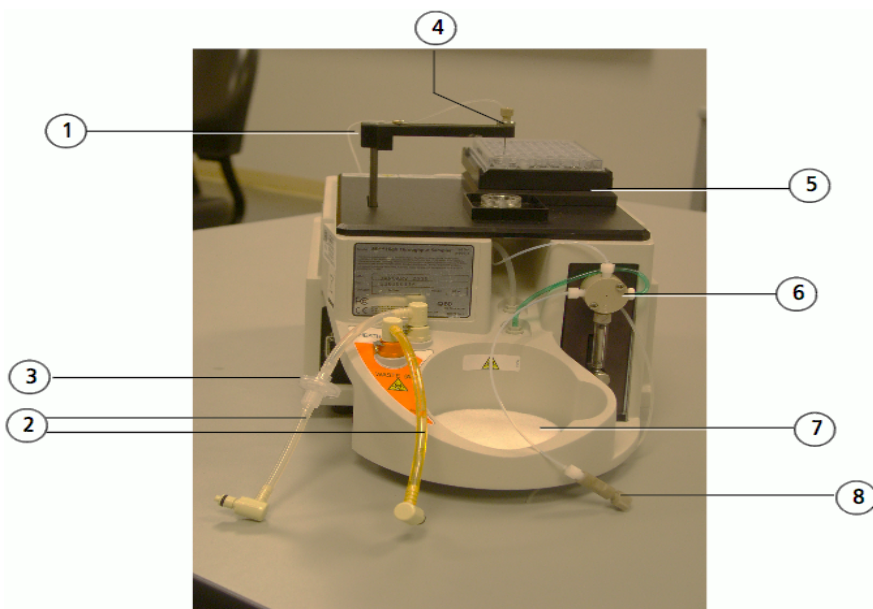


## **High Throughput System (HTS) User Guide (SOP-015)**

This user guide assumes the user has completed Symphony and HTS training, is familiar with BDFACS DIVA software and is deemed an independent user by the TSRI Flow Core Staff-FL. Refer to the Symphony user guide (SOP-) for information on experiments, parameters, compensation, and templates.

This SOP will allow you to properly connect, turn on, prime, clean, run an experiment, shutdown and safely put away the HTS. Ensure that enough time is reserved on the cytometer to run all plates as well as the cleaning procedure.

HTS System Overview:



- |   |  |
|---|--|
| ① | Probe assembly—moves front to back and up and down to transfer sample between plate holder and injection port/wash station             |
| ② | Fluidics tubing: sheath (clear) and waste (orange)   |
| ③ | Sheath filter—filters incoming sheath fluid to HTS unit  |
| ④ | Injection port/wash station—provides interface for sample injection and probe washing  |
| ⑤ | Plate holder—moves left to right and front to back to position plate so the probe can pick up sample                                   |
| ⑥ | Secondary pump and valve—delivers sample to flow cell in high-throughput mode  |
| ⑦ | Absorbent pad—collects potential overflow from the injection port/wash station or drips from the cytometer sample injection tube (SIT) |
| ⑧ | Sample coupler—connector between HTS unit to cytometer SIT   |

- ❖ **Follow the Symphony Startup Procedure, SOP-** (located at cytometer workstation). The system may never run dry, as this will damage the HTS pumps!
- ❖ Verify that the cytometer is in '**STANDBY**' mode.

- ❖ **Standard Tube Setup:** It is recommended to set voltages and compensation for the experiment using a tube setup. Once cytometer settings and compensation are established, proceed with HTS connection and acquisition. Refer to Plate Mode Setup section in this guide if not using a standard tube setup. We suggest this as setting voltages in the plate is cumbersome and there is not much time during plate mode acquisition to allow for you to change your voltages in the setup wells. Once you have established your voltages and settings for your samples and controls, you may be able to use the plate to run everything including setup wells and controls. Let us know if you need help.

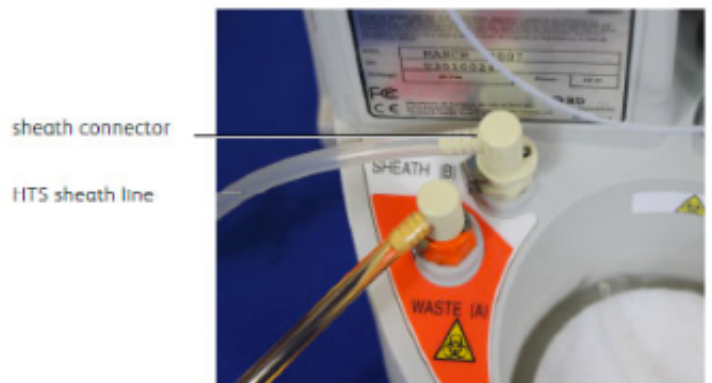
- ❖ **Connect the HTS:**

\*Verify that the cytometer is in 'STANDBY' mode \*

- 1) The HTS unit should already be turned on and connected to the Symphony with the Sheath line and waste line connections.

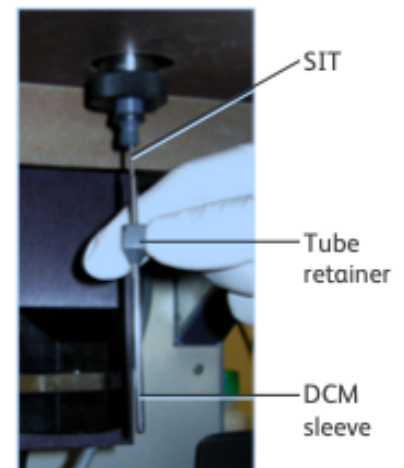
(Verify the connections as show here) →

Sheath line (clear with filter) and the waste line (orange tubing)



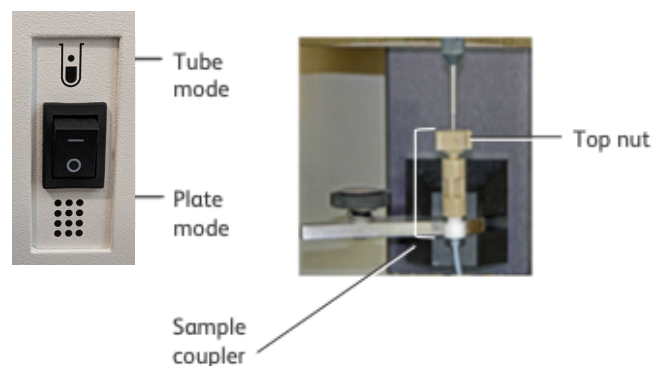
- 2) **Switching to Plate Mode**

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



(Short SIT Sleeve)

- d. Switch the acquisition mode switch to plate mode.



- e. Attach the HTS sample coupler to the cytometer SIT.
- Carefully slide the sample coupler onto the SIT until you reach a hard stop.
  - While holding the sampler coupler with one hand, tighten the top nut (clockwise viewed from the top) with the other hand.
    - There should be a gap between the SIT protector and the nut.
    - Make sure the tubing is not kinked or twisted and adjust as needed.
    - Check for leaks around the connections.

3) **Initialize the HTS Unit.**

- Verify that the HTS power switch is in the ON position.
- Install the HTS cover.
- Press the 'RUN' button on the Symphony control panel.

4) **In BD FACSDiva Software, select HTS > Reinitialize.**

- NOTE:** If the HTS does not initialize, verify that the cytometer is in RUN mode and that the HTS cover is aligned.
  - The HTS probe performs a homing sequence during initialization.

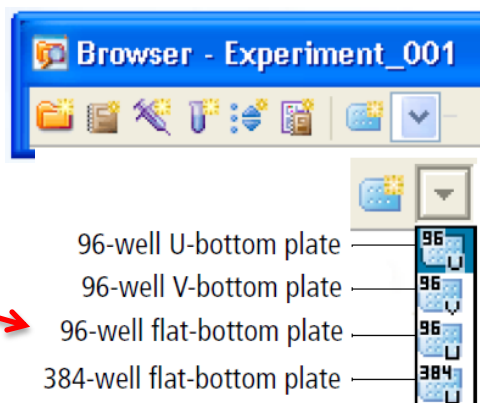
5) **Prime the HTS fluidics.**

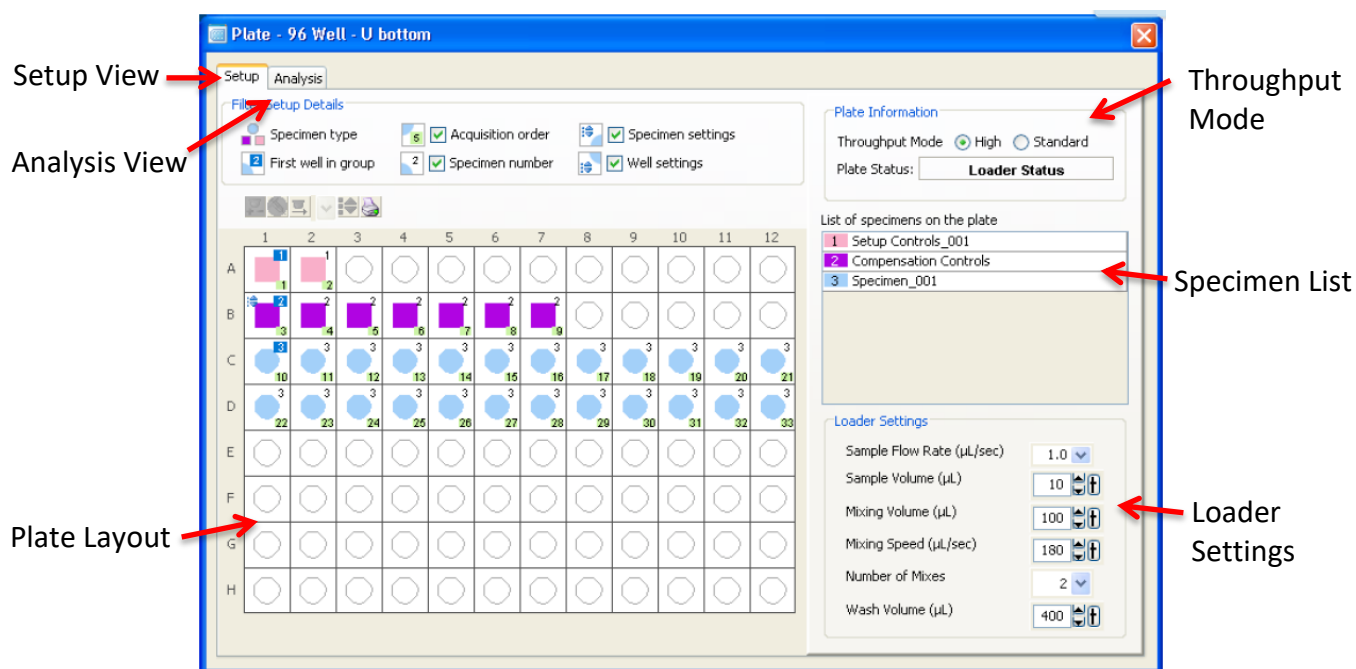
- Select **HTS>Prime**. Prime the system x2. Each prime cycle will last approximately 1 minute. There is a slight delay before the prime cycle begins.

❖ **Plate Selection:** Choose the plate well type in drop down menu. The HTS is only compatible with standard depth 96 or 384 well plates.

❖ **Plate Overview:**

\*96-well flat bottom plates have a greater dead volume and are **not** recommended.






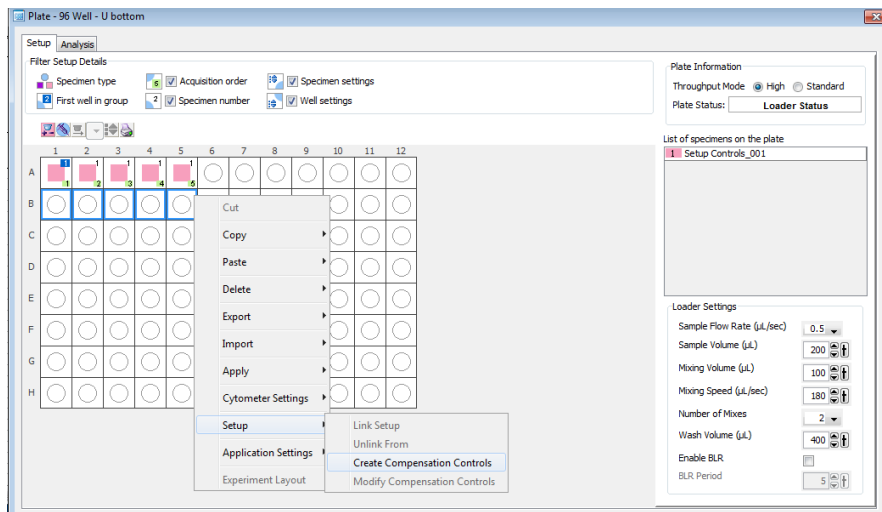
❖ **Select Throughput Mode:** In the Plate Setup View, choose Standard or High throughput mode.

Approximate Total Acquisition time using default loader settings:

- High throughput mode ~15 minutes
- Standard mode ~45 minutes

❖ **Plate Mode Setup (Optional):** Although it is recommended to run all setup controls in tubes before connecting the HTS, it is possible to run setup controls in the plate.

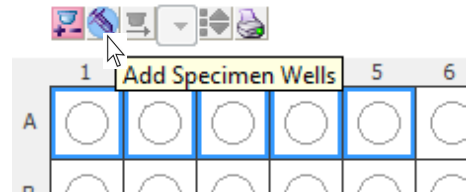
- Setup wells (**Pink Squares**): Click and drag to select a well or group of wells, then click the 'Add Setup Controls' icon  to add well(s) to the plate layout. These wells are for setup of scatter and fluorescent parameter voltages only and are NOT recorded.
- Compensation wells (**Purple Squares**): Click and drag to designate the compensation wells. Right click and select >Setup>Create Compensation Controls. Follow the prompts.




## ❖ Creating Specimens and Wells:

- **Add Specimen:** Select well(s) and click the 'Add Specimen Wells' (syringe icon) to add a specimen. Right click the specimen to rename.

\*Wells will be acquired in the order they are created!

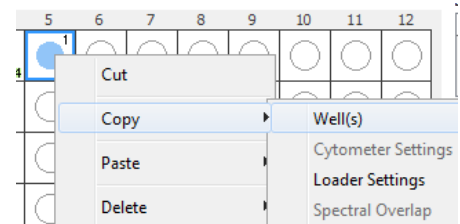


- **Add Additional Well:** Select a well that has already been previously created and click the 'Add Well'  to add another sample to that specimen. Select if you want to add to the right or below the well.

- **Copy and Paste Wells:** Select the well(s) to copy and right click >Copy>Well(s). Select the new wells to create and right click >Paste>Wells(s).

\*Copying a well will carryover all well specific information, cytometer, acquisition, and loader setting details.

- **Delete Specimen or Wells:** Select the specimen(s) or well(s) to delete, right click and select Delete.



❖ **Sample Well Volumes:**

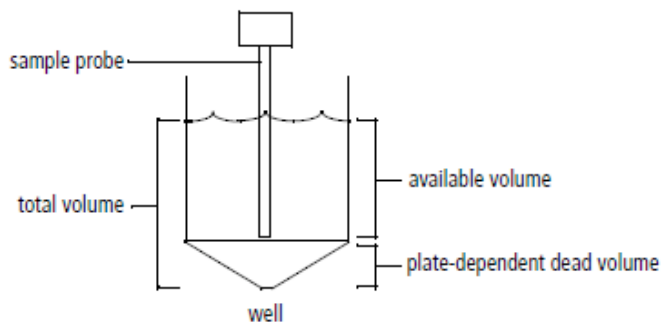


Plate Type	Dead Volume
96-well U-bottom	30ul
96-well V-bottom	TBD
96-well flat-bottom	TBD
384-well flat-bottom	TBD

Volume Type	Definition
Well volume	Volume the well can hold to the brim
Dispensed volume	Volume pipetted into well minus aspirated excess volume
Aspirated excess volume	Standard mode = 20ul
Available volume	Dispensed volume minus dead volume
Minimum volume	50ul for both standard and high throughput mode for 96-well plates
Mixing volume	Approximately ½ the available volume.
Dead volume	Volume in the bottom of the well the probe cannot reach
Sample volume	Amount of sample requested to be analyzed
Total Volume	Available volume plus plate dependent dead volume

❖ **Specify Loader Settings:** In setup view, adjust the loader settings. Loader settings may be varied for each individual well or specimen.

**Loader Settings**

Sample Flow Rate (µL/sec)

Sample Volume (µL)

Mixing Volume (µL)

Mixing Speed (µL/sec)

Number of Mixes

Wash Volume (µL)

Settings*	Standard Mode		High Throughput Mode	
	Default	Range	Default	Range
Sample flow rate (ul/s)	1	0.5-3.0	1	0.5-3.0
Sample Volume (ul)	10	2-200	2	2-10
Mixing volume (ul)	100	5-100	50	5-100
Mixing speed (ul/s)	180	25-250	200	25-250
Number of mixing cycles	2	0-5	2	0-5
Wash volume (ul)	400	200-800	200	200-800

\* Loader settings should be optimized for your cell type, volume, and concentration. The loader settings will impact mixing efficiency, carryover, and throughput. Refer to the Loader Settings Consideration Table for more information.

❖ **Loader Setting Descriptions and Considerations:**

Loader Setting	Description	Considerations
Sample Flow Rate (ul/sec)	Speed the syringe delivers sample from the well.	The greater the value, the higher the throughput but may cause variation of data.
Sample Volume (ul)	<p>Volume of sample aspirated from each well used for acquisition.</p> <p>Standard mode: Aspirates selected volume (2-200ul) plus 20ul excess volume.</p> <p>High throughput mode: Aspirates a fixed volume of 22ul inclusive of selected volume (2-10ul).</p>	<p><b>Important:</b> Each well must contain sufficient sample for the sample volume, aspirated excess volume plus the dead volume!</p> <p>Recommended minimum plate volumes:</p> <ul style="list-style-type: none"> <li>96-well plate: <ul style="list-style-type: none"> <li>Standard mode- 250ul/well</li> <li>HTS mode- 100ul/well</li> </ul> </li> <li>384-well plate- 50ul/well</li> </ul>
Mixing Volume (ul)	Volume of sample aspirated and dispensed during mixing.	<p>This value should be <math>\frac{1}{2}</math> the <b>available volume</b> to avoid introducing bubbles.</p> <p>Mixing volume may not be greater than available volume!</p>
Mixing Speed (ul/sec)	Speed the syringe aspirates sample from and dispenses to well.	Increasing mixing speed may improve throughput, but it may introduce air bubbles, compromise air bolus between samples, result in higher carryover, and could increase the possibility of cell shearing for delicate cells.
Number of Mixes	Number of mixing cycles completed before sample is aspirated.	A greater number of mixes may allow you to decrease mixing volume and speed but may impact sample throughput.
Wash Volume (ul)	Volume of sheath fluid dispensed for rinsing between wells.	A greater wash volume will reduce cross contamination. A lower volume will increase throughput.
BLR Baseline Restore Period	Ignores initial data for a period of time (Settings of min of 5 to max of 150 are equivalent to 50 to 1500 milliseconds).	This setting allows you to delay recording for the specified amount of time which may help to reduce well to well carryover.

Alternating Water Wells	Place wells of water alternating in between your sample wells.	Placing wells with only water on your plate and including those as samples in your run can help to reduce your well to well carryover.
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#### ❖ Assign Loader Settings:

- **Assign Loader Settings to Specimen or Well:** Select the specimen(s) from the Specimen List or Select the well(s) in the plate layout and enter the desired loader settings values.
- **Copy and Paste Loader Settings** Select the specimen(s) or well(s) to copy, right click> Copy >Loader settings. Select the well(s) to paste settings into and right click> Paste>Loader settings.

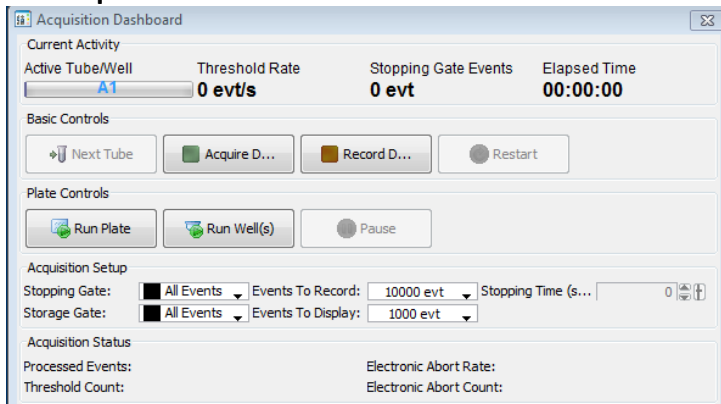
\* If multiple wells are selected, a red highlight will appear around the loader settings that are different.

#### ❖ Experiment Layout: Select Experiment>Experiment Layout>Acquisition tab.

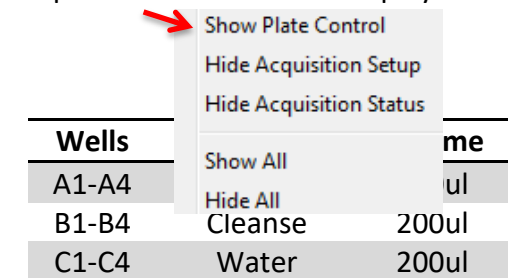
- Use the Experiment Layout to rename specimen or wells, set events to record, assign global worksheet and indicate stopping gate criteria.

#### ❖ Loading a plate: Remove HTS cover; install plate with well A1 on the back-right corner of the stage. Re-install the HTS cover. The HTS will not run if the cover is not installed!

## ❖ Acquisition Dashboard:



\*If Plate Controls are hidden, right click on grey space on acquisition dashboard to display.



- **Running Plates and Wells:** Click 'Run Plate' to run entire plate. Click 'Run Wells' to run selected wells. After homing and priming, acquisition will begin.
- **Pausing the HTS:** Click 'Pause.' The unit completes processing current well and remains paused until you choose 'Resume.' The NEXT well will be aspirated when choosing Resume.
- **Stopping the HTS:** Click 'Stop Well' or 'Stop Well(s)' to stop acquisition.

\*Stopping a well in Standard Mode will result in loss of current well. Stopping a well in High Throughput Mode will result in loss of the current and subsequent well.

❖ **Clean and Disconnect the HTS:** To ensure the HTS unit remains in optimum working condition, users are required to run a clean procedure at the end of each acquisition. Cleaning plates can be found on the shelf below the unit on the utility cart. Please allow 15 minutes for the cleaning process.

- 1) Fill all the cleaning wells, A1-A4, B1-B4, and C1-C4, with deionized water and flick away.
- 2) Completely fill wells on cleaning plate as indicated in table to the right.
- 3) Install the cleaning plate with well A1 on the back-right corner of the stage. Install the HTS cover.
- 4) While in an opened experiment file, Select **HTS>Clean.**
- 5) Select the '**HTS Daily Clean.**' Click OK. The Daily Clean Protocol view will appear.
- 6) Click OK to start cleaning protocol. There is a slight delay before the cleaning process begins.
- 7) When the cleaning sequence is finished, a dialogue window will appear. Click OK.
- 8) Prime the HTS: select **HTS>Prime**

- 9) Remove the plate and flick away the remaining cleaning solution. Rinse all cleaning wells with deionized water, flick away, and return plate to cart.
- 10) Set the fluid control to '**STANDBY.**'
- 11) Leave the HTS unit on.
- 12) Detach the HTS sample coupler from the SIT: Unscrew the top nut with one hand (counterclockwise viewed from top) and with the other hand carefully pull the sample coupler straight down and away from the SIT, ensuring the nut remains attached to the sample coupler.
- 13) Remove the 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.
- 14) Replace the DCM sleeve (Long SIT Cover) by sliding it over the SIT until it reaches a hard stop.
- 15) Push up until you can screw it into the Tube Protector.
- 16) Switch the acquisition mode switch to tube mode.
- 17) Place a **NEW** tube with **~3ml** of water on the SIT and ensure the cytometer is in '**STANDBY**' mode.
- 18) Check SAAS calendar on computer desktop to confirm all instrument reservations. If no one is scheduled on the instrument after you (greater than three hours between appointments), you are responsible for turning off the analyzer. Shutdown the DIVA software, turn off the computer and turn off the monitors.
- 19) Ensure the cover is on HTS.

❖ **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. Select Analysis View, highlight all wells to batch, and right click > Batch Analysis.

❖ **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.  
\* If you have files recorded from different specimens (i.e., control/compensation tubes and samples from plate), you must export the FCS files separately rather than all at once.
- **Export Plate Template:** To export the plate template to use for future similar experiments, highlight the plate in the Browser window, and right click >Export>Plate template. Rename the template using your PI's last name and add an experiment title.

❖ **HTS Troubleshooting:**

Observation	Possible Causes	Solutions
Leaking around sample coupler	Coupler or tube retainer is loose	Check the fittings and tighten them as needed.
Leaking around pump syringes	Fitting is loose or worn	Tighten the fitting. If replacement is necessary, contact Flow Staff.

❖ **Acquisition Troubleshooting:**

Observation	Possible Causes	Solutions
Bubbles in sample wells	Mixing volume too great	Reduce the mixing volume.
	Too many mixes	Reduce the number of mixes.
	Insufficient volume	Increase the sample volume.
Unexpected low event rate, or no events	Clogged probe	Run Clean Cycle Plate. Inspect sample. Re-filter. If problem persists, contact Flow Staff.
	Insufficient sample	Inspect sample. Adjust as needed.