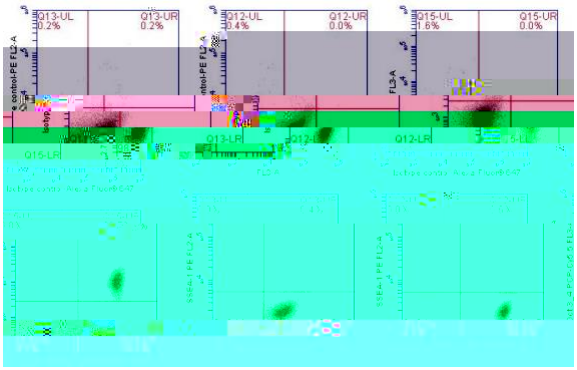
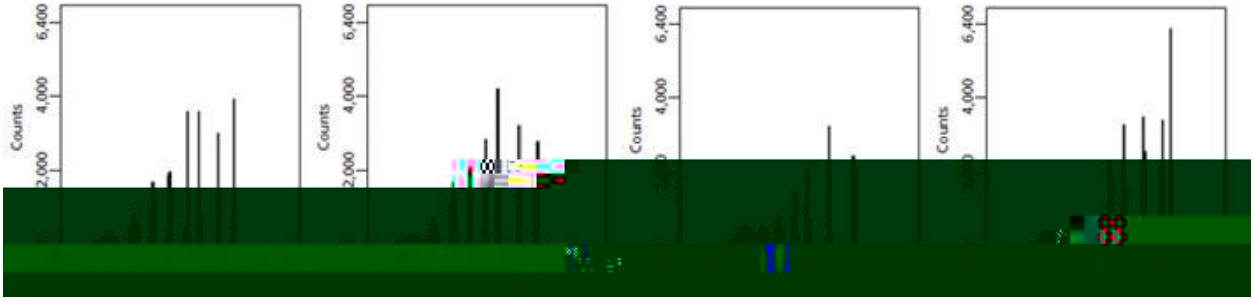
Flow Cytometry

Flow cytometery utilizes fluorescent antibodies to tag proteins on the outside or inside of a cell. Flow cytometry can also utilize fluorescent dyes to stain nucleic acids whether it be DNA or RNA. Although the following protocol starts with cells obtained from blood, one can also stain adherent cells or cells in suspension from cell culture experiments.

While obtaining data from the flow cytometer, the machine is able to accurately count the number of cells acquired and tell you how big (in terms of forward scatter) and how granular (in terms of side scatter) each cell is. There are two main outputs or graphical data sets you can get from a flow cytometer. One is called a scattergram, where each individual cells is shown on a graph.



Or one can obtain a histogram that indicates how many cells fluoresce at a certain level. Higher levels of expression indicate a brighter fluorescence (10^5 to 10^7) while lower expressing cells are not as bright (10^2 to 10^4).



The most important part of flow cytometery is understanding what fluorophore you are using and whether it can be used with other fluorophores. This table will tell you what fluorophores can be used with the C6 flow cytometer:

Laser Excitation
488 nm (rated at 20,000-h life)
640 nm (rated at 20,000-h life)
Laser Profile
10 x 75 µm

Light Scatter Detection
Forward (0°, ±13°)
Side (90°, ±13°)
Emission Detection
4 colors, user-changeable optical filters
Standard set installed: