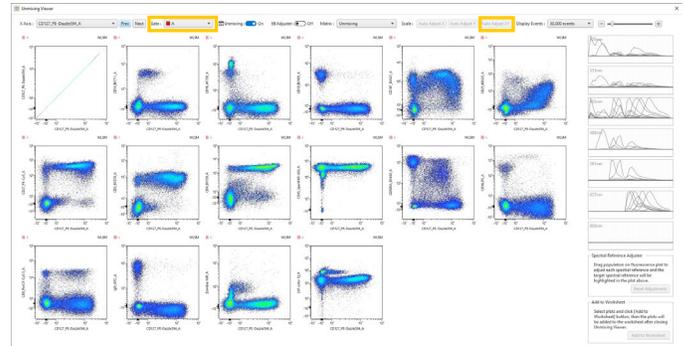# ID7000™: Using the Unmixing Viewer and Spectral Reference Adjuster
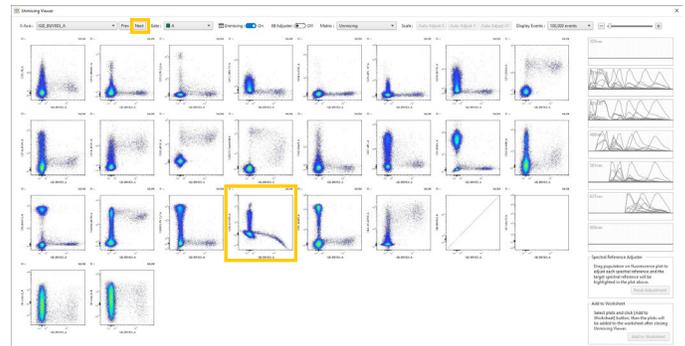
View and Rescale Plots

1. Select a well of a fully stained sample and make sure unmixing is applied and that autofluorescence unmixing is completed.

2. Select the **Unmixing Viewer** icon in the **Unmixing** tab to open the **Unmixing Viewer** window. Choose a gate, for example, a lineage gate, that you want to view. We do not recommend that you use all events.

3. If necessary, quickly rescale all plots by selecting the first plot, pressing **Ctrl + A** on the keyboard to select all plots, and then clicking **Auto Adjust XY**. Fine-tune the scaling for each plot as desired.

Compare the Fluorochrome Combinations

1. The display shows one color on the X-axis vs. every other color on the Y-axis.

2. Click **Next** to review the color combinations or select the desired fluorochrome from the menu.

3. Look for color combinations that show over unmixing or under unmixing patterns.

Adjust the Unmixing Matrix

1. Click the **Adjuster** toggle button. Diagonal lines appear on all the data plots to help delineate the positive populations on the respective axes.

2. Click near the population that you want to correct and drag in the direction that you want it to move. Changes made with the Adjuster will also be reflected in the reference spectra on the right.

3. The software takes a few seconds to make the adjustment, then redisplays the data.

4. When you are satisfied with the results, click **Next** to move on to the next color combination.

5. When you have viewed all the color combinations, and are satisfied with the results, click **Close** in the **Unmixing Viewer**.

6. The software prompts you to save your new matrix. Rename the matrix so you do not overwrite the original matrix.

7. You can now toggle between the original matrix and your adjusted matrix.