

CytoFlex Quick Guide

Start-Up

1. Check the fluidics tanks to see if they need to be emptied or filled.
2. Turn on machine (switch in back on bottom left side)
3. Sign into computer and start CytExpert Software
4. Go to cytometer and sample injection to select Semi-Automatic (FACS Tubes) or plate loader (96 well plate only)
5. The software will complain if the cytometer needs to be turned off and back on again
6. Move the metal flag into the correct position for the fluidics line to the flow cell. It shows P in plate mode, it looks like a narrow version of a FACS Tube in tube mode.
7. Under Cytometer, Run System Startup – follow onscreen instructions. Takes ~8 min.

Running Samples

1. Start New Experiment
2. Save data under your professor
3. Go to Settings and then to select channel
4. Uncheck all unwanted channels, Select the name on dropdown channels you keep
5. Create the plots you would like
6. Pull up acquisition settings and hit recommended for gains
7. If no events occur when running sample, look and see if the events per second is too low. Go to threshold and switch from automatic to manual, then increase the threshold (the default is very low). Watch the events per second to see if the events per second increases while you increase the threshold.
8. Once you have a lot of events per second (1,000-10,000), if you still don't see a lot of cells on your plot do two things:
 - a. Be sure that the plot display is displaying at least 5,000 cells (the default is very low). If your events per second are low, you might just have a dilute sample and will have to wait a few seconds at each change of parameters for the plot to populate. But also do the second thing:
 - b. Move the FSC SSC plot to find where your population is being detected by using the plain hand and the line hand: Pull up the FSCxSSC with the plain hand and then scrunch the plot back down with the hand that has a line next to it to center your population. Alternatively, zoom out as much as you can, then rezoom on the population using the +/- magnifying glass icons.
 - c. Increase the flow rate. The default is "low" so it probably is running slow anyway. Increase the rate, but don't allow the rate to hit 10,000 or it will increase your abort rate, and if your counts are high and your abort rate is high, then your data is not reliable.
9. Once you see your population, adjust the threshold
10. Set gains (start with recommended but adjust to place the population).
11. Setup plots and move the plots in or out based on your sample positive control. You can start with a sample with all colors and if you see positive and negative peaks, then you

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can adjust your gains easier based on whether the peaks are too near an edge of the plot. If they are in the middle of the plot, you should probably leave them there because you can do a digital zoom on your data for analysis. Set your plots with the zoom features (the hand or line hand, or the +/- magnifying glass).

12. When running tubes, always backflush between samples. The Backflush button is just below the Record button in the Acquisition window.

Setup Compensation

- a. Use a multicolor sample first to "Run" but don't record. Use this to adjust the plot area for best viewing, to setup the threshold and to setup all the gains
 - b. Run single stain controls and record each sample
 - c. If you adjust gains, rerun each sample with the new gains setting and re-record
13. Check all positive and negative gates.
 14. Calculate the compensation Matrix.
 15. Save compensation matrix. (You must setup an experiment to run your multicolor sample. Apply the compensation matrix and threshold and gain settings will also apply.)

Flush – Takes about 8 minutes. (Must be done after you are finished running samples).

1. Go to cytometer and select daily clean.
2. Follow instructions to place FlowClean (blue solution) into a tube or plate (3 minutes)
3. Follow instructions to place DI water in a tube or plate (5 minutes)
4. Select standby. Leave the instrument on.
5. When finished close the software.
6. Log out of Windows

Full Shut-down (Done by Technicians at 5pm or you do it if you are working after hours)

– Takes about 25 minutes.

1. Go to cytometer and select daily clean.
2. Follow instructions to place FlowClean (blue solution) into a tube or plate (10 minutes)
3. Follow instructions to place DI water in a tube or plate (15 minutes)
4. Select standby.
5. Turn the cytometer power off.
6. When finished close the software.
7. Log out of Windows

Troubleshooting

1. Adjust scaling!!!!
2. Make sure valve is in the correct place
3. Be sure injection mode is correct
4. Try backflushing the system a few times
5. Call a technician or Dr. Hope

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Notes regarding Start-Up step 5: The Cytometer has two separate fluidics systems and separate internal computers to function for each fluidics system. When the power gets turned on, the cytometer boots into the system that it was previously using. Therefore, when you choose the software from the external computer for tubes or plates, the cytometer complains if it is not in the proper fluidics system. For instance, if it was last used in tube mode and you are using tube mode, there will be no complaint. If it was previously in plate mode, it will have turned on the cytometer fluidics for plate mode and when you switch the software, the cytometer will alert the main computer that it is booted into the wrong system. Turn it off, then turn it on. The cytometer and the computer must talk to each other first before the cytometer will switch systems, so simply having main computer and the software on prior to booting the cytometer will not matter. You need to turn the cytometer on, let it see your software choice, then turn it off and turn it on. Super tedious, I know, but that's how the company made it!

A note about Running Samples step 7: This happens because the cytometer detects nanoparticles. For cell samples, you will have tiny particles from your cell prep and these will all be read as events. The cytometer will then be counting higher than 10,000 events per second and it cannot accurately run that fast, so it becomes conflicted about the readings and begins tossing the data out and not accepting counts as real readings. This tossing of data is called an "abort rate". The abort rate is high and the counts are low because of the problem of too many items to detect. By increasing the threshold, you remove the cytometer's sensitivity to the small sizes. The higher you set the threshold, the more gets blocked from processing and the cytometer then can focus on assessing the cells that are the right size. Once it does this, the abort rate drops and the counts per second increases. As long as you see an abort rate below 5, then you can believe the events per second. If the abort rate is low but the events per second is still low, you probably have a dilute sample. When you prep your cell sample next time, keep the cells very concentrated or suspend the cells in a smaller volume for flow.

A note about Running Samples step 10: Start with the recommended gains, adjust the FSCxSSC gains but probably leave the fluorescence channels unchanged. The recommended gains are based on our weekly QC run. The recommended gains are for the fluorescence channels very good settings and gain changes probably don't need to be extreme if they are changed. The FSCxSSC are more likely to need gain changes.

Other Notes:

Bring concentrated samples! Especially for a plate. 10,000 up to per second. If over 10K, must slow down the event rate to prevent the abort rate from getting too high and to prevent unreliable data.

Abort rate should stay under 5%. It usually runs at 2% or lower.

Plateloader software items:

From a template, the gains and threshold and tables are set and sample wells defined for recording events. Select the file, browse to resave it as something else.

For plates: First brings in the settings (i.e. gain), but not the wells. So you need to select which wells have samples by dragging and then there is an icon above in the 'filled' bucket, it makes it selected for the read. If you change the settings of the next one, then need to select.

Mix can be as short as they want (0.1-3 seconds), can do 0.1 if the sample stays in suspension really well. Backflush is 3-4 seconds to avoid carryover (not lower!!). This makes a plate run about 30 minutes per plate.

Right click lets you set a different settings to other tubes with a selection.

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Laser	Fluorescent Channel	Channel Names/Dyes
488 nm - Blue	525/40	BL1, FITC, GFP, Alexa-488, CFSE, Fluo-3, YFP
	690/50	BL2, PerCP, PI, PC5, PC5.5, PerCP-Cy5.5
	488/8	Blue SSC
	FSC	FSC
638 nm - Red	780/60	RL3, APC-H7, APC-A750, APC-Cy7, APC-eFluor780
	712/25	RL2, APC-A700, Alexa-700
	660/20	RL1, APC, Alexa-647, eFluor-660
561 nm - Yellow	610/20	YL2, ECD, mCherry, PI, PE-Texas Red, PE-CF594
	585/42	YL1, PE, PI, dTomato, DsRed
	690/50	YL3, PerCP, PI, PC5.5, PerCP-Cy5.5, PC5
	780/60	YL4, PC7, PE-Cy7
405 nm- Violet	405/10	Violet SSC (nanoparticle)
	450/45	VL1, Pacific Blue, Brilliant Violet 421, CFP, eFluor-450, V450
	525/40	VL2, CFP-YFP, Krome Orange, AmCyan, V500, Brilliant Violet 510
	610/20	VL3, mCherry, Brilliant Violet 605, Violet 610, Qdot 605

Other optional Filters	Optional Colors
780/60	For Violet laser
660/20	Brilliant Violet 650, Qdot 655
561/10	FSC, SSC for yellow laser

Start-Up – Takes about 8 minutes

1. Check the fluidics tanks
2. Turn on machine
3. Sign into computer and start CytExpert Software
4. Select Cytometer sample injection to Semi-Automatic (Tubes) or plates
5. Possibly turn the cytometer off and on again.
6. Move the metal flag into the correct position P for plate, narrow for tubes.
7. Under Cytometer, Run System Start up.

Running Samples

1. Start New Experiment
2. Save data under your professor
3. Go to Settings and then to select channel
4. Uncheck all unwanted channels, Select the name on dropdown channels you keep
5. Create the plots you would like
6. Pull up acquisition settings and hit “Recommended” for gains
7. Run and set gains. Check: events per second, Threshold, scaling (your view of the plots), population on plots, abort rate, plot display (5,000 cells), sample concentration, run speed
8. Backflush after each sample
9. Create additional plots as needed

Setup New Compensation

1. Uncheck all channels not needed
2. Use a multicolor sample first: “Run” but don’t record. Adjust all items for a run.
3. Run single stain controls and record each sample. Re-record if anything gets changed
4. Check all positive and negative gates.
5. Calculate the compensation Matrix.
6. Save compensation matrix. (You must setup an experiment to run your multicolor sample. Apply the compensation matrix and threshold and gain settings will also apply.

Flush (Must be done after you are finished running samples) – Takes about 8 min

1. Go to cytometer and select daily clean.
2. 3 minutes FlowClean (blue solution), then 5 minutes DI water
3. Select standby. Leave the cytometer on.
4. Close the software. Log out of Windows.

Full Shut-down (Done by Technicians at 5pm or you do it if you are working after hours) – Takes ~25 min.

1. Go to cytometer and select daily clean.
2. 10 minutes FlowClean (blue solution), then 15 minutes DI water
3. Select standby. Turn the cytometer power off.
4. Close the software. Log out of Windows.