Job Aid

BD FACSDiscover™ S8 Cell Sorter: Setting up and recording single-stained controls

This job aid contains instructions for how to set up and record single-stained controls for your experiment in BD FACSChorus™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ 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.
- Ensure that the ROA has been set up on the Adjust Gains page for the specific particles used in your fluorochrome controls.

Working with the Set Up Single-Stain Controls tab

Setting up single-stained controls

1. Click the **Set Up Single-Stain Controls** tab.

   The page is automatically populated with control tubes based on the fluorochromes selected in the Select Your Dyes panel, including Autofluorescence.

2. (Optional) Click **+Add More** to add separate Unstained control tube(s).

   Click the Edit (✎) icon to rename the Unstained control(s), if needed.

3. Load a control tube on the sample loading port.

   NOTE Control tubes can be acquired in any order.

4. Select the appropriate tube from the list and click **Load** in the dashboard.

Adjusting settings

1. Adjust the plot zoom, scatter gains, threshold, plot scaling, 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.

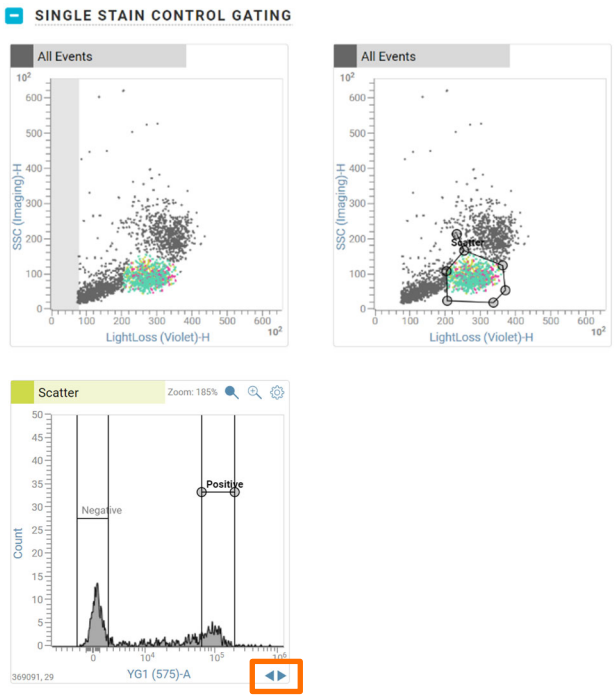
   b. Adjust the scatter gains, if needed. Hover over the scatter plots axes then drag the gain sliders along the axes.

      NOTE If the SSC-Imaging gain is changed, then the ROA must be reset for the control particles before recording data.

   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 plot scaling by clicking the biexponential scale adjustment arrows.

   e. Adjust the gates by dragging the vertices to encompass populations.



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.

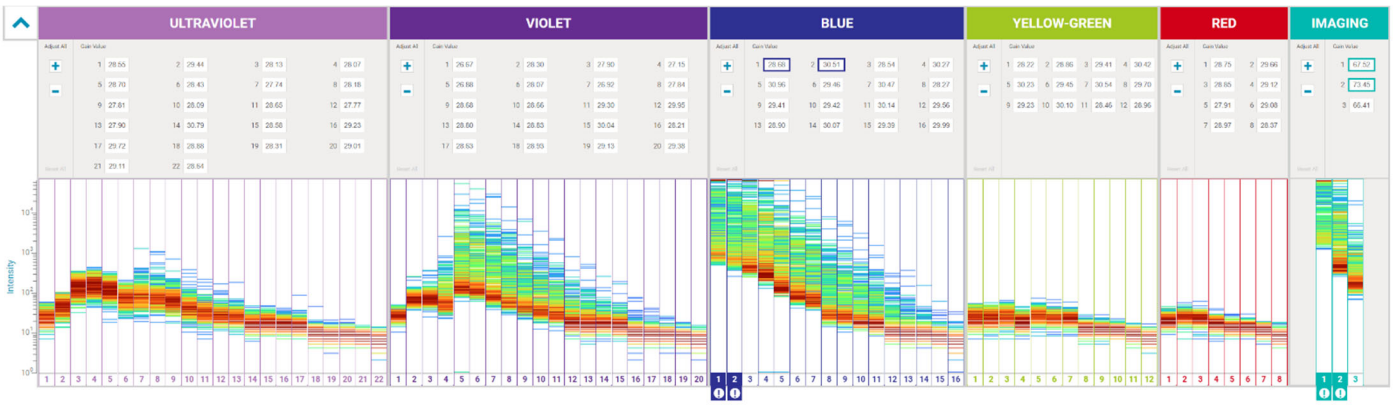
   Lower the gains of any saturated channel.

   a. Click the down arrow on the top left corner of the spectral plot panel.

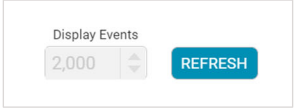
   b. Adjust the spectral plot gains under Gain Value:

      - 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.



Recording controls

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

2. Adjust the scatter and histogram (interval) gates to encompass positive and negative populations of interest.

   If needed, select a previously recorded unstained tube as your negative control.

   Gates can be adjusted at any time before, during, or after recording.

3. Click **OK** under Confirmation.

   NOTE This step is not necessary for the Unstained control tubes.

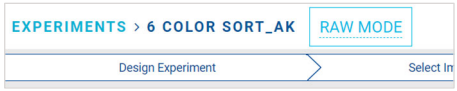
4. Load the next control tube on the sample loading port.

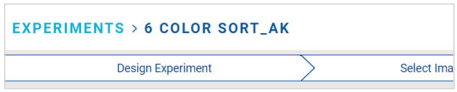
5. Select the appropriate tube from the list and click **Load** in the dashboard.

6. Repeat steps 1 through 5 for all the remaining controls.

   TIP If single-stain controls are not all the same particle type (for example, some control tubes contain beads and some contain cells), we recommend first verifying ROA for one particle type, and recording all tubes containing that particle type. Then verify ROA for the second particle type, and record all tubes containing that particle type.

7. After confirming the last control tube, verify that the Raw Mode indicator next to the experiment name is not displayed.

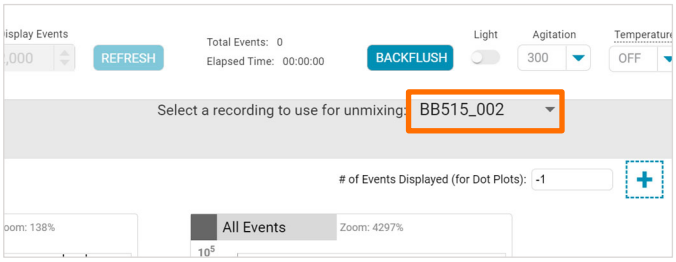




Tips and troubleshooting

Re-recording controls

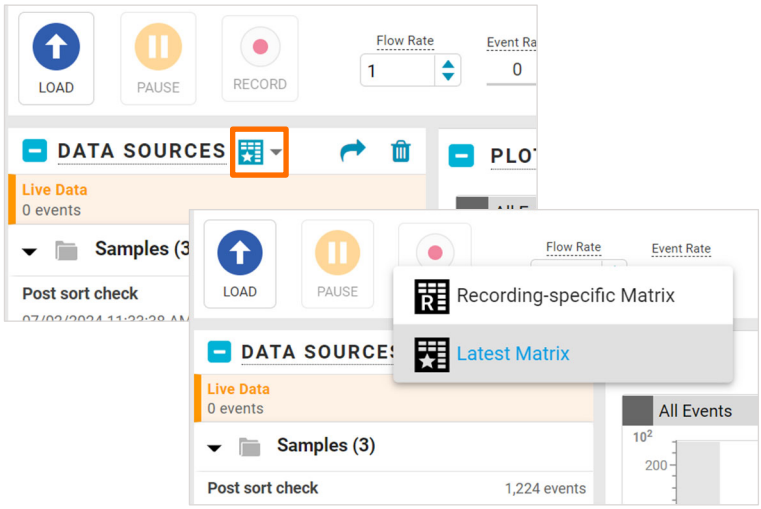
- Fluorochrome and autofluorescence controls can be re-recorded as many times as needed. Previous recordings can be accessed in the **Select a recording to use for unmixing** dropdown list.



- To re-record an unstained control, click **+Add More** to add a new unstained control tube.
- Single-stained controls cannot be renamed or deleted.

Re-unmixing after experimental data is recorded

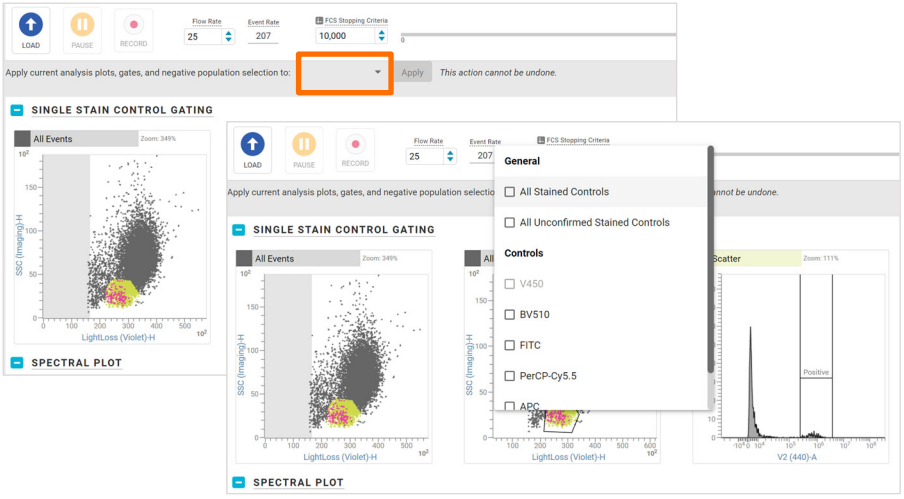
- The spectral unmixing matrix can be recalculated and the new matrix applied to previously recorded data files. To change the spectral unmixing matrix, you must modify the existing fluorochrome controls or record new fluorochrome controls, and then confirm the changes.
- The newest spectral unmixing matrix is referred to as the Latest Matrix, and it will be applied to all samples acquired and recorded after it is created.
- Experimental data that has been unmixed can be viewed with either the Recording-specific Matrix (the matrix at the time of recording) or the Latest Matrix.

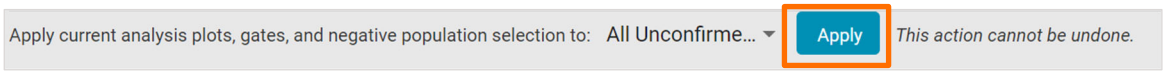


- Experimental data that has not been unmixed (acquired in Raw Mode) cannot be viewed with a spectral unmixing matrix.
- Any sorts performed will use the Latest Matrix for unmixing.
- Statistics exported from the experiment will be calculated using the matrix that is currently selected.
- The Chorus Experiment File (CEF) will contain only the Latest Matrix.

Applying gates to all controls

- To apply the current analysis plots, gates, and negative population selection to some or all controls, click the dropdown menu above the scatter plots to select the controls and then click **Apply**.





- Once overwritten, the default gate positions cannot be restored. However, plots, gates, and negative population selections can be further edited as needed.

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