Protocol cFluor Reagents

Acquisition Protocol for cFluor™ 14 Color Immunoprofiling Kit

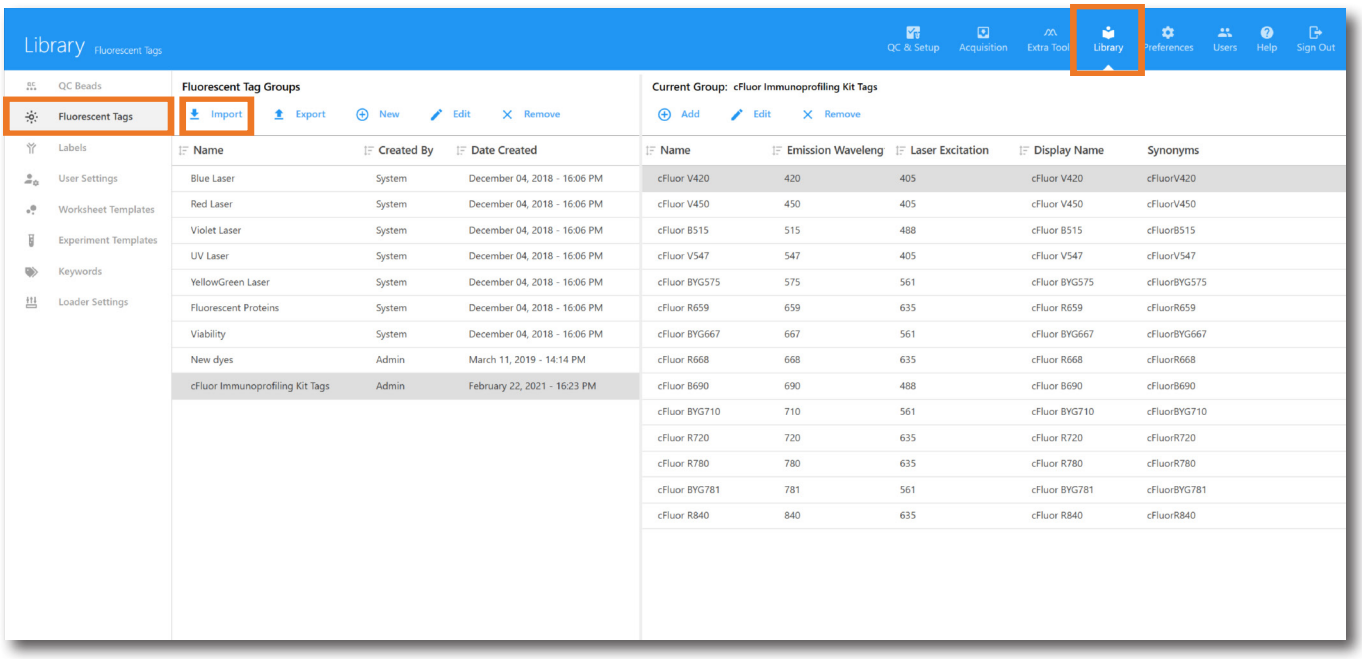
This acquisition protocol provides step-by-step instructions to set up your Cytek® Aurora or Northern Lights system (3-laser V-B-R configuration or higher) for data acquisition of the cFluor™ 14 Color Immunoprofiling Kit (R7-40000). This protocol provides instructions on: 1) preparing the SpectroFlo® software; 2) setting up the instrument; 3) acquiring controls and samples. This kit contains 25 tests.
NOTE: *For suggestions on how to prepare human PBMC or whole blood samples, download the protocol from the cFluor™ kit product page.*

Preparing the SpectroFlo® Software

Adding the 14 cFluor dyes to the library

NOTE: *Adding cFluor dyes to the library is only required for SpectroFlo® versions 2.2 and below.*

1. Download the cFluor fluorescent tag list CSV from the cFluor™ kit product page.
2. Import this CSV file into the **Library** module, under **Fluorescent Tags** to add in the 14 cFluor™ fluorochromes used in this kit.



NOTE: *If any cFluors were previously entered into the SpectroFlo® Library, a warning message will appear. Click* ***Overwrite*** *to overwrite the information in the library with the new fluorescent tag information.*

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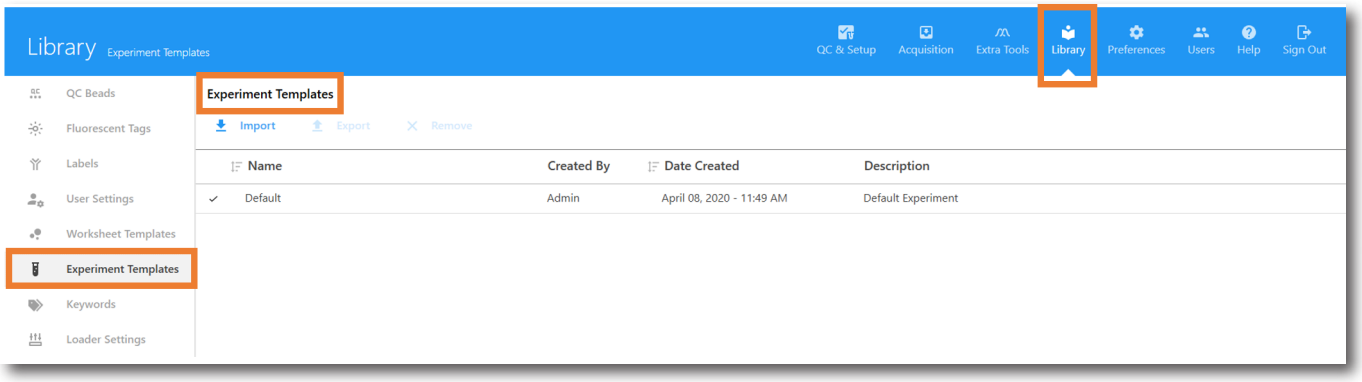
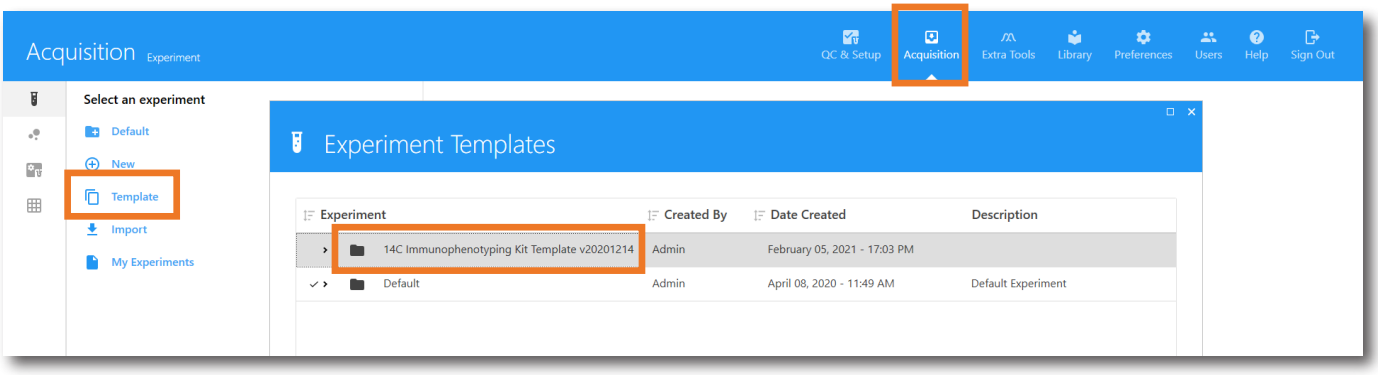
Import the experiment template

1. Download the cFluor Immunoprofiling Kit experiment template from the kit product page. This template includes a reference group with predefined stopping criteria, assigned marker names, as well as recommended acquisition and analysis worksheets.
2. Import the template into the **Library** module, under **Experiment Templates**.



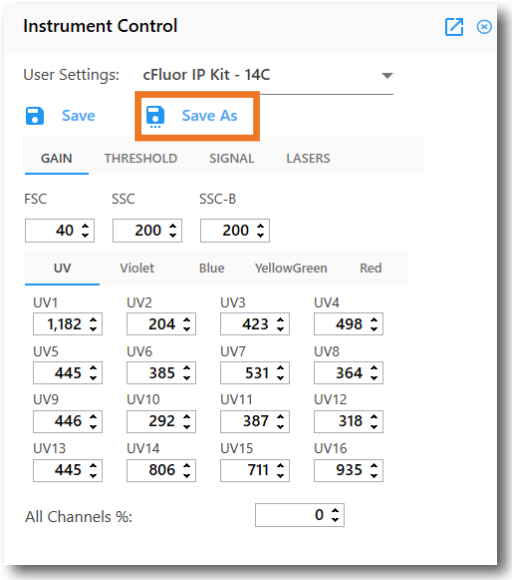
Setting up the Instrument

1. Follow the instructions for instrument startup and **Performing Daily QC** as outlined in the User's Guide.
2. From the **Acquisition** module, create a new experiment by clicking on **Template**, then choosing "14C Immunophenotyping Kit".



3. For best results, we recommend running cells for single color controls. If desired, control type can be changed to beads.
4. The sample tube is set to acquire 500,000 lymphocytes. This can be changed by clicking **Edit**, then changing the Stopping Gate and Stopping Criteria under **Acquisition**.
5. Add sample tubes and groups as needed. Marker labels will auto-populate for any tubes added to the existing sample group. To preserve these markers for additional groups, duplicate the existing group and rename the tube as necessary.
6. Preview unstained cell control at low flow rate to minimize wasted sample volume. Starting from the default CytekAssaySettings, optimize the FSC and SSC gains, as well as the FSC threshold (see Figure 1).

NOTE: *Instrument settings can be saved as "cFluor IP Kit - 14C" for future use by clicking the* ***Save As*** *button in the* ***Instrument Control*** *window.*



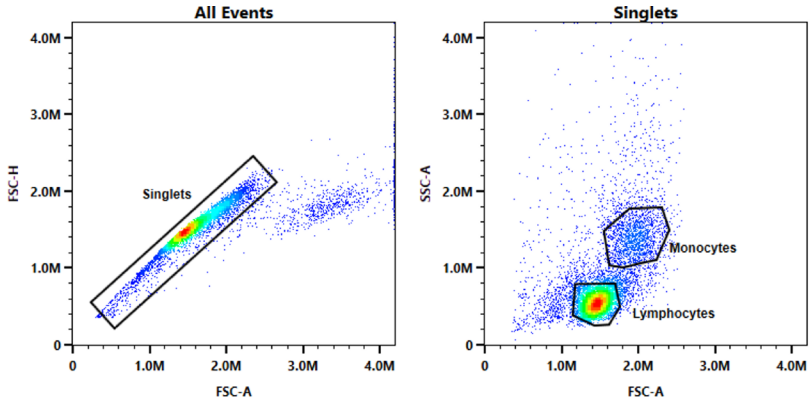
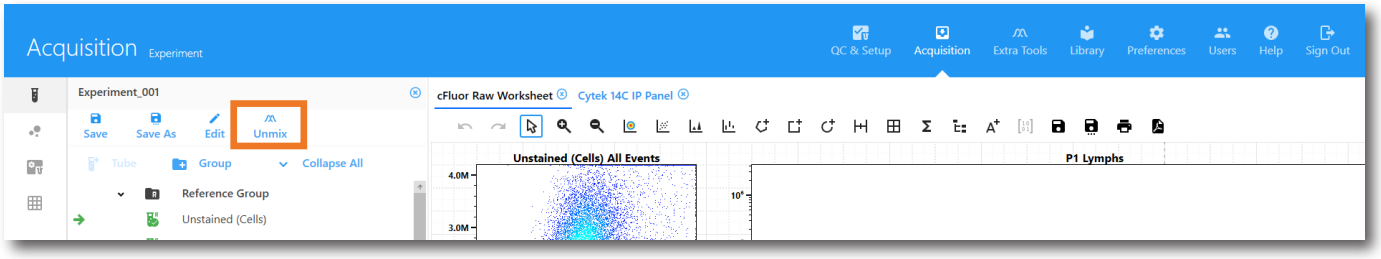


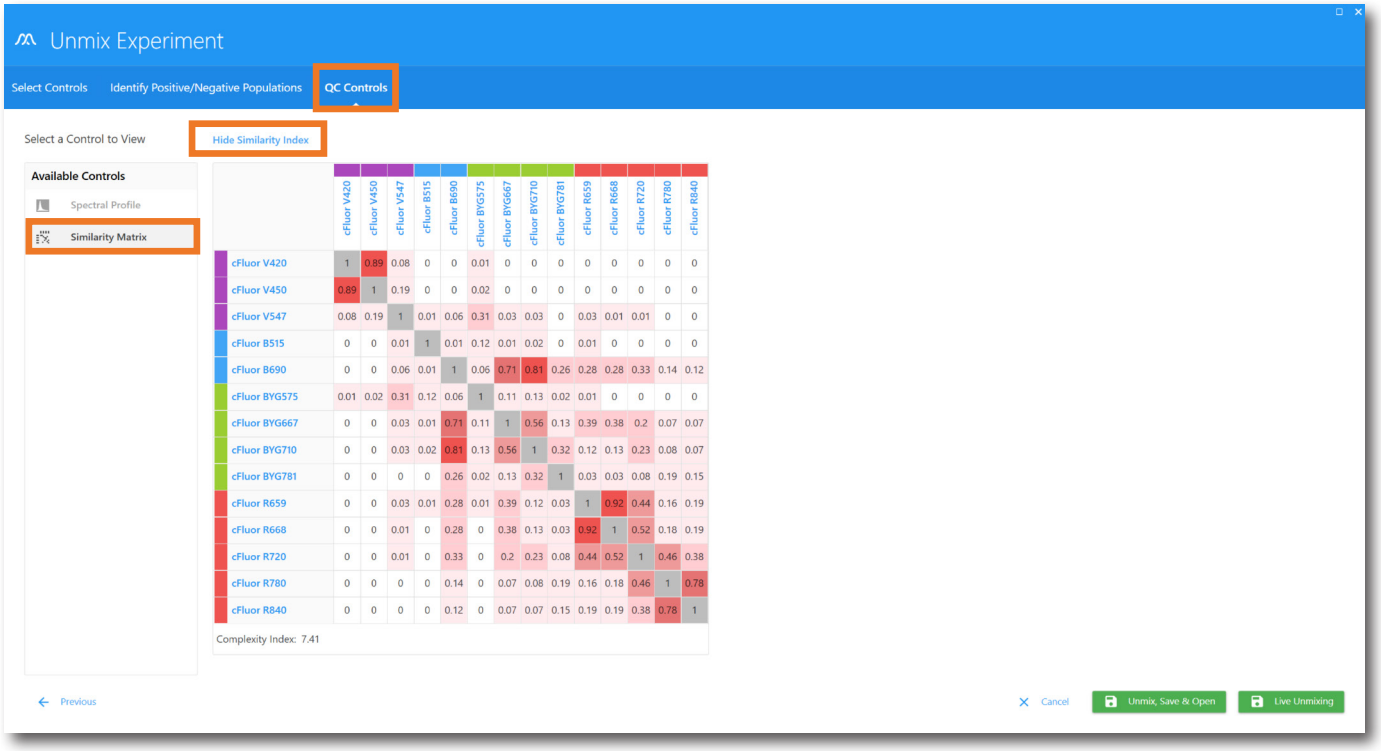
Figure 1: *Example cell resolution of human PBMC samples after FSC and SSC adjustment.*

Acquiring Controls and Samples

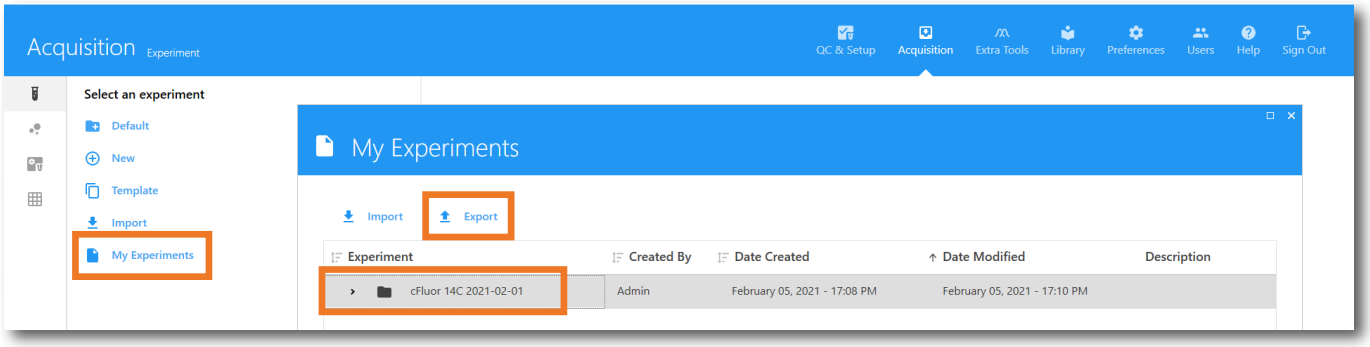
1. When running unstained and single color controls, acquire all tubes in the **Reference Control** group. Click **Unmix**. ***NOTE**: Refer to Appendix A for additional workflows when storing and reusing single color controls.*



2. For each single color control, gate on the appropriate population in the scatter plot and adjust the positive and negative gates in the histograms. Ensure that the signatures for the single color controls match the expected spectrum. Refer to Appendix B for the expected spectra of each cFluor and placement of positive and negative gates in the histograms.
3. Click **Next**. Under the **QC Controls** tab, click on **Similarity Matrix** to visualize how similar the 14 cFluors are in the panel. Click on **View Similarity Index** to compare to expected values found in Appendix C.



4. Click **Live Unmixing**.
5. Acquire all multicolor samples. Adjust the gates in the analysis worksheet as needed.
6. To export the FCS files for offline analysis: 1) close the experiment; 2) click **My Experiments**; 3) select the experiment and click **Export**; 4) choose a directory and click **Export**.



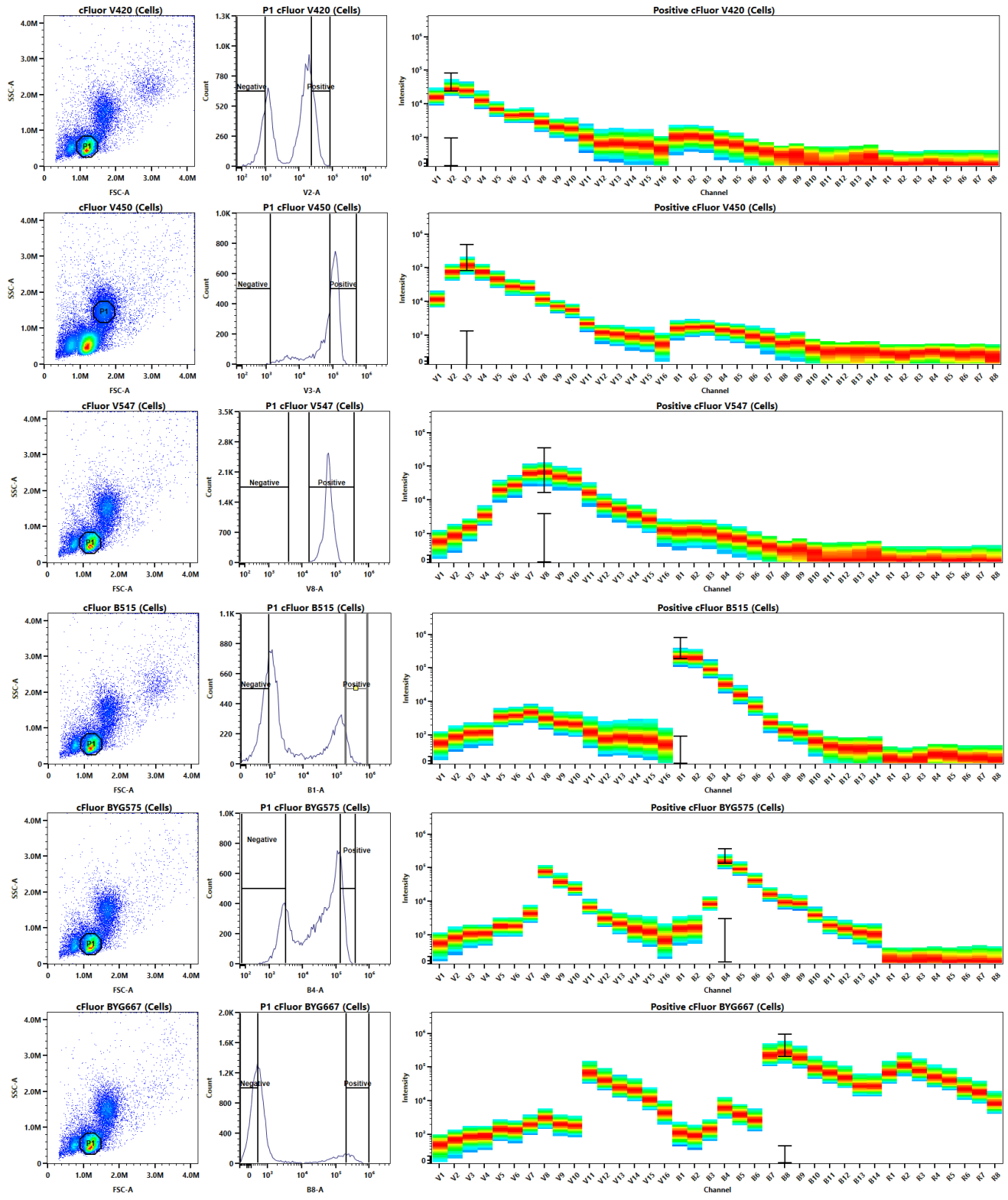
Appendix A: Reusing Single Color Controls

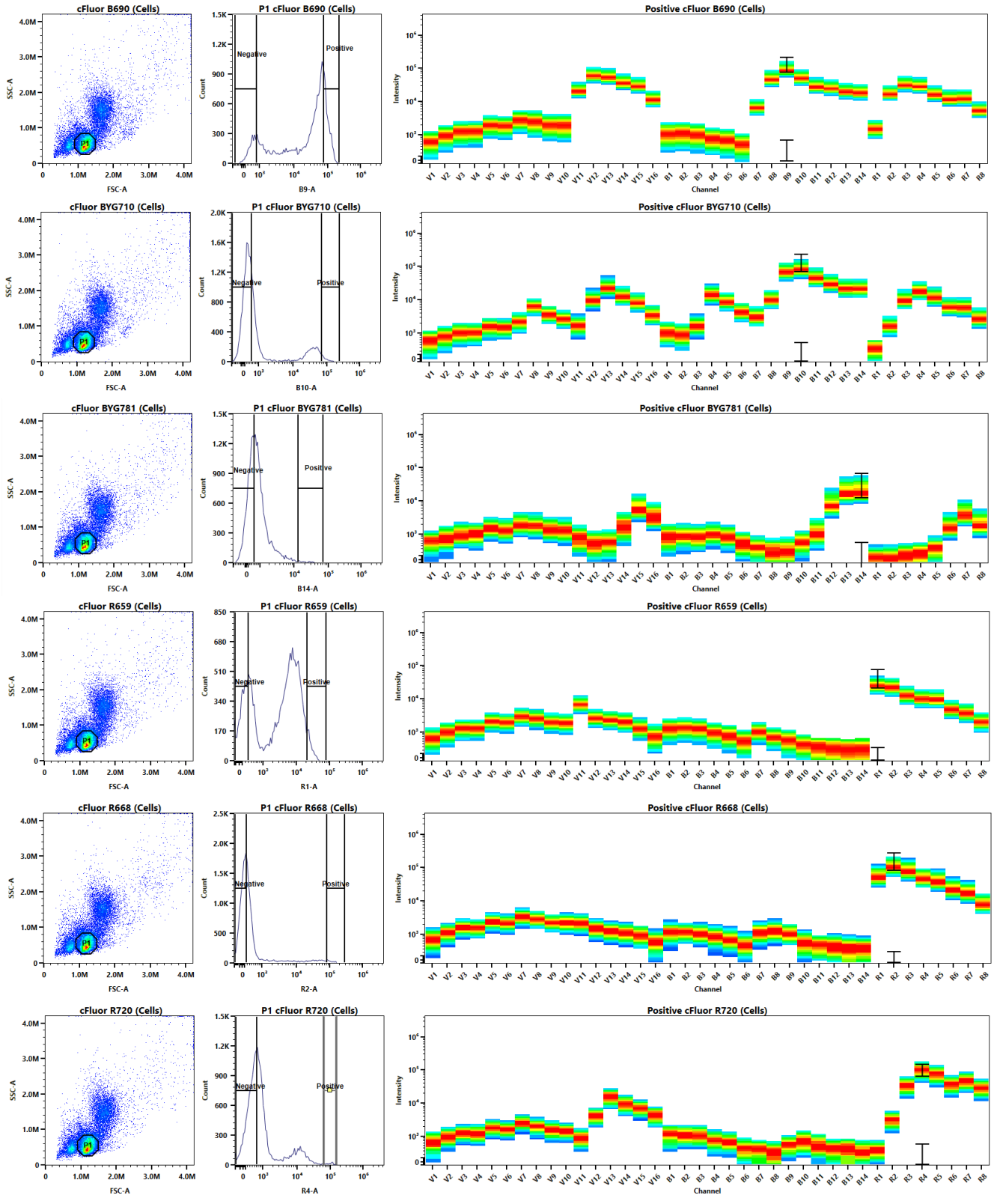
To store controls for reuse in future experiments, follow the instructions below.
***NOTE:** For best results, the instrument should be maintained properly, QC performed daily and the same lot of reagents used.*

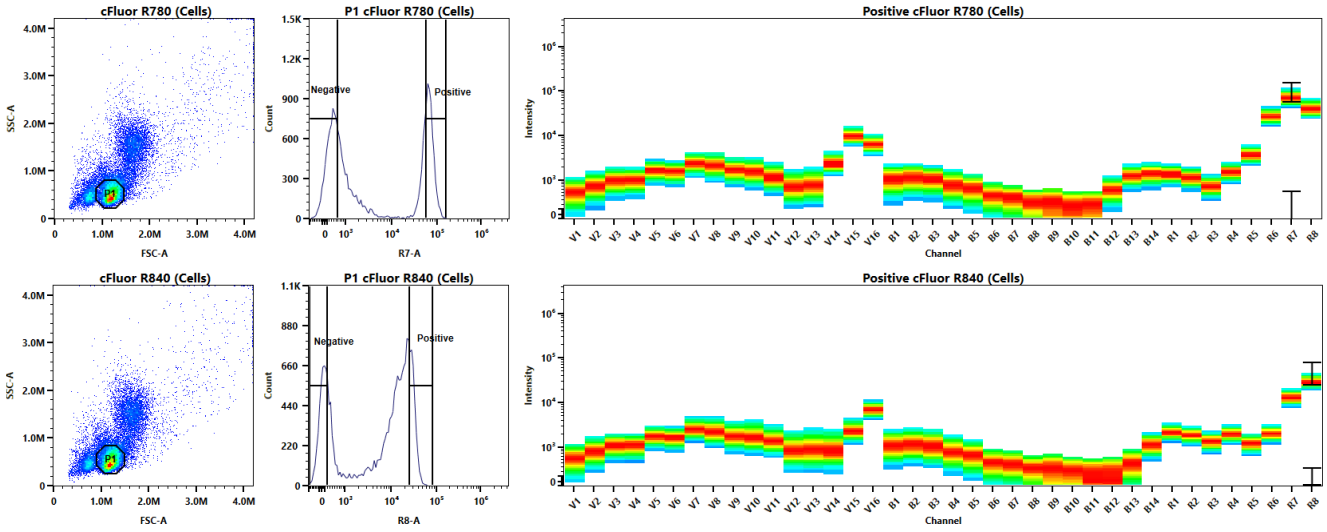
Reusing Single Color Controls From a Previous Experiment

1. From the **Acquisition** module, open **My Experiments**. Right click the saved experiment and select **Duplicate**. This will duplicate the data.
2. Keep all of the FCS files in the **Reference Control** group.
3. Add, delete, duplicate, and/or rename multicolor groups and samples as necessary.
   ***NOTE**: Marker labels may need to be added for new sample groups and tubes.*
4. Acquire all multicolor samples. Adjust the gates in the analysis worksheet as needed. Unmixing will carry over from the previous experiment when references were initially run.
5. To export the FCS files for analysis at a different workstation: 1) close the experiment; 2) click **My Experiments**; 3) select the experiment and click **Export**; 4) choose a directory and click **Export**.

Appendix B: Single Color Control Signatures for 3-Laser (V-B-R) Cytek Aurora and Northern Lights and Suggested Gating When Using Cells as Controls




cFluor B690 (Cells)
P1 cFluor B690 (Cells)
Positive cFluor B690 (Cells)
cFluor BYG710 (Cells)
P1 cFluor BYG710 (Cells)
Positive cFluor BYG710 (Cells)
cFluor BYG781 (Cells)
P1 cFluor BYG781 (Cells)
Positive cFluor BYG781 (Cells)
cFluor R659 (Cells)
P1 cFluor R659 (Cells)
Positive cFluor R659 (Cells)
cFluor R668 (Cells)
P1 cFluor R668 (Cells)
Positive cFluor R668 (Cells)
cFluor R720 (Cells)
P1 cFluor R720 (Cells)
Positive cFluor R720 (Cells)
SSC-A
FSC-A
Count
Negative
Positive
Intensity
Channel
P1



NOTE: *For single color control signatures for other Cytek Aurora and Northern Lights configurations please refer to the fluorochrome guidelines on the Resources Page.*

NOTE: *For cFluor V450 and cFluor V547, the Experiment Template has been formatted to assign the Unstained Cells as the Negative Control, since no negative populations are present when gating on monocytes (CD14 cFluor V450) and lymphocytes (CD45 cFluor V547) for these two controls.*

Appendix C: Example Similarity Matrix for cFluor Immunoprofiling Kit

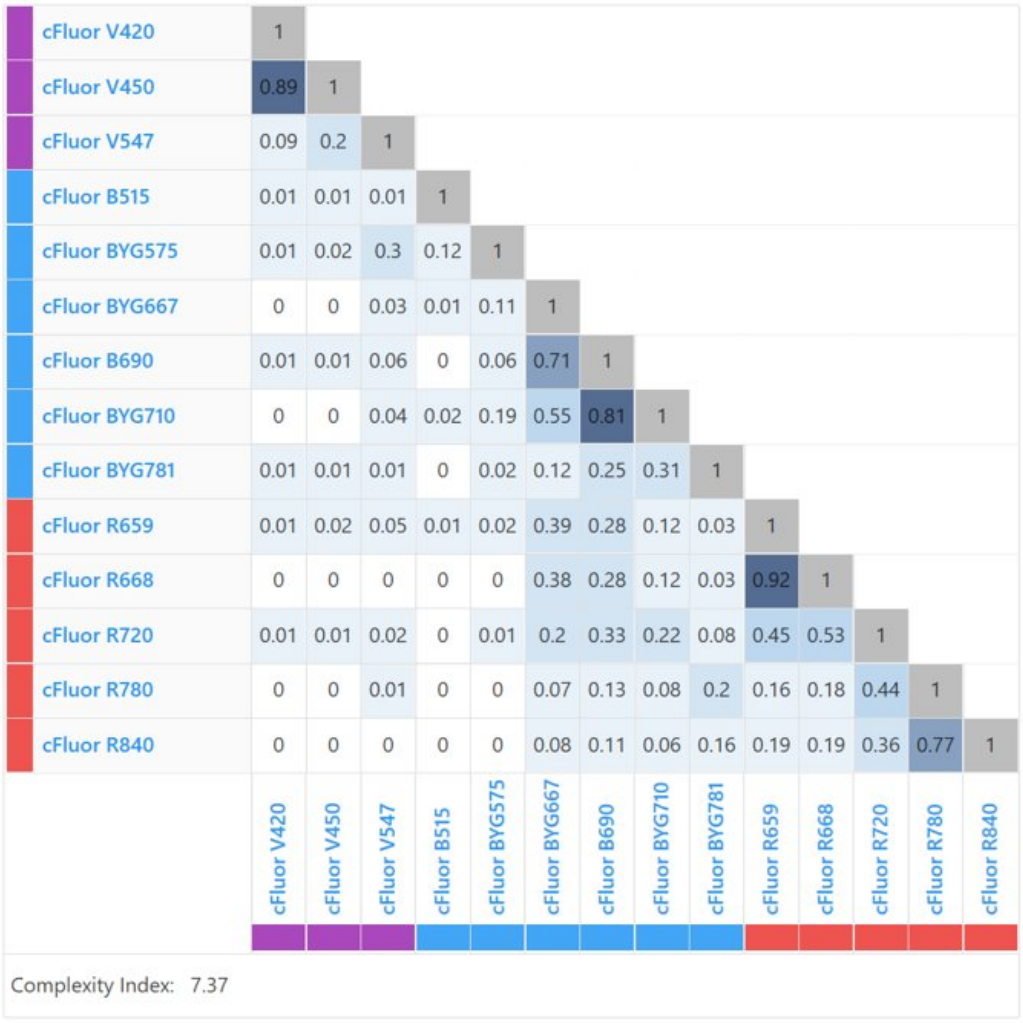


Figure 2: *Expected Similarity™ matrix and Complexity™ index generated on a 3-laser (V-B-R) Cytek® Aurora or Northern Lights. Generating similar values is a good indication that signatures of your single-color controls match those generated by Cytek.*

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