FACSCanto OPERATION GUIDELINES

INSTRUMENT START-UP:

1. Turn on the power supply, cytometer main power (left side of instrument) and computer.
2. Log on to MS Windows using the password "BDIS".
3. Start (double click) the FACSDiva software icon. There is a built-in 10 min warm up period for the instrument and lasers during which one can proceed to step 4.
4. Check fluids and fill/empty as needed. Disconnect tubing and sensor wires and remove fluid level probes while changing container or adding fluid to a container. When the waste tank is emptied, add about 500 ml of bleach before returning it to the cytometer. Reinstall the fluid probes and reconnect tubing and sensor wires.
5. Log on to the Diva software (using your username and password). The software should open with windows for "Browser", "Inspector", "Cytometer" and "Acquisition Dashboard" on the left screen and a window for "Worksheet" on the right screen.
6. Wait for the software to connect to the cytometer. If any fluid containers other than the waste container were serviced, prime those fluidic systems by opening the "Cytometer" drop down menu and then selecting "Cleaning Modes" and then "Prime after tank refill". Select the specific containers serviced and click "OK". This will purge air introduced into the system during the servicing of fluid containers.
7. Run "Fluidics Start-up" under the "Cytometer" drop down menu (takes about 7 min).
8. The instrument is now ready to run samples.

PREPARING TO RUN SAMPLES:

1. In the "Browser" window, create a new experiment by clicking the icon or open a saved experiment template (under the "Experiment" drop-down menu).
2. Check the experiment-level settings and change, add or delete parameters as needed.
3. Edit the experiment and sample names as appropriate.
4. If you have saved instrument settings load these by right clicking the experiment level settings, choosing "Apply from Catalogue", selecting a settings file and clicking "Apply".
5. Select "Experiments" > "Compensation Setup" > "Create Compensation Controls".
6. Run unstained and single-color compensation controls using the normal worksheets created in the compensation specimen folder. Upon completion be sure each positive peak in each histogram is included in the gate for that fluorochrome.
7. Select "Experiment" > "Compensation Setup" > "Calculate Compensation". Name and save your compensation settings by clicking on "Link and Save" to load compensation.
8. Double click on the worksheet icon (top left) to convert the Normal Worksheet (for compensation) back into a Global Worksheet (for data acquisition).

RUNNING SAMPLES:

1. Select your first tube by double clicking on the tube pointer (be sure it changes to a green highlighted arrow).
2. Enter identifying sample information in either of two ways:
   a. Stepwise by adding tubes one at a time and entering information in "Sample ID" and "Patient ID" on the keywords tab in the "Inspector" window; or,
   b. For an entire batch of tubes by entering all labels and information in "Experiment" > "Experiment Layout".
3. Load your first sample on the SIT and click on "Acquire Data". As soon as flow stabilizes

(≈5 sec), and if everything is adjusted to your satisfaction, click on “Record Data”. The sample will run until the stop point is reached.

4. After stopping data acquisition the sample arm will automatically release the tube from the SIT. ***Do not try to manually force the sample arm down or to the side to release the tube: this will damage the cytometer!!*** Remove your sample tube and wait for the SIT cleaning to finish. If entering tubes manually, click on “Next Tube”, enter new sample information repeat steps 3-4.

ANALYZING DATA FILES:

1. Double click on the tube pointer to the left of the file you wish to view (it should turn green).
2. The Global data sheet associated with that data file will be displayed on the right monitor. Gates and regions can be adjusted at this time.
3. You may scroll through your files by clicking on “Next tube” to advance one file or by clicking on the current tube pointer for the next tube.
4. To print your data click on the first tube and then, holding down the Shift Key, click on the last tube file to select all files. Right click on the mouse and select “Batch Analysis” to print your results. Be aware that the batch analysis applies the same gates and regions to all files. If you want to change gates or regions on particular files it would be best to manually advance through the files and print each one individually.

LOG OFF or INSTRUMENT SHUT DOWN:

IF YOU **ARE NOT** LAST USER OF THE DAY (See whiteboard for last user)

Place a tube of 10% bleach on the SIT, click “acquire” and allow to run about 3 min.
Repeat with diH20
Remove tube
Under “File” tab select “Log Out” and then “Log Out **Only**”
It is not necessary to close the software and the cytometer should be left with no tube on the SIT.

IF YOU **ARE** LAST USER OF THE DAY (See whiteboard for last user)

Place a tube of 10% bleach on the SIT, click “acquire and allow to run about 3 min.
Repeat with diH20
Remove tube
Under “Cytometer” tab click on “Fluidics Shutdown”
Press OK for next 2 windows that pop up
After completion, close software and power off instrument

Failure to follow above procedures will result in a slap of the wrist!!!