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Reagent-Driven Reconfiguration/Optimization of a 16-Parameter BD FACSARIA II SORP to Allow Accurate Detection of Violet- and UV-Excited Sirigen Dyes

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Background. Modern flow cytometers can detect emission from a variety of commercially available fluorescent reagents. However, accurate detection of novel dyes is often difficult due to the lack of readily available quality assurance tools, for instrument manufacturer QC and optimization protocols often become available secondary to new fluorescent reagents.

Aims. For standard QC and instrument calibration of the FACSARIA, BD provides a standard 'CS&T' method that includes three-peak beads and a dedicated software module. While this method allows tracking instrument state over time, it does not accurately assess PMT performance on a significant part of the spectrum for violet- or UV-excited dyes. In order to ensure accurate detection of reagents within all 16 channels of the Boston University Flow Core 16-color, 4-laser BD FACSARIA SORP, we created and performed an novel optimization process that allows simultaneous accommodation of as many as nine polymeric Sirigen dyes, including those emitting in both long-wavelength violet- and UV-laser excited channels.

Methods and Results. Firstly, the electronic noise of all PMTs was assessed and information was collected on rSD of non-stained cells within 100-800V range. The derived basal PMT values then provided a starting point for voltage optimization of instrument-specific panels. These values differed greatly from CS&T-deduced PMT voltages for abovementioned channels, for some the CS&T calculation of optimal PMT voltage was not possible due to a poor resolution of CS&T peaks at certain wavelengths. Several PMTs were identified with sub-par performance and were consequently replaced.

Our testing of multiple commercial compensation beads found that the majority demonstrated prohibitively high backgrounds; the eBioscience UltraComp beads performed best and were therefore our reagent of choice. For instrument performance tracking, we also compared multi-peak beads from several manufacturers and found Spherotech Ultra Rainbow beads to be the sole bead type with satisfactory resolution of all peaks on long-wavelength UV channels. Finally, we developed an ergonomic protocol for facility users that includes an experiment template and electronic tables for data processing. With that protocol, a user can: (1) finely tune PMT voltages to accommodate a specific panel, (2) determine antibody concentrations for compensation control preparations, and (3) associate these optimized settings with multi-peak bead target values. Such preliminary setup allows quick panel-specific instrument calibration for each experimental run.

This approach was successfully applied to several 16-color panels used in our Core facility and resulted in vastly improved reproducibility of acquired data over months of use.

Conclusions. Synchronizing cutting-edge reagent technologies with existing instrument QC and maintenance methodology requires development of mix-and-match solutions not necessarily provided by the instrument manufacturer. Creating a user-friendly, accurate QC and calibration protocol that accommodates novel reagents allows dramatic expansion of our userbase's experimental capabilities.