General note

Using the FACSymphony HTS requires a separate introduction. Please contact the facility at info@cytometry.uzh.ch to schedule an appointment.
Do not use the HTS if you need to analyze every cell in your sample because the HTS system uses part of the sample to prime the tubing and has a dead volume.
Injected sample volume is discarded once the FACSymphony reaches the stopping criteria (e.g. # events to record). There is no way to get the remaining sample back into the well.

Sample Preparation

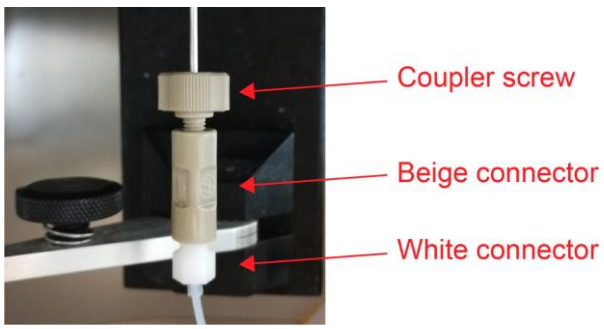
- For initial tests, use a large enough sample volume (≥100 µl/well). To calculate the total sample volume needed, you need to take the dead volume and the excess aspiration volume into account. The dead volume in the well is ~35 µl (varies slightly depending on plate type). This is the volume the needle cannot reach. When sample is aspirated, the HTS will aspirate more than the specified volume:
    - In Standard mode: 20 µl
    - In High mode: fixed aspiration volume of 22 µl
- Filter your cells prior to analysis to avoid sample line clogging.
- To ensure efficient runs optimize your cell density to achieve ~2'000-10'000 events/s ($10x10^6$ cells/ml is a good starting point for PBMCs; start optimizing for cell lines from $1x10^6$ cells/ml).

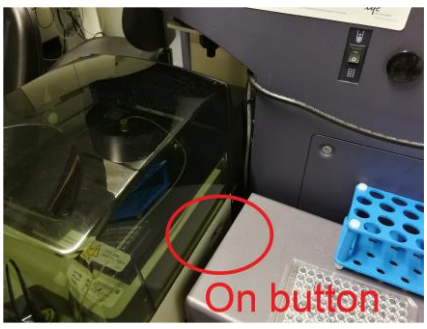
Starting the FACSymphony using the HTS

- Start the FACSymphony as for normal tube acquisition.
- Prime the FACSymphony 2× using the button on the instrument.
- Check the FACS Flow supply and the waste level of the FACSymphony. You want the sheath tank at least ¼ full and half an empty waste so you do not have to interrupt your plate run.
- Check that there is water left in the tank on top of the FACSymphony.
- **Switch Fluidics on by pressing the run button on the instrument.**

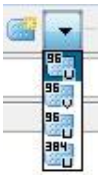
- Set the switch on the right of the SIP to plate mode.
- Attach the "HTS Sampler Coupler" to the bottom of the sample needle. First, ensure that the white connector is securely screwed in to the beige connector. Next, screw the beige coupler screw into the beige connector. Then, unscrew the beige coupler screw ½ turn so that it is loosened. In this loosened state, slide the sample coupler onto the SIT until it reaches a soft stop – THEN push slightly more until it reaches a hard stop (hold on the sample needle to support stability). Finally, hold the lower part of the beige section with one hand while tightening the beige coupler screw with the other hand.

  If the coupler is salty dismantle it and rinse the thread with water.





- Turn on the HTS device via the power switch on the back (see above).
- Place the cover on the HTS and start the FACSDiva software. If the software is already running, close it and restart the software.
- After FACSDiva has started, it should automatically start the initialization and priming process. If the HTS does not start to move, select "HTS" → "Re-initialize". During the initialization, check for leaks on each of the 2 syringe pumps and also at the SIT where the HTS is connect via the sample coupler. For all HTS operations, the cover must be in place or you will receive an error message.
- Define basic settings for the experiment (create experiment, select appropriate channels and parameters).



- Create a plate of the correct type (96/384, flat/round/V bottom) via the dropdown menu next to the "New Plate" button in the browser.
- Generate experimental setup in the plate view window and define "Throughput mode"
    1. *High* → fast because it run 2 pumps in parallel (sample injection/washing). In this mode the HTS aspirates 22 µl/well independent of the sample injection volume you choose. You can define to record 2-10 µl out of this.
    2. *Standard* → slower, but allows sample volumes > 10 ul to be injected. When setting the sample volume, keep the excess aspiration volume (20 ul) and the dead volume in mind.
- Create Specimens in the plate e.g. set up controls, compensation samples and normal samples. **If you need to setup voltages**: we strongly recommend to use tube acquisition instead of plate wells. If you want to record compensation sample in the plate, the compensation wells need to start at the A1 position or they will not be recognized by the software.
- Select at least one well and start measurements via the Acquisition Dashboard by pressing "Run plate" or "Run wells" (only runs selected wells). If the plate control buttons are not shown, right click on the Acquisition Dashboard and select "Show Plate Controls". If the plate controls stay grayed out, make sure that the worksheets are in "Global" mode.
- Prepare the cleaning plate with 250 µl FACSClean in A1-A4, 250 µl FACSRinse in B1-B4.
- In Diva select "HTS" → "Clean" and follow instruction (the cleaning requires about 10 min).
- Perform "HTS" → "Prime" to rinse the HTS with H_2O.

Switching back to Manual Loader for the next user

- Switch the FACSymphony to Standby mode.
- Switch back to tube mode (next to the SIP) and shut down the HTS via the switch at the back.
- Take off the HTS sample coupler from the sample needle (loosen the top screw first).
- Place a dH_2O water tube on the SIT and bring the sample support arm back into place.
- Clean HTS cleaning plate with dH_2O and store next to HTS.

Trouble shooting the HTS and optimization

- Clogs are most likely to happen in the sample needle of the FACSymphony (not within the HTS).
- Check coupler for leakage → disconnect, dry coupler, re-attach until resistance point, tighten.
- Prime HTS 2× to flush out any potential air in the tubing.

- Check that the H_2O line of the HTS is not pinched by the cover of the HTS and that there is still water in the tank on top of the instrument.
- Vortex plate or increase resuspension cycles before injection.
- Optimize sample concentration (remember that the minimum speed of the HTS 0.5 µl /sec equals a MEDIUM flow rate setting in tube mode) → HTS is very sensitive to blockages → if you have "sticky" samples use some Clean & dH_2O wells in between your samples and make sure you clean well in the end!
- Check that you have enough volume in the wells, air bubbles will render the whole system useless → prime if necessary.
- Note: in plate mode it is not possible to append the data acquisition.
- The recommended mixing volume is half the well volume. High mixing speeds can lead to increased carry over.
- To test for carry over, prepare a well with just buffer after a sample well. The cells that you detect for the buffer well are carry over from the well before.
- To reduce carry over, increase the wash volume and adjust your sample concentration. If that is not enough, place wash wells between the sample wells.
- If populations look broader than expected, reduce the sample flow rate. The minimum sample flow rate on the HTS is 30 µl / min (0.5 µl / s), corresponding to the "Medium" flow rate setting for tube acquisition.