

Job Aid

BD FACSDiscover™ A8 Cell Analyzer: Adjusting the settings

This job aid contains instructions for how to adjust the settings for your experiment in BD FACSDiscover™ Software.

NOTE For an imaging experiment, perform all the steps mentioned in the following procedure. For a high-speed experiment, perform the entire procedure except the steps that involve adjusting the Image Wall settings and Region of Analysis (ROA).

For additional information, see the *BD FACSDiscover™ A8 Cell Analyzer with BD CellView™ Image Technology and BD SpectralFX™ Technology User's Guide*.



Before you begin

- Start up the system and run a daily or extended fluidics startup procedure.
- Add and design an experiment, including assigning fluorochromes to imaging detectors, if applicable.

Adjusting the settings

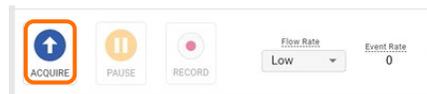
Adjusting the plots, gains, and gates

1. Click the **Adjust Gains** tab.



2. Load a fully stained sample on the manual tube port.

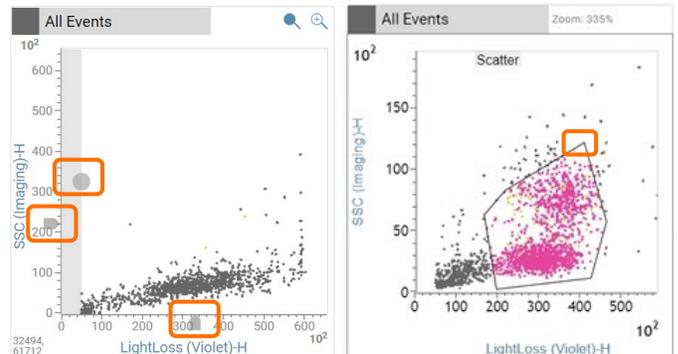
3. In the dashboard, click **Acquire**.



TIP Change the flow rate to Low to avoid sample loss.

4. Adjust the plot zoom, scatter gains, threshold, and gates to encompass cells of interest.

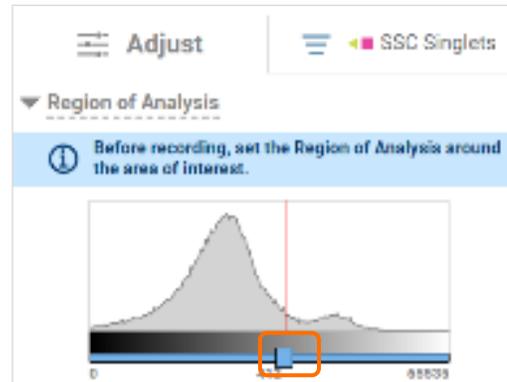
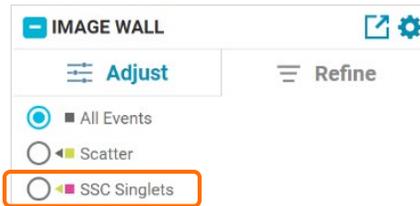
- a. Zoom in on a plot by clicking the plot, then rolling the mouse scroll wheel upwards. Roll downwards to zoom out.
- b. Adjust the scatter gains, if needed. Hover over the scatter plots axes then drag the gain sliders along the axes.
- c. Adjust the threshold by hovering over the gray portion of the first default plot and then dragging the threshold marker (gray dot) horizontally.
- d. Adjust the gates by dragging the vertices to encompass populations.



Adjusting Region of Analysis (ROA) for imaging experiments only

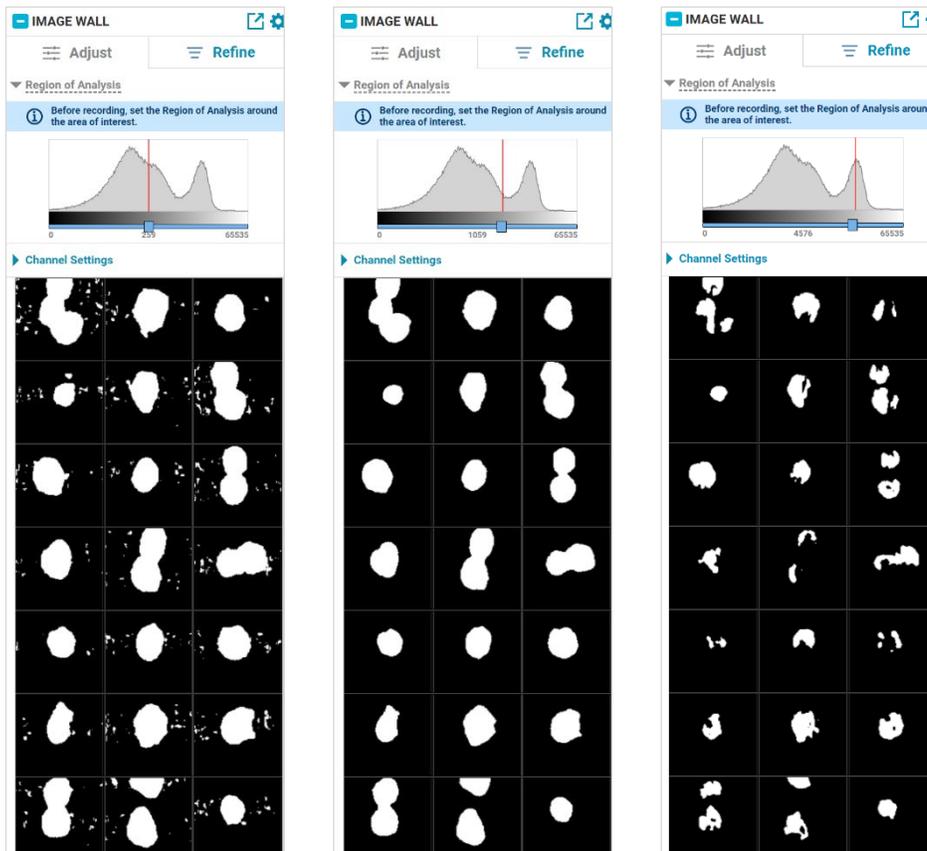
NOTE For a High-Speed Experiment, proceed to Adjusting the spectral gains on the next page.

NOTE For image features to be correctly calculated, you must adjust the ROA before you record your sample. The ROA slider is not available for recorded data because adjustments cannot be made on recorded data.



1. (Optional) To conserve the sample, click **Pause** in the dashboard.
2. Adjust the Region of Analysis (ROA) in the Image Wall.
 - a. In the Refine tab, select **SSC Singlets**. **NOTE** Ensure this gate is adjusted to the population before refining on the image wall.
 - b. In the Adjust tab, move the ROA slider slowly until the white area on the images completely encompasses the events of interest while minimizing background.

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



Example: ROA set too low (left), accurately (center), and too high (right).

Adjusting spectral gains (for both imaging and high-speed experiments)

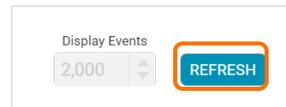
1. If needed, click Resume to continue sample acquisition.
2. Verify that no channels are saturated on the spectral plot.

Saturated channels are indicated by a colored box with the (!) icon, which displays at the bottom of the affected channel.

3. Lower the gains of any saturated channel, if needed.
 - a. Click the down arrow on the top left corner of the spectral plot panel.
 - b. Adjust the the spectral plot gains by using one or more of the following techniques.
 - Enter a new value for the channel, then press **Enter**.
 - Select a channel, then scroll using your mouse scroll wheel.
 - Click the + and – buttons to adjust gains for all channels in that laser.
- Click **Reset All** to set the gains of all channels to the default settings.



TIP After adjusting the gain, click **Refresh** in the dashboard to visualize the change.



4. Click **Stop** and remove the tube.

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