XL FLOW CYTOMETER OPERATION INSTRUCTIONS

1. FILL SHEATH TANK/EMPTY WASTE:

-Press "Run" pad once to get flashing green light- system will depressurize.
-Pull the Sheath drawer out from bottom.
-Unscrew cap on sheath tank (larger tank), and fill with sheath fluid using funnels provided (sheath fluid = Isoflow and is located next to cart in boxes). Tank can be filled completely.
-Replace cap and tighten.
-Loosen cap on waste container (container on side of supply of power supply) and lift cap assembly out of container.
-Empty contents of waste container into sink (while running water) avoiding unnecessary splashing.
-Fill bottom of waste container with bleach-to about 1" depth.
-Place waste bottle back into position and replace cap assembly.
-Press "Run" touch pad to re-pressurize instrument-will run through an initialization step (orange light) followed by a steady green light (means "ready").

2. ACCESS INVESTIGATOR SUBDIRECTORY:

-Highlight "Applications" pull-down menu.
-Select "Utilities" option
-Highlight "File" menu
-Choose "Select" option
-From the list of Subdirectory names, highlight appropriate investigator, then click on "Okay" box on bottom left of screen.
-Check that you are in correct subdirectory by highlighting "Screen" menu and select "Data Management" option. Subdirectory files should have your name displayed for Pro2, Lmd, and Hst (Protocol, Listmode, and Histogram directories, respectively).

3. SELECT ACQUISITION PROTOCOL:

-Highlight "Applications" menu and select "Acquisition" option.
-Highlight "Protocol" menu and choose "Select" option.
-A list of protocols will appear, choose the correct protocol by highlighting it and then clicking on the "Okay" button. The protocol will be displayed on the acquisition screen.

4.SAMPLE ACQUISITION:

-Check the lights on the pad next to the sample stage-the "Run" button should show a steady green light.
-The bottom right hand of screen should have the message "Insert sample tube" flashing.

> **Note:** If these conditions are not present, check the following:
> -Is the light orange? You may not be in the Acquisition menu
> -Is the green light flashing? Press the "Run" pad, a short initialization period (orange) will ensue, followed by a steady green light.

-Place the tube on sample stage-stage will automatically sense it (beep) and raise the tube.
-Observe data acquisition (Screen will report status i.e. tube sensed, acquiring, etc). Data will be acquired until a preset stop count (time) is reached. If no preset stop, click on the "Stop" button on bottom of screen when you feel you have enough events. DO NOT PRESS "ABORT" UNLESS YOU DO NOT WISH TO SAVE LISTMODE DATA. To adjust settings and gates, see below.
-Remove tube from sample stage; proceed to next sample.

5. TO ADJUST PMT VOLTAGE SETTINGS:

-If the voltage setting for a particular fluorescence marker or light scatter is not falling in the appropriate place, the voltage settings can be adjusted in one of the following two ways:

A. Enlarge the graph of interest (i.e. Fl3 PI fluorescence histogram) by clicking on graph. Click on "Display Options" until the PMT signal screen appears on the right side of screen (Lists all parameters followed by current voltages).
Click on the "Fast Set" box-cursor will become a cross-move cursor to population to move (i.e. G0G1 peak), click on population and drag to desired position. Voltages will automatically be adjusted and the sample will automatically be restarted.

Alternatively,

B. Enlarge graph of interest, Click "display options" until the PMT signal screen appears, highlight desired voltage setting (i.e. FL3) and increase or decrease using arrow keys. You must hit RESTART each time you change the voltage in this manner or your histogram will reflect the same population collected at different voltages.

6. TO ADJUST GATING REGIONS WHILE RUNNING:

-Enlarge graph containing region to be adjusted.
-Activate region to be edited by clicking on the letter of the region-to enlarge a region, hold down the left mouse button and move mouse to enlarge or minimize; to simply move region let go of left button and move region to desired location via mouse.

7. GENERAL CAUTIONARY NOTES FOR DATA COLLECTION:

A. Normal position for the NEUTRAL DENSITY filter is with the knob to the right. The left position is meant for acquisition of very large cells. If you start running and don't see events in your FS/SS graph-check the filter which is located under filter cover of instrument (middle front).
B. Check that "List Save" is turned "ON" under protocol: From acquisition menu, highlight "Setup", choose "Protocol". The various features of your protocol will be listed-check for printing and saving options (Turn on by clicking). If this change is required, re-save protocol by selecting "File" and choosing "Save" while in Protocol option.
C. If "Sheath Low" or "Waste Full" light comes on while running, finish collection of your sample (press "STOP" when finished) before doing procedure of filling sheath tank.
D. If sample doesn't look quite right or the event counter goes to zero while you still have sample, press "PRIME" on the instrument control pad. When the prime is completed, hit "RESTART" to start sample fresh.
E. It is helpful to have at least 300ul of liquid in collection tubes, as approximately 50ul of sample is aspirated at start of acquisition.

9. ANALYSIS OF STORED DATA FILES (LISTMODE ANALYSIS):

-To access stored data files, be sure to be in the correct investigator subdirectory (see above).
-Under "Applications" menu, access "Listmode"
-Under "File" menu, highlight "Select". If the files you seek to analyze are not present, click on button labeled "Rebuild"-your files should appear. (Other possibilities-data stored under a different subdirectory, Core manager has backed up data, data files not stored -i.e. "list save" was not "on"-see above).
-Highlight the files you wish to re-analyze and click on button labeled "Okay".
-Check for button on control line (bottom of screen) that toggles between "Runtime protocol/New Pnl/Prot". To analyze the sample as it was collected and then make a few modifications, toggle to "Runtime protocol".

LISTMODE ANALYSIS, CONT.

-Click on “Play next” to analyze the first sample in queue. Make necessary modifications to gates/markers, then click “Play List” in order to play gate changes.
-Print by pressing “F3” key on keyboard. (Assumes print option in “output option” is turned off).
-To analyze a series of data files using a newly defined set of gates/markers, analyze the first sample as described. Then select “Setup” menu and highlight “Protocol”. Finish changes necessary to protocol and click “Save As” under “File” menu. Type in a new protocol name and save.
-Click “Setup” menu and highlight “Analysis”. This will return you to the data file list.
-Click “Reset Queue” button (bottom of screen) and toggle “Runtime Protocol” back to “New Panel/Protocol”
-Highlight “Setup” menu and return to “protocol” screen.
-Select newly saved protocol by highlighting “file” menu and “select”. Highlight protocol name and click “Okay” button.
-Return to “Analysis” screen via the “Setup” menu.
-Proceed with file analysis as above (Click “Play next” and F3 to print).

CAUTIONARY NOTE: LISTMODE ANALYSIS

If you modify/create a protocol in Listmode and save it, the file will be saved with both “List Save” and “Auto Print” off. Therefore, if you opt to use this protocol for acquisition, you must turn on these options and save the protocol file again, or list mode data files will not be stored.

ADDENDUM-HOW TO CREATE AN ACQUISITION PROTOCOL:

-From the Acquisition page, highlight “Setup Screen” menu and select “Protocol”.
-Under “Signal Sources” (screen bottom right), select the parameters needed for acquisition by highlighting the “x” next to the detector name. Be sure to choose linear or log parameters according to your application requirements.

Note: Parameters not selected will not be stored, i.e. you will be unable to include them in later data analysis, so highlight all relevant parameters.

-Edit each parameter name if desired, by highlighting it under “User Name” in “Parameters” box.
-There are eight available graphs for data display. Assign parameters to graphs by highlighting them individually under “Signal” in “Parameters” box, then clicking on the x or y axis of a given graph.

Note: X-axis must be assigned first.

-Within each graph, several options are available, choose as follows:

“No Save”-toggle to “Save” if a histogram file is required (i.e. DNA content for Modfit).
“Autoscale”-toggle to “Manual” to set the scale to a given value.
“No Stop” –toggle to a desired “stop count” to collect a specified number of events.
“No Pos Analysis” – For automated neg/pos discrimination –leave at default.
“128x128”-represents the highest resolution; can change to 64x64 if required
“Print”-Can toggle to “no print” if a selected graph is not needed.

ACQUISITION PROTOCOLS, CONT.

- In the box below the graphs, be sure to change "Listsave" and "Autoprint" to "On". (If Listsave is off, no data will be stored). If desired, set a time stop, and leave the other options at default.
- To acquire data, return to acquisition mode by highlighting "Setup Screen" and selecting "Run". Adjust voltages according to instructions in #5 above.
- Create/edit required gates by clicking on "Regions" box (turns to red), enlarging required graph, and selecting the appropriate gating action (Create, Edit, Copy, Redraw, Erase).
- Assign gates to histograms by highlighting the desired graph and clicking next to the number of the desired graph (while in 8 Hist view).