

Calibur Operation Menu

1. Turn on the cytometer. Wait 1-2 minutes before turning on the computer.
2. In the meantime, check that the Sheath tank is **3/4 full**, waste tank is “empty”. Pressurize the flow cytometer by pushing the **VENT VALVE** forward
3. If you are the first user of the day, **prime twice** with distilled H₂O loading.
4. Sign on to the computer by selecting the appropriate user name and entering the correct password.
5. Double click **CellQuestPro** to start the FACS software.
6. Select ‘**CONNECT TO CYTOMETER**’ from the **Acquire Menu** tab. The **ACQUISITION CONTROL** window will appear with the **SETUP box** checked (Note: Your data will **NOT be saved** with it.).
7. Open or Create an Acquisition document by making the following graphs
 - Density plot: **FSC (x) vs. SSC(y)**. Choose acquisition from the Inspector plot type.
 - Density plot: **FL1 vs FL2** (FL2 vs FL3, FL3-FL4). Choose acquisition plot type.
 - **Histogram** is used for single fluoresce dye analysis. Choose acquisition plot type.
8. Open **DETECTOR/AMPS, COMPENSATION, THRESHOLD, STATUS** from the **Cytometer Menu**, open **COUNTERS** from the **Acquire Menu**.
9. **Before running a sample, give it a few flicks to mix the particles/cells. Ensure the particles/cells are thoroughly re-suspended. Remove any visible clumps or the flow cytometer will be clogged.**
10. To load a sample, remove H₂O, place a control tube in the Sample Injection Port (SIP), and then place the flow cytometer in **RUN** mode. [**Run light** will go from **orange** to **green**].
11. Click **ACQUIRE** in the **ACQUISITION CONTROL** window to begin data acquisition.
12. Run control samples to create and optimize settings (or load a previous setting and do mini adjustments).
 - Unlabeled sample, FL1 labeled sample, FL2 labeled sample.....
13. After setting up, set numbers of events to save in **ACQUISITION AND STORAGE** from **Acquire Menu**.

14. Create a folder to save data in the directory of **PARAMETER DESCRIPTION** from **Acquire Menu** and name the data files (choose Custom Prefix). Label the histogram Axis (option).
15. Uncheck **SETUP box** in the **Acquisition** window and Click **ACQUIRE** to start data collection.
16. If data acquisition is finished, choose **DISCONNECT FROM CYTOMETER** in the **Acquire Menu**.
17. Quit **CellQuestPro**, check on-line booking and log out from the computer
18. **RUN** 10% Bleach 1 min without arm followed by 5 mins with arm **on HIGH**. Repeat with H2O.
19. Pull the **VENT VALVE** back to depressurize. Fill the sheath tank to the level of **3/4 full** and empty the waste if it is more than **1/4 full**.
 - To Fill Sheath – use bottle to transfer FACSFlow from the stock container.
 - To empty waste- dump down the sink and put **2 bleach tablets** into each empty container.
20. Shut down the Calibur if no one is booked after you or the next person is more than two hours after you (especially for the late afternoon users).

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