Scanford Operation Guide
07-11-13

PPE must be worn at all times when in our BSL-2 environment
Lab coat, gloves, closed toed shoes, are mandatory, respirator and goggles are available

All users must have a new Windows account created by staff

1. If off, power on instrument (switch front top left corner).
2. The digital display panel shows fluidics manager status and blue 488 laser status (top right).
3. Check to be sure saline cube has adequate volume (full 45lb/empty 10lbs) and that waste does not need be emptied. Saline cubes are stored in hallway under the counters, and in LSR II room. Lift them carefully to protect your back. The waste tank is located on the floor, if full replace it with an empty prepared cube (located on floor to the left as you walk in room). Flip the sign on the full carton to Full and staff will take care of emptying it. If you empty it remember it has 10% bleach in it so wear gloves, eyewear (goggles or the facemask located above sink).
4. Log in to Windows using your account information
   Username=Sunet ID and password
5. Define BSL-1/BSL-2 level of experiment and fill out log sheet if you are running a BSL-2. Clean up procedures are defined at this time (10% Bleach for 1 min. and Water for 1 minute at the end of each experiment (all users) If running BSL-2 use Cavicide to clean work area. Clean out back flush tray with bleach and water at the sink.
6. Check saline filter and demonstrate air bubble removal and drain fill feature to remove air bubbles and clear flow cell of clogs (pull down panel at air filter gauge)
7. Machine is ready for use
For Calibration of Instrument

11. On the Desktop, open the Flowjo CE icon
   a) In Flowjo pop-up window, click the green icon (acquisition button)

8. You’ll then see the Cytometer Information and Data Scope pop-ups (If the Cytometer pop up fails to appear make sure you data storage path is C:\FACS Data\newfolder you create and name here)

9. a) In Cytometer pop-up, use the click wheel to select Menu function, in drop down menu select “open setting”
b) Navigate to “disk C” and select the SSFF Instrument Setting folder -- open

c) Select the configuration you need for your experiment
   The number of lasers you need will define which
   configuration file you use.

d) Select the template: Click “open” which brings up a
cytometer window, a data scope (workspace area) and
   population statistics window.

e) Check Laser status in the Device area.

f) Green laser has a weird warm up cycle. Define cycle to
   user (starts up super bright and then regulates down)

g) To define your data storage pathway, click on MENU disc
   and select “sample storage and naming”; in that window,
   browse to “C” drive

h) Click “FACS Data”

i) In the ”FACS Data” folder, create a new folder, then left click
   to rename

j) Enter your initials and current date

k) Select “folder”; your storage sample file should now read:
   C:/FACS Data/your newly created folder name

Training note: discuss filename option prefix some users may want to use it

10. Proceed to run 197 bead sample

11. If the Cytometer options functions are hidden hit arrow \( \n \) on
   bottom left of the Cytometer panel

12. In the collection area you define the # of events you want to
   collect. The default is 10,000, assign any collection stopping gates
   your experiment requires.

13. Define sample ID for each sample before you hit play (record)
    function. Enter 197 beads at sample ID space.

14. The Flow rate will appear at the top of Data Scope window
    Adjust flow rate to aprox 200/events per second for beads
    Record Bead data

15. Run the 197 beads and make adjustments to have beads in target
    boxes. New target values are posted on bottom of Display screen.

16. To label your fluorochromes go to the Workspace menu and Edit
    Columns
17. Select the parameters you are using and add columns. You can then click on these in the workspace and edit the names. Using the control E button you can copy these labels to all tubes.
18. Run your experiment, make sure to adjust scatter voltage and thresholds for cells or particles when needed and label your samples as you go along.
19. When done, quit FLowjo and log out of Windows
20. Do not do shut down computer
21. Start the cleaning procedure 1 min with 10% bleach on high speed,
22. Followed by 1 minute of DiH2O on High.
23. Switch machine to STANDBY
24. While the bleach/water is being run, check in your data by selecting, on desktop FACS Data checkin, follow the steps listed.
25. Log out of Computer
26. Do BSL2 cleanup if applicable.
27. Check the updated schedule on the computer (written one is not revised from 7am printing and will not be current) for next user time. Turn off Scanford if next user is more than 2 hrs later. If after hours or weekend, turn off unless next user has asked you to leave it on.
28. Leave Computer on all times.
29. When you leave check machine is in standby and computer is logged out (but left on!!)

Make sure to fill out BSL-2 sheet if you have run Biohazard
Please leave work area clean