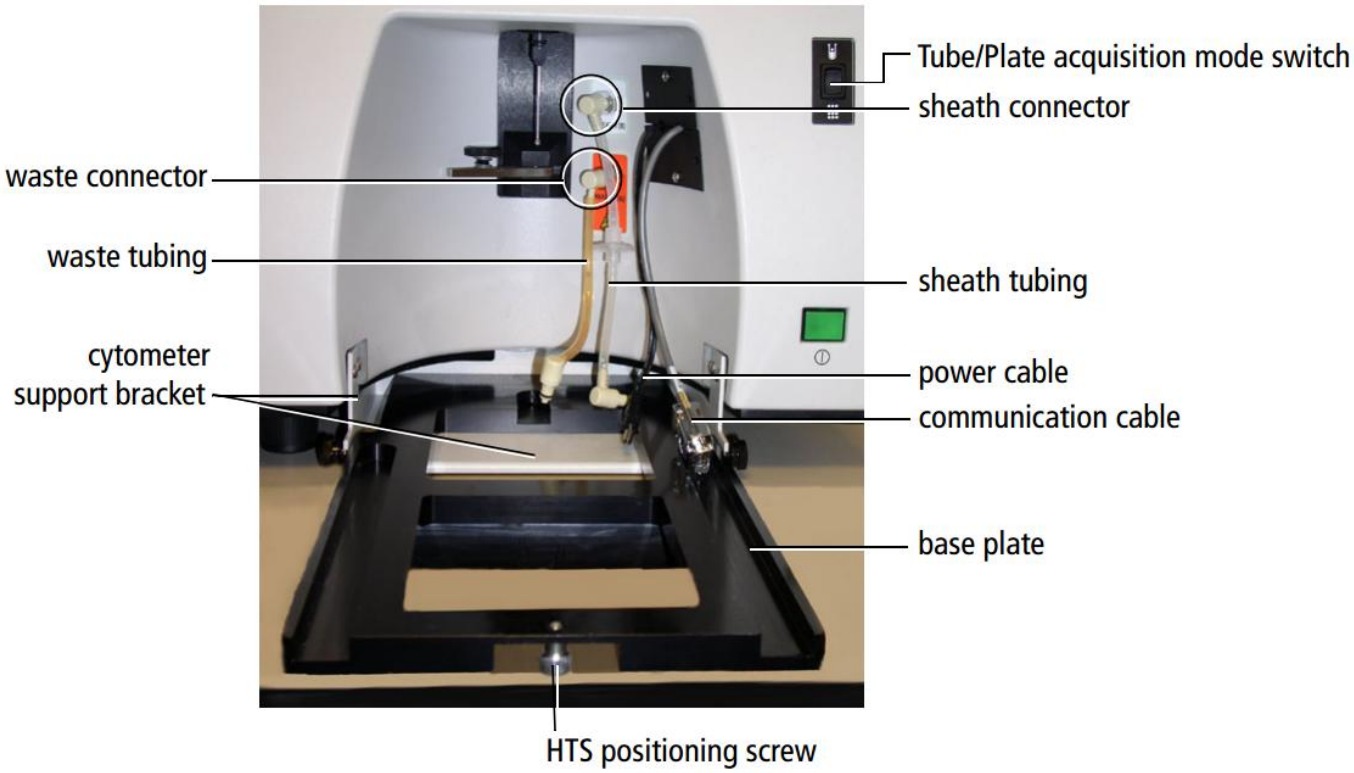
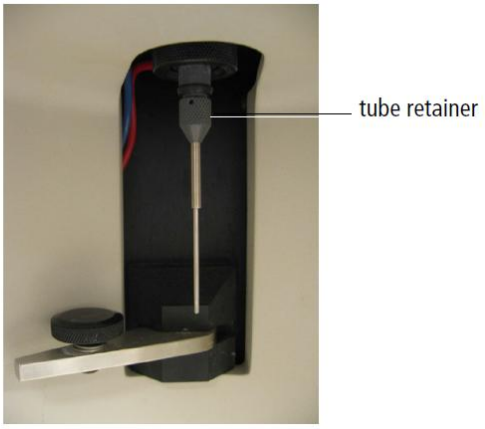
# LSRII HTS Operation

1. **Classic Setup**
   - Using an unstained control, adjust FSC, SSC, and all the fluorescent parameters voltages

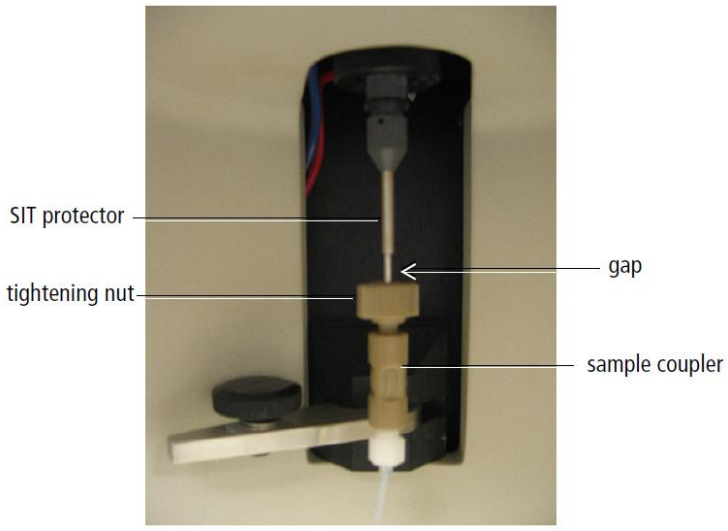
2. **HTS Installation** (base plate, sheath and waste connectors, communication cable, and power cable)
   - Flip the Tube/Plate acquisition mode switch to Plate




   - Replace the DCM (Droplet Containment Module) sleeve with the SIT Protector

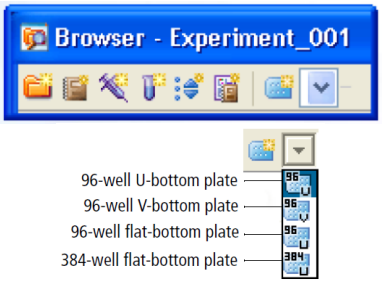
- Attach the Sample Coupler



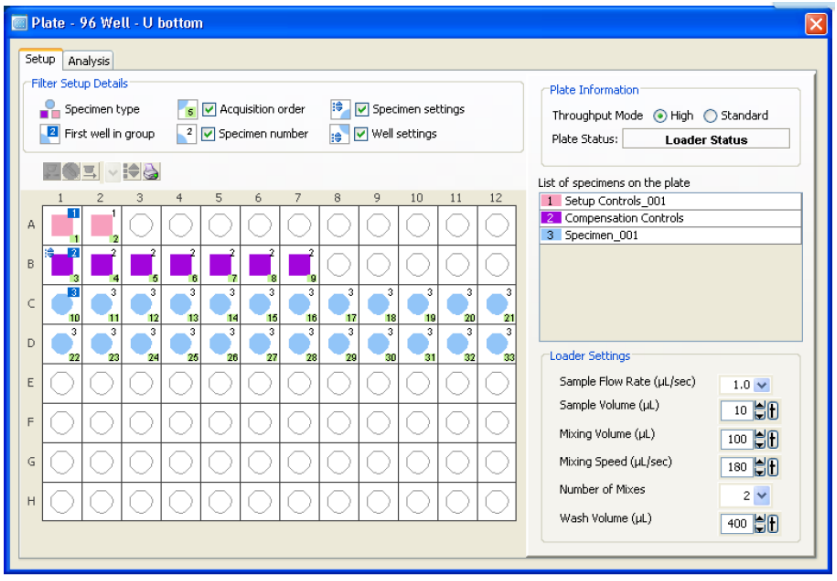

- Turn on the HTS (power switch on the right-hand side of the HTS unit)
- Place the LSRII in Run mode
- Prime the HTS (HTS > Prime)

**3. Plate Running**

- Choose the plate well type (drop-down list in the browser toolbar)




- Select the throughput mode (Standard [2-200µL + 20µL] or High Throughput [22µL])
- Add wells to the plate layout using the plate toolbar
- Check the Loader Settings (R-click a well and copy/paste Loader Settings to another well or multiple wells)



- Label the axis and set the number of events to record (Experiment menu > Experiment Layout)
- Select the first specimen well and click "Run Plate" in the acquisition dashboard

4. **HTS Cleaning** (~15 minutes)
   - HTS > Clean, select the Daily Clean – 96 well U-bottom template
     [std mode, 3µL/s, 200µL sample, 100µL mixing, 200µL/s mixing speed, 4 mixes, 400µL wash]

A1 A2: 200µL 1% Contrad
A3 A4: 200µL FACSClean
B1 B4: 200µL DiH2O

5. **HTS disassembly**

**Miscellaneous**

- Uncheck "Load data after recording" box (Edit > User Preferences)
- Uncheck Specimen number, Specimen Settings, and Well settings (Plate Window > Filter Setup Details)
- If you stop the HTS during a run, the current well will be lost (in HTS mode the next well will be lost as well)
- Cell concentration should not exceed $6x10^6$ cells/mL in 250µL
- Diva will stop acquisition (recording of a well) and proceed to the next well when either the specified number of events to collect is reached or the stopping time is reached.
  ***Stop Time*** (sec) = ***Sample Volume*** (µL) divided by ***Flow Rate*** (µL/sec)
  *Note*: Set a high number of events to collect if you want to record up to the stopping time.
- Do not exceed $1x10^6$ events to collect as it will result in a plate memory error
- Cyto-Fix/Cyto-Perm wash contains flocculates with a wide range of sizes (varies from lot to lot). Filter these solutions (0.22µm) before using them with your cells as these flocculates remain in solution even when diluted in di$H_2O$ or warmed up to 37°C.