

DATA ACQUISITION GUIDELINES

PROCEDURE

1.0 General Guidelines for New Experiment Setup on the Flow Cytometer

- 1.1 Make sure instrument can detect all fluorochromes in your panel by checking the equipment description (on iLabs by expanding descriptions under schedule equipment and on clipboard near instrument).
- 1.2 Select the fluorochromes in your panel. Delete channels you are not using. Make sure you have selected to save FSC and SSC-A, H, and W
- 1.3 Label the parameters to create a record of what marker you are using in each channel.
- 1.4 Set up plots in worksheet as needed. Make sure you are visualizing all the channels from panel.
- 1.5 Use the brightest, fully stained sample to set PMT voltages. Record data after setting voltages.
 - A. Adjust the voltages in each channel to fit the linear range of the PMT. Linearity max on BD systems is approximately 10^5 in log or biexponential scale, and positive populations must be below this value.
- 1.6 Create compensation control tubes. Run and record single stains and verify that gates are set to define fluorochrome+ events.
 - A. Each single stain sample must have positive staining to calculate compensation.
 - B. Single stain samples must be at least as bright as the brightest sample, but not exceed the linear range.
 - C. The core recommends using compensation beads for most antibodies.
 - D. If needed, titrate the antibody volume in compensation control tubes to match the experimental samples.
 - E. Single color controls are required for any fluorescent proteins expressed in cells.
- 1.7 Calculate compensation and apply to your experiment.
- 1.8 You are ready to run your samples. Record enough events to have at least 1,000 events in the least frequent population of interest.
- 1.9 When you are done recording files, export the data to the D: drive in your PI's folder.
- 1.10 Transfer the data to the South Campus Flow Lab server. There is a link on the PC desktop of each machine. Disconnect from the server when you are done using the FLOW DISCONNECT icon on the PC desktop.
- 1.11 Save a template for experiment to keep all settings for future runs. Instructions for this are on clipboard located next to each machine.
- 1.12 Clean the instrument: Run on high 3 minutes each of Contrad, Bleach, and then water. Leave the water tube on the SIP and place in standby (except for Canto II). Logout of FACSDiva.
- 1.13 You're done! Your data can be retrieved from the server when you return to your PC/Mac.

RELATED PROCEDURES

This handout is related to ACSF SOP EX006. It is a handout only.