THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

NEW USER TRAINING

DATA ANALYSIS OVERVIEW

PROCEDURE

1.0 Introduction to Flow Cytometry Data Analysis

1.1. Good data analysis begins with good experiment planning. Carefully consider what question you are asking, and meet with core staff to help plan your flow cytometry experiment.

1.2. **Data Acquisition:** Include critical data such as date of acquisition, cell line name, tissue type, activator/inhibitor concentration, replicate number, etc.

1.3. **Analysis Software:** Choose an analysis platform such as FlowJo (MD Anderson site license), Kaluza (ACSF license), FCS Express (ACSF license, traditional and imaging cytometry), IDEAS (open source, Amnis imaging cytometry), or Cytobank (ACSF license, high parameter analysis).

1.4. **Plots:** Histograms display intensity of one parameter, bivariate plots display intensity of two parameters, and density plots display the percent distribution of particles at the same intensity.

1.5. **Gates:** Gates are applied to isolate populations of interest and generate statistics. Gate types include range, rectangle, ellipses, polygon, quadrant, and spider. Gates can be stacked with hierarchical or Boolean logic to generate more information.

1.6. **Data Modeling:** Some analysis programs contain models for biological processes, such as cell cycle, proliferation, and clustering.

1.7. **Statistics:** Plan to collect enough data so that an appropriate number of events are recorded for your population(s) of interest. Flow cytometry data can be described for the following:

A. Single Group: Assess MFI (Mean Fluorescence Intensity), standard deviation, and CV.

B. Two Groups: Shapiro-Wilk normality test to determine if distribution is Gaussian or non-Gaussian. T-tests can be used for Gaussian distributions, and the Wilcox test can be used for non-Gaussian distributions.

C. Three or More Groups: Shapiro-Wilk normality test to determine if distribution is Gaussian or non-Gaussian. ANOVA or regression analysis can be used for Gaussion distributions, and the Kruskal-Wallis test can be used for non-Gaussian distributions.

1.8. **Preparing for Publication:** MIFlowCyt Standards provide guidelines on publishing flow cytometry data. Please recognize the lab in any publications where data was acquired or analyzed at the flow core by citing grant P30CA16672.

1.9. **Sharing Data:** Aside from publication, high quality flow cytometry data can be shared with other researchers through the Flow Repository or Cytobank.

RELATED PROCEDURES

This handout is related to ACSF SOP TR001. Please see the full SOP for further information.