Flow Cytometry Protocol

We used the BioRad S3e Cell Sorter to carry out our cell fluorescence measurements. We first diluted our culture in 500 µL of Phosphate Buffered Saline (PBS) adding 10uL of culture. On our flow cytometer, we used two channels: one channel configured for measurement of fluorescein (FITC) for GFP and another channel configured for measurement of electron coupled dye (ECD) for mCherry. Both the FITC and ECD channels have a 488nm laser used to excite the cells and a 530/30 filter used to capture the light emission from the cells. To acquire data with flow cytometry, we adjust side-scatter (SSC) and forward scatter (FSC) PMT voltages using bacteria from one of our samples until the distribution of each is centered on the scale and we adjust FITC/GFP PMT voltage until the upper edge of the "bell curve" from the fluorescent population is one order of magnitude below the upper end of the scale. We run the program enough to acquire at least 10,000 events for each biological sample. After running the samples, we acquire at least 10,000 events from a sample of calibration beads with the voltages for FSC at 339 and SSC at 292 and at the voltages applied for the sample collection on the channel(s) used. We used SpheroTech Rainbow Calibration Particles RCP-30-5A for fluorescent calibration beads because they have been calibrated for excitation and detection of the particles in most channels of any flow cytometer. We used FlowCal for downstream analysis and conversion to absolute fluorescence units. The two absolute fluorescence units we used are Molecules of Equivalent Fluorescein (MEFL) for GFP and MEPTR for mCherry.