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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 befollowed to create an objective, optimized setup prior to assay validation. Oncedetermined, CS&T Application Settings can be used to maintain optimized settings.During setup, detector voltages are adjusted to place setup beads at defined targetvalues, sensitivity values are measured, and spectral overlap values are calculatedand applied to compensate data for fluorescence spillover. The Levey-Jenningsfeature in BD FACSCanto clinical software is used to automatically track cytometersetup values over time, and to monitor cytometer performance and see shifts ortrends in parameters as they occur.Run setup once every 24 hours, using BD FACS 7-color setup beads. Thesoftware tracks the time between setups and displays it in the Status window. Asetup age of more than 24 hours appears in red. Running a successful setup resetsthe timer.During optimization, you can adjust thresholds, detector voltages, and spectraloverlap values for a panel type. The software uses BD Biosciences default settingsthe first time you optimize. When you make changes, the new settings apply to alltubes 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 sectionsupervisor, and section pathologist.

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| **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;

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| **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 FACSCantoclinical software |  When prompted,click to save your resultsand continue. | Proceed to Optimizing withBD FACSCanto ClinicalSoftware. |
| Exit setup and save new setup results |  |  |
| (Optional) Save setup results and optimize setupvalues usingBDFACSDiva software |  | Proceed to Optimizing withBD FACSDiva Software. |

 | BDFACS Canto II Operators GuidePages 203-204Setup Wizard Messages The report contains cytometerQC and pass/fail information.You can print the report fromthis 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 acquisitionThreshold and side scatter are the most frequently optimizedparameters for TBNK assays.Spectral overlap values are automatically recalculated when you adjustvoltages.To adjust the optimization gate, click  the acquisitiondisplay stops updating. Move the gate by dragging the gate border, orresize the gate by selecting a corner and dragging. When you are readyto proceed, click To adjust detectors, thresholds, or spectral overlap settings, click on thecorresponding 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 IIOperators GuidePages 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  |  |

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| **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  |
| **Historical Record** |  |  |  |  |
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| 1 | Al Quigley | 08/28/20 | Initial Version |