
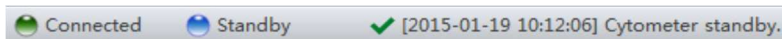


# CytoFLEX – User Instructions

## CytoFLEX Startup Procedure

- ☑ Check whether sheath tank full and waste tank empty.  
If you empty the waste tank, add 400 ml FACS Clean to the tank.
- ☑ Turn on the computer.
- ☑ Start CytExpert software  .  
Enter your personal account name and password to log in, and click OK.
- ☑ Click on CYTOMETER > TURN ON.
- ☑ Check the connection and fluidic status in the status bar.



NOTE If the icon is not green, ensure that the instrument USB is securely connected to the computer and restart the computer.

- ☑ Select CYTOMETER > SAMPLE INJECTION MODE in the Menu to change between the Semi-Automatic Injection mode and the Manual Injection mode.

NOTE The Semi-Automatic Injection mode for tubes is recommended under most circumstances.

NOTE If you change the sample injection mode from Plate mode to Semi- Automatic mode or vice versa, you need to turn the switch in front of the tube holder and switch off and on the cytometer in the software again.

- ☑ Prepare a tube with 2 mL H<sub>2</sub>O.
- ☑ Select CYTOMETER > SYSTEM STARTUP PROCEDURE, click INITIALIZE and follow the software prompts. The system startup program takes approximately 9 minutes.
- ☑ When warm up is finished, select CLOSE to quit the startup program. The system is now initialized.

NOTE In the initialized state, the laser power is on to achieve operating status, and the sheath fluid flows. If the instrument remains idle for 10 minutes, the Cytometer automatically enters the standby state.

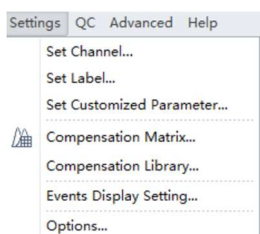
NOTE If you need to execute a task with the Fluid Containers, do so with the instrument in standby state.

- ☑ In order to reinitialize the instrument, select INITIALIZE in the Data Acquisition Control screen. Wait for the beep indicating that the Instrument properly initialized.

## Create an Experiment

CytExpert software separates color compensation and sample acquisition experiments into separate files designated .xipc and .xip, respectively. The experimenter can choose which one to execute first.

- ☑ Select NEW EXPERIMENT in the FILE menu, specify the file path via SETTINGS > OPTIONS and name the experiment or accept the default naming Exp\_YYYYMMDD-1 and select SAVE.
- ☑ Select SETTINGS > SET CHANNEL. Select the desired channel signals in the checkbox. Type in reagent name in the label column if desired.




- ☑ Explore the tool bar buttons to create plots in the experiment workspace. Select the axis name on the plot to change which channel is displayed.




- ☑ To create gates, use the gating control buttons on the toolbar or right click the plot and select the gate type required.



- ☑ Select the heading area of the plot to assign the gates to the required plots.
- ☑ Select the POPULATION HIERARCHY button  from the toolbar to display the population hierarchy.

NOTE You can change color and names of gates by double clicking the color or name in population hierarchy window.

- ☑ Select the STATISTIC button  from the toolbar to display statistics window. Right click the Statistics table and select STATISTICS SETTING and check desired header, statistics and populations of interest.

- ☑ Select the ACQ. SETTINGS on the left side panel to open the Acq. Setting window and display cytometer gains and threshold settings.

NOTE In the Gain tab, you can select Recommended in cases where you do not specify your own default parameter gain settings.


- ☑ Import compensation matrix to apply compensation values to experiment if required by selecting COMPENSATION MATRIX from the SETTING menu to bring up the compensation matrix table. Select Import from Library or Import and locate the compensation matrix file (.comp) to import. Select desired option for importing compensation matrix and gain settings.

## Sampling and Collecting Data on the Tube Loader


Samples can be analyzed manually in the Semi-Automatic Injection mode. If you need to change from Plate mode to Semi-Automatic mode, select CYTOMETER > SAMPLE INJECTION MODE and switch to the corresponding mode, turn the switch in front of the tube holder manually and switch off and on the cytometer again.

- ☑ Place the sample tube in sample tube holder and select RUN to load the sample.
- ☑ Adjust Acquisition settings. In the Acquisition Panel, select the desired acquisition settings for Sample flow rate, Events to Display, Events to Record, and Time to Record.

NOTE High acquisition rate increases the abort rate and measurement CVs. If the event rate is above 10.000 events/second and the abort rate is high, dilute your sample. or adjust the flow rate.

- ☑ Adjust gain settings. Use GAIN tab in the Acq. Setting window to adjust gain settings for the different channels or use the GAIN ADJUSTMENT CONTROL button  on the toolbar for increasing and lowering gain on the plots during data collection.

IMPORTANT To stay in the linear range of fluorescence detection the gain should be above 100 which is normally the case if the negative population is located around  $10^3$ .


- ☑ Select the TRESHOLD tab in the Acq. Setting window. Choose the channel for threshold setting and manually enter a value in Threshold tab. Alternatively, select Threshold button from the tool bar and move the mouse to desired  threshold position in the desired plot.

NOTE Do not use the automatic threshold as it may cut parts of the desired signal !

- ☑ You can adjust axis ranges using the axis display controls from the tool bar  on the plots.




NOTE Alternatively, select Fit with Sample to identify the signal's lower limit, adjusting automatically as warranted. Selecting this item is recommended whenever the signal appears to be relatively low.

- ☑ Select RECORD to save the data and wait for the saving process to finish.
- ☑ Add additional tubes to Tube list if required, load tubes and record data for each tube until all data required has been collected.

- indicates that the tube data was not collected.
- indicates that the tube data was acquired by selecting Run but not saved, and the tube data can be overwritten.
- indicates that the tube data was saved by selecting Record and that this data cannot be overwritten.
-  to the left of the test tube indicates that the sample has been compensated.

## Sampling and Collecting Data via Plate Loader

Samples can be analyzed via the plate loader in the plate mode. Select Cytometer > Sample Injection mode to change from Semi-Automatic mode to Plate mode, turn the switch in front of the tube holder and turn the Cytometer's off and on again by choosing Cytometer > Turn off /Turn on.

- ☑ Select PLATE . The plate window appears.
- ☑ Select ADD PLATE in the Plate dropdown  menu.
- ☑ Select the Plate type and workflow direction from the dropdown menu.
- ☑ Highlight the desired wells and select SET AS SAMPLE WELLS .

NOTE To reset selected wells choose Set as Empty Wells. 

- ☑ Enter all required information or import settings from an FCS file.

NOTE It is recommended to set the Backflush option to 6 seconds.

- ☑ Define "Events to record" *or* "Time to record" under the Stopping Rules tab.

NOTE You should set an acquisition time limit, so the acquisition will not continue if the event limit cannot be reached.

- ☑ Import compensation matrix if required by selecting COMPENSATION MATRIX from the Settings menu. Select Import from Library or Import and locate the compensation matrix file (.comp) to import. Select desired option for importing compensation matrix and gain settings.

- ☑ If any well settings require modification, right click the sample well and select the setting to change.

- ☑ Select Eject.

- ☑ Place the plate flat on the plate holder and ensure that it is secure.


- ☑ TO RUN A SINGLE WELL

Select the well in the Plate window. Select RUN to prompt the system to begin sample aspiratio. Acquisition settings can be adjusted during acquisition.

Select RECORD to save the data, when your are satisfied with the appearance of the data

Select EJECT to prompt the plate loader to eject.

- ☑ TO RUN A SET OF WELLS

Set the selected wells for auto record by selecting the desired wells. Select  or right click and select AUTO RECORD. Select Ax if you want to remove the Auto Record settings from the selected wells.

NOTE Acquisition occurs in the order indicated by the numbers in the botton right corner of each well.

Select  to begin sample acquisition.

## Daily Clean and Instrument Shutdown

After the measurement execute the DAILY CLEAN procedure.

### Daily Clean on the manual Port

- Select CYTOMETER > DAILY CLEAN and follow the cleaning prompts.
- Add a tube of 2 mL FlowClean solution followed by a tube with 3 mL H<sub>2</sub>O.  
Wait for system to finish.

NOTE Do not use Bleach solution during the washing procedure !

- Once completed, select CLOSE.

### Daily Clean on the Plate Loader

- Prepare: 3 wells FlowClean and 5 wells H<sub>2</sub>O: Start the Daily Clean.

NOTE After the measurement of sticky fluorophores like SYBR Green or PI include 3 wells of FACSClean and 5 wells of H<sub>2</sub>O BEFORE the routine Daily clean and define these wells as sample wells.

### Instrument Shutdown

- Turn off instrument via CYTOMETER > TURN OFF.
- Exit software.
- Clean external surfaces and dispose used cleaning materials in biohazard.
- Provide information about your session in the Logbook.

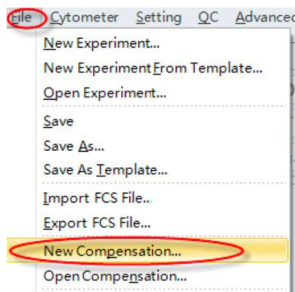
## Compensation

Compensation adjustments can be completed during the data acquisition process or after the data acquisition process is complete. You can create a new Compensation for your experiment or integrate a previously created compensation experiment. For setting up a new compensation, generate as follows:

- ☑ Prepare all necessary unstained and single color controls using compensation beads or cells.


NOTE If a negative population is not present in each single color tube, then an unstained negative control tube is recommended.

- ☑ Select **NEW COMPENSATION** in the File menu, name file and click **SAVE**:



- ☑ Select the channels requiring compensation, and then select sample type. Uncheck tubes not needed for the experiment. Select **OK**.


NOTE Label and Lot No. information can be entered and retained in Compensation Library if desired.


- ☑ Load the corresponding unstained or single color sample fluorospheres or cells and select  in the Acquisition panel.

- ☑ If necessary, adjust the scatter gate so that it encloses the desired population. Adjust the slider scale on fluorescence plot so the negative and positive peaks appear in a suitable position. Move the positive and negative gates to enclose the corresponding peaks.

- ☑ Adjust the threshold settings by selecting the **TRESHOLD** button from the tool bar and move the mouse to desired threshold position in the desired plot.

NOTE Do not use the automatic threshold as it may cut parts of the desired signal!

- ☑ Once the gates are in satisfactory locations and the threshold is manually adjusted, select .

- ☑ After collecting data for all necessary compensation samples, generate the compensation matrix by selecting from toolbar or  **COMPENSATION CALCULATION** from settings menu.

- ☑ The Compensation Matrix window appears displaying the compensation values. Select **SAVE AS** to export the matrix as a .comp file and specify file path. Select **SAVE TO COMPENSATION**

LIBRARY to save the single color compensation values to the compensation library. Select OK and CLOSE.

- ☑ To save or overwrite the compensation matrix in the library, open compensation experiment, run compensation controls. Once completed, calculate compensation and save the comp library using designated button under the compensation matrix (pop-up).  
"SAVE AS COMP MATRIX" , "SAVE AS COMP LIBRARY". Choose a name.
- ☑ To apply compensation retroactively (starting with an experiment first, then applying Compensation), open an experiment, run your samples. Right-click on sample, a box Appears "APPLY COMPENSATION". Next to your tube, there is a clear checkered icon/box (very left), right click on icon to apply compensation matrix. From there, choose your matrix. Then, apply compensation to all tubes.
- ☑ To manually adjust compensation

Select the bivariate plot which the compensation needs to be adjusted. Select from the graphic control area, then click and drag the mouse pointer in the plot to adjust compensation. *OR*

Select Compensation Matrix in the Setting menu to open the compensation matrix. Adjust the compensation value between the primary channel and the secondary channel