Job aid

BD FACSDiva™ Software Determining initial PMT voltages

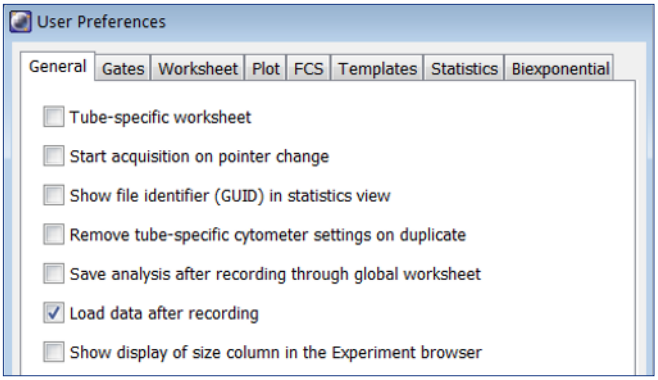
This job aid contains instructions for determining voltage settings that will optimize the stain index (SI) of cells stained with a reference reagent for each detector. After PMT voltages have been determined, they can be saved as Application Settings and used as a starting point for subsequent experiments.

Before you begin

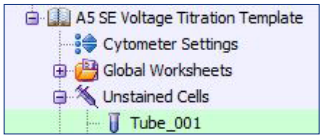
- Prepare cells; for this method only unstained processed cells are required, no single-stained cells are needed.
- Prepare a tube with 1 drop of mid-peak Sphero™ Rainbow Fluorescent Particles (Cat. No. 556298) to be used as the postive control.
- Ensure that the instrument has been started up and a CS&T performance check has been performed.

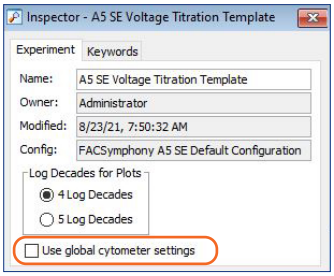
Setting up an experiment

1. Select **Edit** > **User Preferences** and set the following:
   - Under the **General** tab, clear the checkbox for **Remove tube-specific cytometer settings on duplicate.**
   - Clear the checkbox for **Save analysis after recording through a global worksheet.**



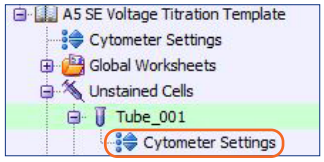
2. Select **File** > **Import** > **Experiments** and select **A5 SE Voltage Titration Template experiment**. This template is only compatible with the BD FACSymphony™ A5 SE Cell Analyzer's default CST configuration, running BD FACSDiva™ Software version 9.3 or later. Skip to the section titled Recording Data Files after importing the BD FACSDiva™ Software template. If you'd like to create your own experiment template then proceed to step 3.





3. Create and rename a new experiment.

4. Select the experiment in the **Browser** and then in the **Inspector**, clear the checkbox for **Use global cytometer settings.**

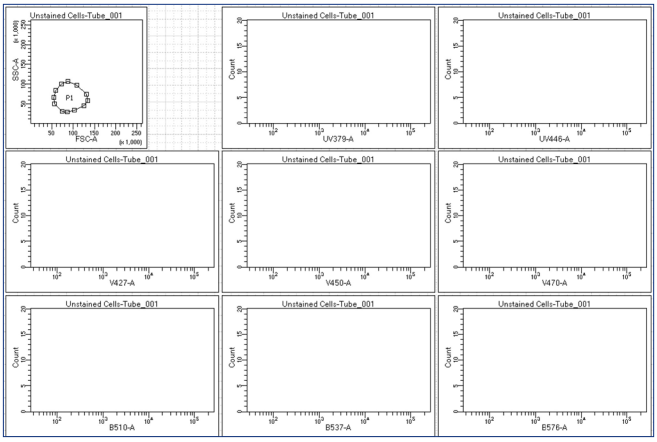


5. Add a specimen and rename it **Unstained Cells**.

6. Select **Tube_001** and click the **New Cytometer Settings** button to add tube-specific cytometer settings to **Tube_001**.

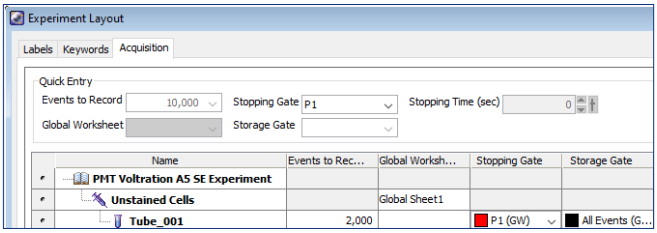
7. Set PMT voltages for each fluorescence detector to 200.

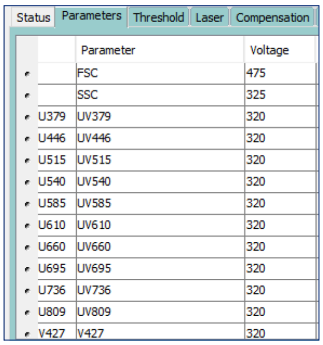
8. On the global worksheet, create an FSC vs. SSC dot plot.
9. Create histograms for each detector on your instrument.
10. Create a P1 gate on the FSC vs. SSC plot and set all histograms to show P1.



Setting voltages

1. Load a tube of unstained cells on the cytometer and start acquiring.
2. Adjust FSC and SSC voltages to place the cells on scale and set FSC threshold, if needed.
3. Load a tube of the mid-peak beads and confirm they are still on scale; adjust FSC and SSC voltage, if needed
4. Adjust P1 to identify the population of interest.
5. In the **Experiment Layout**, **Acquisition** tab, select your global worksheet from the **Global Worksheet** menu.
6. Select **P1** as the **Stopping Gate**.
7. Enter 2,000 in the **Events to Record** field.
8. Duplicate **Tube_001** and increase the PMT voltages for each fluorescence detector by 20 volts.
9. Repeat step 8 to create tubes for voltages up to 800 volts, for a total of 31 tubes.
10. Duplicate the specimen and rename it **Beads**.





Recording data files

1. Acquire the unstained cells and record a data file for **Tube_001** in the **Unstained Cells** specimen.
2. Record data files for all tubes in the specimen using the same sample.
   - Keep the same flow rate for all tubes.
   - Record data for all 31 tubes.
3. Repeat steps 1 and 2 with the beads.

Analyzing data

1. For **Tube_001** of the unstained cell specimen, create a statistics view to display median and rSD for all the fluorescent detectors.

2. Right-click the specimen and select **Batch Analysis**.

3. Make the following selections in the **Batch Analysis** dialog:
   - Select **Auto** and set to 0 seconds.
   - Select **Statistics**.
   - For **Stats Filename**, select a file location for the CSV file and rename it **Unstained Cells**.
   - Deselect all other check boxes

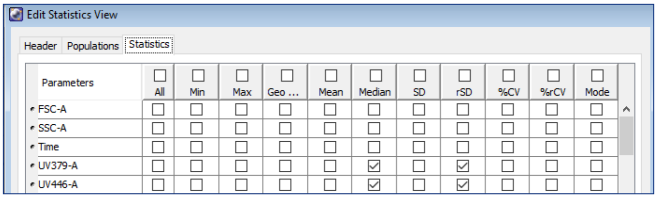
4. Edit the statistics view and deselect the **rSD column**. Repeat steps 2 and 3 with the **Beads Specimen**.

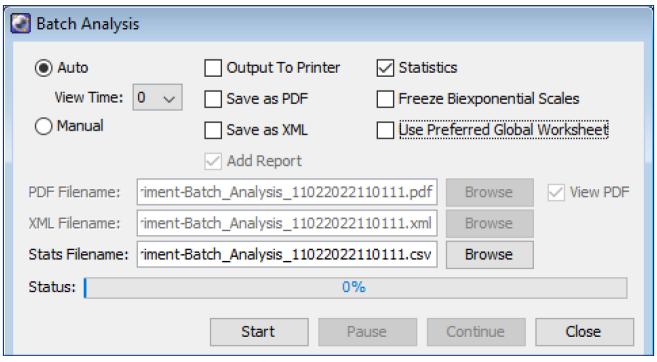
5. Calculate the stain index for each PMT voltage data point using the **A5 SE Voltage Titration Worksheet Template** provided by BD. Open the CSV file from the **Beads Specimen** batch analysis and use shift to select and copy all cells containing values, excluding the top header row. Paste into the highlighted area in the **Positives** tab of the **PMTV Setup Template**.

6. Repeat step 5 for the **Unstained Cells** specimen using the **Negatives tab** in the **A5 SE Voltage Titration Worksheet Template.**

7. Identify PMT voltages where the stain index for a biological sample is at or near the maximum voltage for each detector.

If you need assistance identifying the optimal PMT voltages or have questions about the PMTV Setup Template document, contact **ResearchApplications@bd.com**.

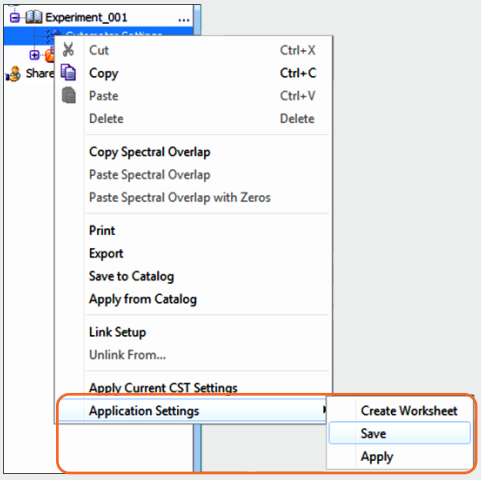




Saving as application settings

1. Create a new experiment in the Browser and enter the voltages determined in the previous section.

2. Right-click the cytometer settings and select **Application Settings** > **Save**.
   - The application settings are saved in the Cytometer Catalog and can be applied to future experiments with the same configuration.
   - Application settings will be updated automatically when a performance check has been run.



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