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| Optimization of Voltage Settings on the FACS Canto II | | | | |
| **Purpose** | Instrument optimization is an often underestimated source of low resolution and high variability. It is important to optimize voltages for each photo multiplier tube (PMT) to determine and maximize the dynamic range available for positivity. An optimal dynamic range provides the best resolution for dim staining, while maintaining the maximum range for very bright staining.  The process described below, using objective values obtained from CS&T, should be  followed to create an objective, optimized setup prior to assay validation. Once  determined, CS&T Application Settings can be used to maintain optimized settings.  During setup, detector voltages are adjusted to place setup beads at defined target  values, sensitivity values are measured, and spectral overlap values are calculated  and applied to compensate data for fluorescence spillover. The Levey-Jennings  feature in BD FACSCanto clinical software is used to automatically track cytometer  setup values over time, and to monitor cytometer performance and see shifts or  trends in parameters as they occur.  Run setup once every 24 hours, using BD FACS 7-color setup beads. The  software tracks the time between setups and displays it in the Status window. A  setup age of more than 24 hours appears in red. Running a successful setup resets  the timer.  During optimization, you can adjust thresholds, detector voltages, and spectral  overlap values for a panel type. The software uses BD Biosciences default settings  the first time you optimize. When you make changes, the new settings apply to all  tubes and samples of this panel type. | | | |
| **Policy Statement**  **Automated Setup Procedure**  **(Using 7 Color Setup Beads)**  **Optimizing for a Specific Panel Type**  **Adjusting Detectors, Thresholds or**  **Spectral Overlap Settings**  **Reviewing Levy-Jennings Reports** | This procedure applies to all laboratory technologists performing Flow Cytometry testing, the section  supervisor, and section pathologist.     |  |  |  | | --- | --- | --- | | **Step** | **Action** | **Related Document** | | 1 | Perform a CS&T Baseline and Performance Check | [FLO-2.9-Analyzing-The-Performance-Setup-on-BD-FACSCanto-II.pdf](https://starnet.childrenshc.org/References/labsop/flow/flow/flo-2.9-analyzing-the-performance-setup-on-bd-facscanto-ii.pdf) | | 2 | Evaluate results;  Incorrect values are saved if you accept a setup that is unsuccessful or setup results that are out of range.  If setup is unsuccessful or if setup results are out of range, **do not** click Finish. Note the message provided by the software and consult BD FACSCanto Clinical Software Troubleshooting.  If setup is successful, the following options will be available;   |  |  |  | | --- | --- | --- | | **To** | **Click** | **Additional Information** | | View Setup results |  | The report contains cytometer QC and pass/fail information. You can print the report from this view. | | Discard current results |  | You will be given the option to use the last setup results. | | **To** | **Click** | **Additional Information** | | Optimize setup values using BD FACSCanto  clinical software | When prompted,  click  to save your results  and continue. | Proceed to Optimizing with  BD FACSCanto Clinical  Software. | | Exit setup and save new setup results |  |  | | (Optional) Save setup results and optimize setup  values using  BDFACSDiva software |  | Proceed to Optimizing with  BD FACSDiva Software. | | BDFACS Canto II Operators Guide  Pages 203-204  Setup Wizard Messages  The report contains cytometer  QC and pass/fail information.  You can print the report from  this view. | | **Step** | **Action** | **Related Document** | | 1 | Select a panel type and parameters from the menus and click      Gate Parameter X and Gate Parameter Y refer to the plot parameters for the first optimization plot, the plot that contains a gate around the cells of interest. |  | | 2 | Install the first optimization tube when prompted, and click |  | | 3 | At the Cytometer Setup Optimization screen, click  Acquisition begins, and events appear in the plots.  Right-click the axis labels on a plot to choose other parameters; |  | | 1 | Optimize settings, as needed.  There are three types of cytometer controls: Detectors, Thresholds, and Spectral Overlap.  **Detectors**; Adjust the signal for events displayed in plots by changing detector voltages.  Higher voltages amplify the signal. Lower voltages decrease the signal.  BD FACSCanto clinical software automatically recalculates spectral overlap when you change detector voltages.  **Thresholds**; Use thresholds to filter out unwanted events: a threshold sets a channel number below which events will not be processed. You can set one or more thresholds at a time, and choose whether any one (OR) or all (AND) need to be met.  **Spectral Overlap**; Fluorochromes emit light over a range of wavelengths. During cytometer setup, fluorescence spillover is automatically determined and corrected. If necessary, you can use the spectral overlap controls to make manual adjustments.  Click on a tab to access the corresponding controls, or choose an option from the View menu;    after Setup, in Wizard    during acquisition  Threshold and side scatter are the most frequently optimized  parameters for TBNK assays.  Spectral overlap values are automatically recalculated when you adjust  voltages.  To adjust the optimization gate, click  the acquisition  display stops updating. Move the gate by dragging the gate border, or  resize the gate by selecting a corner and dragging. When you are ready  to proceed, click  To adjust detectors, thresholds, or spectral overlap settings, click on the  corresponding tab.  Use controls in the tab to adjust the settings.  For clinical applications that use tandem conjugates such as APC-Cy7 or PE-Cy7, spectral overlap varies from lot to lot. Because BD FACSCanto clinical software setup targets the average lot, you might need to adjust spectral overlap settings for these reagents; | BDFACS Canto II  Operators Guide  Pages 71-74 | | 5 | If there are more tubes, click  , and then . When prompted, place the next tube on the SIT, and click . Repeat steps 1-4. |  | | 6 | When there are no more tubes to optimize, click ,  and then . Click . |  | | **Step** | **Action** | **Related Document** | | 1 | The software automatically creates a Levey-Jennings Report from the cytometer setup data. To view the report:  From the main window, select the Levey-Jennings tab.    An  on the tab indicates an  out-of-range value on the report: |  | | **Step**  2 | **Action**  Check the plots in the report;        Parameters outside the limits set by the lab are shown by a red *x* in the affected plot. | **Related Document** | | 3 | To add comments to the report, click Comments:    Enter text into the Comments field (up to 2500 characters), and click OK. |  | | 4 | To print the report, Click |  | | | | |
| **References** | BD FACS Canto II Instructions for Use[**BD\_FACS\_Canto\_II\_Users\_Guide.pdf**](BD_FACS_Canto_II_Users_Guide.pdf)  **bdbiosciences.com**  Part No. 642239 Rev. A  June 2007 | | | |
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| 1 | Al Quigley | 08/28/20 | Initial Version |