

## **BD FACSymphony A3 User Guide (SOP-014)**

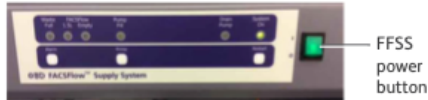
This is intended to be a user guide after training. For the most current instrument configuration please visit our webpage. <https://scripps.ufl.edu/cores-and-technologies/flow-cytometry/>

### **❖ Symphony Startup:**

**1) If not already on, turn on the computer and monitors.**

Computer Login: BDADmin Password: BDIS#2\$\$

**2) Check that FACSSupply System (FFSS), located below the instrument, is switched on then check sheath box level.**



- Check the status of the sheath lights on FFSS.
  - 1.5L light indicates that 1.5L of sheath remains (Full box contains 20L) (This may not be sufficient for long experiments and user should prepare another box if they will be working for a long time.)
  - Empty Light will turn on when empty and will alarm
- Lift box slightly to confirm sheath level.
- If empty, turn off FFSS switch, remove the cap place sensor in holder and replace box with a new box of sheath fluid.
- Place empty boxes on the table in the center of the room for disposal by the flow core staff. Please recap the boxes with the cap from the new box.

**\*Make sure to turn on FFSS switch after replacing cap and sensor.**

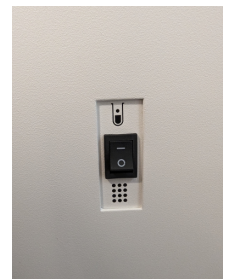
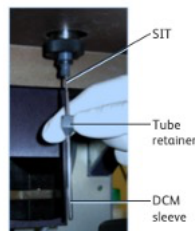
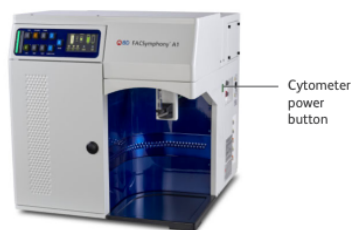
**3) Check Waste Tank Level by lifting tank and empty if near halfway full.**

- Check the status of the waste lights on FFSS
  - If full, the waste light will be lit and alarm will be on
- To empty waste tank:
  - Turn off FFSS switch, remove the sensor cap and place sensor in holder on side of FFSS.
  - Remove the larger waste cap in the rear of the bottle.
  - Empty the contents into the autoclave room sink with hot water running. Add ~500ml of bleach to the waste tank and close the lid. Reconnect the waste cap.

**\*Make sure to turn on FFSS switch after replacing cap and sensor.**

**4) If not already on, turn on the BD Symphony A3 (green button) and that the tube/HTS switch is in tube mode.**

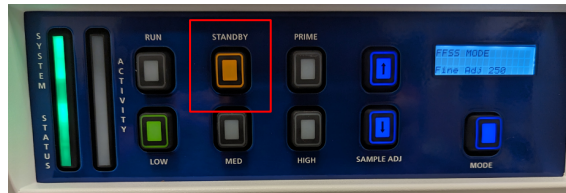
- **IF THE CYTOMETER IS ALREADY WARMED UP SKIP TO STEP 8.**
- For initial setup, make sure tube/HTS switch is set to tube mode
  - Also be sure the long SIT sleeve (DCM sleeve) is in place on the SIT.



**Allow a minimum warm-up time of 30 minutes for all lasers.**

Tube mode is the (1) position on the switch.

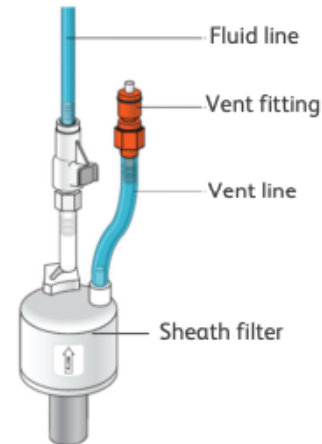
5) Make sure the cytometer is on STANDBY mode and that the FFSS switch is turned on.



6) Purge Air Bubbles:

- **Purge the sheath filter for air bubbles:**  
Purge air by tapping on filter gently and place a kimwipe on top of the orange vent fitting coming out of the filter. Press the top of the fitting until all the air is purged from the filter and fluid seeps out through the valve and into the kimwipe.

❖ Clean up all spills!



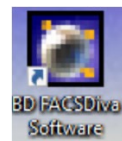
- **Purge the sheath line for air bubbles:**
  - Open the roller clamp located on the sheath line connector at the instrument and drain into beaker for 20 seconds. Close the clamp when the air bubbles have completely bypassed the instrument but leave open for at least 20 seconds or until air is removed from lines.



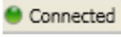
7) Rinsing the System:

- Press Run and High on the cytometer fluidics panel.
- Install a tube containing 3mL of Contrad (1:1 with water) on the SIP. Allow to run for 10 minutes.
- Install a tube containing 3mL of DI water on the SIP. Allow to run for 10 minutes.
- Press the STANDBY button on the fluidics panel.
- Place a tube containing 1mL of DI water on the SIP.

8) Launch DIVA software and login:



DIVA login: User Name      Password: Your password

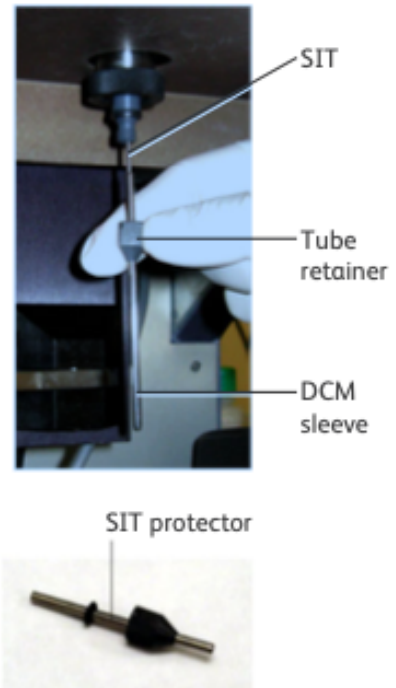
- Verify that the software is connected to the cytometer by looking for the green Connected status icon (  ) in the lower-right corner of the workspace.
- NOTE: It might take a few minutes for the software to connect.

❖ **For users who use microtiter tubes placed inside of the standard 5mL FACS tubes**

\*In order to accommodate this tube setup you will need to change the SIT protector sleeve to the short SIT sleeve as described below:

When using this setup DO NOT change the

- Remove the tube of DI water from the cytometer by placing the tube support arm to the side and pulling down on the tube.
  - Leave tube support arm to the side.
- Remove the droplet containment module (DCM) sleeve by unscrewing the tube retainer and carefully removing the sleeve.
- Attach the short sample injection tube (SIT) protector:
  - Slide the SIT protector up over the SIT until it reaches a hard stop.
  - Push up on the tube retainer until you can screw it onto the SIP.
  - Hand tighten the tube retainer.



Never place more than 1ml of any liquid on the Sit with the short SIT sleeve on because of backpressure issues.

(Short SIT Sleeve)

❖ **Make sure when you are done with your experiment that you change the SIT Sleeve back to the long sleeve (DCM Sleeve) before washing.**

- 1) Set the fluid control to '**STANDBY**'
- 2) Remove the short SIT (Sample Injection Tube) protector by unscrewing the top connector until it is loose and then removing it by pulling straight down to avoid bending the SIT.
- 3) Replace the DCM sleeve (Long SIT Cover) by sliding it over the SIT until it reaches a hard stop.
- 4) Push up until you can screw it into the Tube Protector.
  - a. Hand tighten the tube retainer, being sure not to over tighten.
- 5) Place a **NEW** tube with **~3ml** of water on the SIT and ensure the cytometer is in '**STANDBY**' mode and continue with washing procedure as described below.

## • Control Panel Overview:



- **RUN:** Pressurizes tube and starts fluid flow.
- **LOW, MED, HIGH:** Sample flow rate controls (all are fine for most samples)
- **SAMPLE FINE ADJ:** Fine tune flow rate.
  - BD recommends leaving at 250 at all times.
- **STANDBY:** Stops fluid flow.

- **PRIME:** Removes air bubbles and debris stuck in flow cell or SIP. Priming drains and refills the flow cell with sheath fluid. Unlike on the LSRII this is not recommended unless you have issues with flow rate.

## ❖ Create an Experiment:



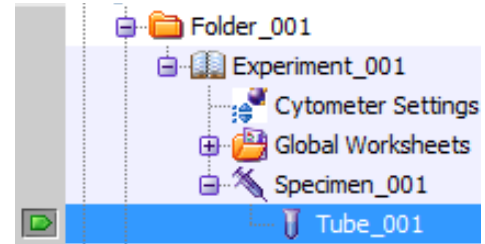
1) To organize experiments, create a new folder (Optional).

Create an experiment .

\*Open Experiment= Open Notebook

- 2) Add a specimen by clicking on the syringe icon. Specimens may be used to separate, organize, or categorize samples of a different type (i.e. cells lines). Click the plus (+) sign next to Specimen to expand and view tube.

- 3) Add additional tube(s) by clicking on the tube icon.



\*To rename a folder, experiment, specimen, or tube, right click on it and select rename or use the Inspector window.

## ❖ Specify the Parameters:

- 1) Click on Cytometer Settings just below the Experiment Name.
- 2) Using the 'Delete' button, highlight and delete all fluorescent parameters in the Parameters tab within the Inspector – Cytometer Settings window.
- 3) Using the 'Add' button, add parameters of interest using the drop-down menu.
- 4) For singlet gating: select 'H'(Height) and 'W'(Width) for forward and side scatter.

Cytometer Settings

Parameter	Voltage	Log	A	H	W
FSC	575	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
SSC	322	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
FITC	537	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PerCP	603	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
BUV395	428	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alexa 350	418	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
APC	472	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alexa 700	587	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
APC-Cy7	552	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
DiRed	479	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE-Alexa 594	605	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE-Cy5	498	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE-Cy5 5	493	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE-Cy7	540	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pacific Blue	430	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Add Delete

- Confirm Log and 'A' (Area) are selected for every fluorescent parameter. Confirm height and width are deselected.

Parameter	Voltage	Log	A	H	W
FSC	432	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
SSC	276	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
FITC	536	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

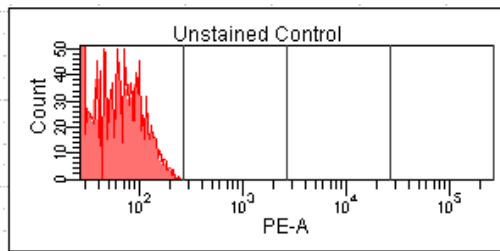
\*Unchecking the Log column will result in data being displayed in a Linear Scale.  
(For a cell cycle parameter, use a linear scale and add width).

### ❖ Acquisition Dashboard Overview:


\*Cytometer must be placed in RUN mode to acquire and record data!

### ❖ Compensation: If you are running a multi-color experiment, you may need to calculate compensation to account for spectral overlap.

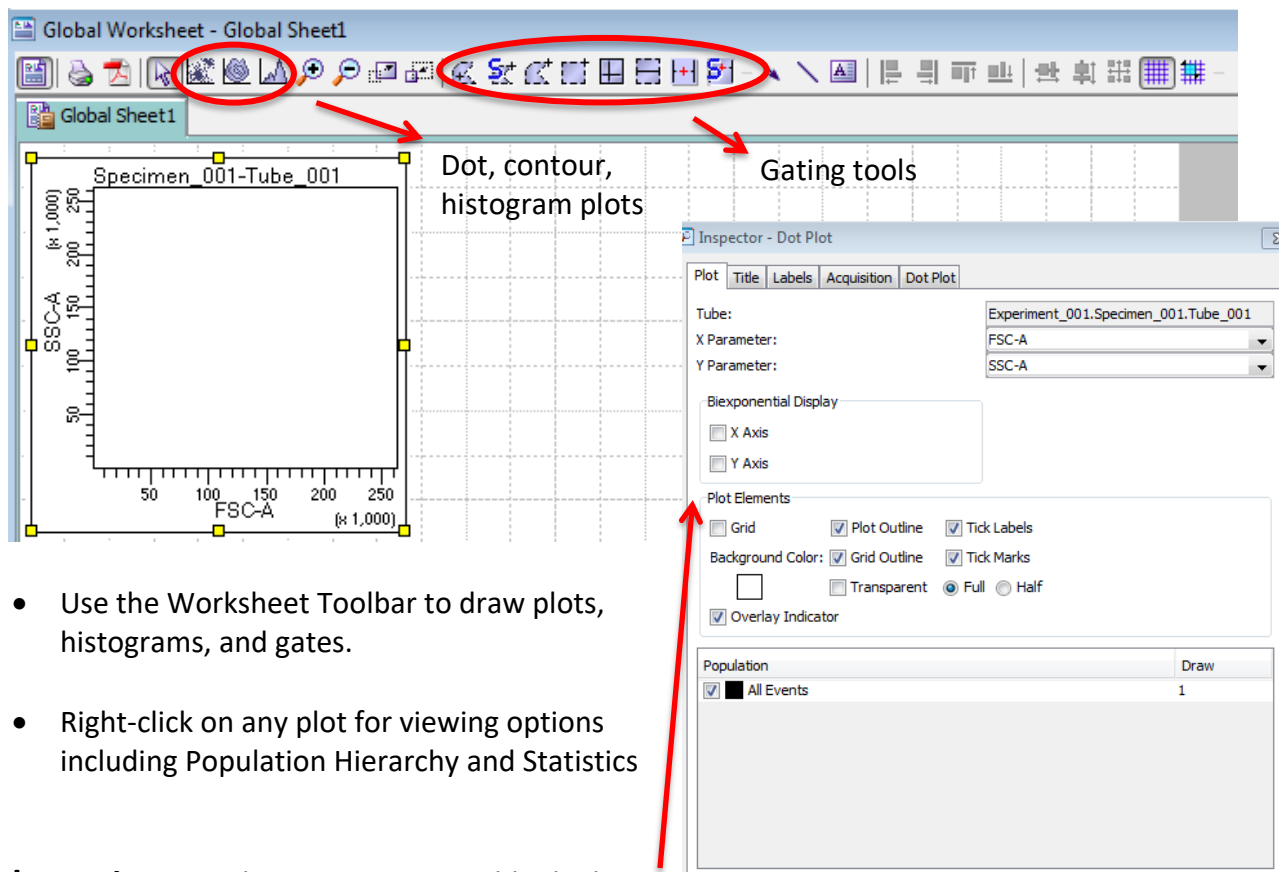
- Select Experiment>Compensation>Create Compensation Controls. Confirm the fluorophores and select 'OK'. A 'Compensation Controls' specimen and tube specific worksheets will automatically be created within a Normal Worksheet template. Open the specimen.
- Click the tube pointer arrow of the Unstained Control Tube (it becomes green).
- Place cytometer in RUN and LOW and load tube. Select Acquire on the Acquisition Dashboard and adjust the forward and side scatter voltages so the entire population is on scale, yet, separated from debris.
- Adjust P1 gate to fit desired population. Right click on P1 gate and select "Apply to All Compensation Controls'.
- Adjust voltages for each fluorescent parameter so the negative population for each fluorophore is set to  $\sim 10^2$ . This is an initial placement and may change during optimization of voltages.



\*Before recording negative control, it is recommended that you run all the single-color controls in the Unstained Control template to confirm the positive peaks for all fluorophores are on-scale. Adjust voltages as needed. It is expected that all antibodies, dyes, and fluorescent reagents have been titrated to optimize signal to noise and eliminate non-specific binding.

- 6) Once all the voltages are set, record unstained control, then record all single-color controls. Adjust the P2 gate over the positive peak.  
\*If the control tubes are a combination of beads and cells (i.e. negative cells and bead single color), add a P3 gate over the entire unstained peak (include axis) for each single color where the background autofluorescence differs than the unstained control.
- 7) After all single-color controls have been recorded, select Experiment>Compensation Setup>Calculate Compensation. Choose Link and Save and name if desired.  
\* An error window will pop up if compensation is done incorrectly. Ensure all fluorescent voltages are identical between each control and that a P3 gate is used when needed.
- 8) Toggle back to the Global Worksheet by clicking on the worksheet icon. 

❖ **Create the Template:** Use a Global Worksheet to apply the same analysis strategy to all tubes.



Dot, contour, histogram plots

Gating tools

Inspector - Dot Plot

Plot Title Labels Acquisition Dot Plot

Tube: Experiment\_001.Specimen\_001.Tube\_001

X Parameter: FSC-A

Y Parameter: SSC-A

Biexponential Display

☐ X Axis

☐ Y Axis

Plot Elements

☐ Grid ☒ Plot Outline ☒ Tick Labels

Background Color: ☒ Grid Outline ☒ Tick Marks


☐ Transparent ☒ Full ☐ Half

☒ Overlay Indicator

Population	Draw
<input checked="" type="checkbox"/> All Events	1

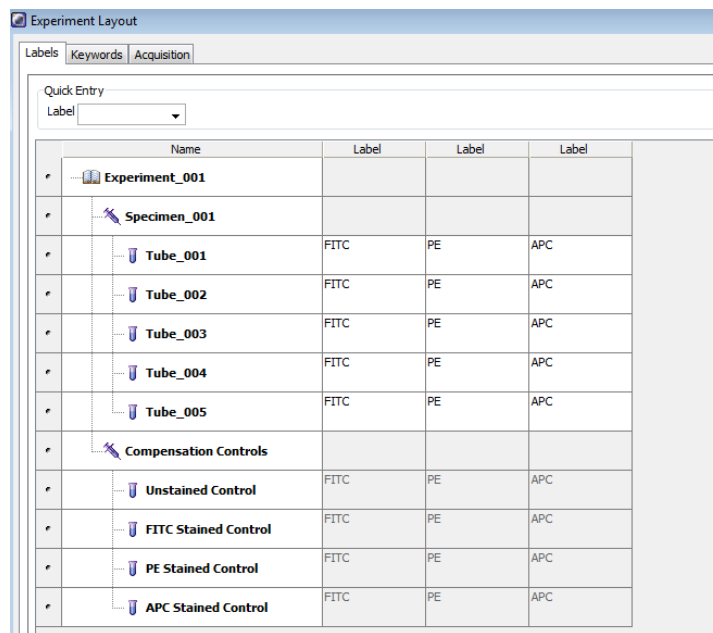
- Use the Worksheet Toolbar to draw plots, histograms, and gates.
- Right-click on any plot for viewing options including Population Hierarchy and Statistics

❖ **Inspector:** Use the Inspector to enable the bi-exponential display, as well other options for the worksheet, plots, and displayed tables.

❖ **Experiment Layout:** Use the  Experiment Layout to label parameters.

- Experiment > Experiment Layout
- Highlight label column and enter label

❖ **PDF Batch Analysis:** For data analysis after acquisition, run a Batch Analysis to create a '.pdf' file: In the Global Worksheet, confirm gates for analysis strategy and check page breaks. Highlight the experiment and first tube of experiment and right click >Batch Analysis. Follow prompts.



Name	Label	Label	Label
Experiment_001			
Specimen_001			
Tube_001	FITC	PE	APC
Tube_002	FITC	PE	APC
Tube_003	FITC	PE	APC
Tube_004	FITC	PE	APC
Tube_005	FITC	PE	APC
Compensation Controls			
Unstained Control	FITC	PE	APC
FITC Stained Control	FITC	PE	APC
PE Stained Control	FITC	PE	APC
APC Stained Control	FITC	PE	APC

❖ **Exporting:**

- **Export FCS files:** To export the raw '.fcs' data files for downstream analysis, highlight the experiment name, and right click >Export>FCS files. Follow the prompts, save to desktop and transfer to server. Please delete any files put on desktop when finished.
- **Export Experiment Template:** To export the '.xml' file to the D: drive, highlight the experiment name, and right click >Export>Experiment Template. Rename the template using your PI's last name, first name and add an experiment title.

❖ **Re-using an Experiment Template:** Select Experiment>New Experiment. Select experiment from list and select 'OK'.

❖ **Symphony Shutdown:**

- 1) After running samples, **CLEAN** with tubes containing ~3ml of bleach, then 1:1 diluted Contrad and then DI water. Set fluid control to **RUN**. Place tube on SIP and Run each tube on **HIGH** for 1 minute first with the support arm to the side (vacuum on), then with the support arm moved under the tube (vacuum off) for 5minutes. **Do not allow cleaning tubes to run dry!**
- 2) Set the fluid control to STANDBY and leave the water tube on the SIP.  
**\*Do not leave more than 1ml of water on the SIP**
- 3) Check SAAS calendar on computer desktop to confirm all instrument reservations. If no one is scheduled on the instrument after you then you are responsible for turning off the analyzer for the day, otherwise leave everything on for the next user of the day.

4) Turn off Procedure for the last user of the day. Shutdown the DIVA software, turn off computer, turn off cytometer.

- **Leave the switch on for the FFSS (fluidics cart) and also leave on the switch for the HTS.**

5) Clean-up work area/spills. Discard sample tubes.

## ❖ Troubleshooting

- If sample is not flowing:

Possible causes:	Solutions:
Air in sheath filter/sheath line	Purge sheath filter. Refer to step 6 in Startup Procedure
Debris in sample	Re-filter sample
Sample tube cracked	Replace sample tube
Clogged SIP	Inspect sample/re-filter. Clean SIP as in shutdown procedure. Prime extensively.
Sheath empty!	Refill and remember to always follow startup procedures
Software frozen	Restart computer

- If Cytometer and Computer disconnect communication, the status window will read “disconnected” and software operation will be restricted. Wait 2 minutes to allow connection to reestablish. If it does not, restart computer and cytometer.
- For other troubleshooting options please get assistance from the Flow Core.
- After hours, please call using emergency contact info on door
- [Scrps-flowcore1@mail.ufl.edu](mailto:Scrps-flowcore1@mail.ufl.edu)

## ❖ Transferring Data

- Always transfer your data to your personal server or dropbox. Experiment data older than 30 days will automatically be deleted **without notice**. USB thumb drives are not allowed. See directions for accessing the network server.

***All independent users are always expected to follow ALL startup/shutdown procedures.***