# Job Aid

# BD FACSDiscover™ S8 Cell Sorter: Recording and analyzing data

This job aid contains instructions for how to record and analyze sample data using imaging in BD FACSChorus™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology User's Guide.*

Start up system ➡ Set up experiment ➡ Establish settings ➡ Sort and analyze ➡ Manage data ➡ Shut down system

## Before you begin

- Start up the system and run a daily or extended fluidics startup procedure.
- Add and design an experiment, adjust your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Perform spectral unmixing by recording data for single-stained controls, if applicable.

## Working with the View Data tab

### Preparing the experiment

1. Click the **View Data** tab.



2. Load your sample tube.

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

3. Clear the doublet discrimination checkbox, if needed.



4. Verify that the spectral or imaging detectors are not saturated in the spectral plot.

5. Verify that your population of interest is onscale in the LightLoss (Violet)-H vs. SSC (Imaging)-H plot.

6. Adjust the plot zoom, scatter gains, and threshold appropriately. Keep the saturated events to a minimum.

   **TIP** In the Statistics panel, verify that most events are Unsaturated.
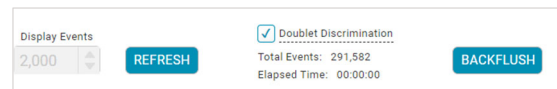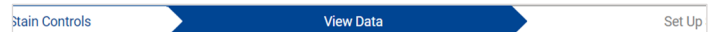
## Preparing the experiment, continued

7. In the Plots panel, adjust the Scatter, SSC Singlets, and FSC Singlets gates to encompass the population of interest.

    The SSC Singlets and FSC Singlets gates are only available if the Doublet Discrimination checkbox is selected.

    **NOTE** The Eccentricity (LightLoss (Imaging)) vs. Radial Moment (LightLoss (Imaging)) plot can be used for additional doublet discrimination. This plot will be removed when the Doublet Discrimination checkbox is cleared.
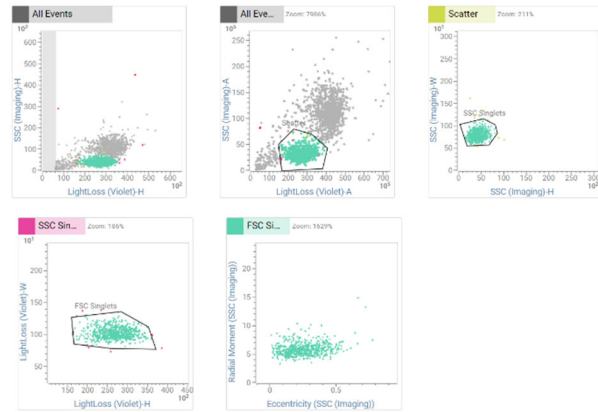


8. Adjust the Region of Analysis and Pixel Threshold for the detectors of interest in the Image Wall.

    **NOTE** The Region of Analysis (ROA) setting can affect the fluorescent data of your sample. You must set the ROA properly for the current particle type before recording.

    - Adjust the Region of Analysis slider until the white area in the images completely encompasses the events of interest while minimizing background pixels.

        **TIP** To adjust the slider in small increments, click the slider then press the arrow keys.

    - Adjust the Pixel Threshold slider for the imaging detectors until the white area in the images completely encompasses the area of interest while minimizing background pixels.
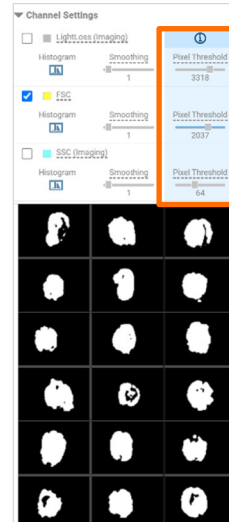


9. Set recording criteria:

    a. Under FCS Storage Population, select **All Events**.

        **NOTE** Selecting children of All Events will only save that population to the FCS file.

    a. Under FCS Stopping Population and FCS Stopping Criteria, select the population and the number of events to record.



    b. Verify that the Images Stored switch is toggled on to save images.

    c. Under Image Storage Population, select the population for image storage.
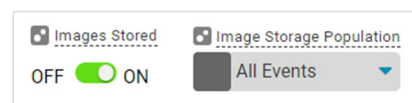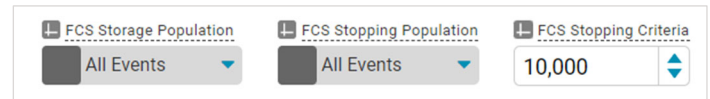
## Recording sample data

1. Click **Record** in the dashboard.

   When the target is reached, acquisition stops.

2. Enter a name for your recorded file and click **OK**.

   The new data file will appear in the Data Sources panel.

   **NOTE** The Data Sources panel displays all previously recorded data files, including Single Stain Control data, if applicable. To view a recorded data file, select it. To view live acquisition, select **Live Data**.
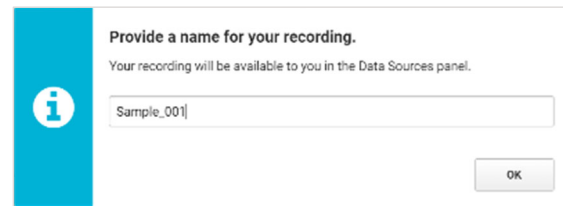
## Analyzing sample data

1. Click the Add new plot (⊞) icon and select a plot type from the menu to create any additional plots.

2. Click the plot axes and from the plot parameters dialog, select parameter and scaling options.

   NOTE Click the Plot properties (⚙) icon on the top right of the plot window to delete the plot or change the parent gate or the plot type.

   **TIP** Drag plots to reorder them in the Plots panel.
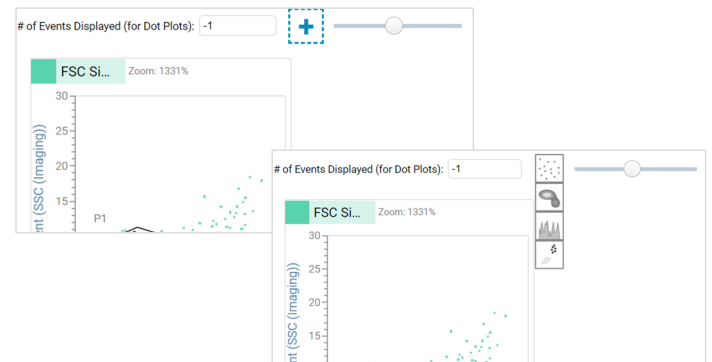
3. Add sort gates.

   Hover over a plot, click the Add new gate (⬚) icon, and select a gate type.

   - For rectangular and oval gates, drag an area on the plot to encompass the population of interest.
   - For polygon gates, click multiple times to create vertices around the population of interest.
   - For quadrant gates, click the plot to place the center of the quadrant gate.
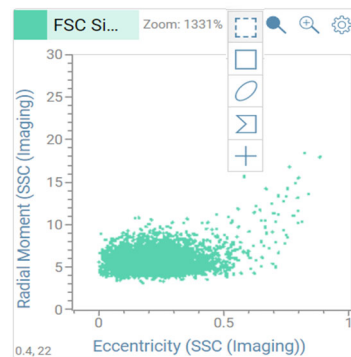
4. In the Population Hierarchy panel:

   - Click the Edit (✏) icon to rename the population.
   - Click the Delete (✖) icon to delete the population.
   - Click the color swatch (■) to change the color of the population.

   **TIP** Drag gates to other locations in the hierarchy, if needed.
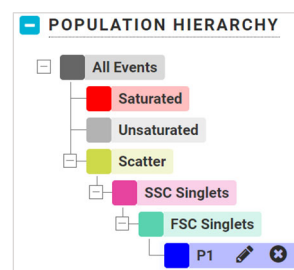
## Analyzing sample images

1.  Adjust the channel settings in the image wall for each detector of interest, as needed.

    **NOTE** The following adjustments can be made at any time before or after recording data. Only ROA and Pixel Threshold must be set correctly before recording.

    a.  Select the checkbox for the channel of interest and clear other channels.

    b.  Click **Histogram** to open the histogram panel.

        i.   Adjust the Minimum (red) bar and Maximum (blue) bar around the signal peak to optimize the brightness resolution of the images.

        ii.  Adjust the Gamma (black circle) as needed to more easily visualize differences in signal intensity between images.

    c.  Adjust the Smoothing slider to reduce blur in the images.

    d.  Select the color swatch to adjust the color.

    e.  Repeat steps a through d for all other imaging channels of interest.
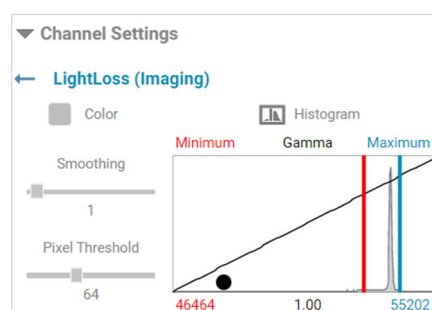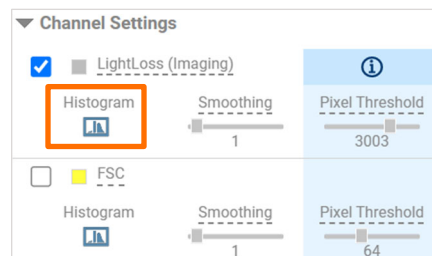




2.  Select the checkboxes for channels of interest to visualize cells appropriately.
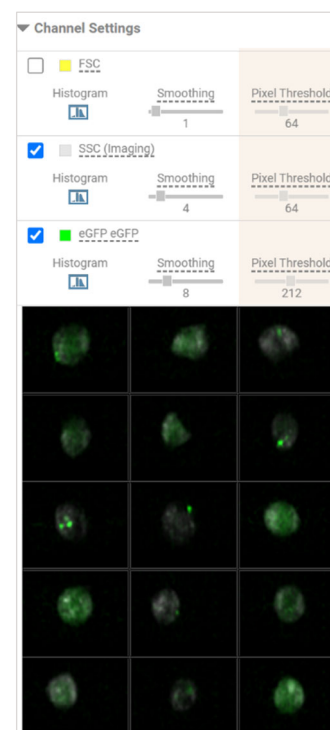


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