Sony SA3800 Spectral Cell Analyzer setup guide for LEGENDplex™ Panels:

Before Starting the Assay:

- For LEGENDplex™ analysis, the cytometer should be equipped with 488nm (blue) and/or 561nm (Yellow-Green) lasers, for PE detection, and 638 nm (red) laser, for APC detection.
- When running LEGENDplex™ bead-based immunoassays, the PE channel is used for reporter and the APC channel is used for beads classification.
- The Raw Beads, FITC Beads, and PE Setup Beads are not required for this instrument setup procedure.

This part of the guide applies to **Sony SA3800 Spectral Analyzer** and its software. This setup procedure is required if you are running LEGENDplex™ kit for the first time.

This setup procedure is not needed if you have run this experiment before or have access to a saved template (can be downloaded from our website biolegend.com). The settings can be saved in the final step of this setup procedure and any settings saved can be imported to a new experiment.

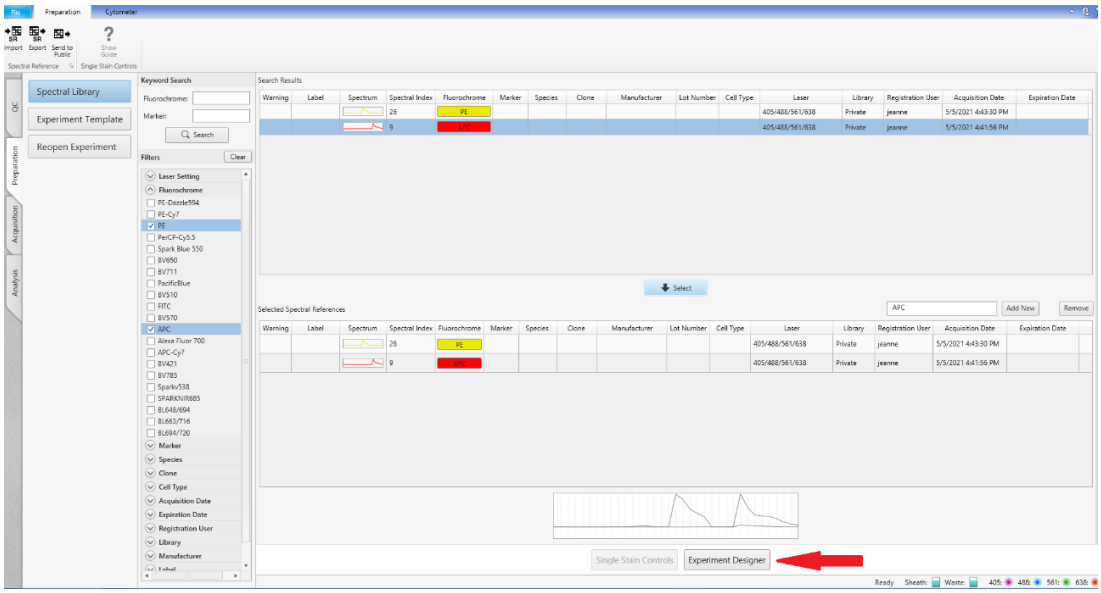
1. **Instrument Setup**
   1.1. Open Sony SA Software application and start up the SA3800 instrument. Allow the instrument to go through set up process and run daily and performance QC, if necessary, following the manufacturer's instructions.

Creating an Experiment
Create an experiment used to acquire measurement data.

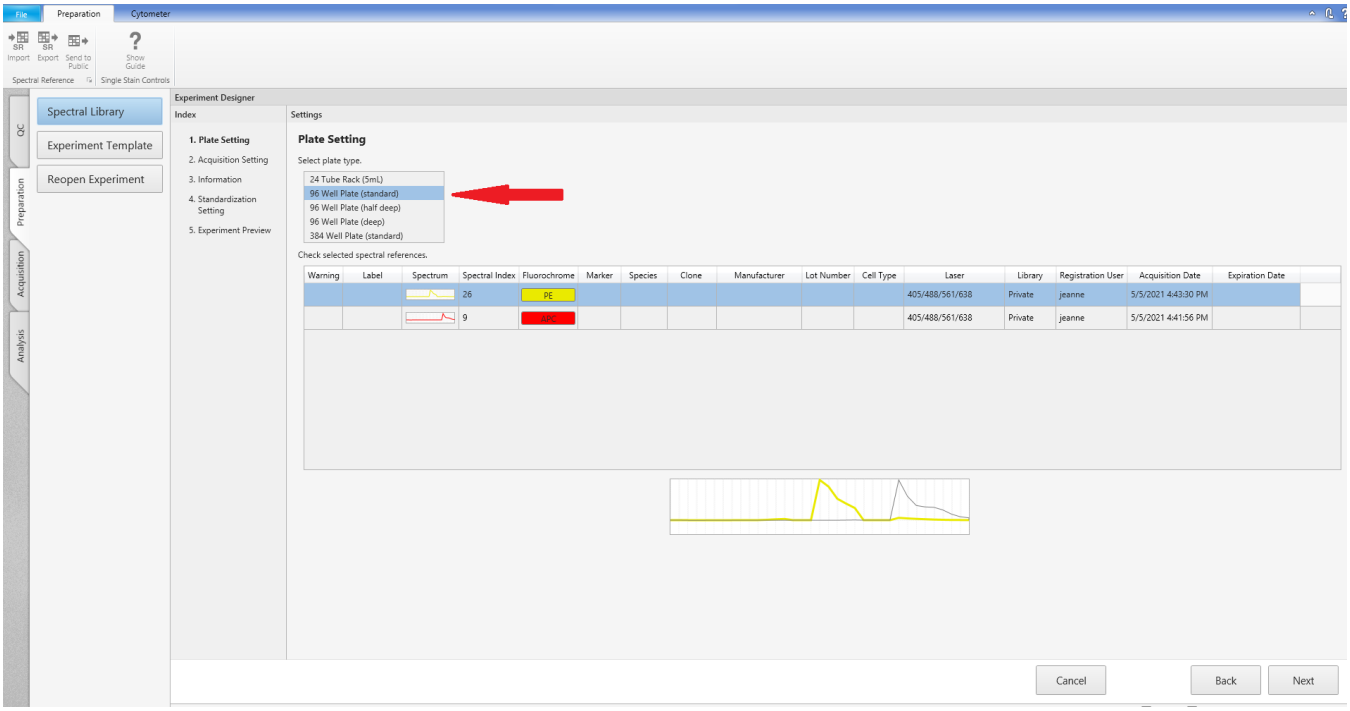
Creating a New Experiment using the Wizard:
This section describes how to create a new experiment using the [Experiment Designer] wizard in the [Spectral Library] window.

2. **Registering a Spectral Reference**

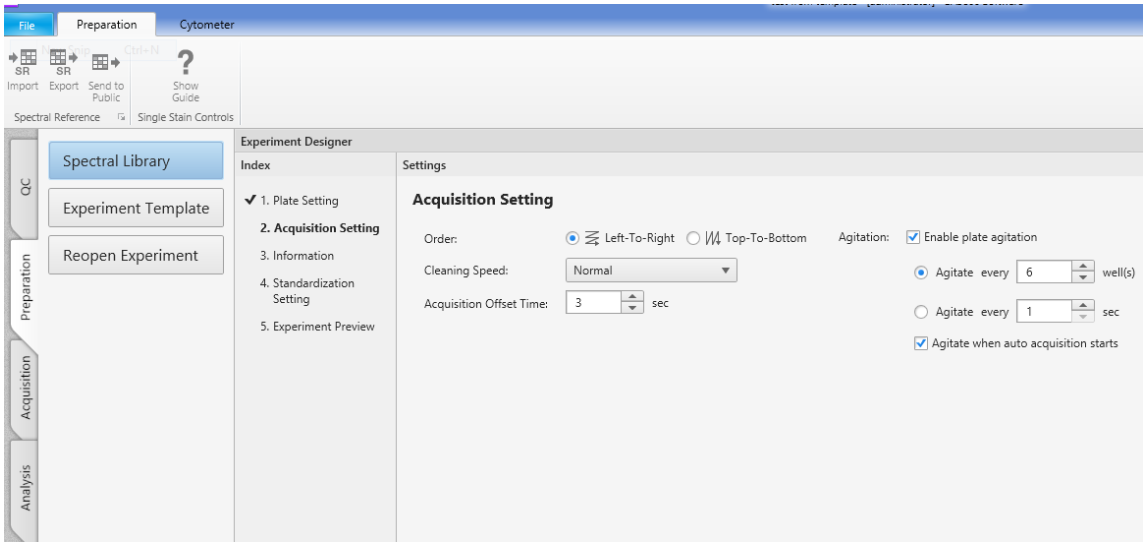
   2.1. Click [Spectral Library] on the [Preparation] tab. The [Spectral Library] window appears.
   2.2. Specify keywords and filter criteria to search for a spectral reference.
   2.3. Select a spectral reference in the [Search Results] list. You can select more than one spectral reference by pressing and hold the Shift key or Ctrl key and clicking other spectral references.
   2.4. Select PE and APC from your spectral library. The selected spectral reference is added to the list in [Selected Spectral References]. You can check the spectral waveform by selecting the row for a spectral reference. If you do not have PE and APC in the library, then follow recommendations of the manufacturer to add PE and APC to your library. You can reach out SONY Biotechnology at SBTcustomerservice@sony.com.
   2.5. Click on [Experiment Designer].



3. **Configure the plate setting:**
   In the [1. Plate Setting] step select the plate type: [96 Well Plate (Standard)] is recommended, then click [Next]:



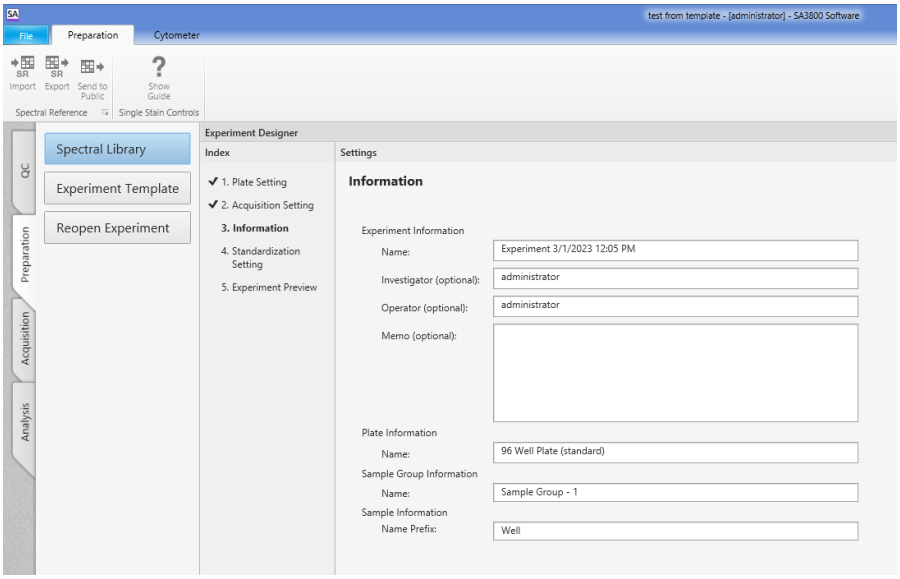
4. **Configure the measurement conditions:**



In the [2. Acquisition Setting] step select the following parameters, and click [Next]:

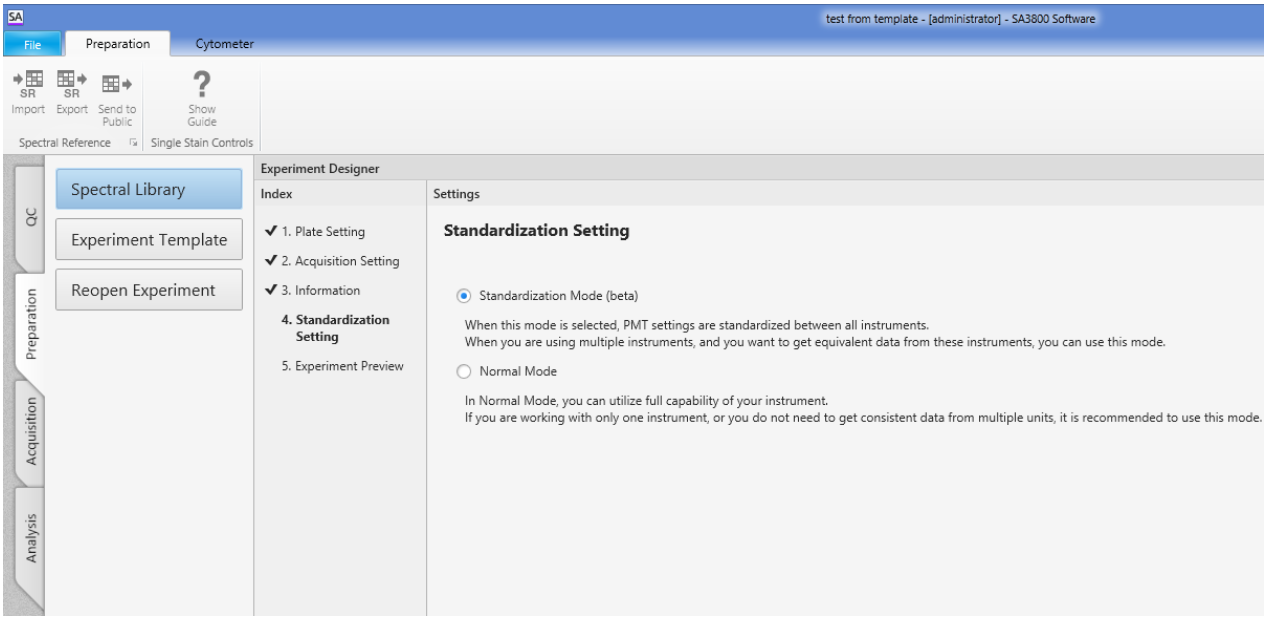
[Order]: Select the well measurement sequence (Top-to-Bottom is recommended)
[Cleaning Speed]: Select the operating speed used during auto acquisition. The operating speed will vary depending on the cleaning method used between measurement of each well ([Normal] is recommended).
[Acquisition Offset Time]: Set the offset time between the start of data acquisition of each well in units of seconds.
[Agitation]: Set agitation settings. Place a check mark in [Enable sample agitation] to enable agitation, and set the agitation duration. (Every [6 wells] is recommended). To enable mixing when auto acquisition starts, place a check mark in [Agitate when auto acquisition starts].
NOTE: These are all pre-set in the user preferences, you could eliminate the details of each setting if you want.

5. **Enter the metadata for the experiment and samples in the [3. Information] step, then click [Next]:**

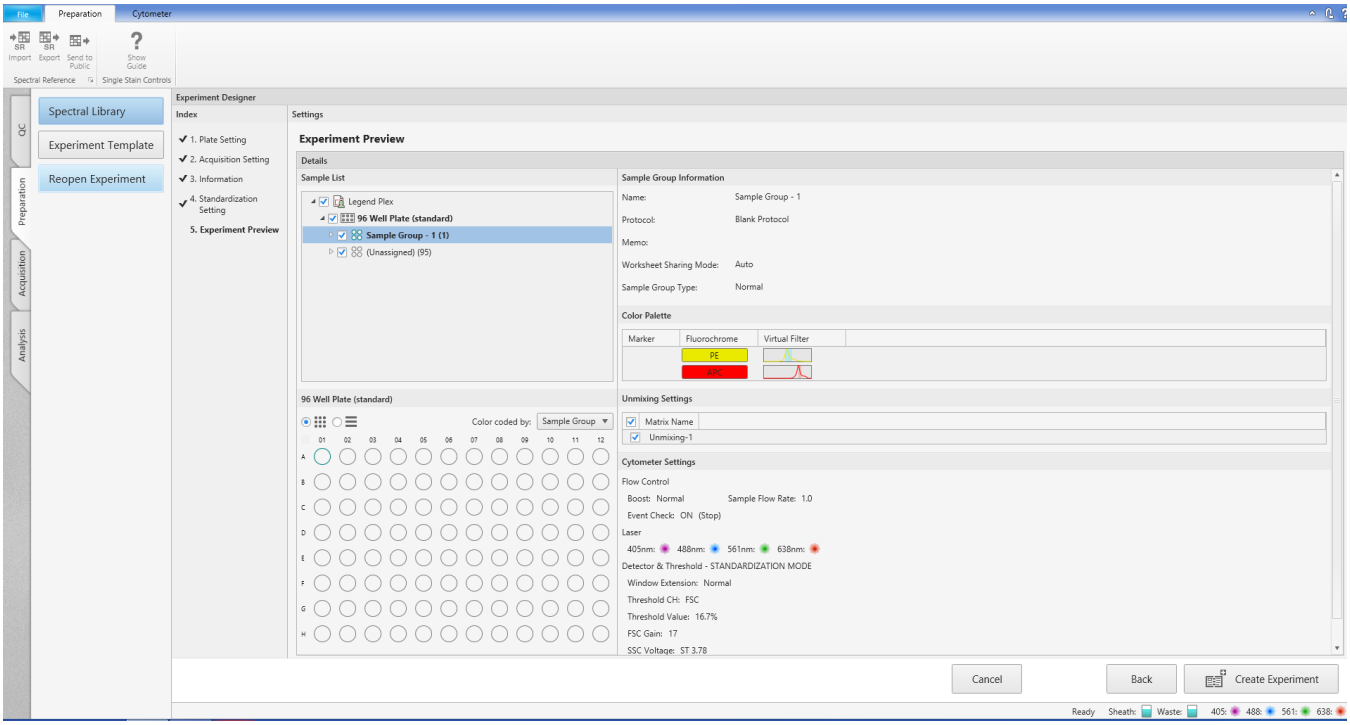


6. Select the instrument settings mode in the [4. Standardization Setting] step, then click [Next].

Select [Standardization Mode (beta)].



7. Check the experiment settings in the [5. Experiment Preview] step, then click [Next]:

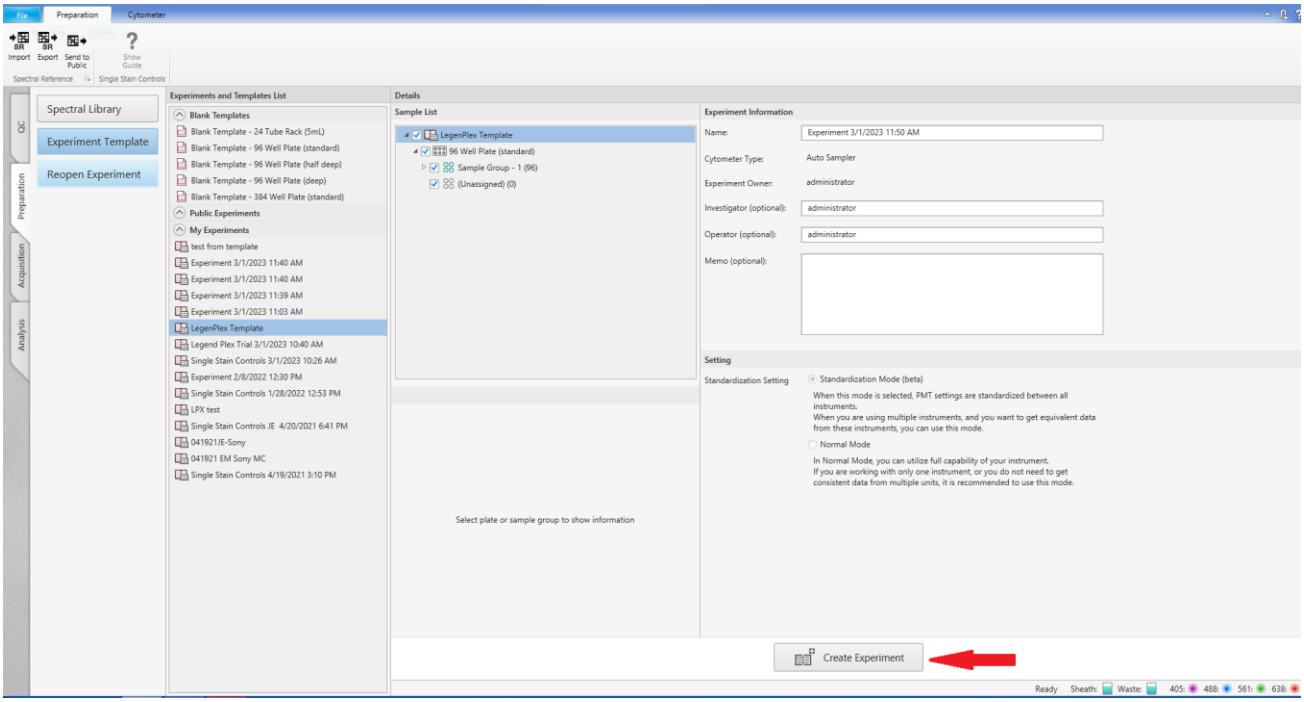


The experiment is created, and the [Acquisition] tab is displayed.

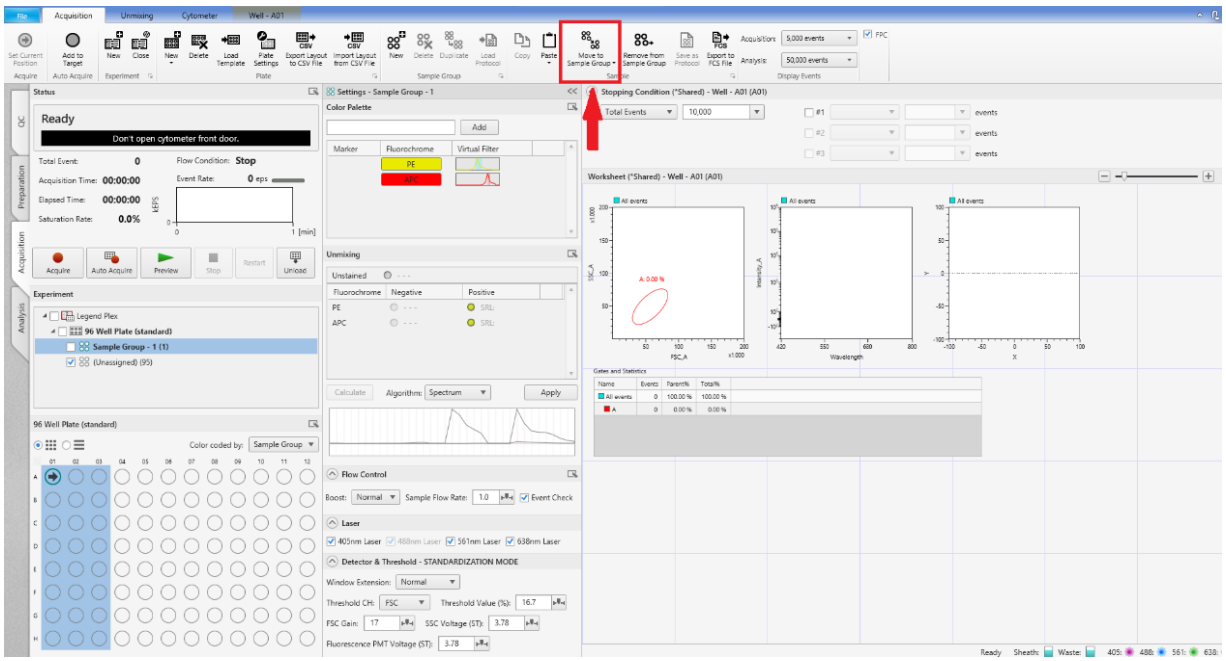
Creating a New Experiment using a Template:

This section describes how to create a new experiment using existing templates and/or recent experiments.

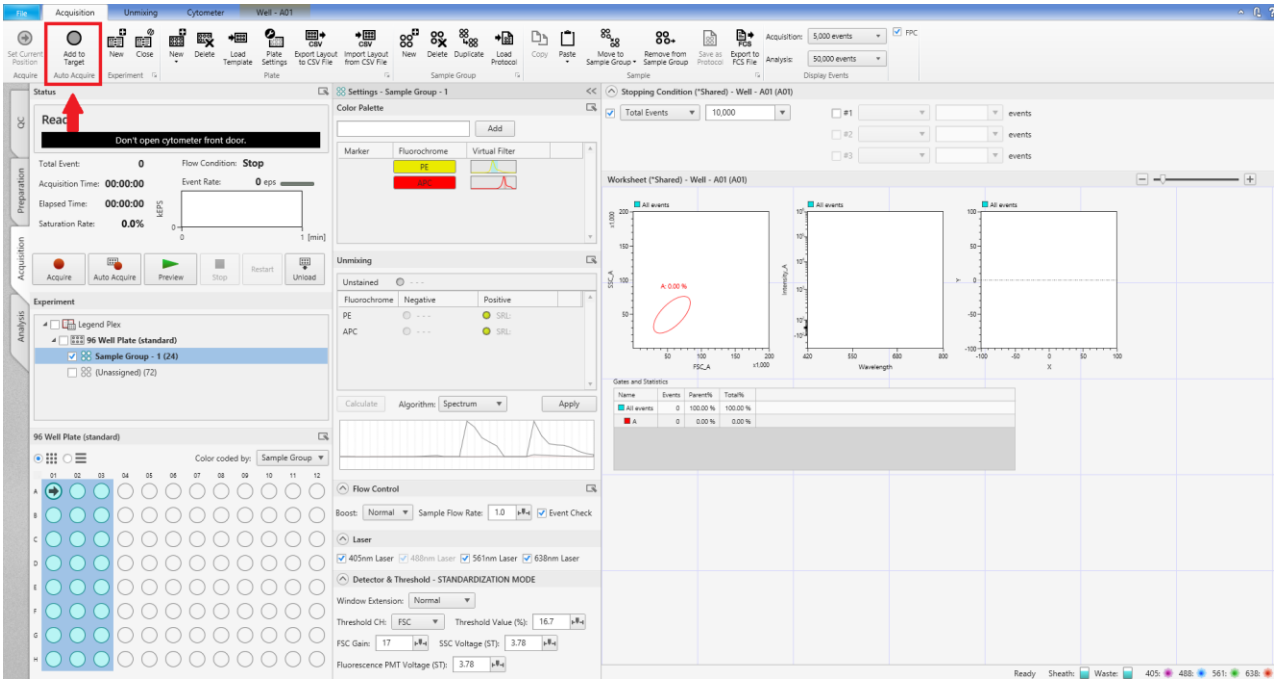
1. Click [Experiment Template] on the [Preparation] tab. The [Experiment Template] window appears. Click [Create Experiment] button.



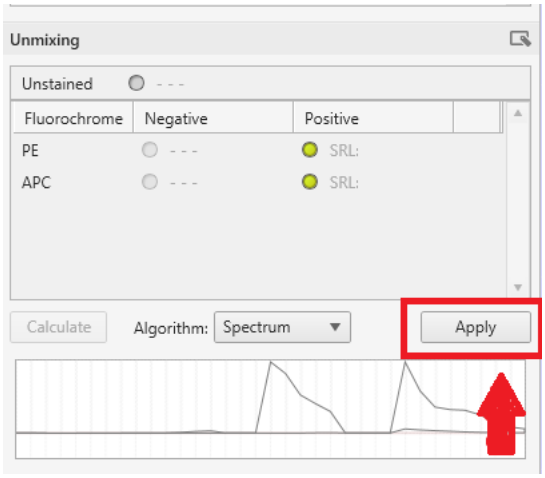
2. Once an experiment is created, a sample group should be selected in the [Sample List] panel.
3. Select the rows used in the experiment on the plate layout. Click on [Move to Sample Group] in the ribbon of the software. Click on [Sample Group – 1].



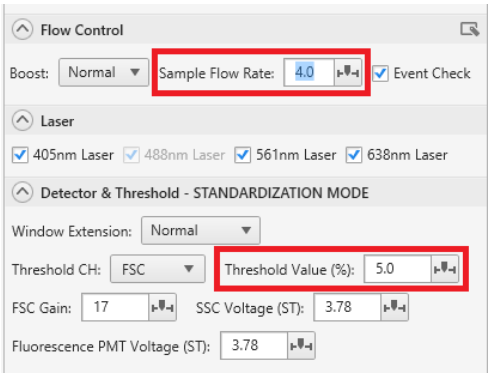
4. Click on the [Add to Target] button on the ribbon of the software.



5. Click [Apply] button in the [Unmixing window].



6. Change the Threshold Value to 5%.
7. Select the sample flow rate (the recommended is 4.0.)
8. The rest of settings in the [Detector and Threshold] section may remain default.

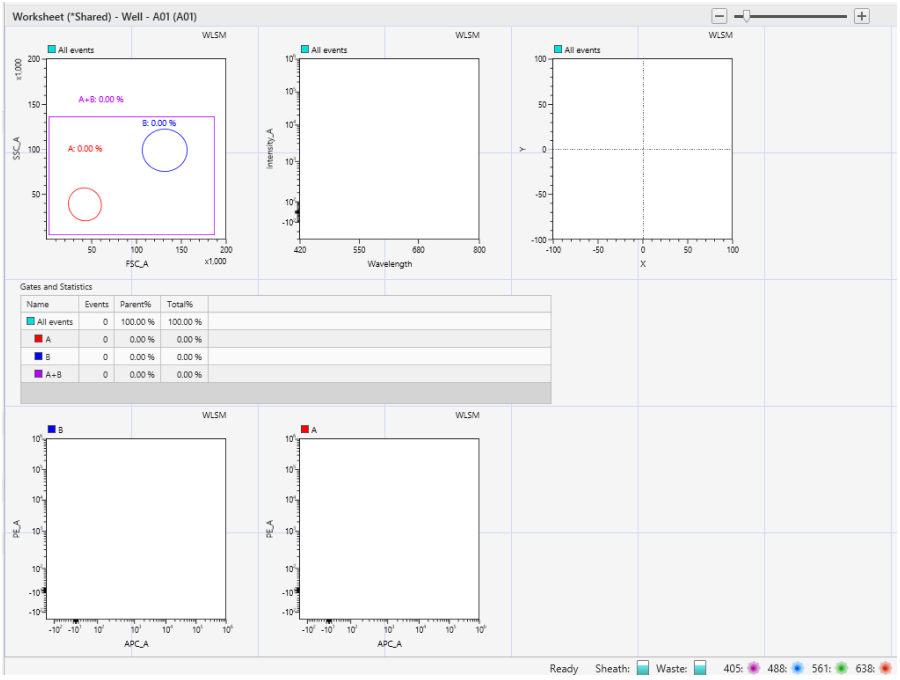


Set up the worksheet:

In the [Worksheet] section you have three plots: Scatter Plot, Spectral Plot, and XY Plot.

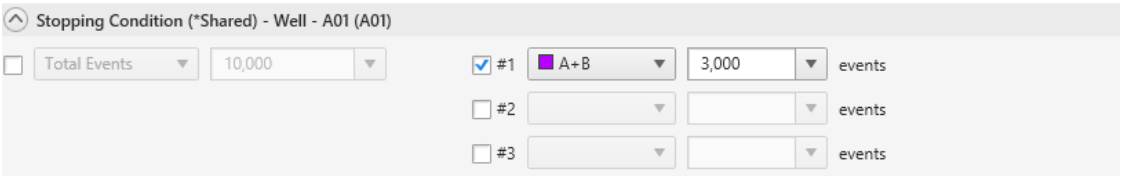
The Scatter Plot has already A gate for A beads. Create another gate for B beads.

Draw a large gate including both A and B beads. Name this gate A + B as shown below:



Set up Stopping Conditions:

1. Uncheck [Total Events]
2. Check #1, and select [A+B], and type in the number of events. Set events to record as 300 events per analyte (e.g., 2400 events for an 8-plex assay) in gate A+B (do not use total events) so that any events that fall out of gate A+B will not be recorded.



Acquisition Step:

1. Put your plate to the plate loader and click [Load] button.
2. Click [Auto Acquire] button to start running the plate.

3. Once the acquisition is done, the machine will stop automatically.

Data Exporting:

1. Click on your [Sample Group].
2. Click [Export to FCS Files] on the ribbon of the software.
3. Select [Export Format] FCS file only.
4. Uncheck [Height] and [Width].
5. Choose the location for saving and press [Export] button.