LIVE CELL ANALYSIS ON CORE ANALYZERS RUNNING MQ WATER AS SHEATH Version 2

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Live, unfixed cells can be analyzed on the core instruments with some relatively minor precautions taken to minimize water introduction into the sample tube. Water dripping occurs for instruments such as LSRII running without the external drip retention sleeve which is a common default sample injection tube (SIT) set up for allowing small volume samples to be run in mini-tubes. MQ water can be used as a sheath buffer because once installed onto the analyzer, no mixing occurs between the sample and the sheath fluid under the hydrodynamic focusing conditions used to introduce the sample into the analyzer (additionally, the transit time from tube to the laser interrogation points is fairly short). Further, unlike for cell sorting applications, cells are not recovered after analysis; cells end up in the waste tank after data acquisition.

When analyzing live, unfixed samples: minimize the effects of water in a sample by:

- **Checking for cracks** on FACS tubes: This is critical as cracked tubes will immediately fill with sheath fluid when installed
- **Resuspending cells in a larger volume:** > 500ul; less sensitive cells (e.g splenocytes) can go down to 200ul if care is taken when loading on the instrument
- **Using separate set up tubes:** take aliquots of the sample to use for set up, only put the final sample for recording on the SIT after detector voltages are finalized
- **Never putting the analyzer in STANDBY if a tube is installed**
- **If needed, ask the core how to install the drip retention outer sleeve** (remember to remove after each use)
- **Changing to 1 x PBS:** For sensitive assays such as calcium flux, use the simple protocol outlined in the following section to change out the sheath buffer for sterile 1x PBS (allow time for changing over and back to sterile MQ water afterwards).

LSRII SHEATH BUFFER EXCHANGE GUIDE

The following procedures described in this document are for exchanging one type of sterile sheath buffer liquid for another (e.g. changing from MQ H2O to 1 x PBS).

Before starting: Obtain (1) 10L carboy(s) of autoclaved buffer needed (check with core staff if you need to prepare it yourself). (2) Waste beaker: for flushing the sheath filter into, during the buffer change over process.

1. **Start up the LSRII according to the Usage Guide** (most recent version in Help Files for the instrument > salk.edu/fccfaccess): Check that the LSRII is clean before starting, as the buffer exchange will initially yields high event rate that will taper off and it is important to be able to distinguish this from the instrument being dirty before you started (refer to Usage Guide on checking cleanliness in the QC template with CS&T beads). Leave Diva open at the QC template after checking cleanliness etc., you will return to it to run MQ H_2O after changing buffers.
2. **Decant buffer from tank:** Depressurize tank (loosen lid and release air using release valve) and remove lid (handle aseptically, do not touch the inside of lid or mouth of tank). Decant into empty labeled sheath carboy: label autoclave tape (buffer, name, and the date).
3. **Fill tank with new sterile buffer and close the lid**
4. **RUN the instrument in HI to start moving liquid through the fluidics:**
    a. Place a FACS tube of fluid on the Sample Injection Port (SIP). Recommended: start cleaning the instrument by running cleaning reagents ending with freshly filtered MQ H_2O).
    b. At the MQ H_2O step, start monitoring the event rate in Diva software using the QC template: events will eventually drop to 0-20evt/s (as it should look, when you confirmed cleanliness before starting).
5. **Flush the 0.2um sheath filter (capsule filter attached to tank) 2-3x over 10-15min:** Release ~200ml buffer from the sheath filter into beaker, using the purging port at the top of the filter (OK to leave the LSRII in RUN).
6. **When event rate is acceptable, proceed to operate instrument according to Usage Guide** (QC instrument/run samples/clean every 30min as directed recording cleaning files).
7. **Return instrument to original buffer and check event rate in Diva:** After using and cleaning, follow steps 2-6. Ensure event rate is acceptable. Record a CS&T file (if directed by staff, re-run CS&T) and export > **select PDF**
8. **Follow/sign off Clipboard** to make sure cleaning, waste is emptied, and data transfer etc. has been completed
9. **Send us pictures/files to** fccf@salk.edu for review:

    a. **Screengrab of Diva browser** with cleaning files showing timestamps (5min cleaning every 30min of use)
    b. **CS&T PDFs and reports** before and after using etc.
    c. **Photo of signed clipboard with Fluidics Panel showing OFF/Standby status**