlets' around a cluster of cells, with a callout: 'Select a gate to adjust its size and position.'

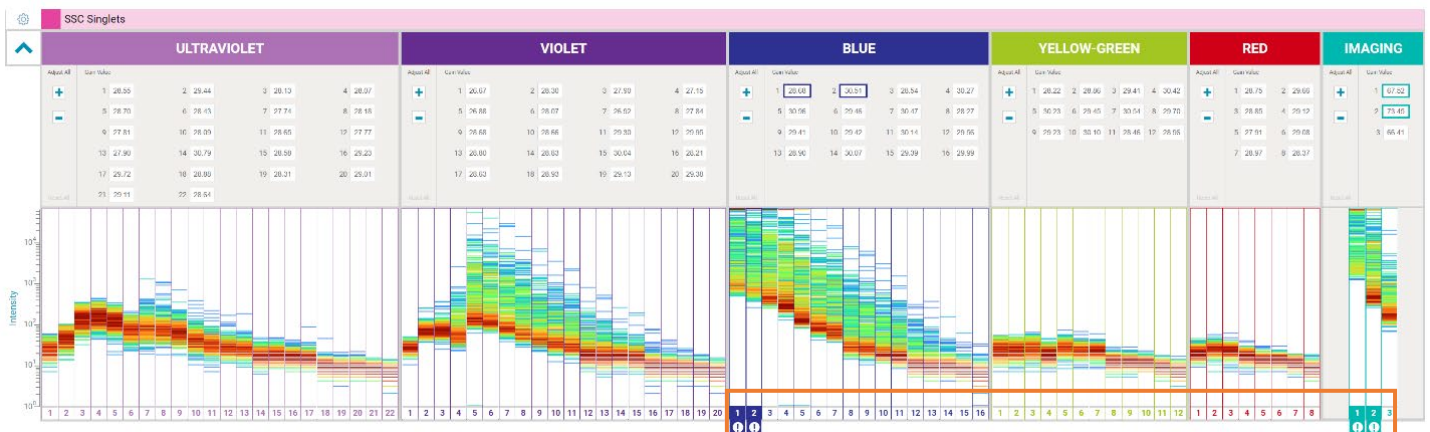
Establish settings, continued

- 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.

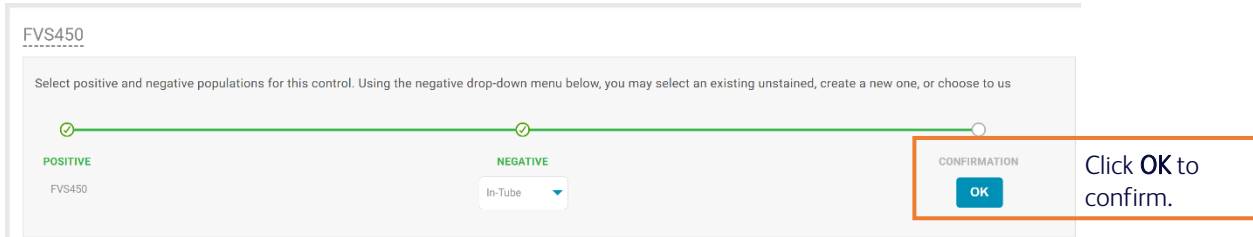
- Use the spectral plot to adjust the gains, if needed.



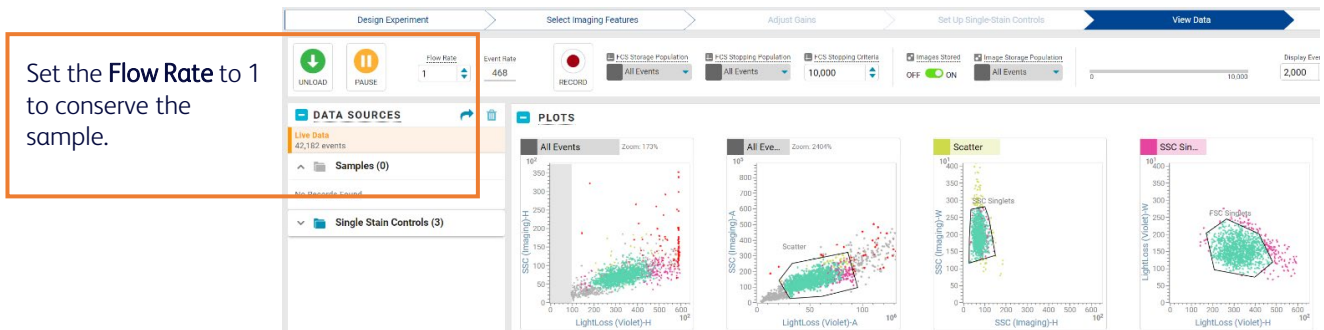
Saturated detectors will be identified with the (!) icon. Lower the gains for these detectors until the icon disappears.

Establish settings, continued

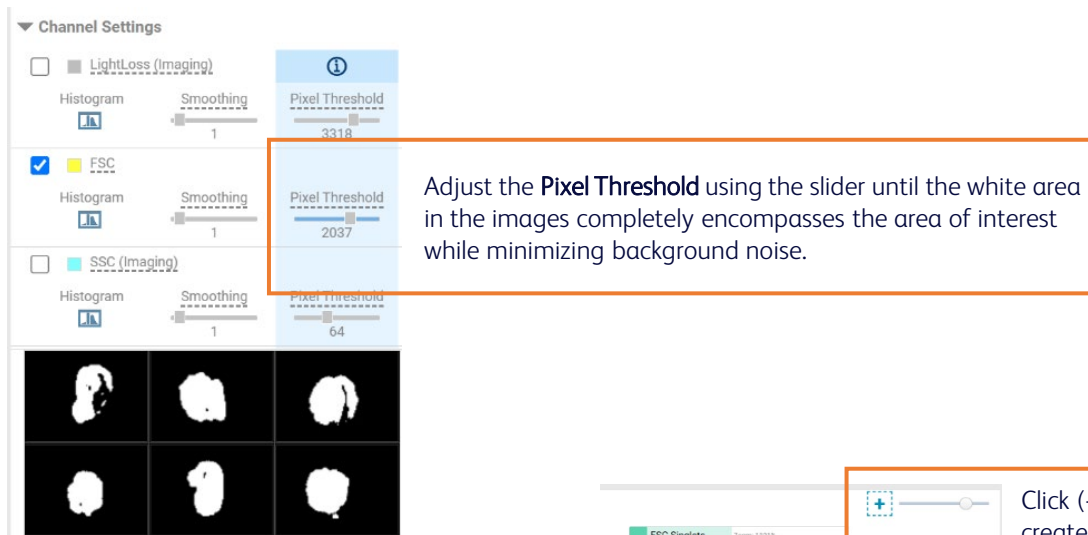
- On the Set Up Single-Stain Controls page, record data for each control tube.
NOTE The Region of Analysis must be appropriately set for the particle type before recording.
- Adjust the gates for each parameter.



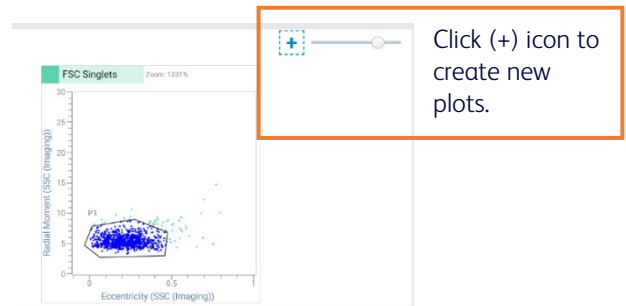
- On the View Data page, load a sample tube and adjust scatter and singlet gates to encompass cells of interest.



- 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 Gamma Maximum

46464 1.00 55202

Adjust the Gamma (black circle) as needed.

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

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: Enable Index Sort

BD-Defined Plate:

Plate Name: [Optimize Plate](#)

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:

Number of Events: Max: 85937 events

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 well.

Select the sort population from the Population Hierarchy.

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.

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 Cytometer software interface during a sort. Key components include:

- Sort Status - SORTING...:** Shows 'Sort Mode: Purity' and 'Purity Threshold: 2.00'. It includes a table with columns for Population, Punctate, Diffuse, and Interned, along with their respective counts and sort rates. Below the table are four vertical progress bars for each population.
- Sort Population Plots:** A grid of plots showing various populations: FSC Singlets (99.76%), Viable Single U2 (99.93%), All Events (99.76%), Scatter (99.93%), SSC Singlets (99.93%), and FSC Singlets (99.93%). Each plot shows a scatter of events with a gate drawn around the target population.
- Statistics:** A table summarizing event counts and percentages for different populations.
- Additional Plots:** A plot for 'All Events' showing a scatter of events.

Annotations on the screenshot:

- A box highlights the 'Monitor the event rate.' area, pointing to the 'Sort Rate' and 'Efficiency' metrics in the Sort Status section.
- A box highlights the 'Monitor the sort efficiency and sort rate.' area, pointing to the 'Sort Rate' and 'Efficiency' metrics in the Sort Status section.
- A box highlights the 'Verify that the sort gates are still capturing the appropriate populations throughout the sort.' area, pointing to the Sort Population Plots.

| Population | Punctate | Diffuse | Interned |
|---------------|----------|---------|----------|
| Target Count: | 50000 | 50000 | 50000 |
| Sort Count: | 3360 | 5334 | 1013 |
| Sort Rate: | 26 | 19 | 4 |
| Efficiency: | 98.29 | 98.49 | 98.73 |

| Population | Events | % Parent | % Total | LightLoss (Violet) A Median | LightLoss (Violet) A %CV | SSC (Imaging) |
|-----------------|--------|----------|----------|-----------------------------|--------------------------|---------------|
| All Events | 2,002 | N/A | 100.00 % | 49,695,006.02 | 41.83 % | |
| Subsorted | 111 | 5.55 % | 5.55 % | 119,012,232.03 | 95.86 % | |
| Unsubsorted | 1,888 | 94.45 % | 94.45 % | 47,582,606.01 | 40.17 % | |
| Scatter | 1,992 | 79.60 % | 79.60 % | 50,172,496.02 | 31.21 % | |
| SSC Singlets | 1,458 | 91.50 % | 72.90 % | 48,566,746.02 | 35.54 % | |
| FSC Singlets | 1,307 | 89.84 % | 65.35 % | 47,527,144.02 | 27.79 % | |
| Viable Singlets | 1,704 | 87.17 % | 69.70 % | 48,566,939.01 | 27.08 % | |

- 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.

BACKFLUSH Light Agitation Temperature

Manage data

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

The screenshot shows the 'View Reports' page in the Cytometer software. Key components include:

- View Reports Page:** A navigation bar at the top shows the current step is 'View Reports'. Below it, there is a dropdown menu for 'Select Sort Report' with 'Tube sort' selected. An 'Export Report' button is highlighted.
- CYTOMETER SETTINGS:** A section showing 'Gains' and 'Spectral Unmixing: Spillover Values'. The 'Export as CSV' button is highlighted.
- Table 1: Gains**
- Table 2: Spectral Unmixing: Spillover Values**

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

| 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 |

