

CytoFlex User Guide

1. Check sheath and waste levels.
 - a) Make sure to remove the sheath container from the vicinity of the instrument for refilling. **DI water should be used.**
 - b) Decontaminated waste should be disposed of down the sink. Fill empty waste tank to the Bleach Fill Line (0.5L) for a final concentration of 10%.
- 2) Switch on the instrument power (back left, above plug).
- 3) Confirm computer is on or turn on. Log into Windows with username




- 4) Open CytExpert Software
- 5) Click Initialize to wake up cytometer
 - a) The cytometer will revert to Standby if left idle for more than 5 minutes. Clicking Initialize will be necessary to wake the computer up again if left idle.

To begin with a compensation setup: (if needed)

*This auto-comp procedure will only work with very good controls. It is recommended to do actual compensation in off-line analysis software. Auto-comp should only be used to get a quick idea of your staining. To use it:

- 1) Select New Compensation in the File menu.
- 2) Select the corresponding tubes in the pop-up Compensation Setup window, identify them as bead or cell and click OK. CytExpert automatically creates a list of matching empty tubes in the Tube panel as well as all the plots needed to gate the positive populations.
- 3) Record the appropriate negative and single color controls; adjust the positive population gates in corresponding histograms. (Negative gates can be added if necessary).
- 4) After collecting data for all necessary compensation samples, generate the compensation matrix by selecting Compensation Calculation in the Compensation menu.
- 5) Save compensation and acquisition settings by opening the Compensation Matrix window







(Settings>Compensation Matrix or the  button on the worksheet ribbon) and clicking "Save As...". this will create a ".comp" file that you can use to import Gains and Compensation into an experiment.

- 6) To export .fcs files from a Compensation you must click the Folder icon at the top of the Tube window in order to open up the containing folder. REMEMBER - do not move these files. Instead, copy and paste them to an export folder for your experiment. The files can also be exported individually by right clicking and exporting, but there is no bulk method.

Experiment creation (with or without a previous compensation setup):

- 1) Select New Experiment or Open from Template
- 2) Name saved file with initials, date, etc.
- 3) Create plots and histograms using the tools in the panel on top.
- 4) Specify sample flow rate, numbers of events to display and to record.
- 5) Name the first tube (double click the name).


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- 6) **Click Initialize in the acquisition panel; load the tube.**
- 7) Adjust gains and compensation depending on whether an auto-compensation has been run:
 - a) If auto-compensation has already been run:
 - i) Click on the Compensation Matrix button  and click Import at the bottom.
 - ii) Select the .comp saved earlier and select "Import both gains and compensation matrix".
 - b) If no auto-compensation has been run:
 - i) Click Run. Adjust voltage gains in the Acq. Settings panel to bring the desired populations on scale.
 - c)  Threshold can be seen by clicking this button (see top ribbon).
- 8) Use scaling  and compensation  tools (on the top ribbon) to optimize the plots. If needed, there is also a rectangular icon towards the bottom of each plot that allows adjustments of the linear scaling
* Compensation with the hand tool produces very large changes in compensation when it is applied closer to the negative population. When applied near the brighter edge of the positive population, it produces more manageable changes.
- 9) Once the optimal voltage, scaling, and compensation are determined, load the first sample tube and click Record. The fcs file is saved once the recording reaches the specified number of events or when it is stopped manually.
 - a)  Threshold can be seen by clicking this button (see top ribbon).
- 10) Create next tube. The voltage, compensation, and scaling are automatically copied from the last tube. To apply to previous tubes or a set of already created tube, use the "Apply to.." options.
* Recorded tubes (green) cannot be overwritten.
- 11)  Population hierarchy can be visualized (see top ribbon).
- 12) Export specific graphs if needed (select, right-click). Use print screen if need an image of the entire workspace.
 - a) Experiments can be saved as templates - good idea to backup templates and take them with you.

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- 13) Clean:
 - a. In Cytometer menu, select Daily Clean
 - b. Run Coulter Clenz (blue cleaning solution) if cells were fixed or 10% bleach if cells were live for 3 minutes.
 - c. Run DI water for 5 minutes.
- 14) Close software.
- 15) Turn off the instrument power and leave computer ON.

Changing to plate reader from semi-automatic tube loader

- 1) Cytometer > Sample injection mode > plate reader
- 2) Turn off cytometer
- 3) Change to plate reader probe while cytometer is off
- 4) Turn cytometer back on and software will reflect mode change
- 5) Mode changes are done in Standby mode so you must select 'Initialize' afterwards.
- 6) Once mode change is complete, run a Daily Clean. ***Please Note:*** Daily clean must be done before **and** after any mode change in order to ensure clear sample lines and probes. For fixed/non-biohazardous material, the first three wells of the cleaning plate (located next to the machine) should be Coulter Clenz. For unfixed human cells/any biohazardous material, **bleach** should be used.
- 7) In order to set up plate for analysis:  plate setup → ***select plate type*** → setup sample wells, preferences, etc.

Troubleshooting

- For clogs, air in the system or other fluidics problems use the cytometer menu to Backflush > Prime > System startup program, listed in order of the extremity of the response. Prime can only be done from a Standby state.