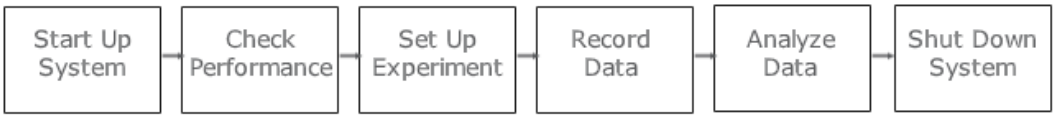
Instrument: BD FACSAria™ III



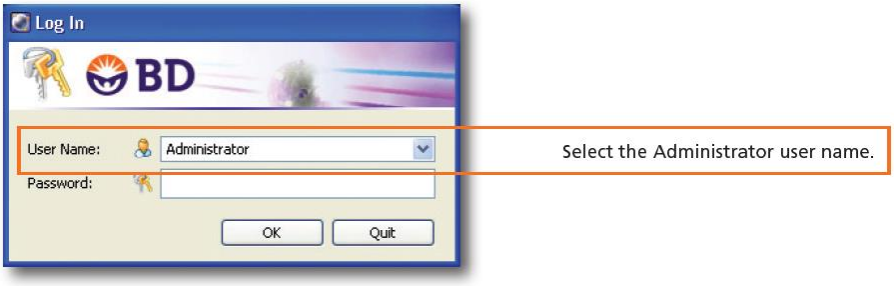
1. Turn on:

Stablizer → Computer → Power of the machine → Software

2. Software:

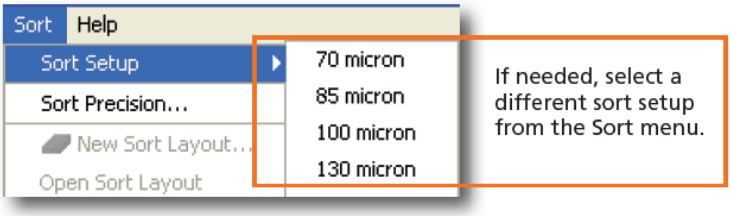
a. Log in

Password: BDIS#1



b. "Use CST setting"

c. Choose appropriate sort setup: "Sort" > "Sort Setup"

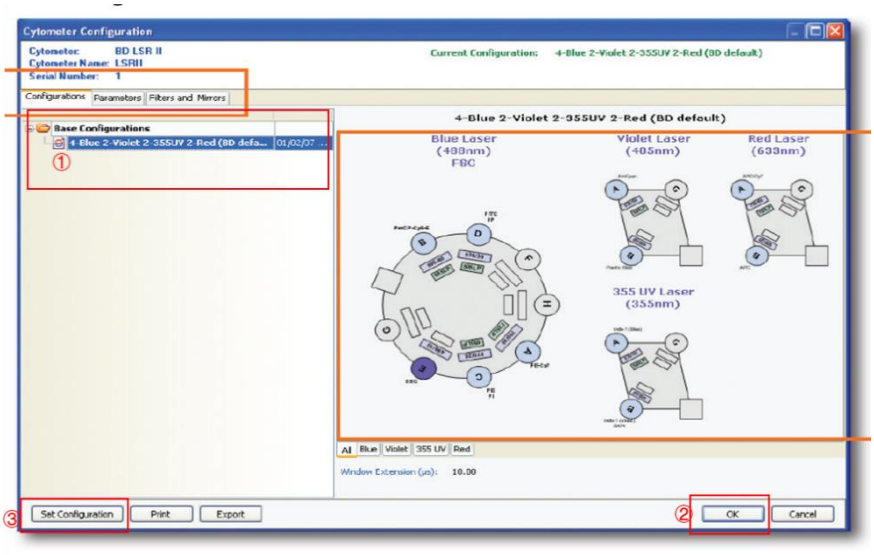


d. CST – Cytometer Setup & Tracking

(1) Define Cytometer Configurations (every time change the nozzle, choose the correct configuration)

i. Open the "Stream" (not to adjust the stream)

ii. Select "Cytometer" > "View Configurations" > Choose correct configuration > "OK" > "Set configuration"



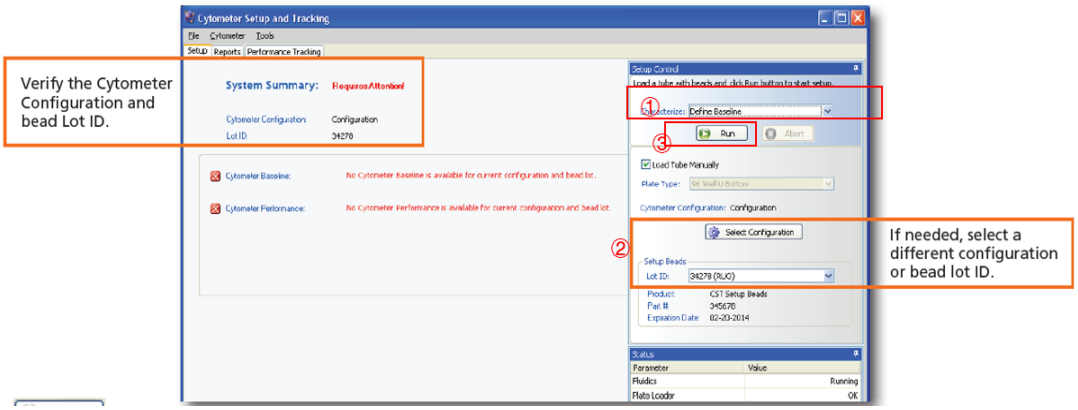
(2) Define a baseline: Select Cytometer > CST (if the baseline was not adjusted before for this selected configuration)

i. Prepare the BD FACSDiva™ CS&T research beads: 3 drops of beads + 500µl sterilized water/PBS.

ii. Open all the lasers.

iii. Choose “Define Baseline”.

iv. Install the file into the software with same number as the bead lot.



v. Click “Run” to perform the baseline adjustment.

vi. Follow the steps shown in the screen.

vii. Click “View report” to view the Cytometer Baseline Report.

(3) Check performance (if the baseline was adjusted before for this selected configuration)

i. Prepare the BD FACSDiva™ CS&T research beads: 1 drops of beads + 350µl sterilized water/PBS.

ii. Open all the lasers.

iii. Choose “Check performance”.

iv. Click “Run” to check performance.
v. Follow the steps shown in the screen.
vi. Click “View report” to view the Cytometer Performance Report.

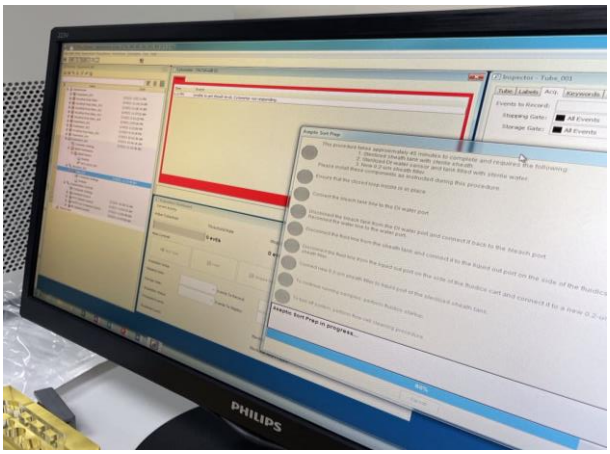
e. Aseptic cleaning (needed before aseptic sorting)

(1) preparation

i. Verify that the pressure has been vented from the sheath tank and the sheath probe has been removed.
ii. Disconnect the fluid and air lines from the sheath tank. Disconnect the DI water(plastic) tank. Disconnect the ethanol cleaning tank.
iii. Empty all the tanks, and clean them with 2-3L 70% ethanol one time and rinse them with autoclaved water 3 times.
iv. Autoclave the sheath tank and ethanol tank at 125°C and 15 psig for 30 minutes with a 7.5 minute warmup and shutdown cycle.
v. Make sure all the tanks are refilled.

(2) operation

i. Select “Cytometer” > “Cleaning Modes” > “Prepare for Aseptic Sort”.
ii. Close the “Stream”.
iii. Follow the steps on the screen.
iv. At the end, to complete the process, select one of the two options:
• To continue running samples, perform fluidics startup.
• To turn off the system, perform the flow cell cleaning procedure.



f. Fluidic startup

(1) Click on the bar “Cytometer” to perform “Fluidic startup”.
(2) Follow the steps on the screen.
(3) Put closed loop nozzle in the flow cell, the red ring is upwards. (Don’t touch the red ring!)
(4) Take out the closed loop nozzle.

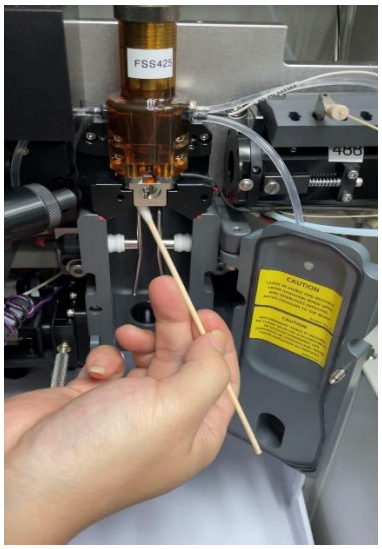
(5) Choose the proper size of the nozzle and put it into the flow cell, the red ring is upwards. (For bacteria, better to choose 70µm) (Don't touch the red ring!)

g. **Flow cell/camera/plate cleaning**: Clean the flow cell if the stream windows image looks scattered or blurred

(1) Closed "Stream".

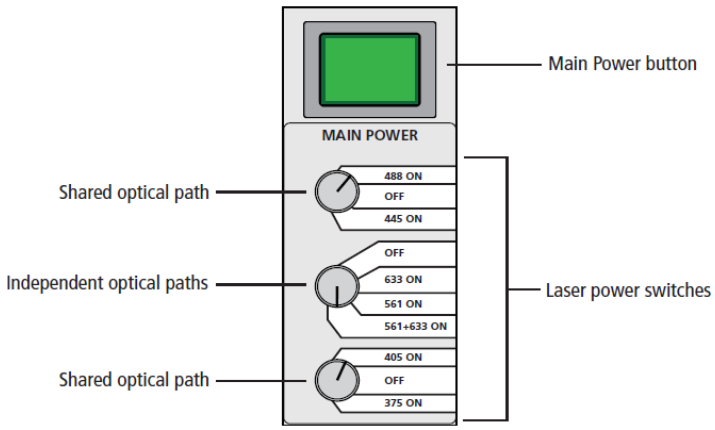
(2) Remove the closed-loop nozzle from the cuvette flow cell. Turn the nozzle-locking lever counterclockwise to the 6:00 position. Remove the nozzle by pulling it straight out.

(3) Use sticks to gently clean the flow cell with sterilized water and then dry with sticks.



h. Close the flow cell access door, turn on "Stream", turn on laser (blue laser – 488nm must be on)

Figure 4-1 Power panel



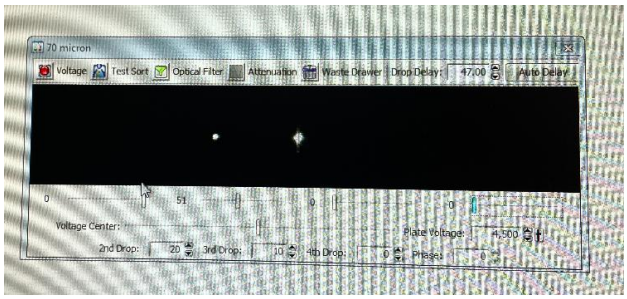
i. Adjust droplet position

(1) Only adjust "Ampl" to make the drop 1 in the 1/3 position of the window, and make the gap stable between 6-7 for 70µm nozzle.

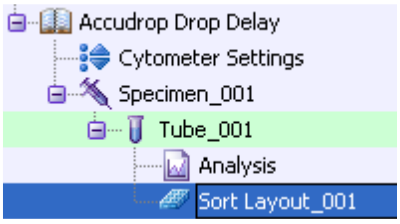
(2) Once it is settled, input the values of “Drop 1” and “Gap”.
(3) Select “Sweet spot” to lock the parameters.

j. Adjust delay time

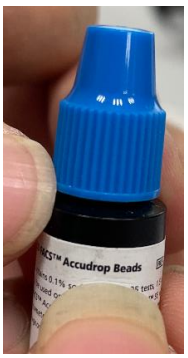
(1) Turn on “Voltage”, “Test sort”.
(2) Change the 2nd and 3rd drops function to make sure the middle drops look focused
(3) Only use the second drop to adjust the delay time.



(4) Turn on “optical filter” to show the gate, and change the voltage to make sure the 2 drops are inside the gate.
(5) Turn off “Voltage” “Test sort”.
(6) Select your experiment folder and select “Experiment” > “New Experiment”.
(7) Select the “Accudrop Drop Delay” experiment and click “OK”.
(8) Expand Specimen_001.
(9) Expand Tube_001.
(10) ActivateTube_001.
(11) Open the Sort Layout by double-clicking it.



(12) Load a tube filled with a suspension of 1 drop of the bead and 1ml PBS/sterilized water



(13) Adjust the flow rate to achieve the event rates specified in the following table.

Sort setup	Events per second
70 micron	1,000–3,000
85 micron	800–2,000
100 micron	600–1,500
130 micron	400–1,200

(14) Click “Sort” in the Sort Layout window and click “Cancel” in the Confirm dialog.
(15) Turn on “Voltage”, “Optical filter”.
(16) In the “Initial mode”, adjust “delay” until larger than 95.
(17) In the “Fine tube” mode, adjust “delay” until larger than 95.
(18) After the adjustment, click “Sort” and click “Cancel” in the Confirm dialog. Turn off the “Voltage”, “Optical filter”, and “Unload” the beads.

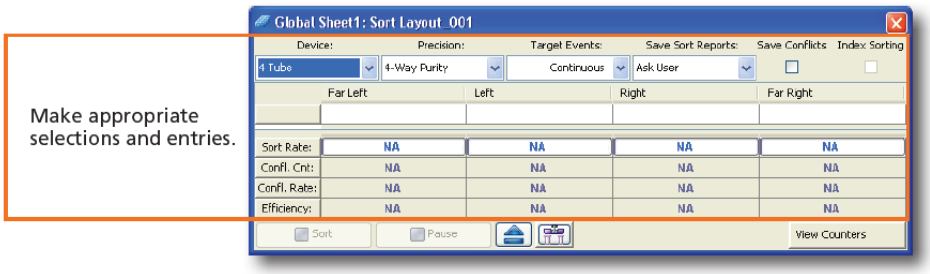
k. Adjust position of the collection tube

(1) Connect the tubes with water to the adaptor.
(2) Connect it to the machine.
(3) Turn on “Voltage”, “Test sort”, “Waste drawer”.
(4) Change the tube position visually, make sure the stream hit the liquid level instead of the test tube wall.
(5) Turn off “Voltage”, “Test sort”, “Waste drawer”.

l. Analysis & Sort

Before sampling, put in sampling tube and clean with rinse 2 mins, water 5 mins.

(1) Analysis
   i. select “New Experiment” > “New Specimen”.
   ii. Prepare blank (negative control), single positive controls, and sample.
   iii. Use negative control to adjust voltage and click “record data”. (before recording data, choose “sorting gate” and “record event”.
   iv. Use single positive controls to adjust compensation and click “record data”. (can use auto compensation)
   v. Use sample to gate the separate groups and click “record data”.
(2) Sorting
   i. Put the tubes with medium in the adaptor and put into the machine.
   ii. Click “Create a new sort layout”.
   iii. Select the parameters, “device”, “purity” “target events”.



iv. Choose the selected groups in the window.

v. Load samples and click “Load”.

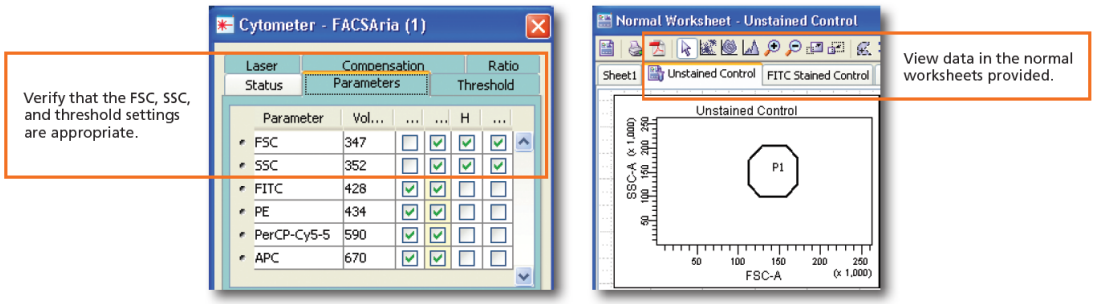
vi. Click “Sort” and click “OK” in the Confirm dialog.

vii. After the sorting, click “Unload”.

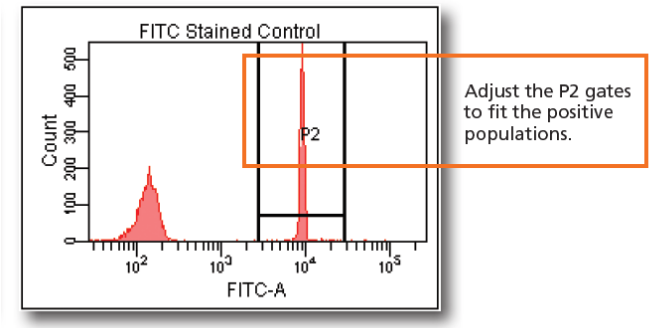
m. Auto compensation

(1) Select “Experiment” > “Compensation Setup” > “Create Compensation Controls”.

(2) Load the unstained control tube and adjust the voltage and click “apply to all compensation controls”. Select “Record data”.



(3) Load the single positive control to adjust the gates. Select “Record data”.



(4) Select “Experiment” > “Compensation Setup” > “Calculate Compensation” > “Link and Save”.

n. **Daily cleaning**: Perform a fluidics shutdown or clean the flow cell.

(1) Fluidic shutdown

i. Unload the sample tube, if one is loaded.
ii. Turn off the stream.
iii. Check the waste container and empty it if needed.
iv. Check the ethanol shutdown tank and refill if it needed.
v. Select “Cytometer” > “Fluidics Shutdown”. Follow the steps on the screen.
vi. Click OK when you see a message informing you that the system can be turned off.
vii. Exit BD FACSDiva software and shut down the computer.
viii. Turn off the cytometer main power and the stabilizer can be on.

(2) Clean the flow cell

i. Load the CLEAN tube for 10 mins, water tube for 10 mins.
ii. Turn off the stream.
iii. Remove the nozzle and install the integrated closed-loop nozzle.
iv. Select “Cytometer” > “Cleaning Modes” > “Clean Flow Cell”. Follow the steps on the screen.
v. Click OK when you see a message informing you that the system can be turned off.
vi. Exit BD FACSDiva software and shut down the computer.
vii. Turn off the cytometer main power and the stabilizer can be on.