



# FACSCalibur 255 User´s guide (CELLQUEST)



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## **IMPORTANT: If computer is ON and FACSCalibur system is OFF:**

Shut down and turn OFF the computer, then perform start up as listed below. Otherwise the computer will not recognize that the cytometer is connected!!



Sheath and Waste Tank Drawer



FACSCalibur Front Control Panel

## **Turning on the FACSCalibur**

1. Open Drawer, Check sheath and waste bottles (fill sheath container with BD FACS flow under the narrow, verify sheath box is not over 3/4 full, and empty waste container properly add undiluted bleach 50ml-100ml into waste reservoir)
2. Turn FACSCalibur system power switch ON (green button on right side of the cytometer)
3. Wait 30 seconds and turn computer ON
4. LOG IN on computer: Introduce the user name and password of your lab
5. Open Cell Quest software
6. Connect the computer to the cytometer: from CellQuest menu choose

**Acquire ⇒ Connect to cytometer\***

\*Acquisition Control window will be opened and cytometer options from the menu bar will be activated

## Sample acquisition

1. Open/Create experiment pattern (planilla) that **will be save first at the desktop**

**File ⇒ Open ⇒ Desktop ⇒ BDFiles ⇒ Planilla**

2. Visualize all the windows you can need:

In ***Cytometer***

- Detectors/Amps
- Compensation
- Status
- Threshold

In ***Acquire***

- Counters (rate)
- Parameter description
  - Folder: create a folder with data inside your lab folder in DATOS hard disc
  - File: define your file name prefix-suffix...

**YOU HAVE TO TRANSFER YOUR DATA FILES WITHIN 24 HOURS FROM THIS COMPUTER TO ANALYSIS COMPUTER: FOLLOW THE INSTRUCTIONS PLACED IN THE SIDE OF ADQUISITION COMPUTER**

3. Choose the appropriate "settings" of cytometer to acquire your sample

**Cytometer ⇒ InstrSettings ⇒ Open ⇒ Desktop ⇒ BDFiles ⇒ Settings ⇒ Open ⇒ Set**

**CHECK THAT SETTINGS HAVE BEEN LOADED!!!!**

**THEN CLICK ⇒ Done**

4. Check in Acquisition and *storage* ⇒ *parameter saved* that the signals you want to analyze are selected.
5. PRESSURIZE sheath fluid tank: Flip Vent-Tank Change toggle switch located between sheath and waste tanks inside the fluidics drawer towards front of instrument (towards you).
6. Press PRIME button on Front Control Panel of cytometer (this prepares the fluidics to begin a run) and wait for instrument to go to standby. Repeat this stage.

7. Allow about 10 minutes for instrument warm up: Acquire samples when the status of the cytometer is in standby or ready

**Cytometer ⇒ Status ⇒ Standby**

8. Run your samples:

- a) Swing the arm out and remove the tube of water, install your sample tube so the top of the tube is snug with the bal seal swing the arm into place under the tube.
- b) Push RUN button on the cytometer (check the button change to green)
- c) Click setup mode and then Acquire in Acquisition Control Window ⇒ you are viewing a real-time acquisition without saving the data to a file.
- d) If your sample looks ok, pause and abort acquisition in the software
- e) Inactivate setup mode: in acquisition window your data file name and destination appears ⇒ Check your data file destination is correct.
- f) 10.000 total events will be save in your data files, if you want to modify this go to Acquire Parameter menu
- g) Press RUN in the cytometer and acquire in Acquisition control window
- h) When acquisition is finished you hear an acoustic signal, no more events are saved BUT YOUR SAMPLE IS STILL RUNNING, PUT A DISTILLED WATER TUBE AND PRESS STANDBY BUTTOM ON THE CYTOMETER!!
- i) Change the sample tube (swing out the arm put your sample snug it and restore arm position) change sample name in parameter description and repeat the steps **g)** and **h)**
- j) WHEN YOU ARE FINISHED WITH CALIBUR and you aren't the last user for the day:

- IMMEDIATELY place a distilled water tube in the cytometer and allow water to clean sample line **with support arm to the side** (about 2 ml or **1 minute**).
- Swing support arm under the tube and **RUN** on **HI** mode (high speed) for **5 minutes**. Open the document LIMPIEZA that is in the desktop, connect to cytometer, put the settings LIMPIEZA and acquire in set up mode at HIGHT speed during 5 minutes. If there aren't events (data rate 0-15 events/second) and there are less than 300 events, you can **log off the cytometer from Fileguard password**

- DO NOT REMOVE TUBE FROM INSTRUMENT when run completed
- PUT THE CYTOMETER ON STANDBY (Press Standby switch on Front Control Panel) leaves dH<sub>2</sub>O tube on instrument (less than 2ml)
- TURN OFF VENT VALVE TOGGLE SWITCH
- FILL SHEATH tank with FACSTFlow sheath fluid under the narrow (not more then  $\frac{3}{4}$  full)
- EMPTY WASTE tank put 100ml of bleach.

k) **If you are the last user** of the day you HAVE TO CLEAN DEEPLY THE MACHINE:

- Fill with about 3ml a tube with clear CLEAN (check it) swing out the arm, put the CLEAN tube and allow to pass 2ml of clean solution
- Put the arm under the tube and pass this solution during 5 min
  
- Fill with about 3ml a tube with clear RINSE (check it) swing out the arm, put the RINSE tube and allow to pass 2ml of rinse solution
- Put the arm under the tube and pass this solution during 5 min
  
- Fill with about 3ml a tube with clear DISTILLED WATER (check it) swing out the arm, put the RINSE tube and allow to pass 2ml DISTILLED WATER
  
- Put the arm under the tube and pass this solution during 5 min (press acquire in set up mode at high speed). Open the document LIMPIEZA that is in the desktop, connect to cytometer, put the settings LIMPIEZA and acquire in set up mode for 5 minutes. If there are no events (data rate 0-15 events/second) and there are less than 300 events, you can turn off the cytometer ⇒ PUT THE CYTOMETER ON STANDBY  
TURN OFF VENT VALVE TOGGLE SWITCH

**YOU HAVE TO CLEAN PROPERLY THE  
CYTOMETER**

## Turning off the FACScalibur

IF THERE ARE A RESERVATION IN THE NEXT 30 MIN:

- **VERIFY IT** AND THEN:
- DEPRESSURIZE the sheath tank: Flip vent toggle switch in opposite direction, toward rear of drawer
- QUIT CELL QUEST SESION (FILE ⇒ QUIT)
- LOG OFF YOUR FILEGUARD SESION (FILEGUARD ICON (TWO ARROWS CROSSING A WHITE DOCUMENT) THAT IS LOCATED IN UPPER RIGHT CORNER IN MENU BAR SELECT IT AND LOG OFF YOUR SESSION)

IF THERE ARE NOT RESERVATION IN THE NEXT 30 MIN

- DEPRESSURIZE the sheath tank: Flip vent toggle switch in opposite direction, toward rear of drawer
- QUIT CELL QUEST SESION (FILE ⇒ QUIT)
- TURN OFF THE COMPUTER (SPECIAL ⇒ SHUTDOWN)
- PUT THE CYTOMETER ON STANDBY AND WAIT 5 MINUTES
- TURN OFF THE CYTOMETER (GREEN BUTTOM RIGHT SIDE)

**YOU HAVE TO REGISTER IN THE NOTEBOOK YOUR NAME  
DATA, HOUR, LAB AND EXTENSION NUMBER, AS WELL AS ANY  
INCIDENCE YOU HAVE DETECTED**