Scanford Users Guide

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

An Instrument Configuration is located on the website in the Instrument Overview area
*The configuration lists the lasers and filters for each machine*

All samples must be in POLYSTYRENE round bottom tubes.
Fisher: BD Falcon 352052 (no cap) or 352058 (with cap)

What samples do I need to bring?
• All users **ALWAYS** need to bring single stained compensation controls
• If you have a 7 color panel you should bring: 8 compensation controls
  o One tube stained with each individual antibody
  o One unstained tube
  o No PI or viability dye in the single color control

Don’t have enough cells for compensation?
User can purchase compensation beads from many different suppliers
• eBioscience UltraComp Beads 01-2222-42 *(for panels including UV and Violet dyes)*
• eBioscience OneComp Beads 01-1111-42 *(for panels that do NOT include any UV or Violet dyes)*
• BD CompBeads 552845 ‘Anti-Rat/Hamster’
• BD CompBeads 552843 ‘Anti-Mouse’
• ThermoFisher Amine Reactive Compensation Beads A10628 *(for LIVE/DEAD Fixable stains)*

All user must have a Windows account created by staff
Starting Up the System
1. Check to see if the power is on. If off turn the power on with the switch in the front top left corner of the instrument.
2. The digital display panel shows the fluidics manager status and the **BLUE (488) LASER** status in the top right corner of the instrument.
3. Log in to Windows using your account information
   a. Username: SUNet ID
   b. Password: Name
4. Define BSL-1/BSL-2 level of experiment and fill out log sheet if you are running a BSL-2 experiment.
5. Check the saline filter. Remove any bubbles from the filter. The drain and fill feature will remove any air bubbles and clear flow cell of clogs.
6. IF you are using the **VIOLET (405) LASER** it must be turned on manually.
   a. The **VIOLET (405) LASER** adds two new measurement parameters VioFL1 and VioFL2, allowing up to 12 measurements on this instrument. The fluorochromes that are available are shown in the accompanying table.
   b. To use the **VIOLET (405) LASER**, you should choose the 4 Laser Setup from the SSFF instrument Setting folder. Although the settings will have the violet laser **ON** in the Device Panel, it must also be turned on manually.
   c. NOTE: IF you are NOT using the **VIOLET (405) LASER** this switch must be off to get accurate measurements from the **RED (640) laser**.

<table>
<thead>
<tr>
<th>VoF11</th>
<th>Best</th>
<th>Possible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pacific Blue, Alexa 405, Brilliant Violet 421,V450, DAPI</td>
<td>CFP, mCerulean, DyeCycle Violet, Cascade Blue</td>
</tr>
<tr>
<td>VoF12</td>
<td>live/dead Aqua Amine, BV510</td>
<td>Qdot 525</td>
</tr>
</tbody>
</table>

Scanford 20L Pump System Instructions (Cytek)
A fluid manager is in use on all our analyzers. All users must know this to get after hour use. Using the pump offers the following advantages:

**To use:**
1. The pump system turns on automatically with the Scan.
2. Verify that the pump is on. The Scan will not work properly otherwise.
   *Its switch is located on the blue box labeled “Cytek” under the Scan with the black lever switch.*
3. Check the sheath tank. Fill the sheath tank if there is not enough for your run.
4. Check the waste tank. Empty the waste tank if full.
5. Please email [facs-issues@stanford.edu](mailto:facs-issues@stanford.edu) with any problems or questions

**Changing the tanks:**
1. The cube is on a scale, so you can check the weight of the cube.
2. If the sheath cube is empty replace it with a new one (it is heavy so lift carefully) New cubes are located in either the hall or in the room.
   *Note: The cube closure just screws on – there is no pressurization of the cube. Be careful not to kink the tubing. Turn the outer ring only so you do not stress the wires for the sensors.*
3. Empty waste in sink in hallway when full, using the protective face shield, gloves and lab coat.

updated: 17 January 2018          by: MEW
Remember: Add bleach to line marked on carboy.

4. Alarm sensors are connected to the cube and the waste, indicating when they need attention if you forget. 
The Scan won’t run samples properly if the pump is turned off. 
*The pump should NEVER be turned off manually!!*

**Setting Up Your Experiment**

1. On the Desktop on the FLOWJO CE icon
2. A flowjo workspace will open up in the window, click the green icon *(acquisition button)*

![FlowJo Workspace](image)

**Laser Warm Up**

1. You’ll then see the Cytometer Information and Data Scope pop-ups (If the Cytometer pop up fails to appear go to step 2)
2. Go to C:\FACSData→create a new folder and select the new folder
3. Navigate to C:\ and select the SSFF instrument setting folder and click open
4. Select the configuration you need for your experiment. The number of lasers you need will define which of the template files you use.
5. Select the template: Click **OPEN** which brings up a cytometer window a data scope (workspace area) and population statistics window.
6. If the Cytometer options functions are hidden hit the arrow ↓ on the bottom left of the Cytometer panel
7. Check laser status in the device area.
8. Turn off laser you will not be using by checking the box next to them in the Device area.
9. The **GREEN (561) LASER** has a weird warm up cycle. It starts up super bright and then regulates down.

**DATA**

1. To define your data storage pathway click on the **MENU** in the disc and select “sample storage and naming” In that window, browse to C:\FACS Data\“your initials and current date and experiment name”
2. Select the folder: your storage sample file should now read:
   C:\FACS DATA\your initials and data and experiment name then select OK
3. In the collection area you need to define the number of events you want to collect. The default is 10,000
   assign any collection stop gates your experiment requires.
4. Label your sample using the SAMPLE ID

Calibration
1. Remove the water tube from the SIT. Place the machine fluidics control knob in the run position. Watch for
   a drop of saline to back flush of the SIT line.
2. Enter 197 beads at the sample ID space.
3. The flow rate will appear at the top of the Data Scope Window
4. Run the 197 beads
5. Adjust the flow rate to approximately 200 events per second of beads
6. The Flow rate will appear at the top of Data Scope window
7. Make adjustments to the PMT Voltages to match the target values. Target values are posted on the
   side of the monitor.
8. Record the Bead data. By hitting the PLAY button on the disc.
9. To label your dye names go to the Workspace menu in FLOWJO.
   a. Select edit columns
   b. Delete the unneeded parameters and remove column.
   c. In the workspace click the column and add your label.
10. Run your experiment, make sure to adjust the scatter voltage for your cells or particles as needed.
    FSC Gain: 10x Beads, 1x Cells and .1x Cells
11. If running compensation controls: acquire each of your single stained controls making sure they are on scale,
    adjust your PMT voltage if needed. Do this before recording any data.
12. Differential Pressure must be in the gray. If RED you are running to fast.
13. Label your samples before hitting the PLAY button.
14. Create a new tube by pushing >>| button. Red tubes are empty and yellow tubes have data.
15. Save the layout you have created by clicking the Menu button and save settings in C:\User Templates
    a. To bring in another template go to Menu and choose open settings and find your template
    b. Saved templates come in with previous PMT voltages so you will need to manually enter correct PMT
       voltages from the 197 beads QC.

Clean-up
1. Run BLEACH on HIGH for 5 minutes
2. Run WATER on HIGH for 5 minutes
3. Leave the machine in STANDBY and LOW with a tube of WATER on the SIP.
IF you ran BSL-2 samples follow the additional cleaning steps below:
4. Spray cavicide on all surfaces. Contact time for cavicide is 5 minutes unless your APB requires a longer period. *Please let staff know if different contact times are needed.*
5. Fill-out the biosafety clipboard next to the machine. List the biohazard, user, lab and clean up procedure.
6. Empty and flush the tupperware catch basin under the sample tube with bleach, then water, and dry with paper towels; dispose of the paper towels in the biohazard waste container.

**Finishing Up Steps**
1. Check in your data by selecting **FACS DATA CHECKIN** on the desktop
2. Turn off the **VIOLET (405) LASER** if you turned it on
3. Log out of Windows, but do not shut down the computer
4. The instrument should be left with 1mL of water in a tube
5. Switch the machine to **STANDBY**
6. Check the Sheath and Waste tanks. If the Sheath is empty you will need to fill it. If the waste is full you will need to empty it. Follow the directions on page 2.
7. Check the updated schedule on the computer for the time of the next user. Turn off the instrument if no one is scheduled for the next 2 hours. If it is after hours or on the weekend, please turn off the instrument unless you have been asked to leave it on.
8. Make sure you have filled out the BSL-2 sheet if you ran BSL-2 samples.
9. Please clean up your area and leave the space clean.

**Rules**

**Data Rules**
1. Do NOT use USB sticks, thumb drives, external hard drivers or any other form of USB with the computer.
2. Do NOT connect to the internet from this computer.
3. You MUST export your experiment.

**Running the Machine Rules**
4. You must FILTER your cells before running.
5. You MUST follow the cleaning procedure.
6. Leave the area neat and tidy.

**Notes on Data**

- Check in your data to protect it
- Data is deleted monthly. If it is not checked in it is gone.
- USB and personal back up devices are not allowed on our machines.
- Discs may be burned when alternatives are needed.
- A .jo file for MAC (legacy Flowjo 9.75 and older) and a .wsp (version 10 both Mac and PC). Will be sent to you through the data archive once your data is checked in
- Currently we are recommending FlowJo version 10.2
- You will be prompted for Username: ‘flowjo’ Password: ‘314159’.
Scanford Configuration

<table>
<thead>
<tr>
<th>488 nm Blue 30 mW (all detectors have 560 SP filter in optical path)</th>
<th>PMT</th>
<th>Splitter</th>
<th>BP filter</th>
<th>Possible Fluorochromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BluFL1</td>
<td>560 SP</td>
<td>525/50</td>
<td>FITC, Alexa 488, GFP, CFSE</td>
<td></td>
</tr>
<tr>
<td>BluFL2</td>
<td>640 LP</td>
<td>615/25</td>
<td>PI</td>
<td></td>
</tr>
<tr>
<td>BluFL3</td>
<td>640 LP</td>
<td>710/50</td>
<td>PerCP, PE-Cy5.5, PerCP-Cy5.5</td>
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<table>
<thead>
<tr>
<th>640 nm Red 100 mW (all detectors have 645 LP filter in optical path)</th>
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<th>Splitter</th>
<th>BP filter</th>
<th>Possible Fluorochromes</th>
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<tbody>
<tr>
<td>RedFL1</td>
<td>655 LP</td>
<td>661/16</td>
<td>APC, Alexa 647</td>
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<tr>
<td>RedFL2</td>
<td>655 LP &amp; 680 LP</td>
<td>710/50</td>
<td>APC-Cy5.5, Alexa 700</td>
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<tr>
<td>RedFL3</td>
<td>655 LP &amp; 747 LP</td>
<td>780/60</td>
<td>APC-Cy7, Alexa 750, Alexa 790</td>
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<table>
<thead>
<tr>
<th>561 nm Yellow/Green 100 mW (all detectors have 571 LP filter in optical path)</th>
<th>PMT</th>
<th>Splitter</th>
<th>BP filter</th>
<th>Possible Fluorochromes</th>
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</thead>
<tbody>
<tr>
<td>YelFL1</td>
<td>600 SP</td>
<td>590/20</td>
<td>PE, DsRed, tdTomato</td>
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<tr>
<td>YelFL2</td>
<td>600 SP</td>
<td>615/25</td>
<td>PE-Texas Red, PE-CF594, PE-Dazzle 594, mCherry</td>
<td></td>
</tr>
<tr>
<td>YelFL3</td>
<td>725 SP</td>
<td>690/40</td>
<td>PE-Cy5, PI</td>
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<tr>
<td>YelFL4</td>
<td>725 SP</td>
<td>740 LP</td>
<td>PE-Cy7</td>
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</table>

Use of YelFL3 for PI recommended when using FITC or Alexa 488.

<table>
<thead>
<tr>
<th>405 nm Violet Laser (Co-linear with Red Laser)</th>
<th>PMT</th>
<th>Splitter</th>
<th>BP filter</th>
<th>Possible Fluorochromes</th>
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</thead>
<tbody>
<tr>
<td>VolFL1</td>
<td>480 SP</td>
<td>450/50</td>
<td>Pacific Blue, Alexa 405, Brilliant Violet 421, CFP, mCerulean, DyeCycle Violet, DAPI, Cascade Blue</td>
<td></td>
</tr>
<tr>
<td>VolFL2</td>
<td>480 SP</td>
<td>545/30</td>
<td>Qdot 525, live/dead Aqua Amine, BV510</td>
<td></td>
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</tbody>
</table>

Filters:

LP - Long Pass filter: passes light with wavelength >= number shown.
SP - Short Pass filter: passes light with wavelength <= number shown.
BP - Band Pass filter: passes light with wavelength centered on first number and passband second number.