

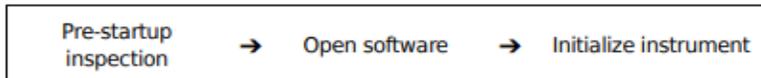
CytoFlex Quick Guide

<http://www.pedsresearch.org/uploads/blog/doc/Cytoflex-Manual.pdf>

<https://www.beckman.com/search#q=Cytoflex%20LX&t=coveo-tab-allresults>

Detectors' Configuration for Cytoflex LX (6 laser default):

Laser	Color	Detector/filter order (top to bottom, left side then right side):
638nm	Red	638, 763/43, 660, 712
561nm	Yellow	561, 610, 763/43, 585, 675/30, 710/50
488nm	Blue	488, 525, 610, 690, 840/20, 885/40
808nm	Infrared	840/20, 885/40
405nm	Violet	405, 450, 525, 610, 660, 763/43
375nm	Near UV	450, 525, 675/30



Starting up

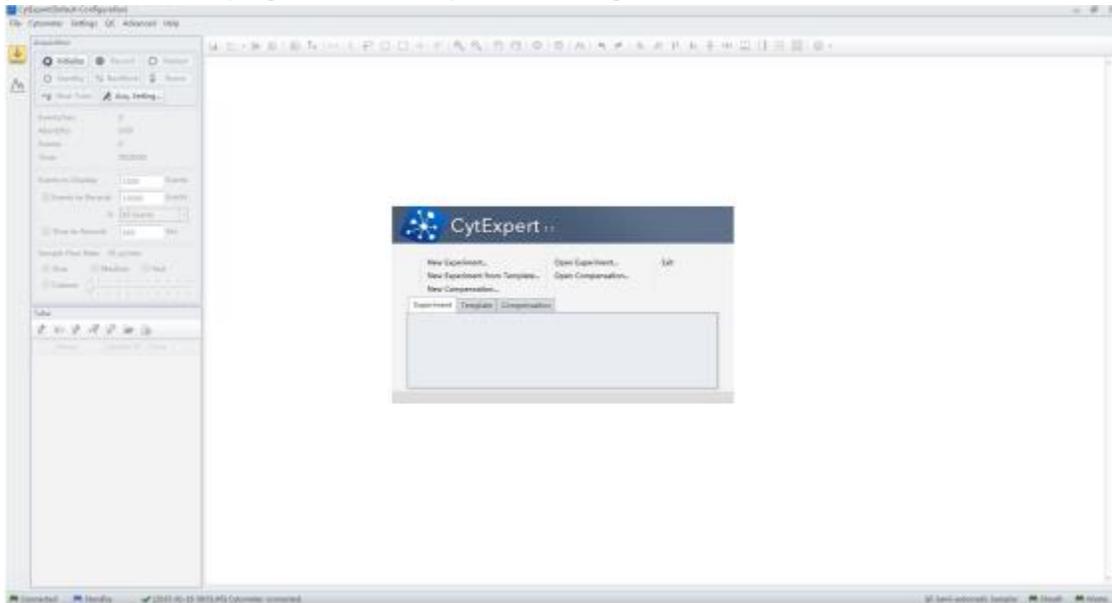
1. Check the sheath fluid level every time you use the cytometer. This ensures that you do not run out of sheath fluid during an experiment. Replenish fluids if needed as follows:
 - The sheath tank is the bladder labeled DI only attached to blue umbilical.
 - Fill the bladder with DI water from sink. This is the faucet line with filter attached to it.
 - Replace Sheath tank onto the instrument and replace the blue umbilical.
2. Check the waste container, empty if necessary, and add a small amount of bleach to inactivate pathogens. (Upon filling the Sheath Bladder, it is best to empty waste to avoid any spillover).
 - The waste tank is attached to the yellow umbilical.
 - Empty the waste into the sink. Use eye protection and avoid splashing.
 - Add a small amount of bleach into the bottom of the tank.

- Replace the Waste tank onto the instrument and replace the yellow umbilical.

(**Note:** The machine will beep at you when waste or sheath is an issue. They will turn red at the status bar at the bottom of the screen. There will also be a muter to stop the alarm while you are filling or empty the respective containers. Cytotflex runs on DI water and not sheath fluid or 1x PBS or 10% bleach.



3. Turn on the computer.
4. Log in to the computer. Use user BIDFLOW. There should be no password. If login issues occur, then use user Beckman Coulter with password of password no caps.
5. Open CytExpert software using the shortcut. Please login with the username and password. This page will then open after login occurs.



6. Check the bottom left of the screen to verify that the Cytometer is connected. If the computer was already on, you may have to initialize the instrument through the software. If the instrument is not responding please make sure that the switch on the back of the instrument is on.
7. Check to see if the instrument and computer is set up in tube or plate mode. If not in the mode you need, refer to “**Changing to tube mode**” section below to switch the instrument or computer to tube or plate mode.

8. From the Cytometer Menu, select System Startup Program. The Startup program takes approximately 8 minutes.

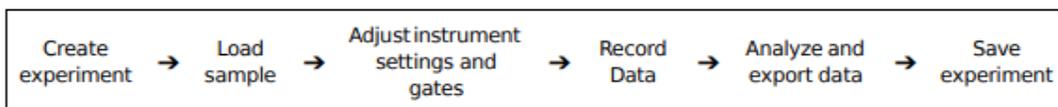
9. Click Initialize and place a tube of 2 mL deionized water in the sample loader. Click Start

10. System will initialize and prime. At the end of priming, the remainder of the time the system will be warming up. Close to quit when warm up is finished.

(Note: QC is run by the flow core if you have any questions in regards to this please contact us. Please do not adjust our QC settings, change filters, or try to calibrate without flow core assistance.)

Changing to Tube Mode from Plate Mode or Plate Mode from Tube Mode

1. In the Cytometer menu select Sample Injection Mode. Select Semi Auto for running tubes. Select Plate mode for running plates.
2. A Restart warning prompt will appear on the screen. Select OK.
3. Turn the silver switch from P or T, depending on if running plates or tubes, to the correct setting for your experiment.
4. Turn the cytometer's power off from the cytometer menu.
5. Turn the cytometer's power on from the cytometer menu.
6. Initialize the machine to begin running.



Creating your experiment

1. View the Detector Configuration in the top left corner to verify it is set on default configuration. If another configuration other than default is needed, please contact the core flow staff before proceeding.
2. Create a new experiment. From the file menu, select New Experiment.
3. Use the default path as the location for your saved data, rename the experiment as you see fit, and save the experiment.

4. Save experiment as a template. This allows for other experiments to be open from this template with the same set up.

Creating New Tube Experiment Settings

1. Select Set Channel in the Settings menu. Uncheck all parameters you will not be using. Unselected parameters will not be saved into the data files. Select Apply to all empty tubes, and OK. Select close once done with channel set up.

2. Uncheck all parameters you will not be using, and apply labels if you desire. Unselected parameters will not be saved into the data files.

3. Create a worksheet to view your cells as they are acquiring and recording. There are many types of plots to use. Right click on plots to change properties. Axis ranges can be adjusted also. (**Note:** Nothing will be seen on the plots until you begin to

acquire.) 

4. Select Acq Settings in the Acquisition Window to bring up the gains.

5. Use the Population hierarchy tool to bring up a population hierarchy.

6. Add a tube to the experiment.

7. Adjust the sample flow rate in the Experiment Window. Default is slow at 10 μ L/min.

8. Place the unstained cells tube onto the cytometer.

9. Optimize the FSC and SSC gains to place the population of interest on scale. Optimize the fluorescent gains so that the negative populations are visible on the right corner area. Add gates to select the population of interest. Select plots title to change the view to include only gated parameters if desired. Select the statistics or

Statistics 

population hierarchy to be display if desired. Hierarchy 

10. Place the color stained cells tube onto the cytometer.

11. Verify that the positive populations are on scale. If a positive population is off scale, first try adjusting the number of decades shown on the plot. If a population is still off scale, lower the gain for that parameter until the positive population is entirely on scale. Note that lowering gain settings below the default settings for fluorescence channels may decrease resolution on that channel. Gains below 25 might impact rare populations.

12. You now have your gain and fluorescence settings.
13. Continue with compensation as necessary.
14. Save as a template.

Creating New Plate Experiment Settings

1. Select Set Channel in the Settings menu
2. Uncheck all parameters you will not be using, and apply labels if you desire. Unselected parameters will not be saved into the data files.
3. Create a worksheet to view your cells as they are acquiring and recording. There are many types of plots to use. Right click on plots to change properties. Axis ranges can be adjusted also. (**Note:** Nothing will be seen on the plots until you begin to acquire.)



4. Select plate settings to make the window appear. Click on then add a plate. Choose which type of 96 well plate and the direction that the plate is to be run.
5. The white wells are empty. To add a well to be water select the water icon for the well. The same can be done with bleach or your sample. To select multiple wells at a time click each desired well while holding the crt button on the keyboard.
6. Setting up your sample well will bring up a menu that you can change all the gains, channels to be used, compensation, and stopping rules. To apply these setting to multiple tubes if you did not select them all at the start or to apply a made change, you can select the sample tube and click **apply well setting** to. Under the apply well setting to select all sample well or all empty well or crt click desired wells that these setting will be applied to. You are able to save this plate as a template using the save as a plate template option.
7. To run your plate after please press the eject button to be able to load your plate onto the analyzer.

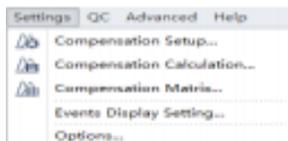
Manual Compensation

1. Ensure that you have saved a file of the correct gain settings and channels first.
2. Make sure that you have single stain controls and unstained.

3. Make a dot plot that has the channel you are currently looking at on an axis. Then look at it versus all the other colors one at a time. You can use the compensation hand or use the matrix with typing in the number to adjust your compensation. The compensation hand only works on dot plots. Histograms help with this to visualize that channel bleed over moving back into the negative range with the single cells controls.
4. Duplicate without date so that the compensation carries. Repeat step 3 for all your colors of your panel.
5. You can then save as to export the matrix and specify where to save it.

Auto Compensation

1. Ensure that you have saved a file of the correct gain settings and channels first.
2. Create a compensation experiment by selecting New Compensation in the File menu. Use the default file path and select Save.
3. Select the channels requiring compensation and the sample type. The unstained negative and single stains should be used for this.
4. Select OK. A compensation control experiment is generated. Import the gain settings of your experiment and apply to all tubes. This will ensure that the compensation samples are run with the same settings as your experiment.
5. Install the unstained cells tube onto the cytometer. Move the FSC SSC gate so that it encloses the desired population.
6. Select Record. Unload the tube.
7. Install the next tube onto the cytometer.
8. If necessary, move the positive gate in the plots so that it encloses the positive population. Select Record. Repeat for all compensation tubes.
9. Select Compensation Calculation in the Settings menu.



10. The matrix displays. Save as to export the matrix and specify where to save it.

Recording Sample Tube Data

1. Open your previously created experiment (From the File menu, choose Open Experiment. It should be listed as your “experiment name”.xit.)
2. If compensation was performed import it (using the compensation icon in the tube window) and apply to all tubes (Import>Compensation). Select Compensation Matrix in the Settings menu. **Select Import compensation matrix and gain.** Select OK.
3. Add as many tubes to the experiment as necessary. Rename them by right clicking on them.
4. Adjust the sample flow rate in the Experiment Window. Default is slow at 10 µl / min.
5. Click the first tube, install the tube, load it and record it. (It will turn green on the test tube location. If it is still blue you only acquired) You may need to initialize to bring the tube holder out again if set up process took longer.
6. Continue for all tubes you wish to run.

Recording Sample Plate Data

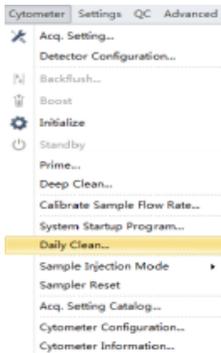
1. Open your previously created experiment (From the File menu, choose Open Experiment. It should be listed as your “experiment name”.xit.)
2. If compensation was performed import it (using the compensation icon in the tube window) and apply to all tubes (Import>Compensation). Select Compensation Matrix in the Settings menu. **Select Import compensation matrix and gain.** Select OK.
3. Add as many plate and wells to the experiment as necessary. Rename them by right clicking on them.
4. Adjust the sample flow rate in the Experiment Window. Default is slow at 10 µl/min.
5. Selecting run to being aspiration of your sample. Press record to save the data. (Note: if any of the gains are changed please apply your new well setting to the rest of your plate by following how to use **apply well setting** seen in step 6 of plate setup.)
6. To run auto record just select auto record to being. You can skip the current well with the skip button. Pause recording with the pause button. Please remember to resume with the resume button. The auto record will not run your plates that already have the green checkmark or the red “X” that appears on your plate if you were doing the run manually.

(**Note:** Overlay of histograms and dot plots are only available in the analysis portion of the software after samples have been run.)

Clean the instrument → Turn the instrument off

Shutting down

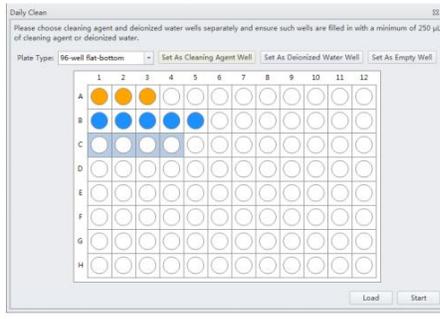
1. Select Daily Clean from the Cytometer menu.



2. Load a tube of 2mL 10% Bleach solution into the sample holder and select Run. Cleaning time should be at least 3 minutes.

3. Repeat with a tube of 2mL DI water for at least 5 minutes and select Run.

(**Note:** For plate use cleaning plate and follow the well set up as shown total time of



about 12 min.)

4. After cleaning process is completed close the Daily Clean window.
5. Exit the Cytoflex software.

Clog / Debris Clearing

Problem with sampling without events registering is often a clog/debris issue. Lines may be purged of debris/clogs by fluidics. The clog is usually in the probe but sometimes elsewhere in the system.

Prime performs high pressure run to purge lines of debris and detergent.

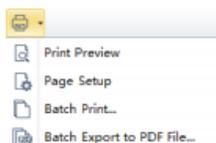
- Cytometer and click the prime button. Perform prime 2-3x times.
- Back flush on the plate mode does this purge of the line.

Deep Clean

1. Top-off distilled reservoir and empty waste collection containers.
2. Set physical switch to sample tube.
3. Go to Cytometer. Click Standby. Click Deep Clean and proceed. (**Note:** Deep clean pulls enzymatic detergent into working lines of the machine and suggests **30 min wait.**)
4. Immediately proceed to Cytometer and click the prime button. Perform prime 2-3x as time permits.

Data Export

1. Navigate to your experiment. Save a copy of your experiment.
2. Two ways to export:
 - In the top left select file. Go down to export fcs files. Select your save path by following the screen prompts for which samples are selected.
 - Right click the desired samples. Go down to the export FCS file that appears on the screen. Select your save path by follow the screen prompts for which sample is selected.
3. For pdf exports please go to the bar that has the plots creation on it. Go across to the little printer. Select the drop down arrow for the printer icon. Select pdf export. Select your save path by following the screen prompts for which samples are selected.



(**Note:** Always save and copy your data as data logs are removed periodically.)