CyAn User Manual

Flow & Mass Cytometry Center – Biomedical Core facility

Technion Faculty of Medicine

For assistant please call:

Amir Grau	**054-210-0778**
Yaakov Sakoury	050-749-8586
Ofer Shenker	052-456-8846

Notes

- These instructions are intended only for users who have been trained and are certified as independent operators of this instrument.
- Do not turn on/off or operate the instrument without authorization.
- Use ALL precaution measures needed to work with the instrument.

Starting Up

Note: *Turning ON/OFF the CyAN is done through the computer. The CyAN and cart main power switch is always ON. Please DO NOT touch them!*

1. Make sure the sheath fluid tank (left one, fig 1)) is filled at least up to ⅓ with DDW. The led above it should be green.
2. Make sure the waste tank (right one) is not full. The led above it is green. ***Please let us know if this is not so!***
3. Turn on the computer. During work days it is already on – only the screen is off.
4. Log-in into BookIt.
5. The application (Summit 4.3) is activated by BookIt. Wait for it to finish loading. If it does not load automatically, please click the summit icon (fig 2).

Figure1 Fluid Cart

Figure2 Summit icon

6. On the prompt dialog box (fig 3) select *CYAN* and create a *New* database with today's date and hit OK.

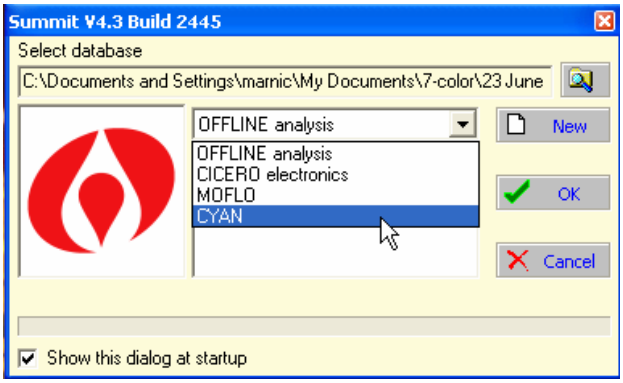


Figure3 New Database

7. Open the control panel of the instrument, click *Instrument* on the main menu → *CyAn* → *Control Panel*.
8. Click *View* in main menu → *Control Panel* (this is not the same as the above; fig 4).
9. Remove the tube from the sample tube holder and close it. Press on the key marked '*Startup*' (fig 4). A window pops-up; follow instruction. The cart will turn on. You'll see 3 green LEDs lit (fig 5); any other color means trouble – please let us know.
10. Meanwhile, fill a tube with about ~ 3 ml of DDW.
11. When *Startup* has finished, verify all "LED"s in the SMS are green.

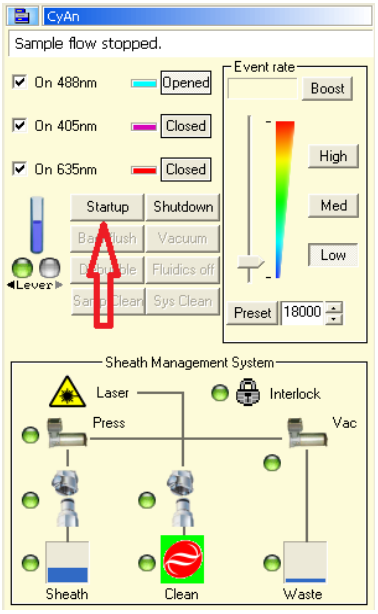


Figure4 Control Panel

SMS

Figure5 Sheath Management System (SMS) on cart

12. Place the DDW tube in the CyAN.
13. In the Acquisition panel (fig 6), set the *Limit* to 10 minutes and *event rate* to *Med* (at least 22,000).
14. Press run (F2).
15. Verify that the instrument is clean. If not, run DDW again.

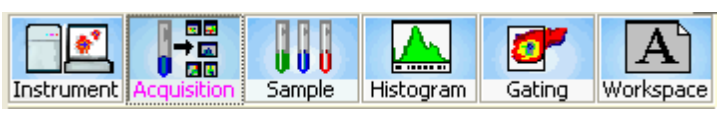


Figure6 Acquisition panel

Running an Experiment

A. Preparations

1. If you are the first one to open the CyAn or opened a new database, you will get an empty protocol with one empty page.
2. Load your protocol from *File* → *Protocols* → *Load*.
3. Browse to the location where you have saved it (…*Users Data*).
4. Otherwise, you need to create an experiment from scratch.
5. Choose *Acquisition* in the toolbar.
6. Within the *Acquisition Parameters* panel (fig 7), select the parameters you need.
   Note: *these names are common known fluorochromes channels. You can change them to fit your fluorochrome by double clicking their name.* ***Caution:*** *changing GFP to BV421 does not mean you will read BV421. If not certain, please seek for advice.*

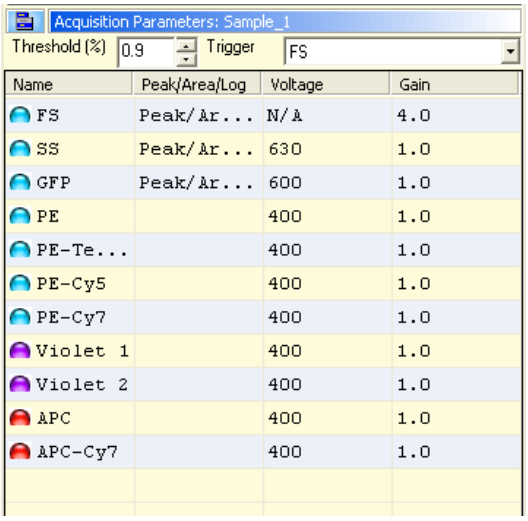


Figure7 Acquisition Parameters Panel

7. Adjust *Threshold* to 0.9 and make sure FS is the *Trigger* channel.
8. Create graphs:
   a. Choose *Histogram* in the toolbar (fig 8 and 9).

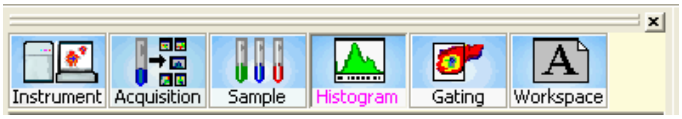


Figure8 Histogram

   b. To create:
      i. A histogram: double-click the X parameter.
      ii. A dot-plot: click the X parameter and double-click the Y parameter.

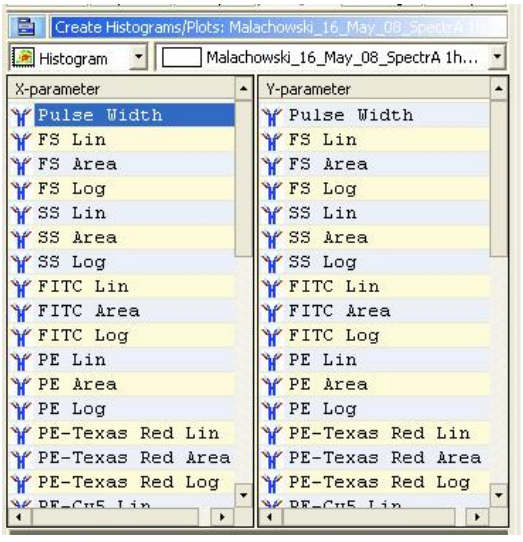


Figure9 Histogram plots

   c. Right-click the workspace and choose →*Arrange Windows* → *Horizontally…*
9. In the *CyAn* window (fig 10), choose *Low* in the *Event rate* zone.
10. Select the lasers you need. The 488 laser line is mandatory.

11. Choose *Acquisition* in the toolbar.
12. In the *Acquisition Sample* panel (fig 11), setup the following fields by double-clicking the text box on the right. A pop-up window will appear.
    a. *Sample Name*: Choose the parameters you want to appear in the name of the *fcs* file.
    b. *Number*: enter 1.
    c. *Sample Description*: Type the name of your sample. Double click *Number* and hit Enter to force a refresh in *Sample Name*.
    d. *Limit*: Choose the way you want the acquisition to stop. In the pop-up window:
        i. Click *Free Run* if you want to stop manually (fig 12).
        ii. Otherwise *Limit* and check the box of interest:
            - *Event Limit:* type desire cell number.
            - *Gate Limit*: choose a gate and type desire cell number.
            - *Time*: type number of seconds.
    e. *Save Path*: Choose your lab folder.
    f. *File Name*: The name of sample as appears in the folder. Select it to be the same as the *Sample Name*.

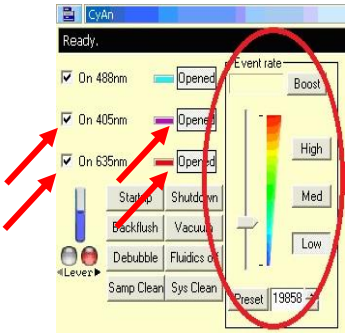


Figure10 CyAn Control panel

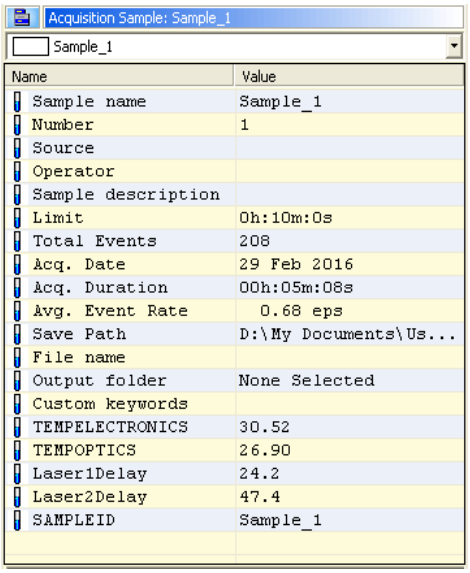


Figure11 Acquisition Sample

13. To get a reminder for saving the result of the current sample (Save file dialog), click *Acquisition* →*Auto Save* (fig 13).
14. If you collected data and didn't do the above #13, you can still save the data manually by choosing *Acquisition* →*Save*. This will save the current displayed results. The same dialog will open.

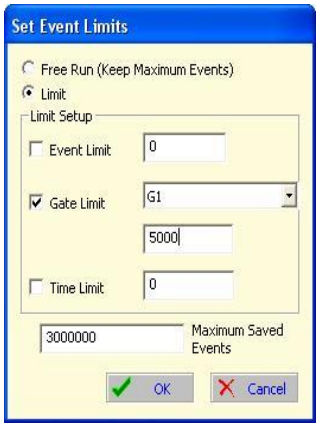


Figure12 Event limit

Note: *For optimal laser readout wait at least 10 min for the lasers to warm up.*

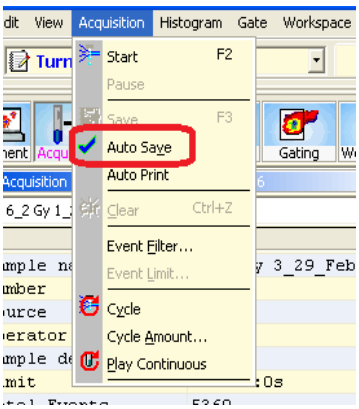


Figure13 Auto save

B. Running a Sample

1. Position the control tube on the SIP and close the lever. Make sure that in the *Instrument* window the blue tube appears (fig 14) (When a tube is not present, an empty tube appears). and the left lamp of 'levers' is green. (When the lever is opened, the right lamp is red).
2. Press F2 (Run).
3. The events will show up in a few seconds. If not, use the slider under the CyAn control panel zone to raise the pressure in the tube and hence the event rate.
4. If no events show up, press *Boost* for a few seconds. When you see events press Ctrl+Z to restart acquisition.
5. While the sample is running, adjust *Voltages* and *Gains* of the parameters you have chosen (#6 in the section above) in the *Acquisition Parameters* window (fig 15 and 16). Center most of the population in the FS-SS dot plot.

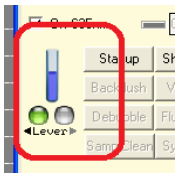
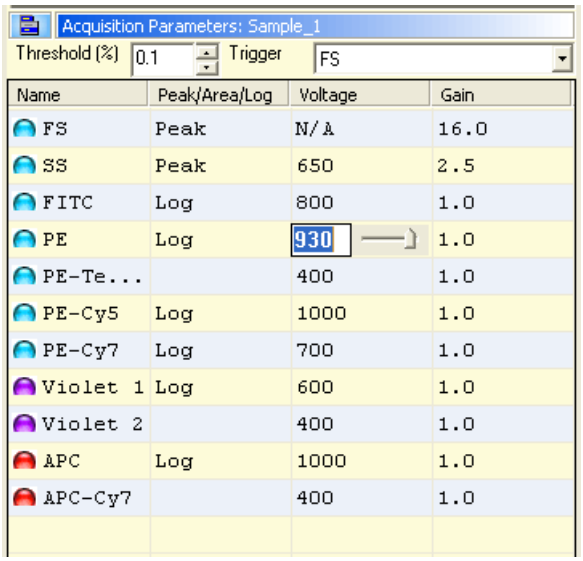
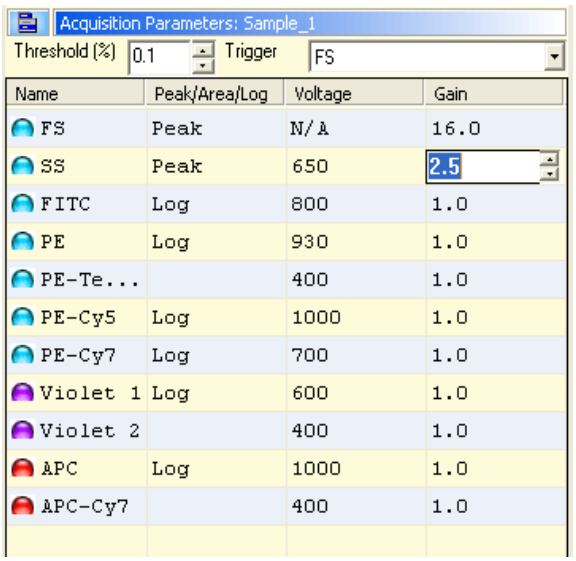


Figure14 Lever



Name	Peak/Area/Log	Voltage	Gain
FS	Peak	N/A	16.0
SS	Peak	650	2.5
FITC	Log	800	1.0
PE	Log	930	1.0
PE-Te...		400	1.0
PE-Cy5	Log	1000	1.0
PE-Cy7	Log	700	1.0
Violet 1	Log	600	1.0
Violet 2		400	1.0
APC	Log	1000	1.0
APC-Cy7		400	1.0

Figure15 Set Voltage for a Parameter



Name	Peak/Area/Log	Voltage	Gain
FS	Peak	N/A	16.0
SS	Peak	650	2.5
FITC	Log	800	1.0
PE	Log	930	1.0
PE-Te...		400	1.0
PE-Cy5	Log	1000	1.0
PE-Cy7	Log	700	1.0
Violet 1	Log	600	1.0
Violet 2		400	1.0
APC	Log	1000	1.0
APC-Cy7		400	1.0

Figure16 Set Gain for the Voltage

6. Create a region (gate) around the population of interest (fig 17):
   a. Right-Click in the area of the graph you chose.
   b. Select how you want to draw the region.
   c. Position the region and adjust its shape and size to fit the population.
7. When the cell limit you have setup is reached, acquisition stops.

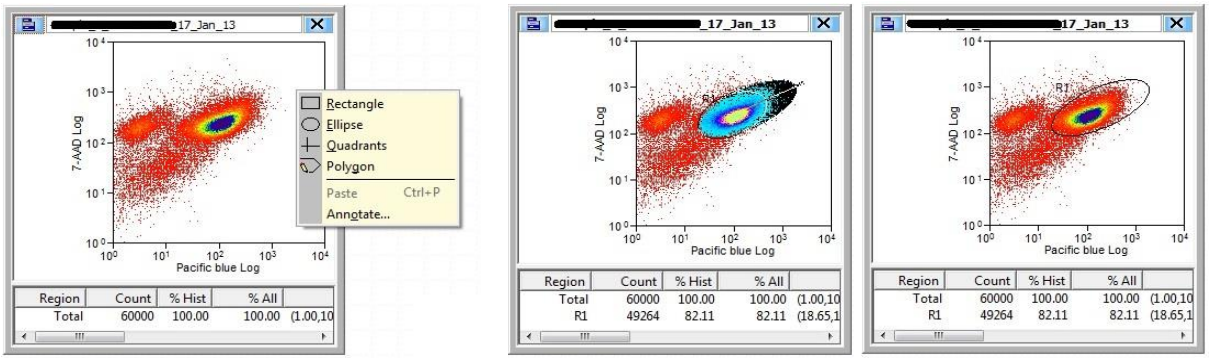


Figure17 Gating Population

8. Remove the tube and close the lever.
9. Allow auto backflush to clean the instrument from leftovers of the sample you've just run (30 sec).
10. Insert next tube and change the S*ample Description* for that tube as explained above.
11. Run all your samples.
12. When finished, perform the cleaning procedure.

Cleaning Procedure

1. Close the lever without a tube, wait until the auto-backflush is finished.
2. Load a FACS tube marked FACS clean (or 'C') and run (F2) for 4 min on high rate.
3. Repeat step 1 and 2 with FACS rinse (or 'R') and DDW tubes.
4. Run *Shutdown.*
5. Follow instructions in the popped-up window. Fill the same tube as before when you get the message to insert a tube with DDW.
6. The cart is turned off.
7. Close Summit.
8. Log off bookit.
9. Press simultaneously on Ctrl+Alt+Delete. Choose *Lock Computer* from the popped-up window.
10. On weekends and holidays – shutdown the computer.

Verify that there is a tube with DDW (~1 ml) in the instrument before leaving it.

Troubleshooting

Problem: No events show up on my plots.

Possible reason 1: Events are stuck against the axis in FS Lin and/or SS Lin.

Solutions:

1. Reduce FS voltage. Use initial values between 400 and 600.
2. Reduce SS voltage. Use initial values between 400 and 650.

Possible reason 2: Threshold too high.

Solution: Reduce Threshold as explained (See Preparation #7).

Possible reason 3: System clog.

Solution: Run 5 minutes of Clean ('C'), while boosting a few seconds each time.

Problem: Lights on the fluidics cart are not green.

Possible reason: Sheath fluid tank ran out and/or Waste tank is full.

Solutions:

1. Press the button *Fluidics off* in the instrument's window. Follow instruction.
2. Disconnect tubes from the tanks. Exchange the sheath tank with a full one. If no tank is ready, empty the tank and fill with 16 liters of DDW, using the system in the student's lab.
3. Decant the waste tank box into the sink. Add 0.5 liter of bleach (Found under the sink).
4. Connect tubes to the tanks. Press the 'Reset' button on the cart.