FACSCALIBUR USER TRAINING CHECKLIST

Customers must sign into the Training log book so we can add them to the S Users list.
S Schedule for customers to sign up for machine use. Phone numbers on Calibur for help.

TURNING ON THE S FACSCALIBUR:
- Turn on the machine first then the computer.
  Fluidics:
- Filling and emptying sheath and waste tanks
- Preparation and location of the 1X PBS and 8X PBS sheath fluid
- Pressurizing/depressurizing the sheath tank
- Priming
- Run water while lasers warm up, green light/orange light on RUN for pressure
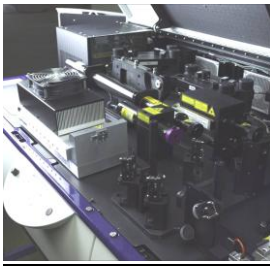
RUNNING FACSCOMP:
- Check the sample voltage
- CaliBRIGHT bead preparation and location in the refrigerator
- Running the beads in automatic mode
- Check to make sure it passed, quit program to print

CELLQUEST:
- Basics of making protocols
- Preference for acquisition to analysis dot plots and histograms
- Parameter selection
- Height parameters for general use vs. area and width for DDM parameter
- Using the toolbar, statistics markers vs. gating regions
- Gating: Toolbar or inspector. Saving gated, and displaying gated
- Saving a protocol to their user folder
- Using our protocols as templates
- Calling up old instrument settings

CYTOMETER:
- Acquisition dashboard, voltages, threshold, status (cover pressurization troubleshooting) and compensation. Use of the shortcut keys.
- Collecting Data: Use single color beads of all 4 colors
- Setting voltages on the unstained control sample
- Explaining spillover and why compensation may be necessary
- Setting compensation visually or mathematically with means, or leaving data uncompensated to do in FloJo
- Maintain voltages/compensation for the rest of the experiment
- Run, Standby. Do not leave on run. Do not have a sample tube on when in standby.

STORAGE OF DATA:
- Folder where data should be stored
- File name: unique with date and initials
- Write all info in the S logbook – time, folder, file, and PI, name
- Data is deleted after one month off the computer, but not protocols
- Turning off the setup checkbox to save data
- Allow sample flow rate (sample voltage) to stabilize before recording data
- Using the Fetch program to transfer data

CLEAN UP:
- Squirt bottles with 20% bleach and water
- Run each for 5 minutes
- Fill sheath and empty waste tanks
- Check the S Calibur schedule to see if it should be shut down
- Put in standby or depressurize, shut down computer and turn off cytometer.

For researchers interested in using Cytometric Bead Array (CBA) Assays
- Templates are in the BD folder

For researchers doing DNA analysis:
- Changing the DNA parameter to linear (FL-2)
- Turning FL-4 off
- DDM set to FL-2
- Threshold set to FL-2
- Putting G0/G1 peak to channel 200