

# Job Aid

## BD FACSDiscover™ S8 Cell Sorter: Adjusting the settings

This job aid contains instructions for how to adjust the settings for your experiment in BD FACSDiscover™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology User's Guide*.



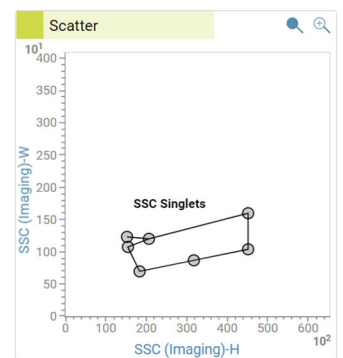
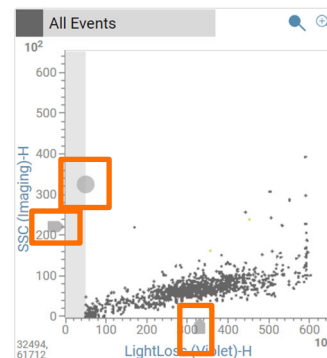
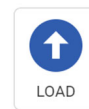
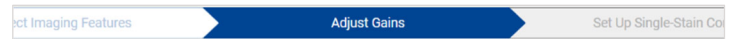
### Before you begin

- 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

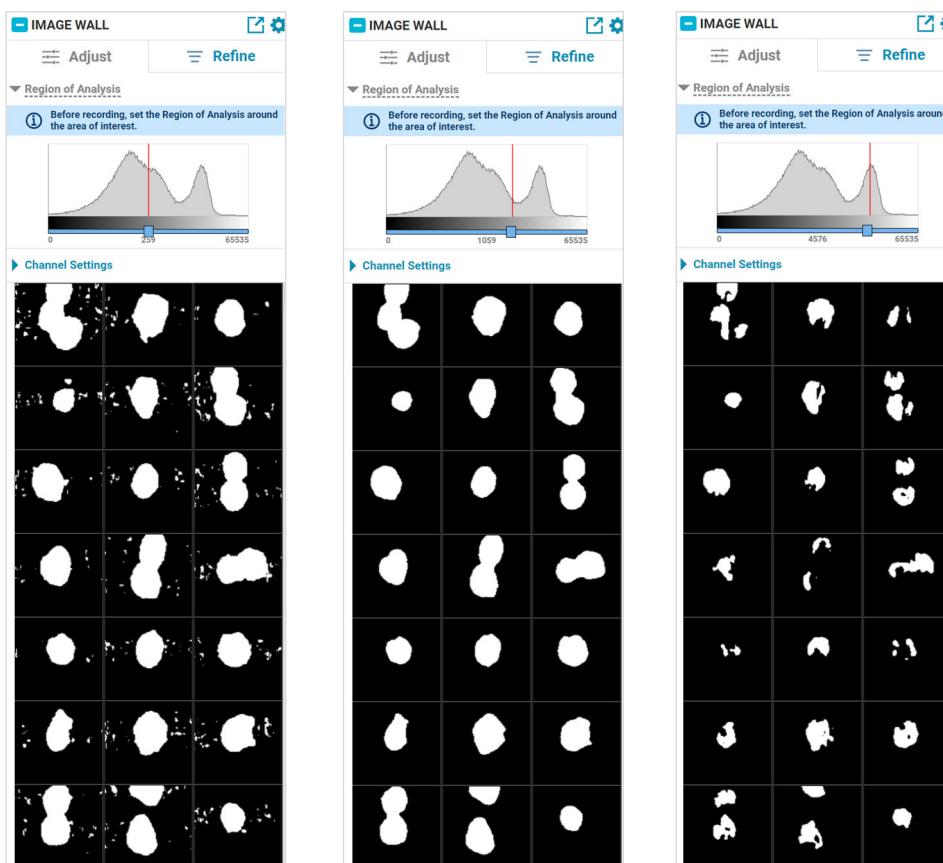
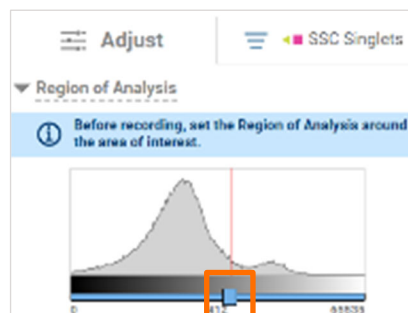
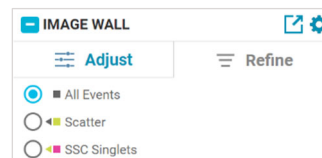
1. Click the **Adjust Gains** tab.
2. Load a fully stained sample on the sample loading port.
3. In the dashboard, click **Load**.
4. Adjust the plot zoom, scatter gains, threshold, and gates to encompass cells of interest.
  - a. Zoom in on a plot by clicking the plot, then rolling the mouse scroll 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) and spectral gains

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**.
  - 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).

3. If needed, click **Resume** to continue sample acquisition.

## Adjusting Region of Analysis (ROA) and spectral gains, continued

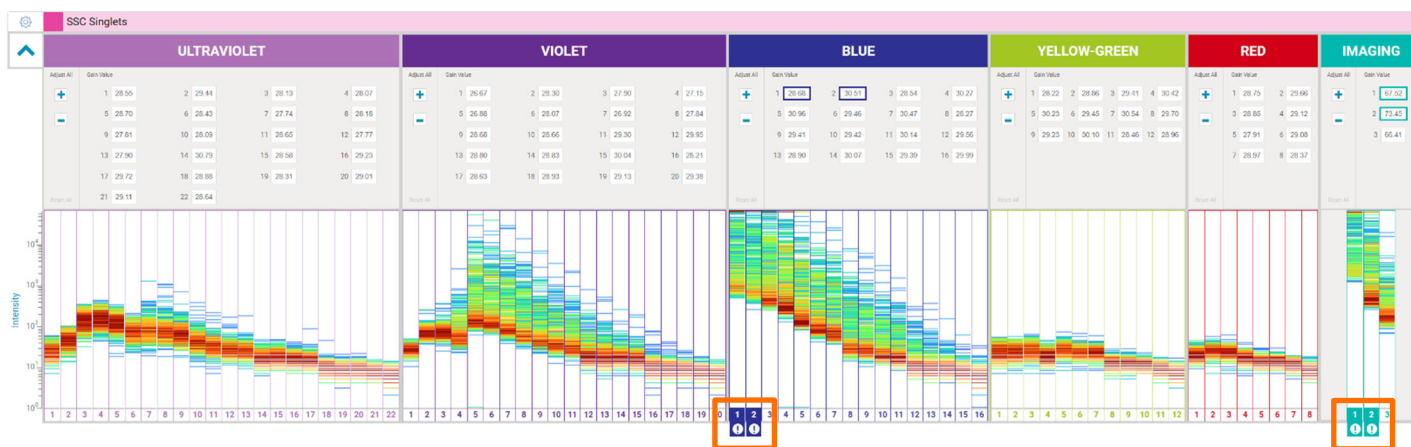
- Verify that no channels are saturated on the spectral plot.

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

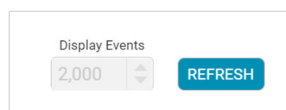
Lower the gains of any saturated channel.

- Click the down arrow on the top left corner of the spectral plot panel.
- Adjust the spectral plot gains under Gain Value:
  - 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.



- Click **Unload** in the dashboard.

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