



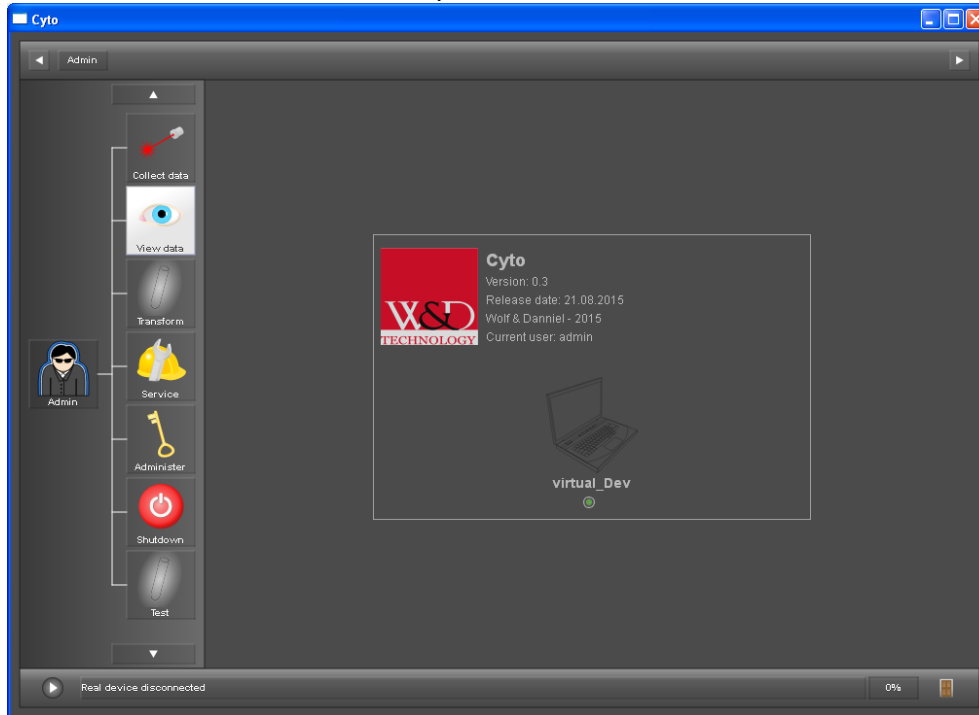
For questions and comments please contact us at: support@wolf-daniel.com

Content of the manual:

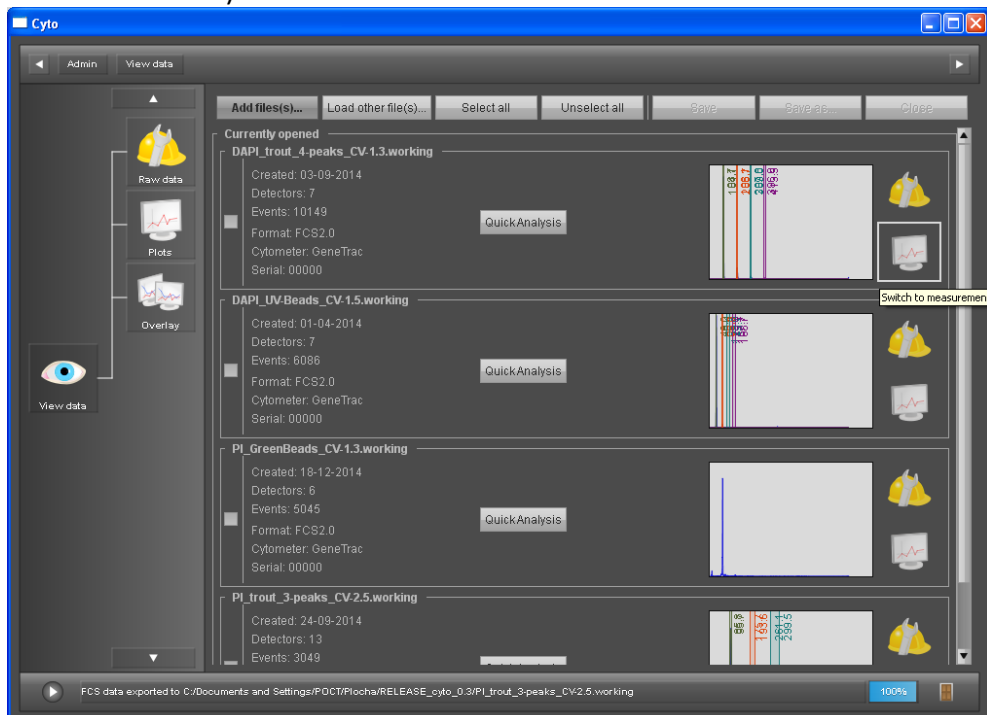
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|---|---------|
| 1. Starting the software and opening FCS files | page 2 |
| How to start the application and create plots from the FCS data. | |
| 2. Graph options | page 3 |
| How to edit the graph parameters (detectors) and adjust the appearance. | |
| 3. Histogram analysis | page 4 |
| How to compute the position and CV of peaks of interest within the histogram. | |
| 4. Histogram overlay | page 6 |
| How to merge more histograms together for easier data comparison. | |
| 5. Data filtering | page 8 |
| How to filter out unwanted background signal or remove temporary fluctuations. | |
| Option 1 – histogram filtering (= selection of peaks or intensity intervals of interest) | |
| Option 2 – time filtering (= selection of some periods of time with stable conditions) | |
| 6. Histogram transformations | page 12 |
| How to perform a simple math functions on FCS data. | |

1. Starting the software and opening FCS files

- Start the application by a double click on „cyto.exe“
- Logon as „admin“ with the „admin“ password
- Click on section „View data“ - white square in the screenshot

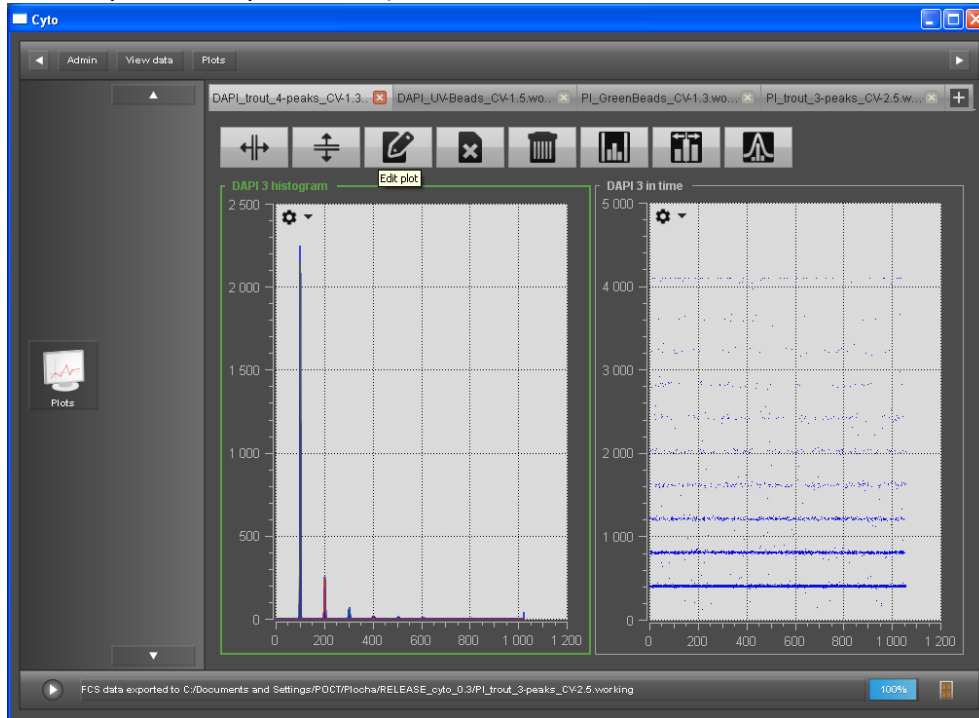


- Click on the button „Add measurement(s)“, which will open a file dialog window
- After selecting one or more FCS files to view, click on the „Graph“ icon (white rectangle in the screenshot below)

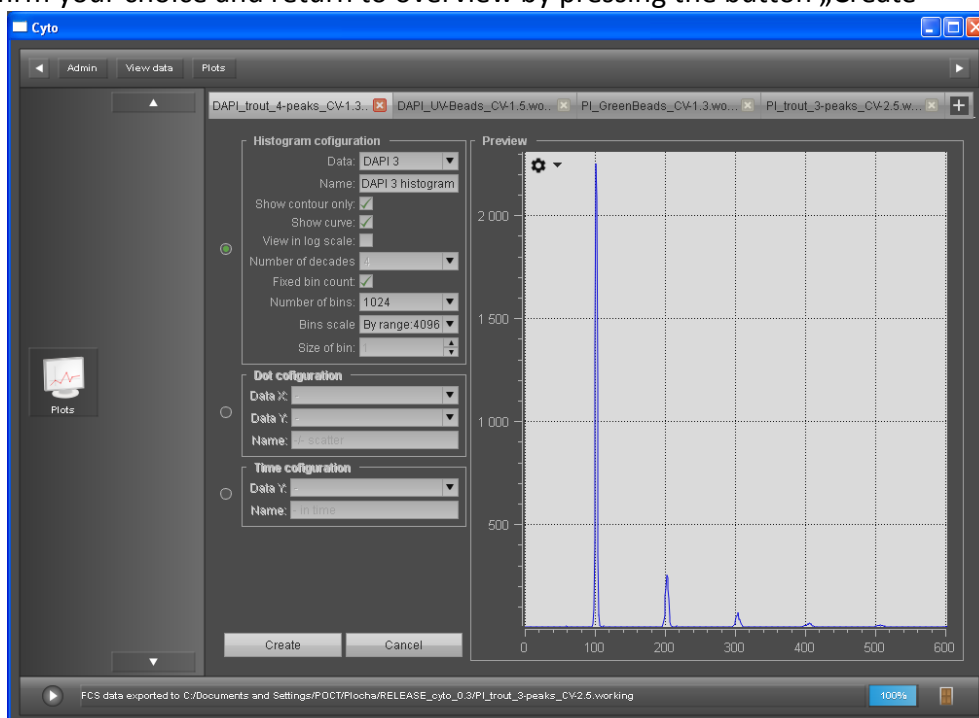


2. Graph options

- In case you need to change detector / channel or properties of the active graph, select the graph (indicated by a green rectangle around it) and press the 3rd button from the left (called „Edit plot“ with pencil icon)



- In this sub-window you can edit different options of the graph like detector or change the visual appearance of the data by choosing the options „Show contour only“, „Show curve“, „Log scale“, „Number of bins“ etc.
- Confirm your choice and return to overview by pressing the button „Create“

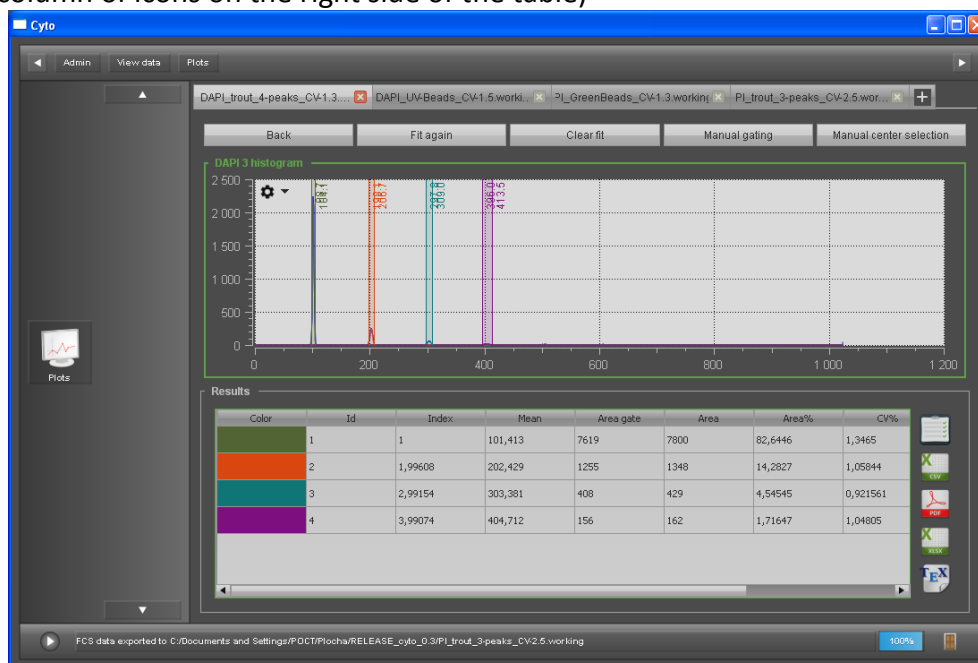


3. Histogram analysis

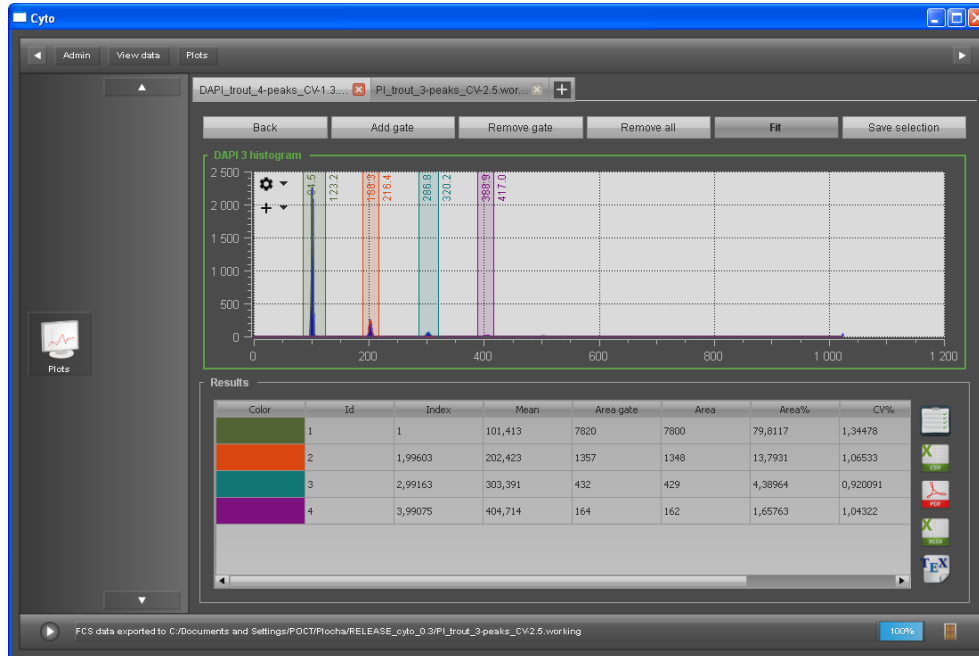
- Make sure that you display the proper signal/detector in the active histogram
- Analysis (fit of peaks) could be done by 3 rightmost buttons above the graphs (representing 3 different modes of analysis: Manual gating, Manual centers, Automatic fit)



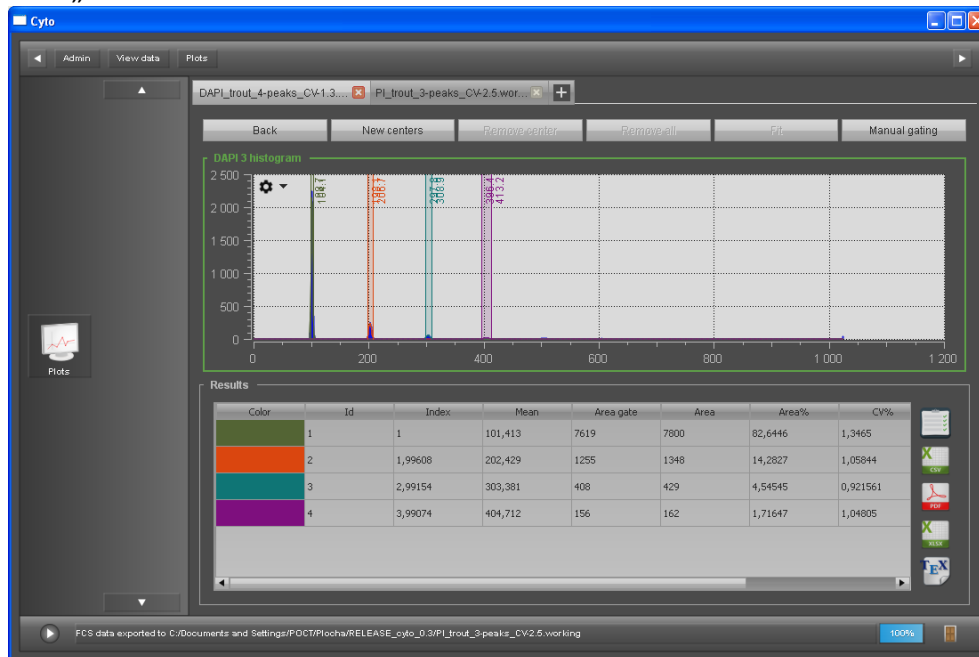
- Example of the „Automatic fit“ routine
- Data of the fit could be exported into different formats like CSV, PDF, XLSX or Clipboard (see the column of icons on the right side of the table)



- „Manual gate“ fit routine – sequence of steps:
 - Press „Add gate“ button, click on the left side and on the right side of the peak
 - Repeat previous step for all expected peaks
 - Press the „Fit“ button



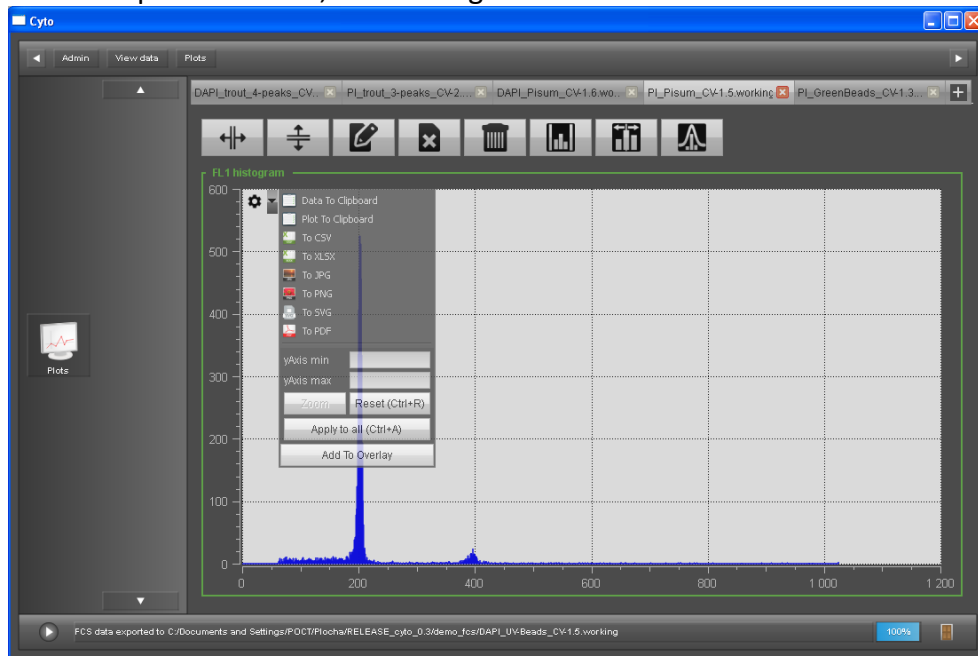
- „Manual center“ fit routine – sequence of steps:
 - Press „Add center“ button, click on the expected center of the peak
 - Repeat previous step for all expected peaks
 - Press the „Fit“ button



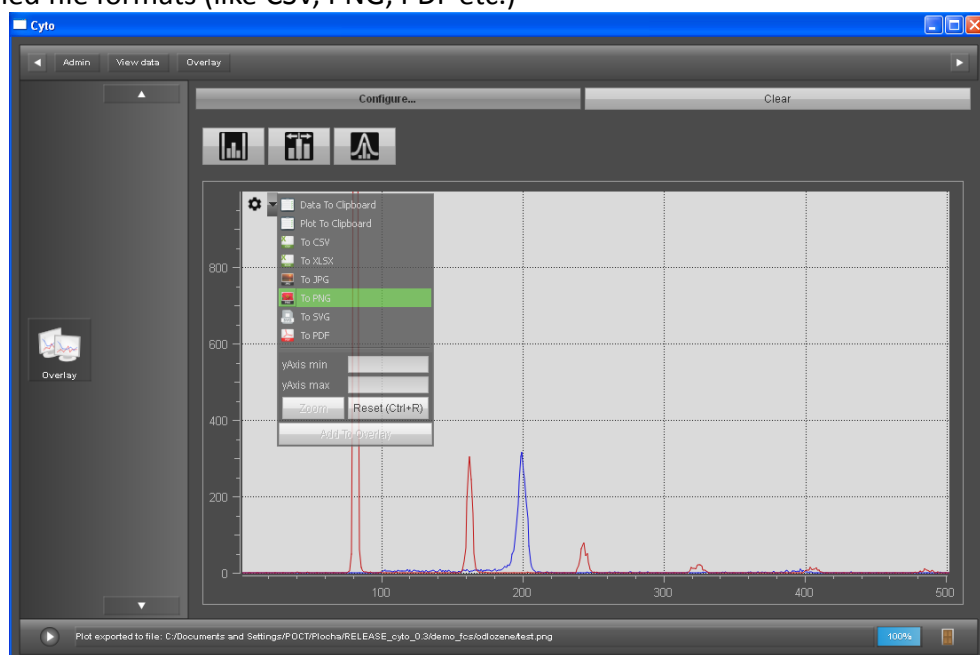
- For both fit routines, data of the fit could be exported into different formats like CSV, PDF, XLSX or Clipboard (see the column of icons on the right side of the table)

4. Histogram overlay

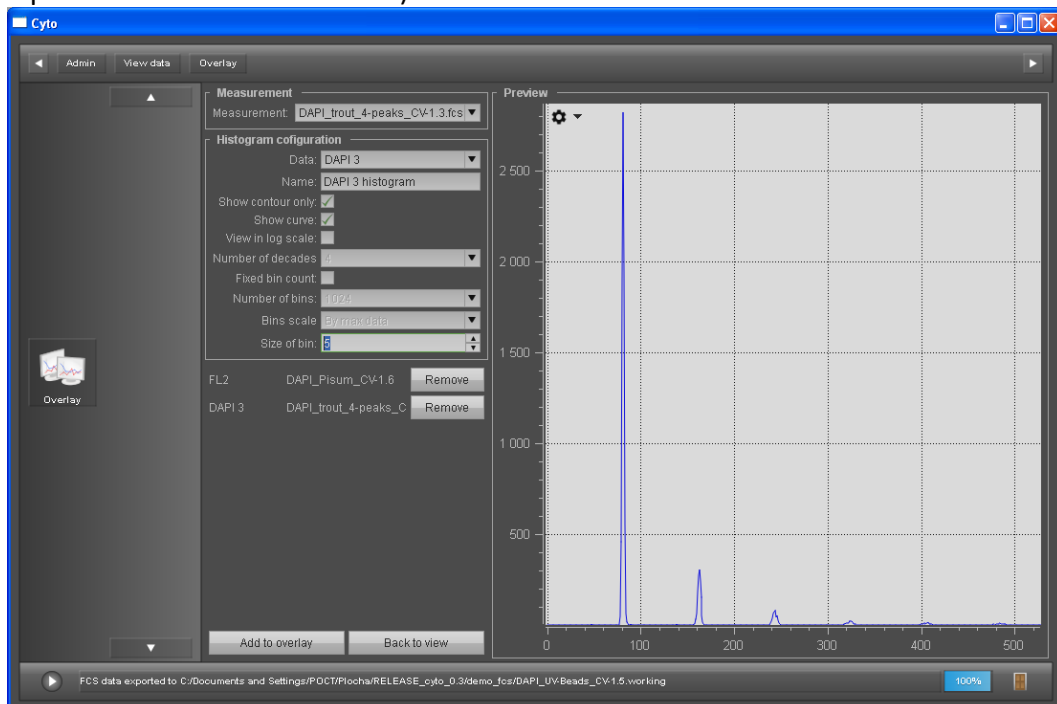
- Make sure that histograms have the desired graphical settings (as described in Section 2).
- Open the context menu of the histogram and press the „Add to Overlay“ button
- Repeat this step for a second, third histogram...



- Return one level back by pressing the square button „Plot“ on the left side
- Enter the Overlay section by pressing the „Overlay“ square button (see page 2)
- In the Overlay histogram window, use free Zoom by dragging the cursor with the mouse over the graph or use the context menu for specific Y-axis range (see the screenshot below)
- For the export to other Windows application use either Clipboard (data or plot) or some predefined file formats (like CSV, PNG, PDF etc.)



- In case you need to add, remove or change histograms, use the button „Configure“.
- Make sure that you use choose the proper combination of „Measurement“ = FCS file and „Data“ = detector/channel.
- Additionally, you can modify the histogram features using the options:
 - „Contour“
 - „Show curve“ = fine smoothening function
 - „Fixed bin count“
 - „Bins scale“ = 4096 or „max“ for transformed data
 - „Size of bin“ = stronger smoothening by merging and averaging (leads to a shift of the peak on the absolute X-scale)

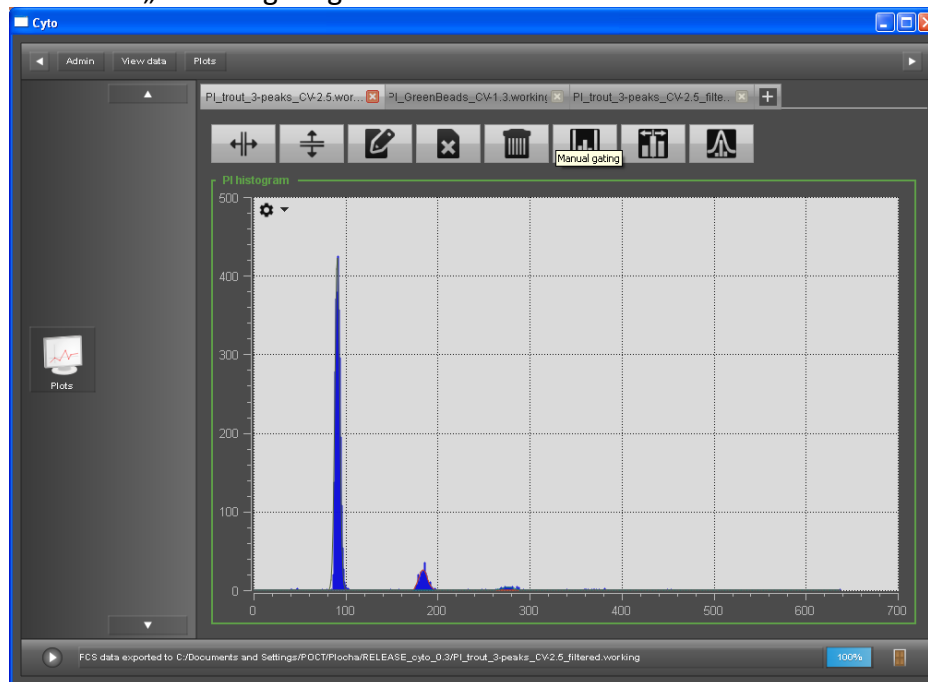


5. Data filtering

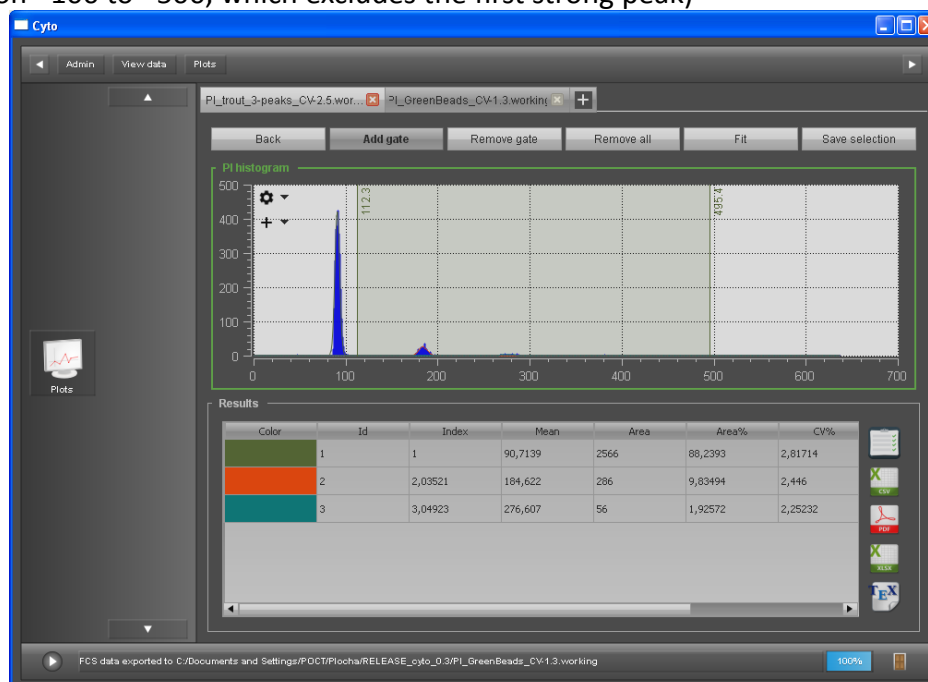
Option 1 – histogram filtering (= selection of peaks or intensity intervals of interest)

This option will include only the selected intervals, which might be good for removal of the unwanted signal from the original data (e.g. strong background peak) or which allows you to separate peaks of different samples within FCS file into more FCS files.

- Use the function „Manual gating“

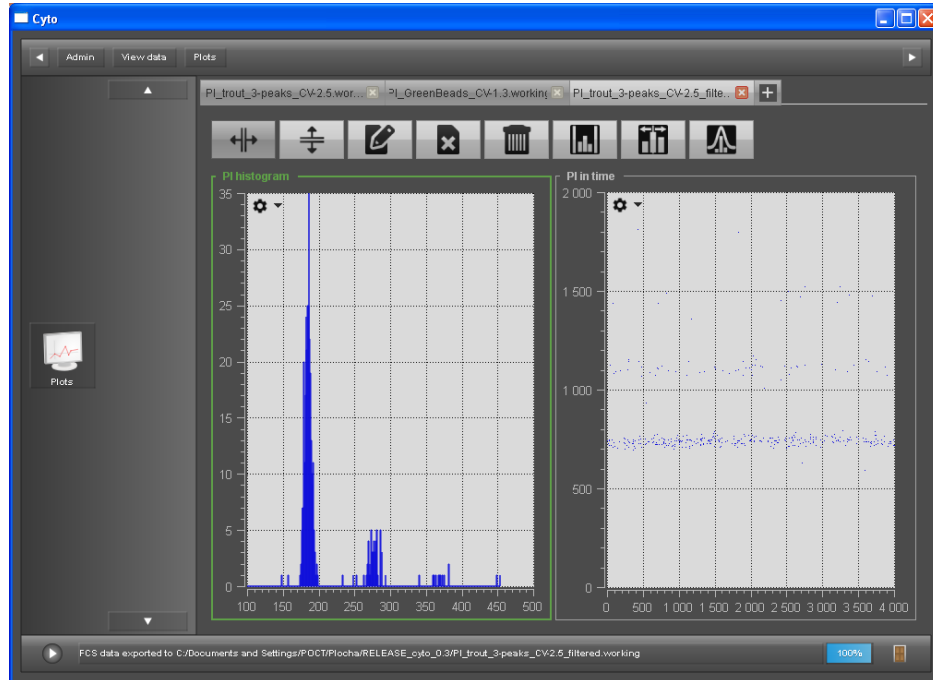


- Select the range (or peaks) of your interest (for example here, we choose the interval from position ~100 to ~500, which excludes the first strong peak)

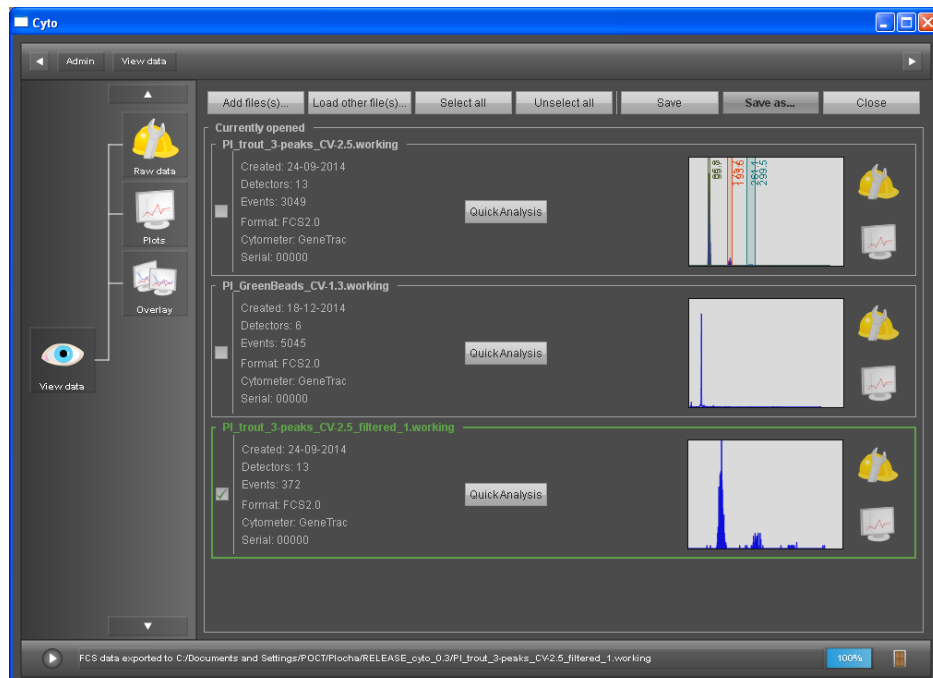


- After defining the gate(s), press the button „Save selection“ and a new tab will be opened

- Now you can continue with the analysis of the reduced file:



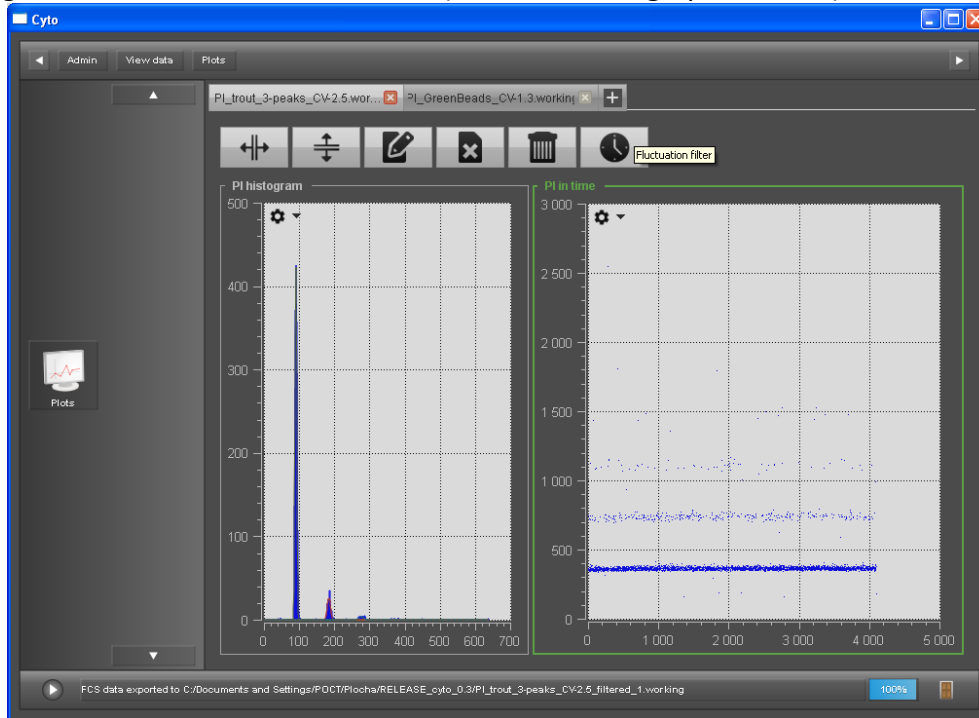
- In order to manually save the new file to a FCS file, go back to the „View data“ menu, select the file with the checkmark and press the „Save as“ button
- Alternatively, you can leave the tab(s) open and when exiting the program completely, you will be asked to save them



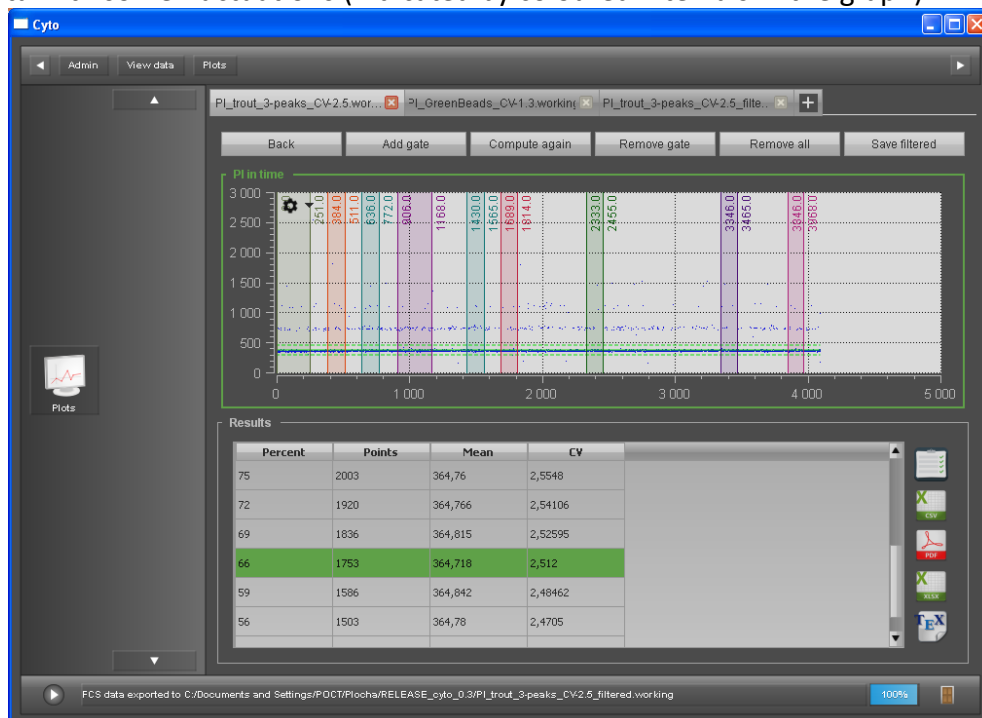
Option 2 – time filtering (= selection of some periods of time with stable conditions)

This is a time-based reduction filter, which will keep all peaks in the histogram, but will just reduce the amount of counts for each peak depending on the specified level of filtering. This option is designed to remove fluctuations (like a bubble in the tubing) from longer measurements that contain valuable data.

- Using the function „Fluctuation filter“ (when a „time“ graph is active)

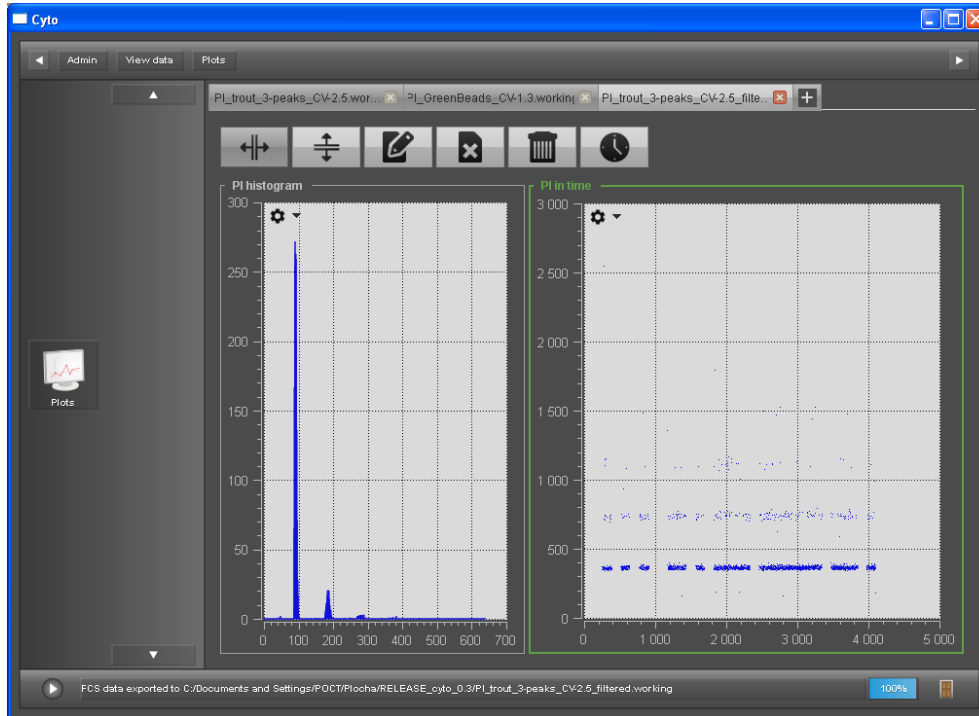


- Automatic routine will start and will suggest a table of intervals with different filtering level
- For example, a line with 66% will keep 66% of original data and suggest to filter out 34% of the data with some fluctuations (indicated by coloured intervals in the graph)

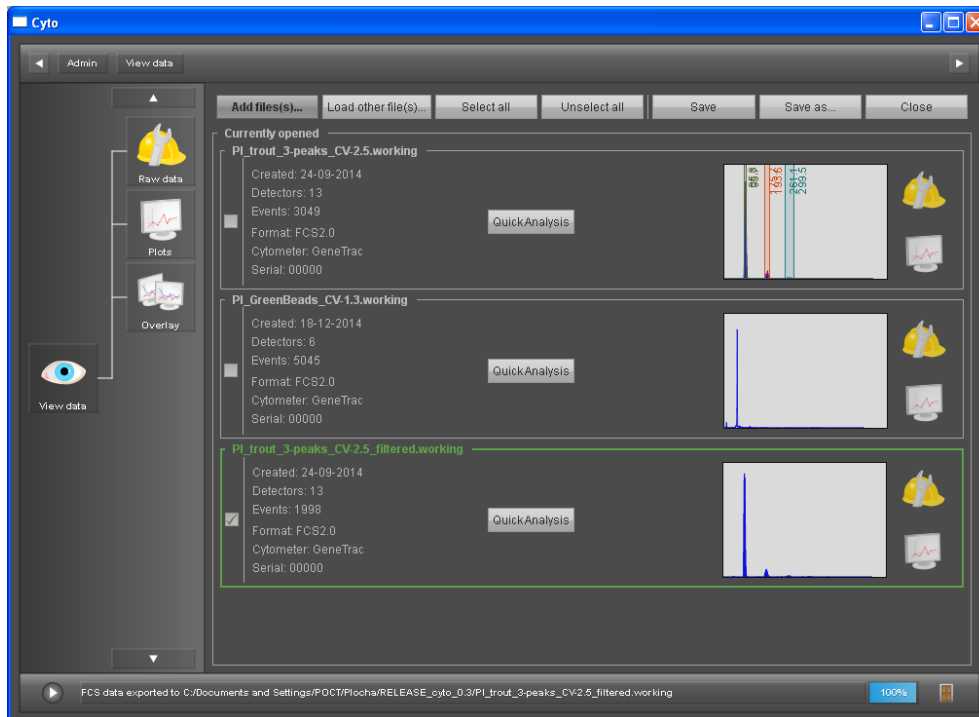


- After specifying the filter, press the „**Save filtered**“ button and a new tab will be opened

- Now you can continue with the analysis of the reduced file:



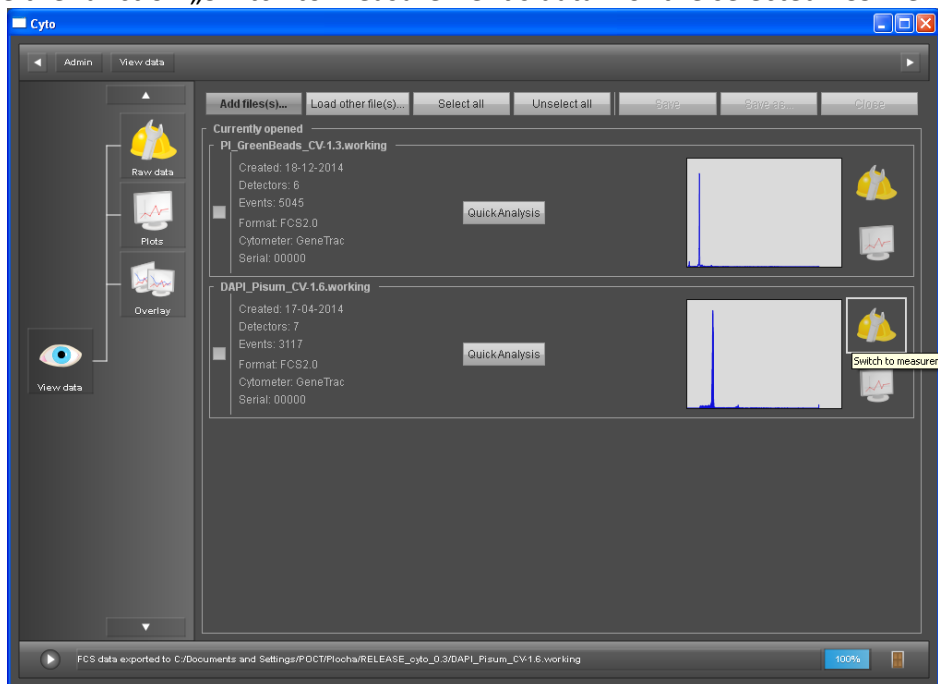
- In order to manually save the new file to a FCS file, go back to the „View data“ menu, select the file with the checkmark and press the „Save as“ button
- Alternatively, you can leave the tab(s) open and when exiting the program completely, you will be asked to save them



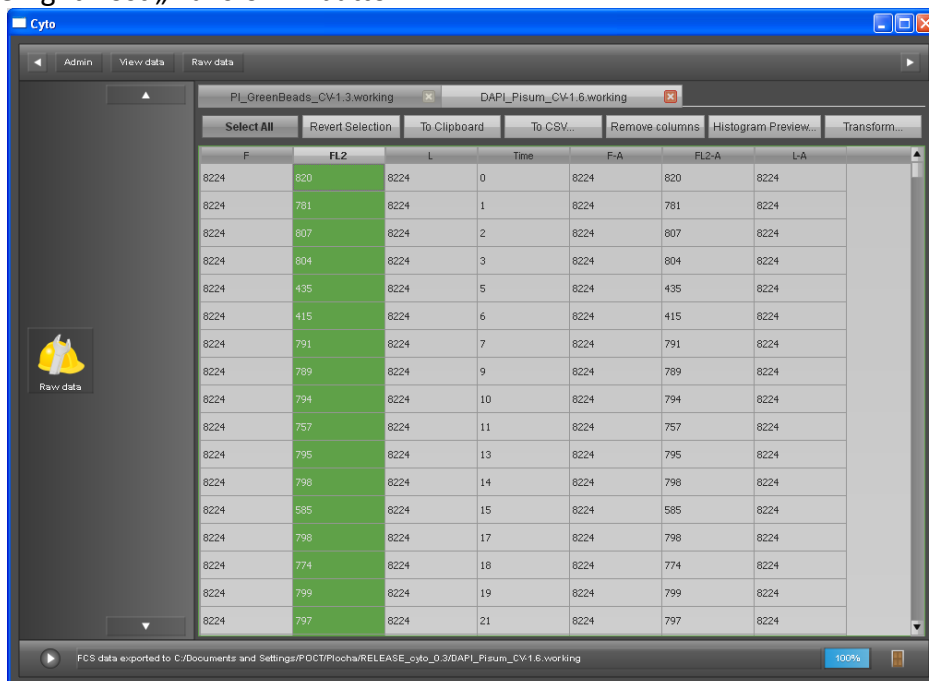
6. Histogram transformations

This function allows you to create a new column within the FCS data file based on simple math functions applied to an existing column.

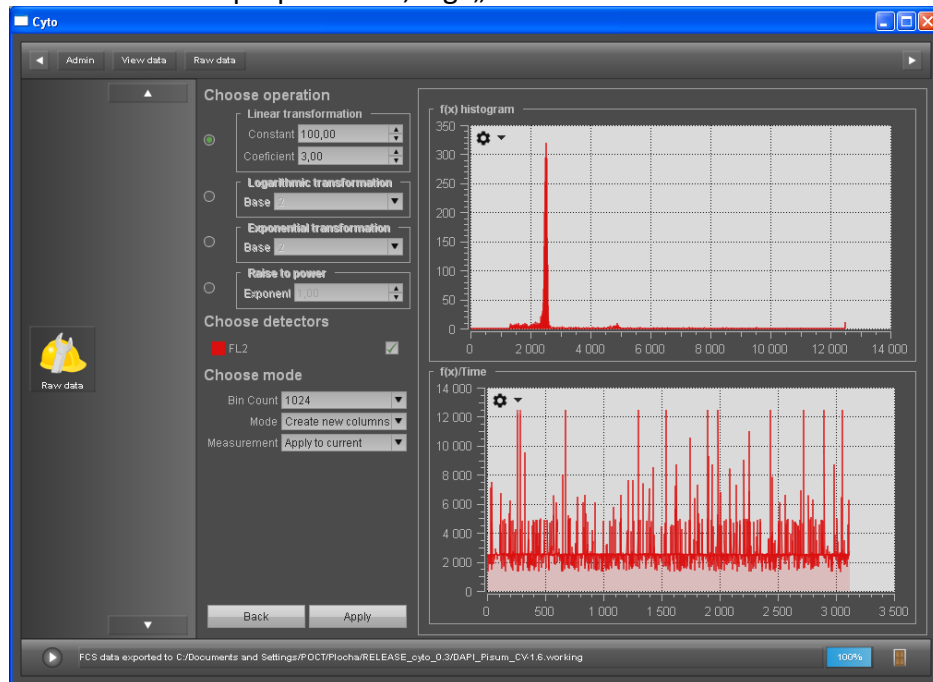
- Choose the function „Switch to measurement's data” for the selected FCS file



- Select the column (representing the detector) that you want to transform (here FL2) and press the rightmost „Transform” button



- Specify the constant (offset) and coefficient (multiplier) for the linear transformation or select a different transformation method
- Make sure to select the proper mode, e.g. „Create a new column“ within the FCS file



- After the computation, a new column will be created

Cyto

Admin View data Raw data

PL_GreenBeads_CV1.3.working

DAPI_Pisum_CV1.6.working

Select All

Revert Selection

To Clipboard

To CSV

Remove columns

Histogram Preview

Transform...

F	FL2	L	Time	F-A	FL2-A	L-A	3*(FL2) + 100
820	8224	0	8224	820	8224	2560	
781	8224	1	8224	781	8224	2443	
807	8224	2	8224	807	8224	2521	
804	8224	3	8224	804	8224	2512	
435	8224	5	8224	435	8224	1405	
415	8224	6	8224	415	8224	1345	
791	8224	7	8224	791	8224	2473	
789	8224	9	8224	789	8224	2467	
794	8224	10	8224	794	8224	2482	
757	8224	11	8224	757	8224	2371	
795	8224	13	8224	795	8224	2485	
798	8224	14	8224	798	8224	2494	
585	8224	15	8224	585	8224	1855	
798	8224	17	8224	798	8224	2494	
774	8224	18	8224	774	8224	2422	
799	8224	19	8224	799	8224	2497	
797	8224	21	8224	797	8224	2491	

Raw data

FCS data exported to C:/Documents and Settings/POCT/Plots/RELEASE_cyto_0_3/DAPI_Pisum_CV1.6.working

100%

- It is recommended to save the file (back to „View data“ menu, select the file with the checkmark and press the „Save as“ button) before continuing with other analysis operations (like Overlay histogram of original and transformed data etc.)