Transferring application-specific target values from the BD FACSCanto™ II Flow Cytometer to the BD FACSLyric™ Flow Cytometer

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The ability to transfer application-specific target values from one flow cytometer to another is an important tool that can be employed to enable standardization within a laboratory and across the globe. In 2012, we published a Technical Bulletin titled "Standardizing Application Setup Across Multiple Flow Cytometers Using BD FACSDiva™ Version 6 Software."

Here, we expand upon that technical bulletin to include the transfer of target values from the BD FACSCanto™ II Flow Cytometer to the BD FACSLyric™ Flow Cytometer. The procedure in this document uses BD® FC Beads to collect the target values from the BD FACSCanto™ II Flow Cytometer and establish the target values on the BD FACSLyric™ Flow Cytometer.

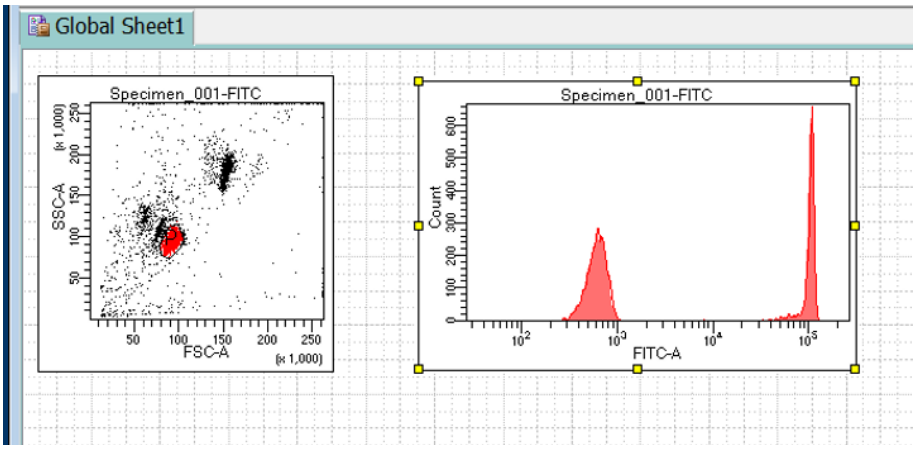
Materials:

- BD FACSCanto™ II Flow Cytometer
- BD FACSLyric™ Flow Cytometer
- BD FACSDiva™ CS&T Beads
- BD® CS&T IVD Beads or BD™ CS&T RUO beads, as appropriate
- BD® FC Beads
- BD™ FC Bead Dilution Buffer
- BD® CompBeads Particles (optional)

Acquiring target values on the BD FACSCanto™ II Flow Cytometer

1. Begin by starting up your BD FACSCanto™ II Flow Cytometer and running daily QC, following the instructions in "Instructions for Use."
2. Create an experiment and apply the settings intended to be transferred.
    a. Disable compensation if needed.
3. Within that experiment, create a FSC vs SSC plot to view the bead population.
4. Draw a polygon gate on the scatter plot to encompass the bead population.

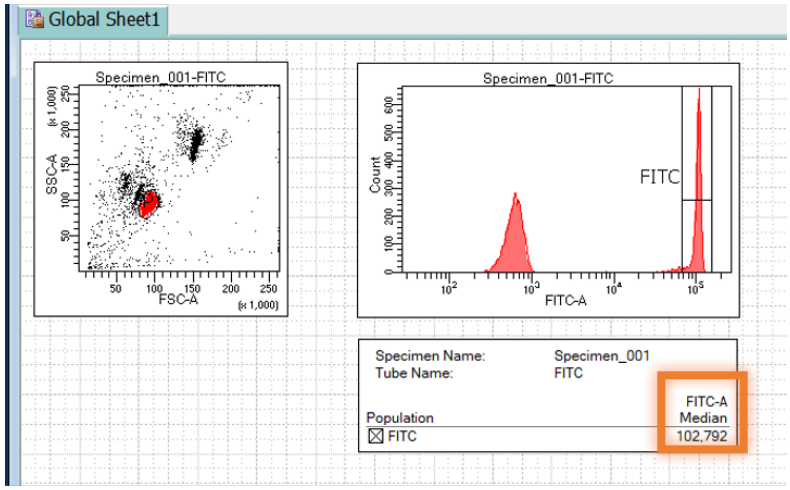
5. Create a histogram for each fluorescence parameter to be measured gated on the bead population.



6. Prepare BD® FC Beads for each fluorochrome you intend to transfer following the "Instructions for Use."
   a. Remember that BD® FC Beads are light sensitive and should be placed in the dark when not in use.

NOTE: If the desired fluorochrome is not available on BD® FC Beads, stained BD® CompBeads Particle can be used. If the dye that you are staining with can only be viewed on cells (i.e., propidium iodide, GFP), then cells may be used to collect and transfer the target value.

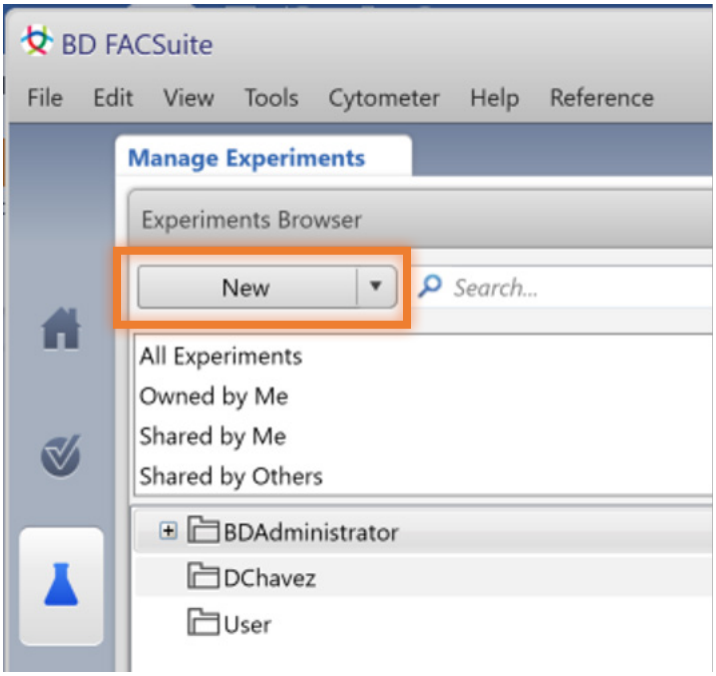
7. Acquire each BD® FC Bead, or alternative control sample, on a Medium flow rate, acquiring at least 10,000 gated events.
   a. Repeat for all fluorochromes to be transferred.
   b. Adjust FSC, SSC and threshold as needed to bring the beads on scale.
8. On each histogram, draw an interval gate around the bright bead population.
9. Create a statistics view displaying the median value for each interval gate drawn



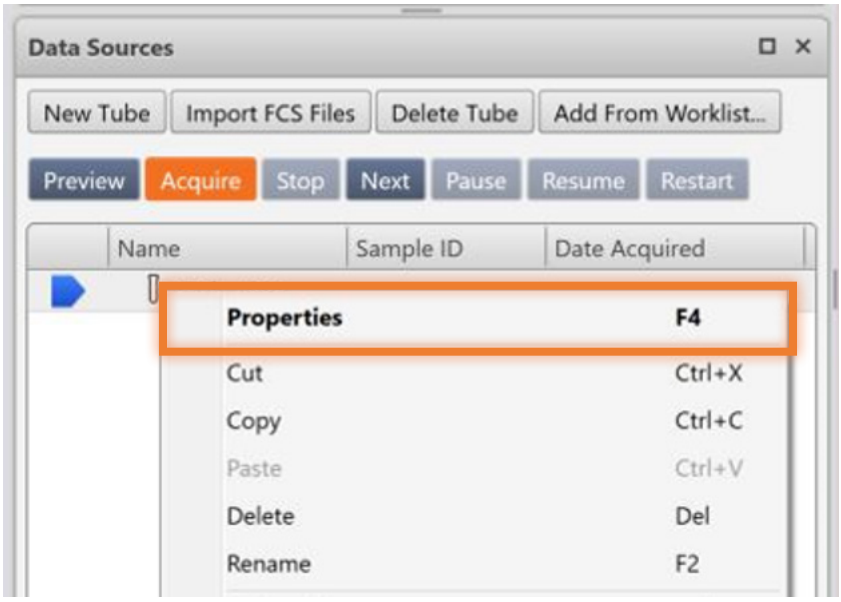
These median values will be your target values for each fluorochrome when transferring to the BD FACSLyric™ Flow Cytometer.

Transferring target values to the BD FACSLyric™ Flow Cytometer

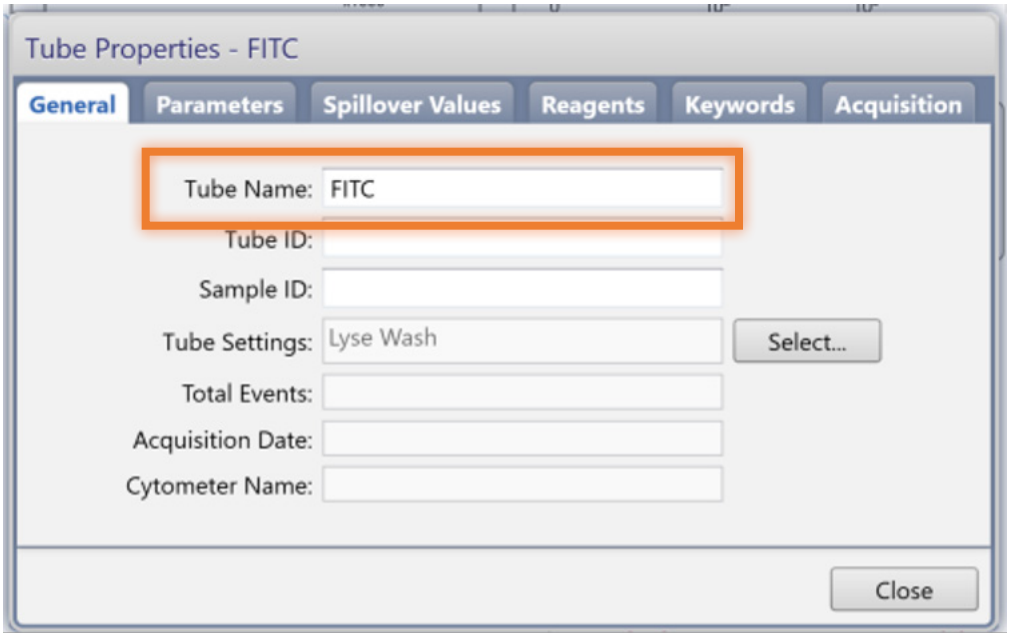
1. Start up your BD FACSLyric™ Flow Cytometer and run daily QC, following the instructions in the "Instructions for Use."
2. Create a new experiment.



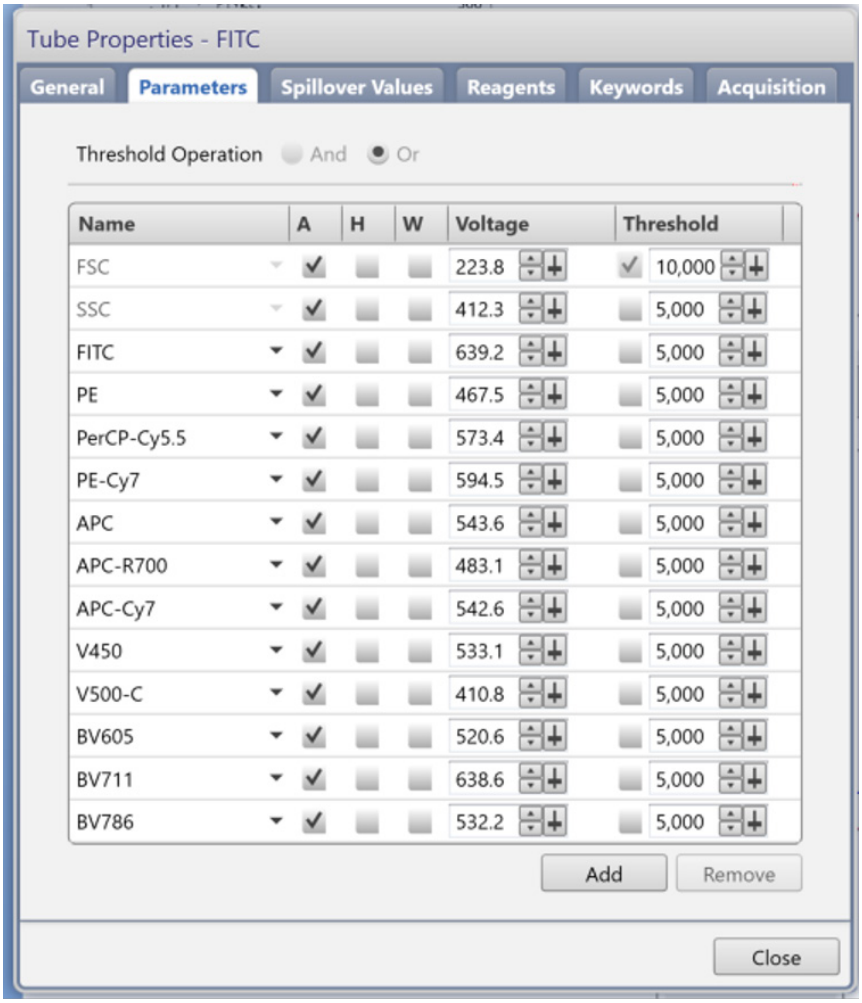
3. Select the tube in the experiment, right click to open the menu, then click on **Properties**.



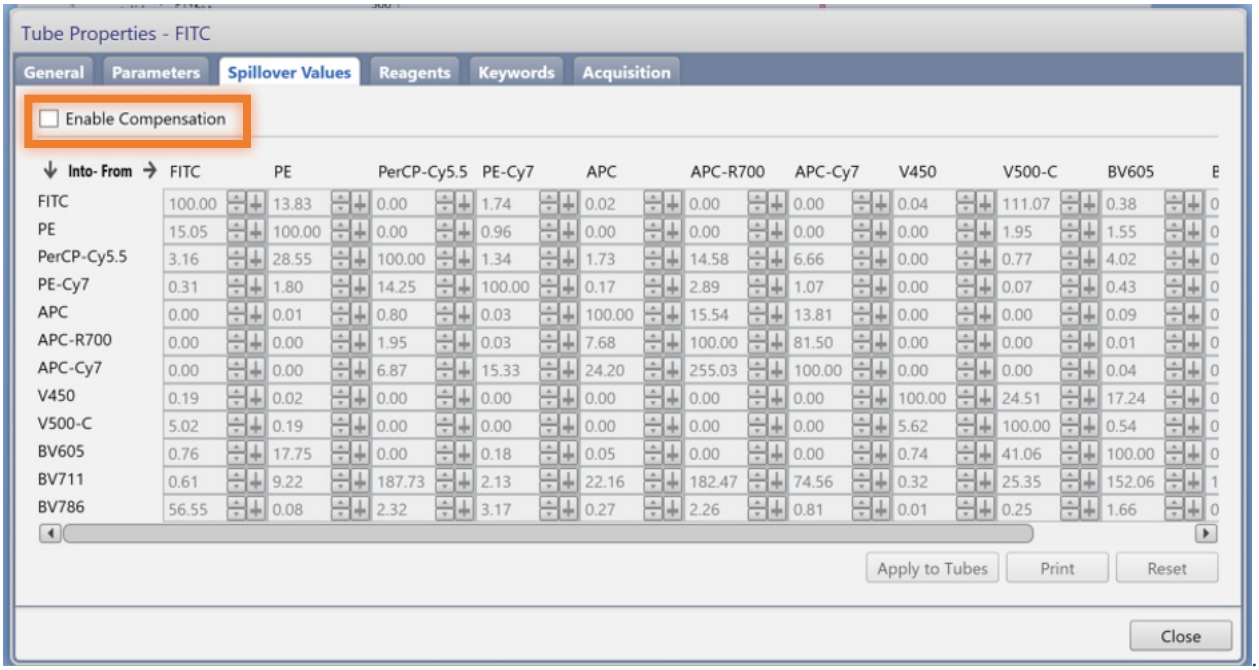
4. On the **General** tab, rename the tube to match the name of the fluorochrome.



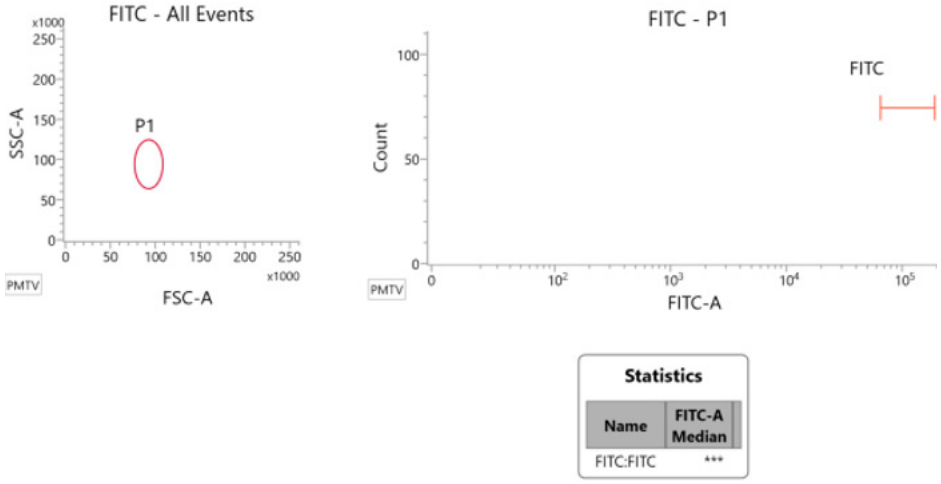
5. In the **Parameters** tab, select the parameters you will be using, if different than the default.



6. On the **Spillover Values** tab, uncheck **Enable Compensation.**

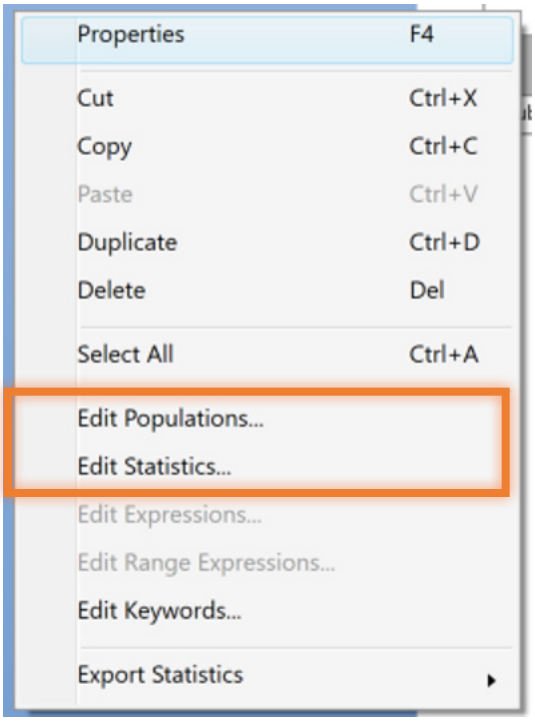


7. Use an ellipse gate to encompass the bead population in the scatter plot. Create a histogram for each fluorescent parameter, adjust the histogram to display events from the ellipse gate, then draw an interval marker on each plot where you anticipate the bright bead population to appear. The gate can be adjusted later, if needed.



8. You will create one statistics box for each histogram as you acquire tubes. First, create a multitube statistics box.

9. Right click on the statistics box, then choose **Edit Populations** and choose the interval gate to match the plot. Right click on the statistic plot, then choose **Edit Statistics** to display the median value for that gate.

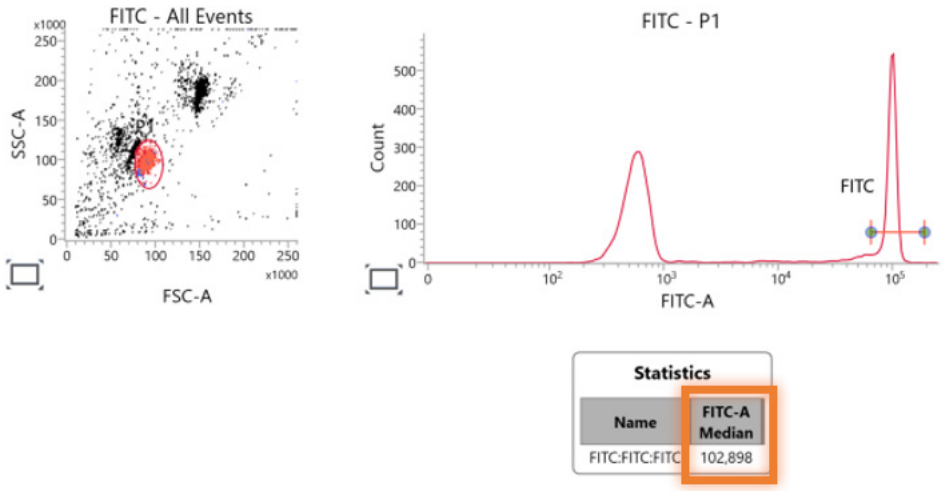


10. Load the matching BD® FC Beads on the manual tube port. Begin Previewing with the flow rate set to Medium.

11. Adjust the PMT voltage (PMTV) until the bright bead median value matches the corresponding bright bead median target value from the BD FACSCanto™ II Flow Cytometer.

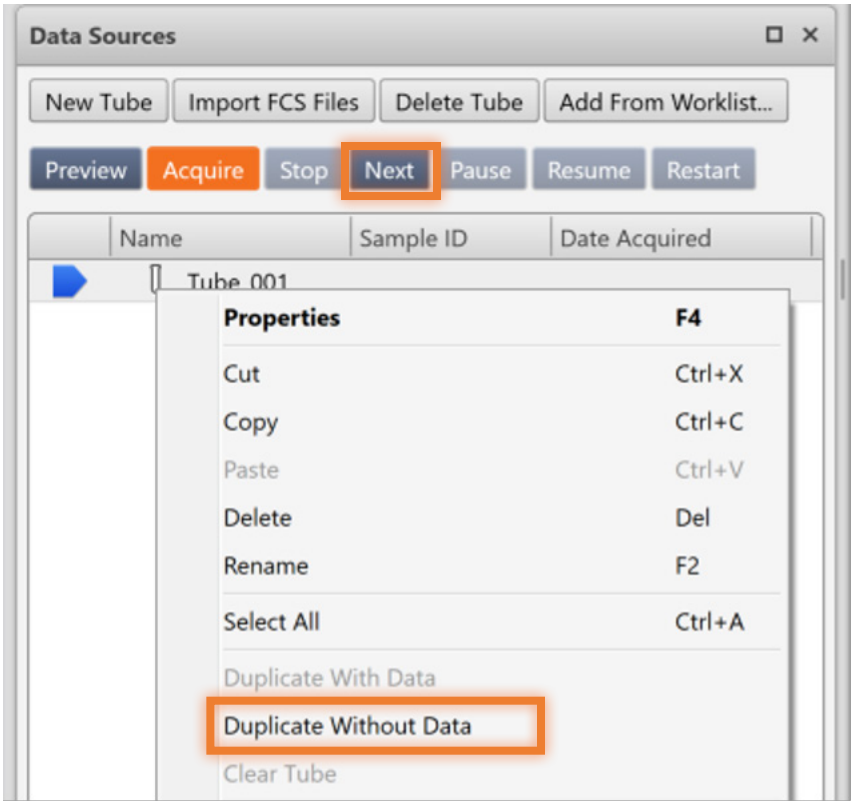
    a. The median value will be based on all the events acquired during the preview, so you can click Restart as many times as you need to refresh the plot view and ensure that the bright bead median value matches the target for each fluorochrome.

NOTE: You will need to determine the acceptable variance between the value achieved on the BD FACSLyric™ Flow Cytometer versus the target value from the BD FACSCanto™ II Flow Cytometer. For guidance, we recommend viewing CLSI H62 "Validation of Assays Performed by Flow Cytometry", 1st edition.



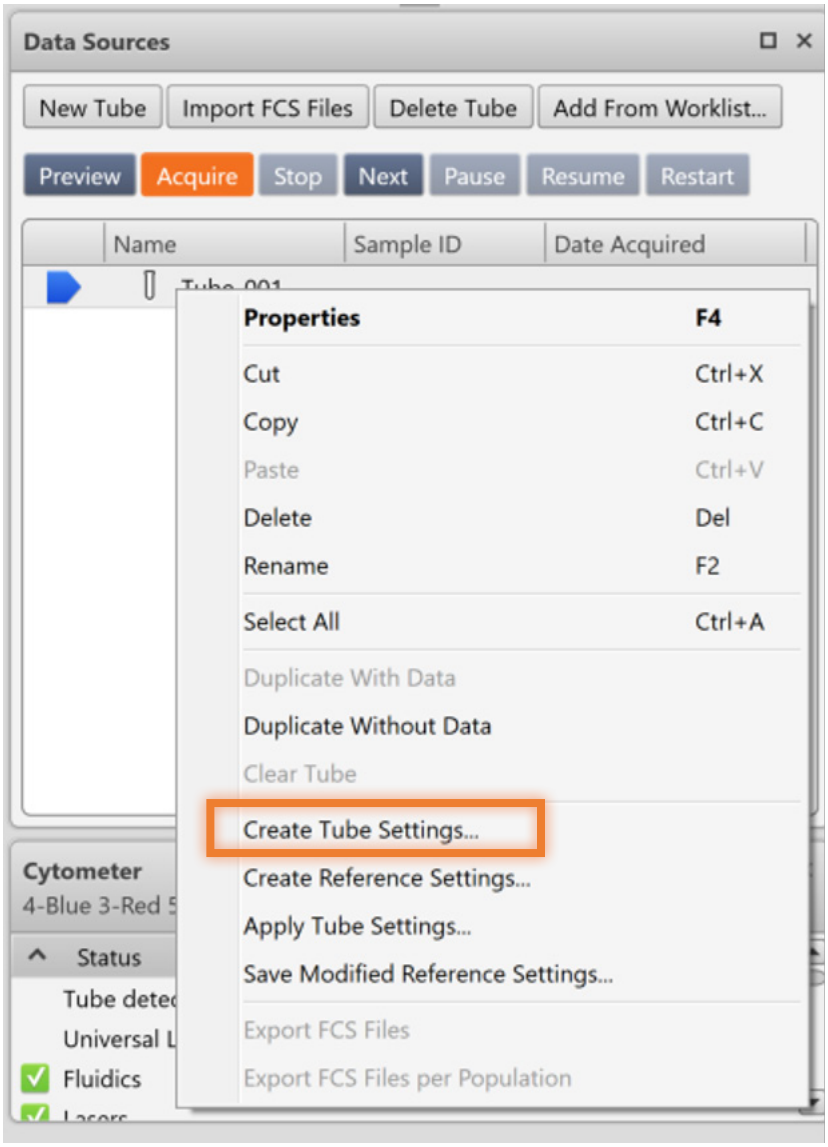
12. Once the target value has been achieved, acquire 10,000 gated events.

13. Select the acquired tube. Right click and select **Duplicate Without Data**. Click **Next** in the data sources window to move the run pointer to the new tube or click on the run pointer next to the tube to be acquired.



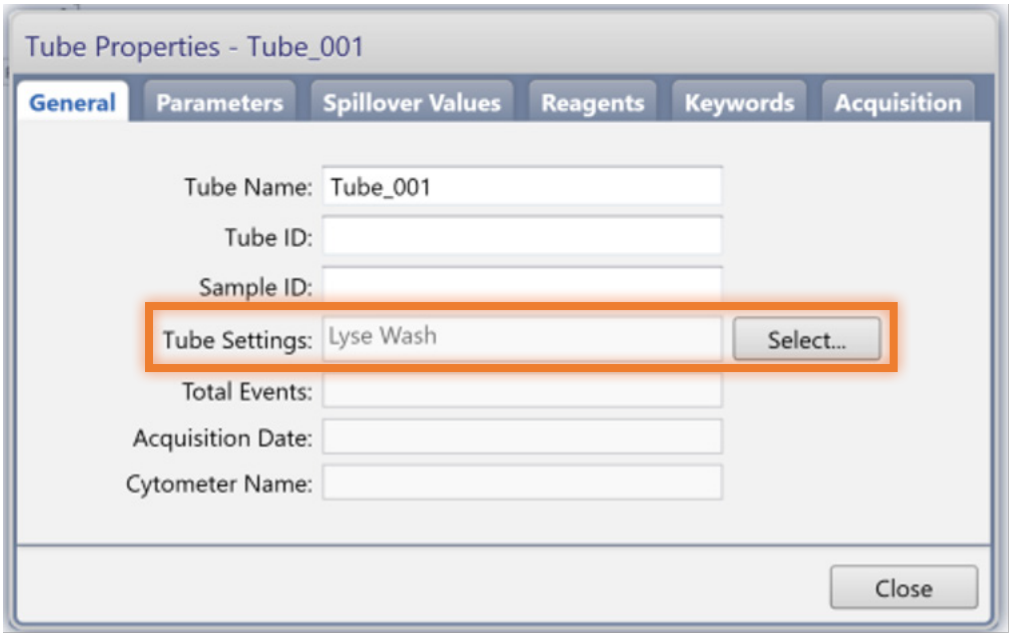
14. Select the newly created tube, rename it to match the next fluorochrome to be run, and repeat steps 10–13 for each BD® FC Beads or other control sample for each fluorochrome. Modify the corresponding multitube statistics box to display the statistics from the newly created tube.

15. Once you have acquired all the tubes, repeat step 13, right click on the last, empty tube and select **Create Tube Settings**.
    a. Follow the on-screen instructions to create and save the tube settings.

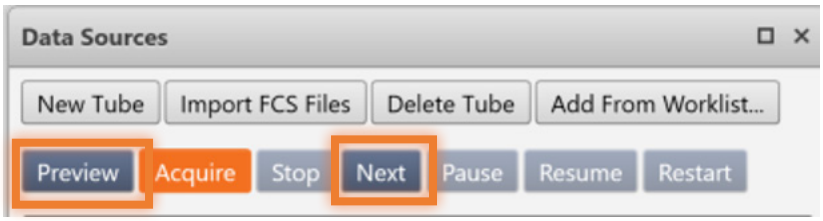


Using your saved tube settings to create an application-specific assay.

16. When you are ready to create an assay using cells, open a new experiment and apply these tube settings to the current tube.



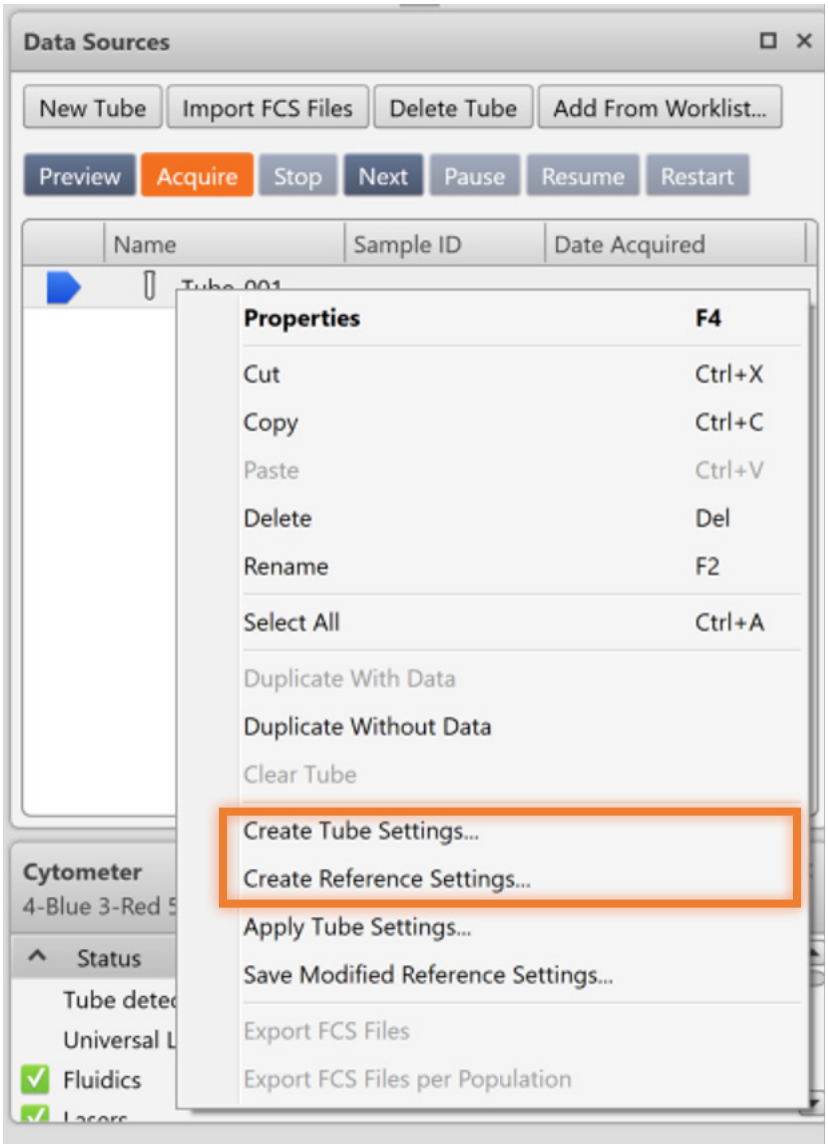
17. Load your cell samples onto the manual tube port. Click **Preview** to begin sampling. Adjust FSC, SSC, threshold, and flow rate, as needed.



18. Click **Stop** to stop sampling and remove the sample tube from the manual tube port.

19. Right click on the tube in the data sources window and select **Create Tube Settings** or **Create Reference Settings**, as appropriate.

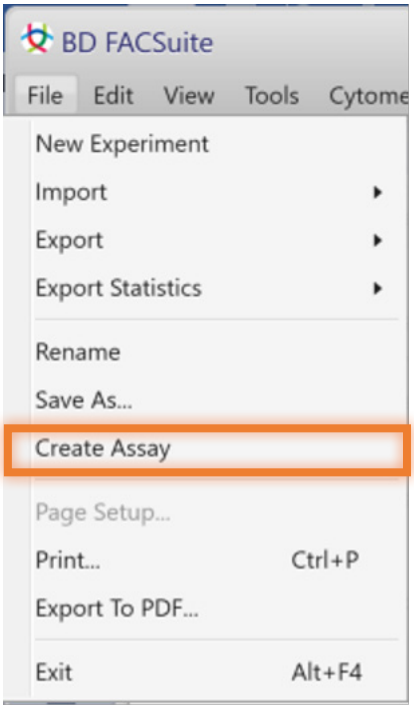
    a. Follow the on-screen instructions to create and save the settings.



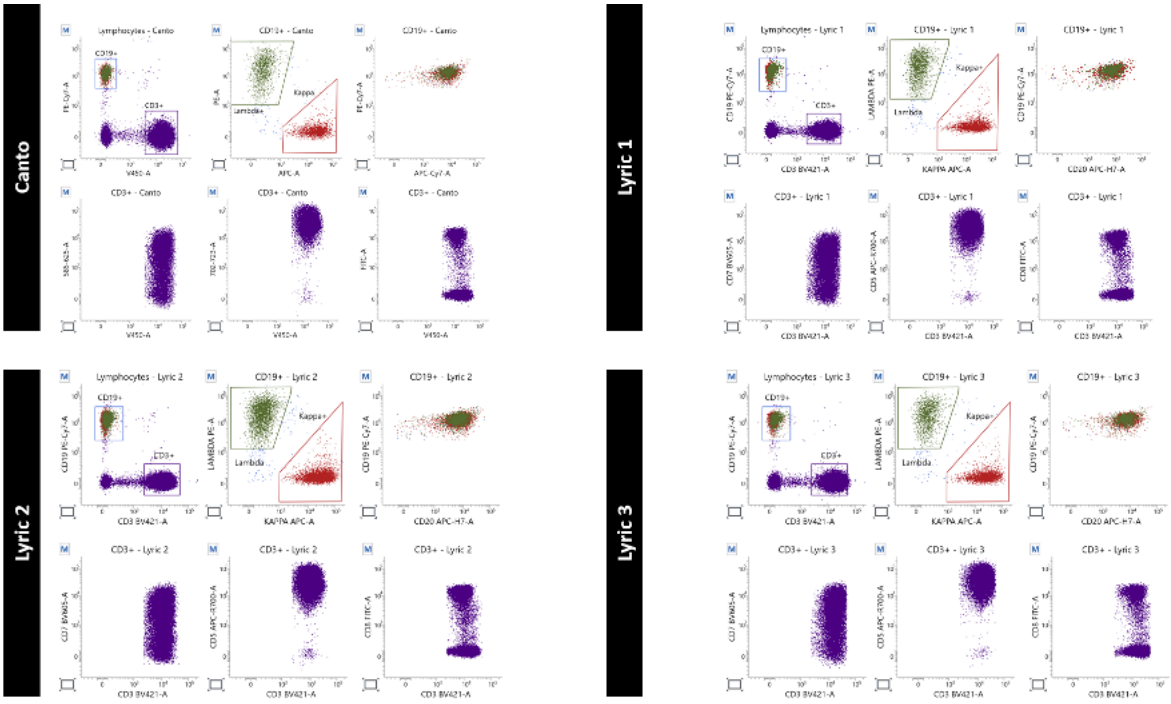
20. Create additional tubes, worksheet, reports or analysis elements as needed.

21. Choose **File**, **Create Assay**.

    a. Follow the on-screen instructions to create and save the assay.



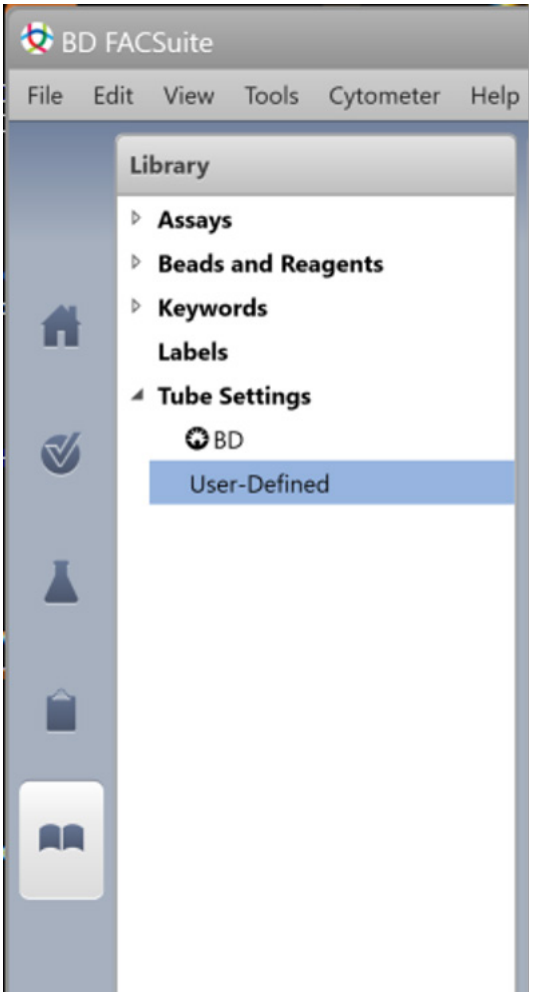
Example



(Fig) Representative data. Target values were created on the BD FACSCanto™ II Flow Cytometer (Canto) and transferred to a BD FACSLyric™ Flow Cytometer (Lyric 1). Using assay transfer feature in the BD FACSuite™ Application, the settings were then transferred to Lyric 2 and Lyric 3. Data from the BD FACSCanto™ II Flow Cytometer are analyzed in BD FACSuite™ Application for comparison.

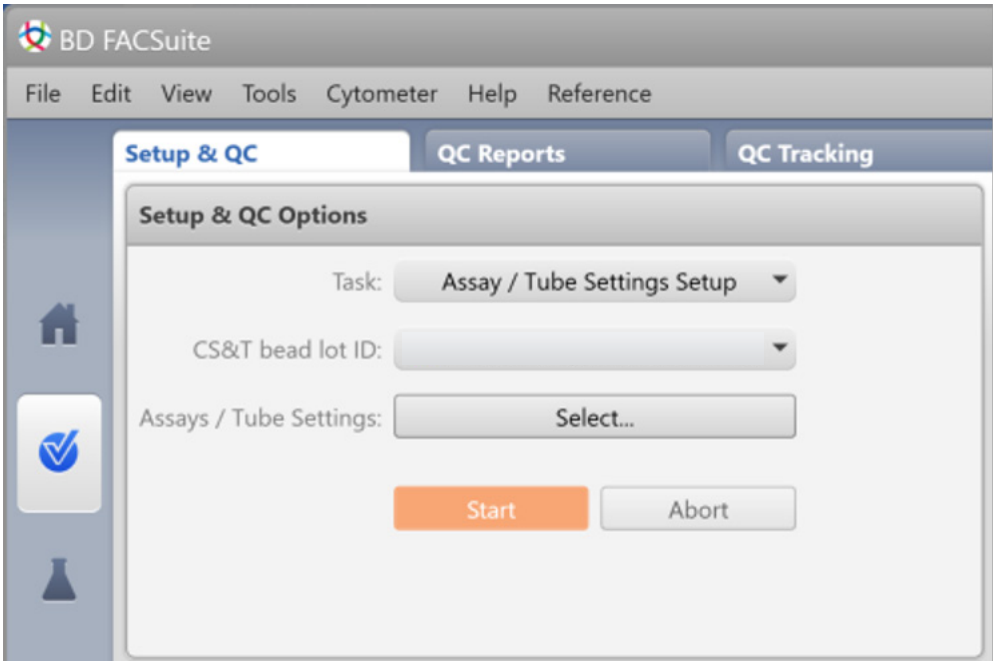
Transferring settings between BD FACSLyric™ Flow Cytometers

1. In the BD FACSuite™ Application, go to the **Library**, select **Tube Settings** then **User-Defined.**



2. Select the setting to transfer, then choose **File**, **Export**.
3. The exported file can be transferred electronically or via a USB to any existing BD FACSLyric™ Flow Cytometer, regardless of BD® CS&T Beads lot number.
4. Go to the next BD FACSLyric™ Flow Cytometer and run daily QC, following the instructions in the "Instructions for Use."
5. In the BD FACSuite™ Application, go to **Library**, **Tube Settings**, **User-Defined**, then choose **File**, **Import**.

6. Go to the **Setup & QC** workspace and select **Assay/Tube Setting Setup**.



7. Click **Select**, then choose the settings that were imported. Follow the on-screen instructions for running Assay/Tube Setting Setup.
8. If the transferred settings are Reference Settings, open a new experiment, assign the settings to a tube, then right click and select **Create Reference Settings**.

a. Follow the on-screen instructions for creating and saving Reference Settings.

9. The system is now ready for acquisition.

Maintaining your settings

The saved tube or reference settings are updated each day that you run Performance QC (PQC) and Assay Tube Setting Setup (ATSS), depending on the setting. This set up will continue in perpetuity, regardless of BD® CS&T Bead lot if the following conditions are maintained:

1. When switching lots of BD® CS&T Beads, a Bead Lot Transfer (BLT) is performed using the old lot of BD® CS&T Beads and the new lot of BD™ CS&T beads.
2. You can run a CQC using an existing lot of BD® CS&T Beads and that lot of beads was previously used to run a CQC or BLT.

NOTE: If a CQC is run with a new lot of BD® CS&T Beads that does not match the criteria above or a new CQC is run after your instrument has undergone a major repair to the optical system, it is recommended that you verify your target values using FC beads and recreate the settings if needed.

BD flow cytometers are Class 1 Laser Products.

In the U.S., the BD FACSCanto™ II Flow Cytometer is for In Vitro Diagnostic Use for up to six colors. Seven and eight colors are for Research Use Only.

In the EU, the BD FACSCanto™ II Flow Cytometer is no longer available for sale.

In the U.S., the BD FACSLyric™ Flow Cytometer is for In Vitro Diagnostic Use with BD FACSuite™ Clinical Application for up to six colors.

In the U.S., the BD FACSLyric™ Flow Cytometer is for Research Use Only with BD FACSuite™ Application for up to 12 colors. Not for use in diagnostic or therapeutic procedures.

In the EU., the BD FACSLyric™ Flow Cytometer with the BD FACSuite™ Clinical and BD FACSuite™ Applications is an in vitro diagnostic medical device bearing a CE mark.

FC Beads, CompBeads and FC Bead Dilution Buffer are for Research Use Only. Not for use in diagnostic or therapeutic procedures.

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BD-87191 (v1.0) 0523