Miltenyi Biotec

MACSQuantify™ Tyto® Software 1.1

User manual



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MACSQuantify™ Tyto® Software 1.1

User manual

Original instructions

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Read the chapter **Important safety information** and all other information in this user manual before using the instrument

⚠WARNING Before using the MACSQuant Tyto Cell Sorter, read the chapter **Important safety information** in the MACSQuant Tyto Cell Sorter user manual and all other information contained in this software user manual, including any safety and operating instructions. Pay special attention to all warnings displayed on the instrument. Failure to read and follow these guidelines could lead to improper or incorrect usage and result in damage to the instrument. Improper usage could also cause severe personal injury, death, unpredictable results, instrument malfunction, and premature wear to components shortening the lifetime of the instrument. Such actions may void your warranty. Keep the user manual and any other safety and operating instructions provided with the instrument in a safe place accessible to all users for future reference.

If you have a serious concern regarding the safe use of your instrument, contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Technical Support.

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1 Basic functions

⚠WARNING Read the chapter **Important safety information** in the MACSQuant Tyto Cell Sorter user manual as well as all safety information in this manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

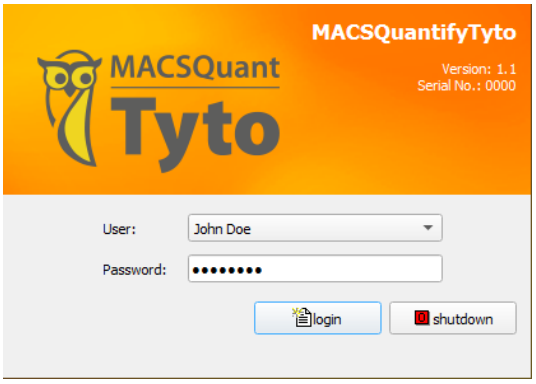
This MACSQuantify Tyto Software user manual applies to the MACSQuant Tyto Cell Sorter running MACSQuantify Tyto Software version 1.1.

1.1 Switching on the instrument

1. Switch on the power switch to turn the instrument into stand-by mode. A red LED on the orange touchscreen confirms stand-by mode.
2. Tap the touchscreen to power up the system. Wait until Windows is booted completely.
3. Double-click the MACSQuantify icon on the desktop to launch the MACSQuantify Tyto Software. This can take 1-2 minutes.
4. A login window will appear.

1.2 Login

1. Enter a user name or select it from the drop-down list.
2. Enter your password.
3. Click **login**.



1.3 Logout



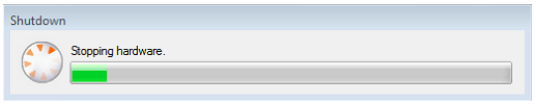
To log out, click the **Logout** button.

1.4 Switching off the instrument

The software should always be shut down before switching off the instrument.



1 To shut down the software, click the **Shutdown** button at the upper right side. The MACSQuantify Tyto Software and all processes on the MACSQuant Tyto Cell Sorter will shut down. This can take 1-2 minutes.



2 Use the **Windows**® icon to shut down the computer. The instrument is now in standby-mode. Tap the orange touchscreen to power up again.

3 Ensure that the MACSQuantify Tyto Software and Windows® software has shut down. Switch off the instrument. The power switch is on the rear side of the instrument.

2
The user interface

Access most functions on the monitor via the menu bar under **File**, **Edit**, **Mode**, **Analysis**, **Window** and **Help** (refer to **The menu bar on the next page**). Frequently used functions can also be accessed directly from the toolbar (refer to **The toolbar on page 15**). The currently logged-in user is displayed at the upper right side. Via the side panel, experiment and instrument settings can be set as well as the cartridge status is displayed. The **Real time sort statistics** window opens on the right side of the user interface when the software is started (refer to **The Real time sort statistics window on page 22**). Via the status bar, access the **Clear**, **Sort**, **Start/Stop measurement** and **Pause** buttons. In addition, use the orange touchscreen to modify instrument settings (refer to **The touchscreen on page 26**).

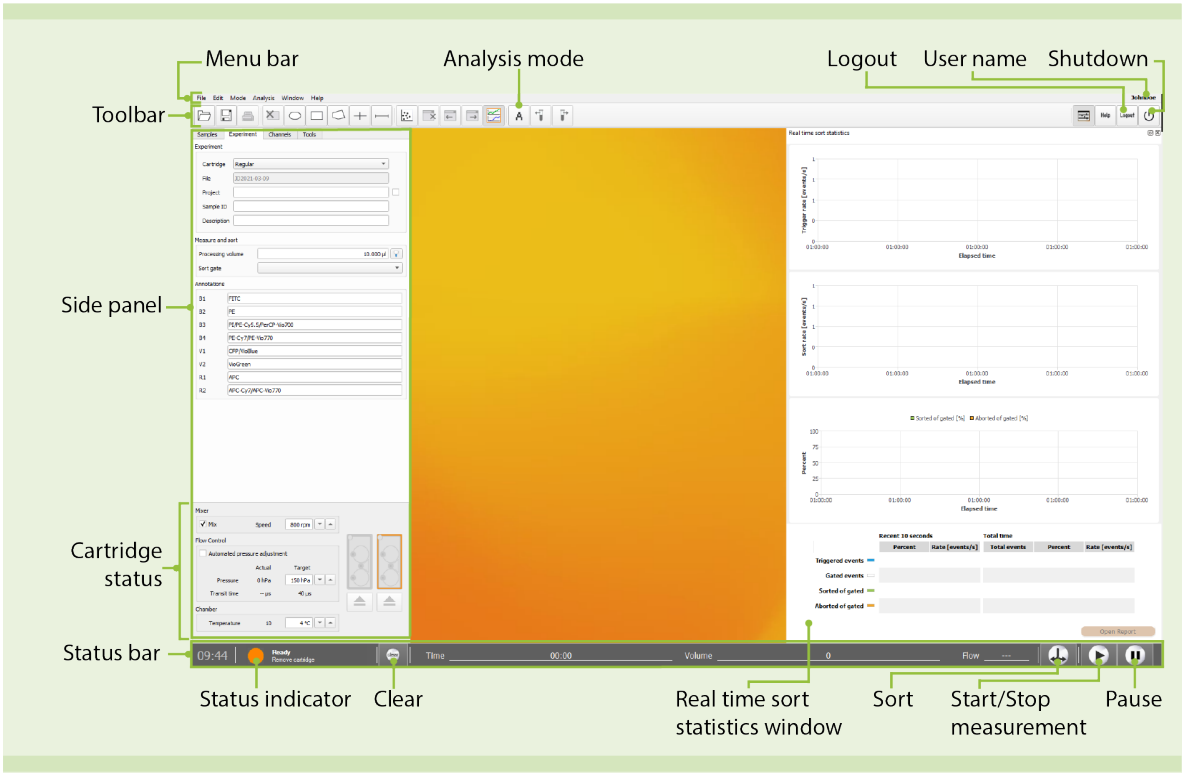


Figure 2.1: The MACSQuantify Tyto Software user interface.

2.1 The menu bar

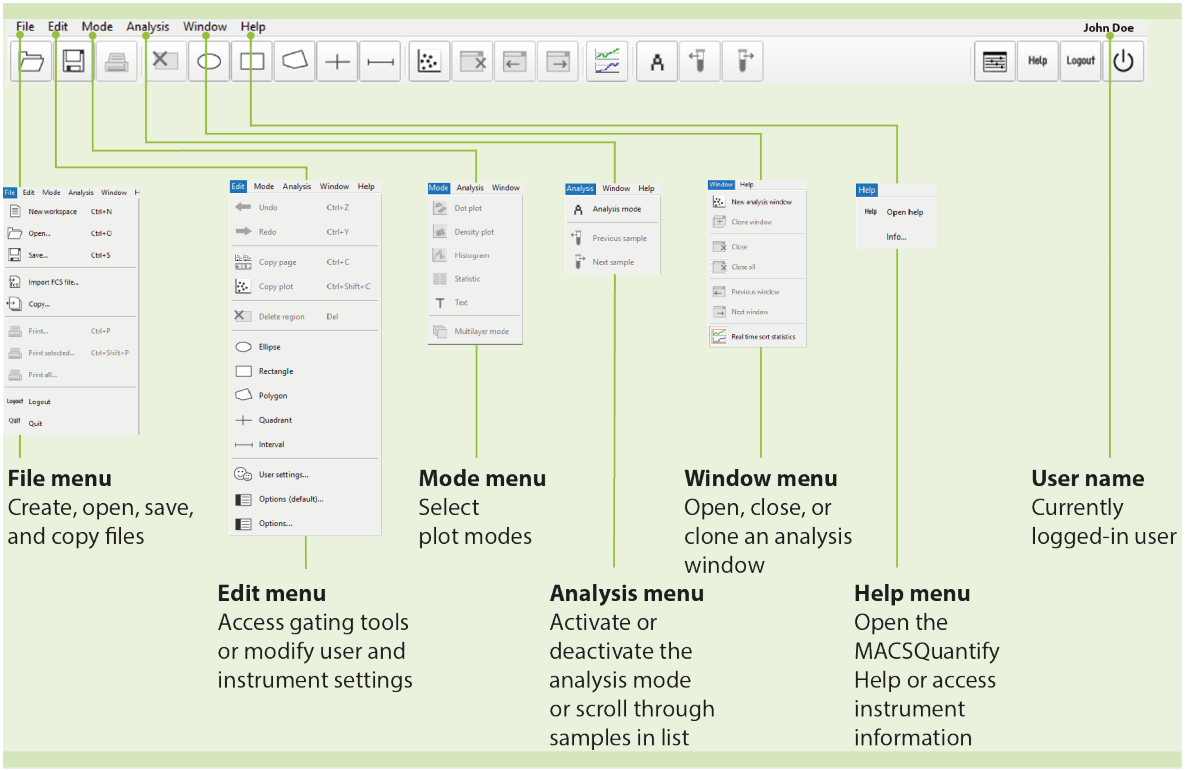


Figure 2.2: The menu bar.

2.1.1 The File menu

New workspace	Create a new workspace. The new workspace is initialized with the default instrument settings.
Open	Open workspaces, instrument settings, experiments, analysis templates, data files. or report files.
Save	Save workspaces, instrument settings, experiments, or analysis templates.
Import FCS file	Import data files of FCS format.
Copy	Transfer workspaces, instrument settings, experiments, analysis templates, data files (MQD or FCS files), log files, or other files (bitmap or excel files) to and from the instrument.
Print	Print active analysis windows (refer to **Printing data files on page 69**). The default page range will include all active analysis windows. Use the option **Pages** to print specific pages of an analysis window. The number of printed analysis windows per sheet can be selected under **Edit > Option > Software > Print** (refer to **Printing on page 88**).
Print selected	Print selected samples in the sample list (refer to **Printing selected windows on page 70**).
Print all	Print all samples in the sample list (refer to **Printing all windows on page 69**).
Logout	Logout of current user without shutting down MACSQuantify Tyto Software.
Quit	Shut down the MACSQuantify Tyto Software.

2.1.2 The Edit menu

Undo	Undo the last action.
Redo	Redo the last action.
Copy page	Copy the entire content of an analysis window to the clipboard.
Copy plot	Copy a single selected plot/histogram.
Delete region	Delete a selected region of interest.
Ellipse	Draw an elliptical gate.
Rectangle	Draw a rectangular gate.
Polygon	Draw a polygonal gate.
Quadrant	Define a quadrant.
Interval	Define an interval.
User settings	Define user settings. Only available for administrators (refer to **The Administrator on page 79**).
Options (default)	Customize global settings that apply to all users. Only accessible for administrators (refer to **The Administrator on page 79**).
Options	Customize user-specific settings (refer to **Changing default settings on page 85**).

2.1.3 The Mode menu

Dot plot	Display current data as a dot plot.
Density plot	Display current data as a density plot (color-gradient).
Histogram	Display current data as a histogram.
Statistic	Display current data in a statistics table.
Text	Open a text box.
Multilayer mode	View data in a multilayer format.

2.1.4 The Analysis menu

Analysis mode	Activate/deactivate the analysis mode. Activated analysis mode automatically deactivates **Edit** and **Mode** in the menu bar.
Previous sample/ Next sample	Browse through samples in list.

2.1.5 The Window menu

New analysis window	Open a new analysis window using predefined templates. The currently visible window is the active window. The total number of opened windows and the active window are indicated in the upper left corner of the screen.
Clone window	Create an exact copy of the analysis window.
Close	Close an analysis window.
Close all	Close all currently opened analysis windows.
Previous window/ Next window	Browse through open analysis windows.
Real time sort statistics window	Open and close the **Real time sort statistics** window. Refer to **The Real time sort statistics window on page 22** for more information about the statistics shown.

2.1.6 The Help menu

Open Help	Open the software help.
Info...	Display information about currently installed MACSQuantify Tyto Software version.

2.2 The toolbar

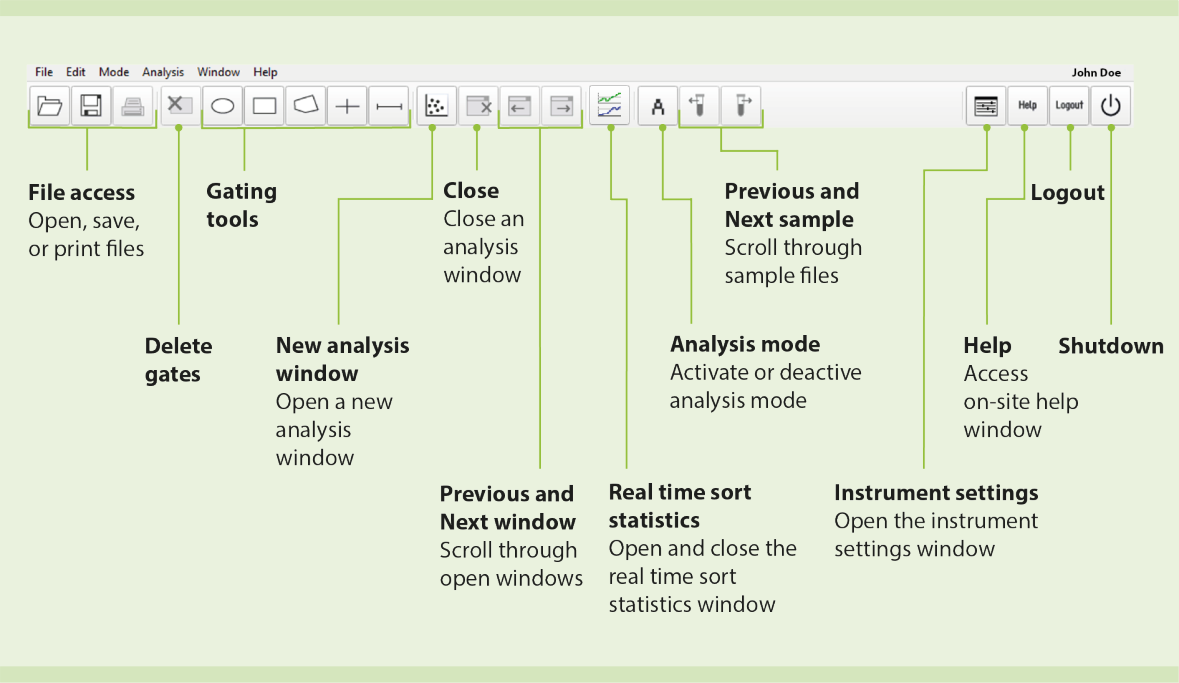


Figure 2.3: Frequently used buttons are located in the toolbar.

2.2.1 Toolbar functions

Open	Open workspaces, instrument settings, experiments, analysis templates, or data files. Refer to **Data management on page 73** for more information about file types and storage.
Save	Save workspaces, instrument settings, experiments, analysis templates, or data files. Refer to **Data management on page 73** for more information about file types and storage.
Print	Open a print dialog box. Only active if an analysis window is open (refer to **Printing data files on page 69**).
Delete	Delete a gate. Click on an existing gate (manipulators will be visible) and click the **Delete** button.
Ellipse **Rectangle** **Polygon** **Quadrant** **Interval**	Draw a gate of the respective shape. An **Interval** gate can only be used for histograms (refer to **Available gating tools on page 48**).
New analysis window	Open a new analysis window.
Close	Close an analysis window.
Previous and Next window	Browse through open analysis window pages.
Real time sort statistics window	Open and close the **Real time sort statistics** window. Refer to **The Real time sort statistics window on page 22** for more information about the statistics shown.

Analysis mode	Activate (light grey) or deactivate (dark grey) the **Analysis mode** button. Activate to modify analysis templates and plots.
Previous and Next sample	Browse through the files if several sample files are open.
Instrument settings	Edit instrument settings.
Help	Access the software help.
Logout	Logout.
Shutdown	Shut down the software and all processes on the instrument.

2.3 The side panel

The side panel has four tabs: **Samples**, **Experiment**, **Channels**, and **Tools**. It also shows the cartridge status box, which is always visible regardless of the tab that is currently open.

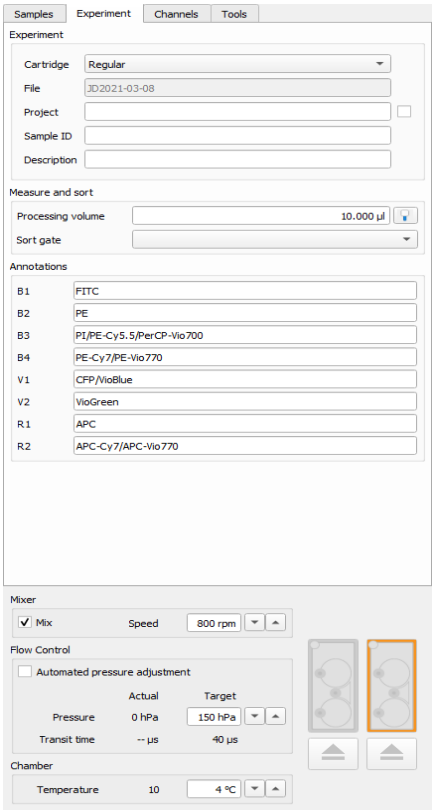


Figure 2.4: The side panel.

2.3.1 The Samples tab

Go to the **Samples** tab to view a list of opened data files. If a gating hierarchy was applied, it is also displayed.

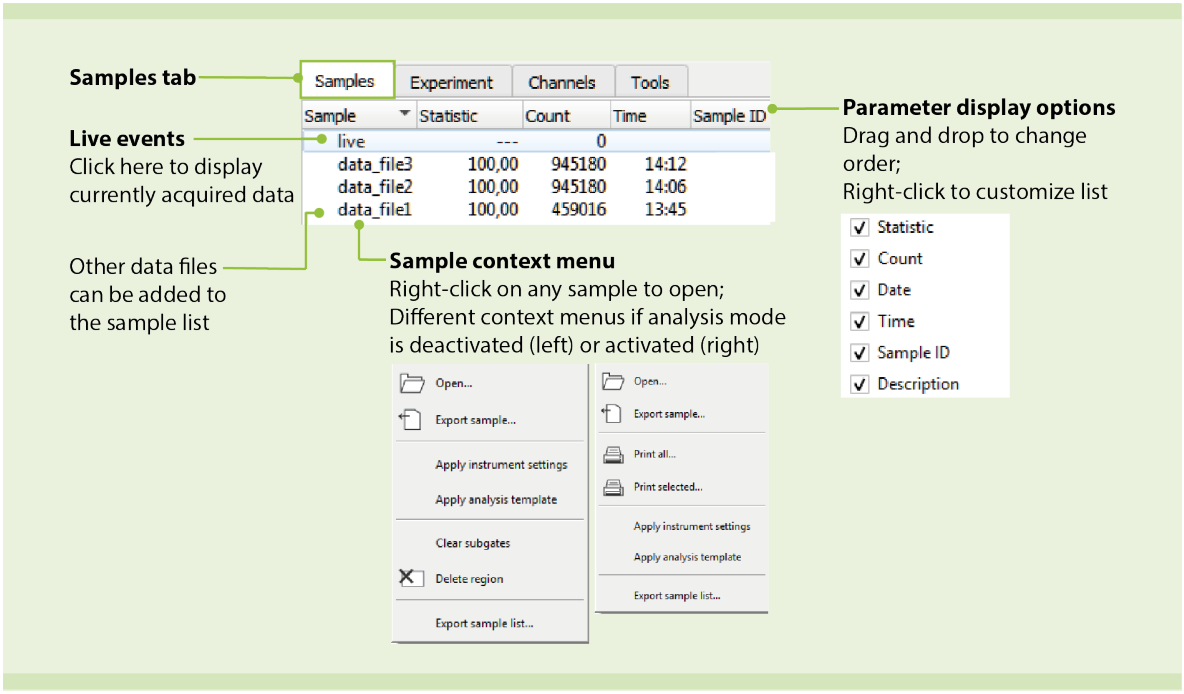


Figure 2.5: The side panel: **Samples** tab options.

The sample list contains the following columns: **Sample**, **Statistic**, **Count**, **Date**, **Time**, **Sample ID**, and **Description**. Move the scrollbar at the bottom of the sample list to view all categories. Moving the pointer over a sample displays a ScreenTip that contains the information of all categories.

Customizing the sample list

Sample order	Left-click on the column header arrow to sort a list in ascending or descending order.
Column width	The width of a column can be changed. When the pointer becomes a double-headed arrow, drag the pointer to move the split line to the width of choice.
Column order	The order of the columns can be changed by clicking a column header and dragging it to the desired position.
Columns displayed	Which columns are displayed can be customized by a right-click on a column header and checking or unchecking columns in the context menu.

Samples tab parameter

Sample	Name of the data file.
Statistic	%-T
Count	Number of events.
Date	Date of data acquisition.
Time	Time of data acquisition.
Sample ID	As defined in the **Experiment** tab.
Description	As defined in the **Experiment** tab.

Samples tab context menu

Right-click on the sample list to access the context menu. The number and type of functions available in the samples tab context menu may vary. It depends on whether you are logged in as an administrator or a custom user and whether analysis mode is activated or not. Refer to the table below for all samples tab context menu functions.

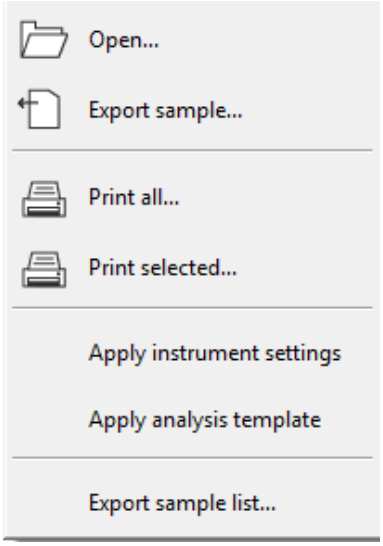


Figure 2.6: The **Samples** tab context menu when analysis mode is activated for custom users.

Open	Open files such as workspaces, instrument settings, experiments, analysis templates, data files or report files (refer to **Opening files on page 73**).
Export sample	Export samples in CSV and FCS format (refer to **Exporting FCS files on page 75**).
Print all	Batch print option only accessible in analysis mode. Select **Print all** from the **Print** dialog box to print the current analysis template for all samples in the sample list. The current analysis has to be applied to all samples in the sample list prior to print.
Print selected	Batch print option only accessible in analysis mode. Select **Print selected** from the **Print** dialog box to print the current analysis template for the selected samples in the sample list. Ensure that the current analysis is applied to all selected samples in the sample list prior to print.

Apply instrument settings	Apply the instrument settings that were run with a selected data file. Only available for administrators. For more information about using instrument settings, refer to **Applying PMT voltage and compensation settings on page 58**.
Apply analysis template	Only available for files acquired with a MACSQuant Tyto Cell Sorter. Right-click on a data file or files within the **Samples** tab. Select **Apply analysis template** to display the analysis template used during acquisition. The **Analysis Mode** is automatically activated when opening the analysis template. To modify the gating strategy or the display window of the analysis template, deselect the **Analysis Mode** and then modify as needed. For more information about analysis templates, see **Applying analysis templates on page 58**.
Clear subgates	Available if a sample with associated gates is selected. Clear all associated gates and regions.
Remove	Remove selected samples from the sample list.
Export sample list	Export the sample list and associated statistics to Microsoft® Excel (refer to **Exporting the sample list to Microsoft® Excel on page 70**).

2.3.2 The Experiment tab

On this tab, set parameters unique for your experiment.

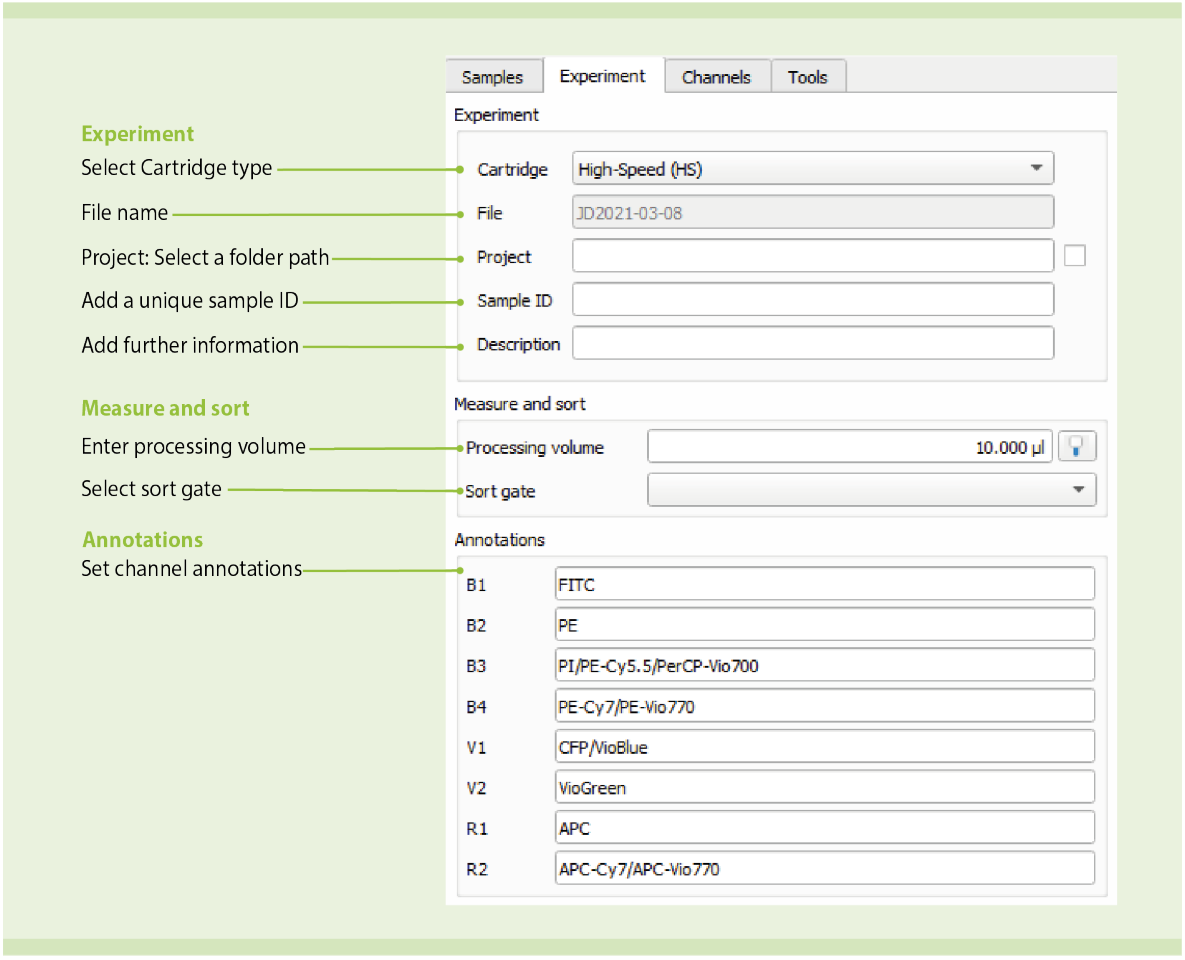


Figure 2.7: The side panel: **Experiment** tab options.

Experiment

- **Cartridge** – Displays the cartridge type. By scanning a cartridge, this information is automatically entered in this text box. Alternatively, the cartridge type can be selected from the drop-down list. If it is unclear which cartridge type is used and whether it is suitable for, e.g., high-speed sorting, refer to the data sheet of the respective cartridge.
- **File** – Name of the current file.
  File names are generated automatically. The administrator can modify the default file name (refer to **Modifying the default file name on page 85**).
- **Project** – Select a **Project** folder where to save files.
  Check the checkbox to select an existing project folder from the drop-down list. To create a new project folder, uncheck the box and enter a new name.
- **Sample ID** – Enter alphanumeric name for the sample ID if desired.
- **Description** – Additional information can be added into this text box if desired.

Measure and sort

- **Processing volume** – Enter a number or use the slider to the right. A sort stops automatically once the volume is processed.
- **Sort gate** – Select a sort gate from the drop down list. Events within the sort gate are sorted into the positive selection chamber of the cartridge during the sorting process.

Annotations

To set annotation values for the different channels go to **Edit > Options > Instrument > Annotations**. Default values for the annotations can be set for every experiment.

Annotations are automatically set to default values. Clearing the entries will set the annotations back to the default values.

2.3.3 The Tools tab

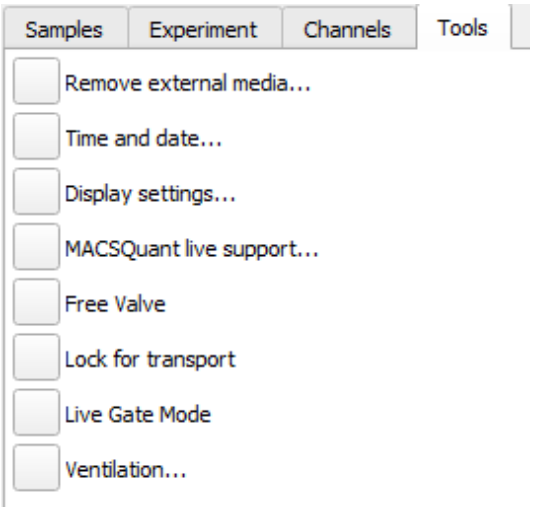


Figure 2.8: The **Tools** tab.

Remove external media	Safely eject removable media from a MACSQuant Tyto Cell Sorter to prevent data loss or corrupted data after writing to a USB stick.
Time and date	To set time and date (refer to **Setting the time and date on page 81**). Only available for administrators.
Display settings	Adjust display properties of an external monitor (refer to **Installation of an external monitor on page 81**). Only available for administrators.
MACSQuant Live Support	Open a dialog box to start a MACSQuant Live Support session (refer to **MACSQuant Live Support on page 91**).
Free Valve	Used to free blocked sort valves (refer to **Troubleshooting** in the MACSQuant Tyto Cell Sorter user manual).
Lock for transport	Locks the optical system of the instrument to avoid damage during transport. Contact Miltenyi Biotec Technical Support for instructions to prepare the instrument for transport.
Live Gate Mode	Only events within the sort gate are shown (refer to **Live Gate mode on page 53**). This improves handling for rare cell sorting.
Ventilation	Start and stop ventilation of the internal hardware reservoirs during decontamination of the MACSQuant Tyto Cell Sorter by gas. Refer to **Decontamination of the MACSQuant Tyto Cell Sorter** in the MACSQuant Tyto Cell Sorter user manual.

2.3.4 The Channels tab

Open the **Channels** tab to view and modify optical channel settings (refer to **Adjusting the event detection settings on page 59**).

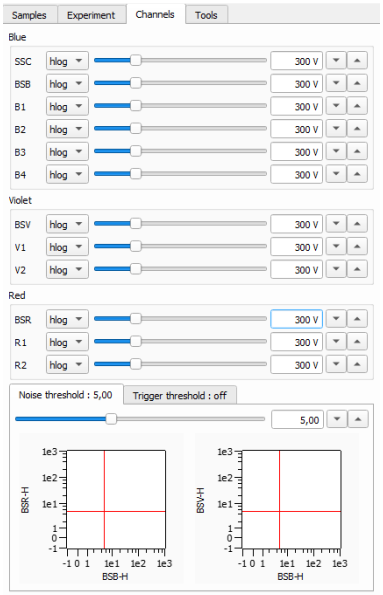


Figure 2.9: The **Channels** tab.

Blue/Violet/Red – Adjust scale and voltage of the channels.

Noise threshold – Define a noise threshold in the backscatter channels to cut off background signals, noise and debris.

Trigger threshold – Define an optional, additional trigger threshold to discard events that are not relevant. It can be set on a fluorescent or side scatter channel to only display events with intensities above the trigger threshold.

2.3.5 The Cartridge Status box

The **Cartridge Status** box in the side panel displays current mixer speed in the cartridge, flow control parameters (refer to **Flow control on page 65**) and temperature of the cooling chamber. It also displays the current cartridge status (refer to **The status indicator on page 25**).

The mixer speed, pressure applied to the input chamber of the cartridge and temperature of the cooling chamber can be set in the **Cartridge Status** box (refer to **Defining experiment parameters on page 31**).

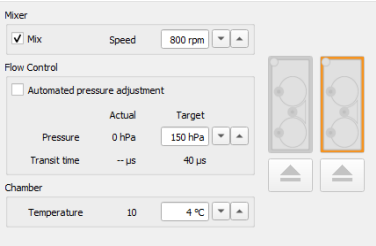


Figure 2.10: The **Cartridge Status** box.

2.4 The Real time sort statistics window

The **Real time sort statistics** window shows the event rate over the course of the sort process, after a measurement is started. It also shows statistics for the sort rate, sort count, abort rate, and abort count over time and in total. Data and statistics shown are from the newest measurement and sort.



The **Real time sort statistics** window opens when the MACSQuantify Tyto Software is started. If the window is closed, click the **Real time sort statistics** button in the toolbar to open the window again.

The graphs and statistics for a sort are summarized in a **Run Report** file after the sort is finished (refer to **MACSQuant Tyto run report on page 67**). Click **Open Report** at the bottom of the window to open the **Run Report** for the finished sort.

The sort statistics are shown in three graphs and two tables (**Figure 2.11**). The three graphs depend on the elapsed measurement and sort time on the x-axis. The first two graphs depict event rates per second:

- total trigger event rate (**Trigger rate**, first graph, blue line)
- rate of target events inside the sort gate that technically trigger a sort pulse (**Sort rate**, second graph, green line)

The third graph relates only to events inside the sort gate (sort targets) and shows:

- sort frequencies (**Sorted** , green line)
- technical abort frequencies (**Aborted**, orange line)

It is possible to zoom in the graphs by drawing a square into the respective graph or right click and select **Rectangular Zoom**. To zoom out again double click on the graph or right click and select **Auto Scrolling**. Alternatively, zoom in and out by selecting the plot and rolling the mouse wheel, pressing **+** or **-**, or using the cursor buttons.

The tables below the graphs shows total event numbers, percentages, and event rates for:

- all triggered events (**Triggered**)
- events in the sort gate (**Gated**)
- sorted events (**Sorted**)
- aborted events (**Aborted**)

The numbers are shown for events measured or sorted in the last 10 seconds (table **Recent 10 seconds**, **Figure 2.11**) and for the total measurement and sort duration (table **Total time**, **Figure 2.11**). In the table **Total time**, the numbers in the rows for**Triggered** and **Gated** events refer to periods of measurement and sort, the numbers in the rows for **Sorted** and **Aborted** events refer only to sort periods.

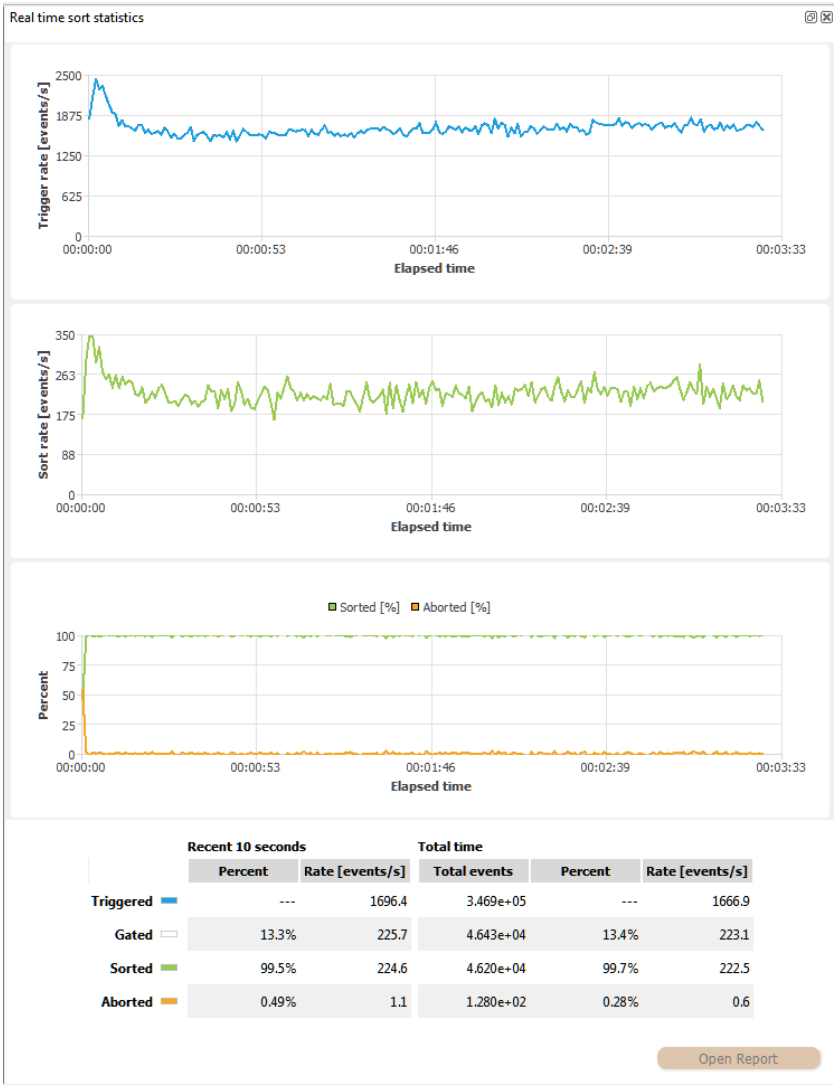


Figure 2.11: Example of **Real time sort statistics** for a sort.

2.5 The status bar

The status bar at the bottom of the user interface indicates the current status of the instrument. On the far right, the **Sort**, **Start/Stop measurement**, and **Pause** buttons are located.

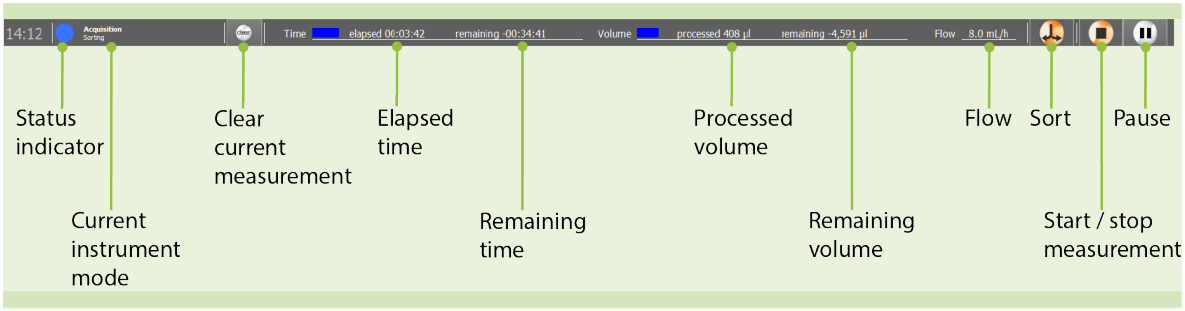


Figure 2.12: Elements of the status bar.

Status indicator – Displays the color code of the current status of the instrument and changes its color depending on the current status (refer to **The status indicator on the facing page**).

Current instrument mode – Shows the current status of the instrument in text format.

Clear current measurement – Deletes the current data from the dot plots.

Time – elapsed – Shows the elapsed time since start of sorting.

Time – remaining – Displays the estimated remaining time based on the processing volume entered in the **Experiment** tab.

Volume – processed – Shows the volume processed by the microchip.

Volume – remaining – Displays the estimated remaining volume based on the processing volume entered in the **Experiment** tab.

Flow – Shows the current volume running through the microchip in mL/h.

Sort – Activates the use of the sort valve of the instrument and initiates the sorting of the sample. Button may be inactive during certain processes.

Start/Stop measurement – Initiates/stops the complete alignment routine and data acquisition of the cartridge and starts/stops the flow of the sample through the microchip.

Pause – Pauses the acquisition/sort. Button may be inactive during certain processes.

2.5.1 The status indicator

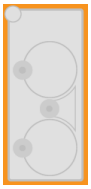
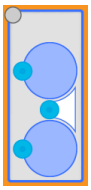
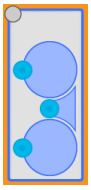
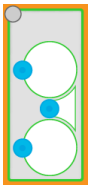
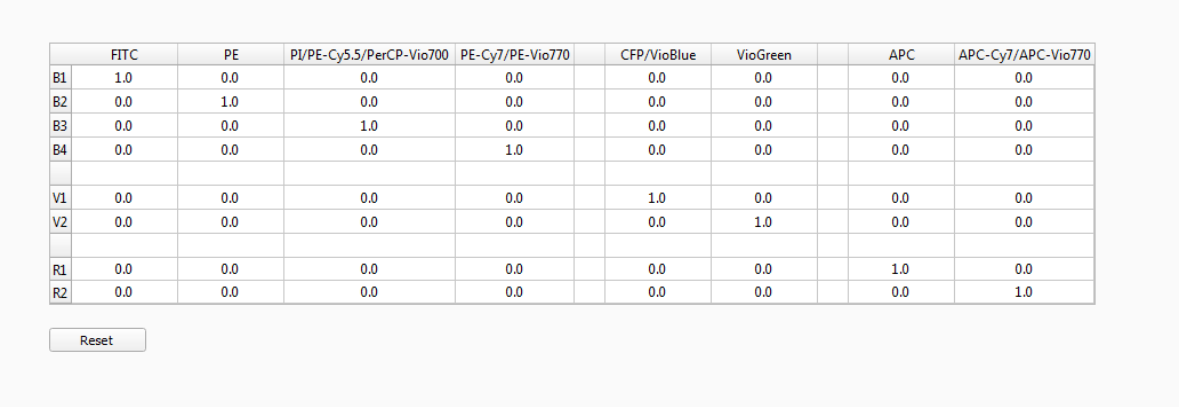
Status indicator	Cartridge status	Combined status
Green		No cartridge is inserted. Scan and insert a cartridge.
Blue		Cartridge inserted and recognized.
Blue		The instrument is processing a sample.
Blue		The instrument is sorting a sample.
Orange		Measurement paused. Measurement is finished but cartridge is still installed.
Red		Error alert. Refer to the chapter **Troubleshooting in the MACSQuant Tyto Cell Sorter user manual** or contact Miltenyi Biotec Technical Support.

Table 2.1: Overview of combined statuses.

2.6 The touchscreen

2.6.1 The Compensation tab

The **Compensation** tab shows the compensation matrix, which organizes the detection of individual fluorescence signals in columns. The matrix values can be adjusted to compensate the specific channel (refer to **Compensation matrix on page 64**). To display the compensation matrix (**Figure 2.13**), click the **Instrument settings** button in the toolbar and then check the **Matrix** checkbox.



	FITC	PE	PI/PE-Cy5.5/PerCP-Vio700	PE-Cy7/PE-Vio770	CFP/VioBlue	VioGreen	APC	APC-Cy7/APC-Vio770
B1	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B2	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
B3	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
B4	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
V1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
V2	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
R1	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
R2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0

Reset

Figure 2.13: Compensation matrix on the orange touchscreen.

2.6.2 The Alignment tab

The **Alignment** tab on the touchscreen displays the camera live view of the sorting mechanism in the cartridge. It also shows the alignment of the camera and the chip of the cartridge. If they are aligned correctly, the yellow crosshair is centered on the fiducial mark (**Figure 2.14**). The alignment proccess is automatic and continously maintained during the sort. If no camera image is available, a plain gray image is shown. The status of the built-in camera is visualized by a colored frame around the camera image:

- Green frame: Camera is connected and images are displayed (cartridge alignment is working).
- Yellow frame: Camera is connected but images are not displayed (cartridge alignment is still working but it is not depending on incoming images).
- Red frame: Camera failed to connect and images are not displayed (cartridge alignment is not working, refer to the chapter **Troubleshooting in the MACSQuant Tyto Cell Sorter user manual**).

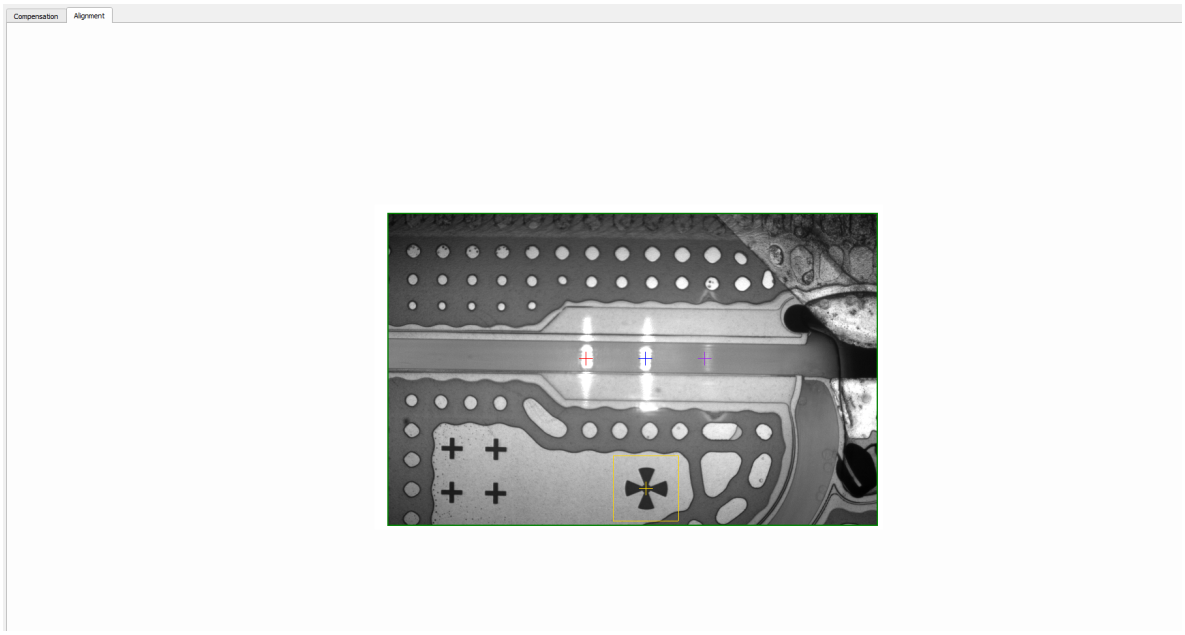


Figure 2.14: Camera alignment on the orange touchscreen, example for a regular speed cartridge.

3
Workflow of a sort process

The MACSQuantify Tyto Software enables users to create different preset files for efficient use of the MACSQuant Tyto Cell Sorter. There are two types of preset files: analysis templates and workspaces. Analysis templates store predefined analysis layouts and are created by defining views (plots, histograms, statistics, and tables) and gating strategies. A workspace comprises of the analysis template and all experiment data parameters for a particular experiment, calibration settings (PMT voltage values, channel scales), compensation matrix, trigger selections, and sample volumes. The sort process can be based on a pre-established workspace. Thus, the MACSQuant Tyto Cell Sorter allows for cell sorting without the need for specialized technical expertise for daily operation. Laser alignment, flow control, and speed detection are all fully automated.

3.1 Creating a workspace

1. Select an analysis template (refer to **Opening an analysis window on page 35**).
2. Annotate fluorescence channels (refer to **Annotations on page 32**).
3. Set up a gating strategy (refer to **Gating strategies on page 52**).
4. Define a sort gate (refer to **Make sort gate on page 32**).
5. Set photomultiplier tube (PMT) voltages (refer to **Adjusting the event detection settings on page 59**).
6. Set noise threshold (refer to **Setting the noise threshold on page 60**).
7. Optional: Set trigger threshold (refer to **Setting the trigger threshold (optional) on page 61**).
8. Optional: Set up a compensation (dependent on fluorochrome panel, refer to **Compensation for spectral overlap on page 62**).
9. Save the workspace (refer to **Saving workspace files on page 56**).

3.2 Setting up a sort

1. Load a workspace (refer to **Opening workspace files on page 57**).
2. Optional: Adjust sample volume in the **Experiment** tab according to the particular sample (refer to **Measure and sort on page 32**).
3. Load a cartridge into the instrument (refer to the **MACSQuant Tyto Cell Sorter user manua**l).



4. Click the **Start measurement** button in the status bar to initialize data acquisition.
5. Adjust settings: PMT, noise threshold, trigger threshold, and gating (refer to **Setting up the instrument on page 59**).



6. Click the **Sort** button in the status bar to start the sort process.
7. The **Real time sort statistics** window shows the statistics for the current sort (refer to **The Real time sort statistics window on page 22**).



8. The measurement stops automatically after the set sample volume is sorted or the input chamber of the cartridge is empty. Optional: Click the **Stop measurement** button in the status bar to stop the measurement manually. Take out the cartridge (refer to the **MACSQuant Tyto Cell Sorter user manual**).

If unexpected or no events are detected in the **Real time sort statistics** window during measurement or sort, adjust **Flow Control** settings (refer to **Flow control on page 65**).

4

Setting up an experiment

⚠WARNING Read the chapter **Important Safety Information** in the MACSQuant Tyto Cell Sorter user manual as well as all safety information in this manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

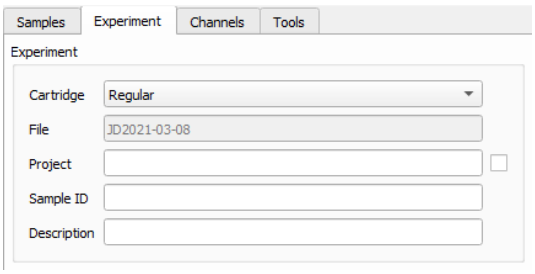
Before operating the MACSQuant Tyto Cell Sorter for the first time, the instrument has to be calibrated by your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support for an optimal outcome of your experiments.

This chapter describes how to set up your basic experiment. On the **Experiment** tab, you can set up parameters, such as the general experiment settings, channel annotations, and set a sort gate.

4.1 Defining experiment parameters

4.1.1 General experiment settings

1 Go to the **Experiment** tab.



2 To set the cartridge type, it is advised to scan the 2D code of the cartridge using the 2D code reader at the front of the instrument. Alternatively, the cartridge type can be selected from the **Cartridge** drop-down list.

3 Enter alphanumeric text for the **Project**, **Sample ID**, and **Description** if desired.

4.1.2 Measure and sort

The section **Measure and sort** displays the volume to be processed by the instrument and the different gates from which the sort gate can be chose.



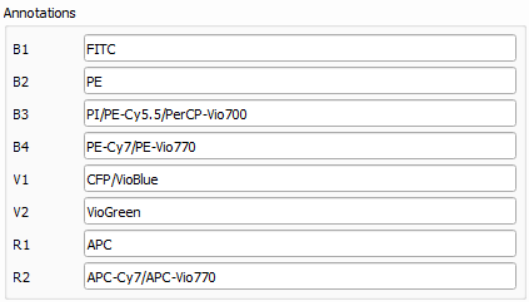
Figure 4.1: The section **Measure and sort** in the **Experiment** tab.

- The value entered in the **Processing volume** box sets the volume to be processed by the instrument. Once this volume has been processed, the acquisition is stopped, data files are finalized and the cartridge is ejected automatically.
- Different gates can be selected as sort gate from the **Sort gate** drop-down menu. By default no sort gate is selected.

4.1.3 Annotations

1 Go to the **Experiment** tab.

2 Modify the annotations for the fluorescence channels, if required.

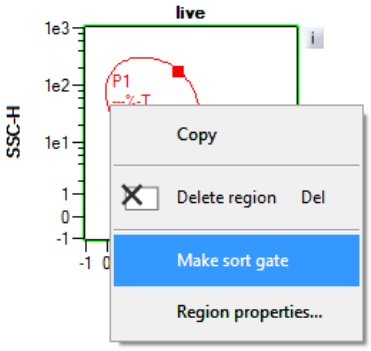


4.1.4 Make sort gate

Select the sort gate appropiate for your target cells and your gating strategy (refer to **Gating strategies on page 52**). Events within the sort gate are sorted into the positive selection chamber of the cartridge during the sorting process.

1 Right-click on a gate within a dot plot.

2 Click on **Make sort gate**.



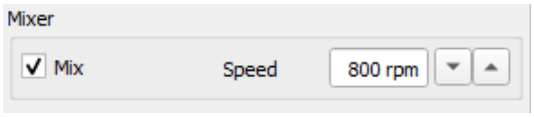
Alternatively: Select the sort gate via drop-down in the **Experiment** tab (refer to **Measure and sort above**).

4.1.5 Mixer

The mixer of the cartridge keeps the cells in the sample in suspension. If needed, adjust the propeller parameters of the cartridge under the **Mixer** section in the side panel.

1 Type a number between 100 and 800 rpm (step size of 10) in the **Mixer speed** box in the side panel.

2 Activate/deactivate the **Mix** checkbox to initiate/stop rotation of the propeller.



The preset mixer speed of 800 rpm is recommended for most cell types.

4.1.6 Chamber

The temperature of the cooling chamber where the cartridge is located during sorting can be set. Enter a target temperature for the cooling chamber (4-25 °C) under **Chamber** in the side panel.



Figure 4.2: The section **Chamber** in the **Experiment** tab.

5
Setting up a sorting strategy

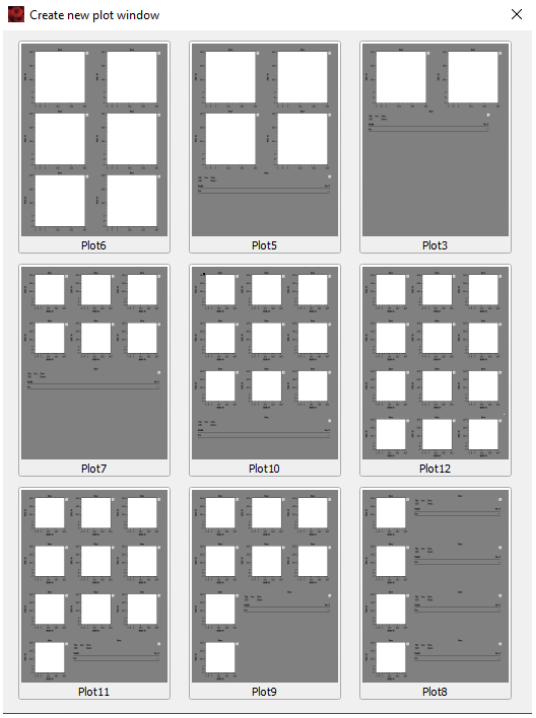
This chapter explains how to create, modify, and save analysis templates. An analysis template is a file type that contains all information related to the visible dot plots. In addition, all functions related to modify dot plots are explained in this chapter.

5.1 Opening an analysis window



1 Click the **New analysis window** button in the toolbar or go to **Window > New analysis window**.

2 Select the desired analysis template from the plot options.





3 If multiple analysis windows are open, use the **Previous and Next window** buttons in the toolbar to access hidden windows.

Acquired data is displayed and analyzed in an analysis window. Analysis templates consist of a plot template and a gating strategy. Depending on which template is applied, the analysis windows may contain dot plots, density plots, histograms, statistics, and text tables. Several analysis windows can be opened at one time. These can contain several experiments or represent a single experiment with a complex gating strategy. Gating strategies can be created during sample acquisition (live gating) and saved for future use, or they can be created after data acquisition. Deselect the **Analysis mode** button before modifying plots or analysis templates.

	Analysis mode deselected	Display settings and analysis templates can be modified.
	Analysis mode selected	Display settings are locked.

5.2 Plot properties

Click the **'i'** button in the upper right corner of each plot to change its properties. Double-click on a plot to view it full screen. Double-click again to return to the original view. Click on the axis label to modify the displayed parameters.

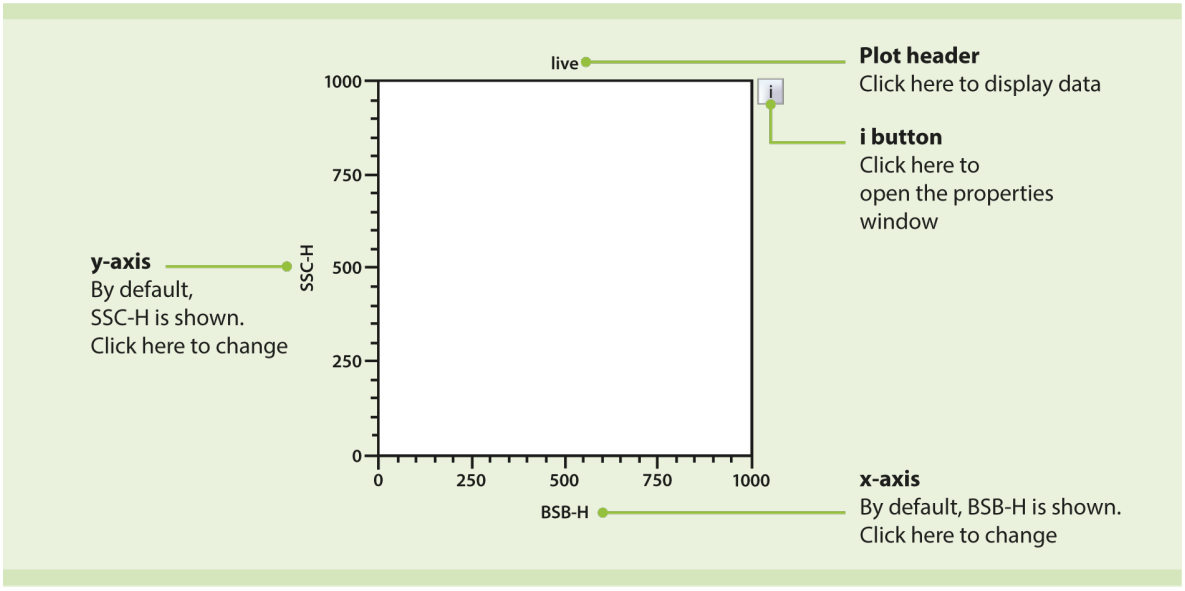


Figure 5.1: Features of a plot. To modify its appearance, click the **'i'** button to the right of the plot.

5.3 Plot types

Flow cytometry data can be displayed in different formats: as a **Dot plot**, **Density plot**, **Histogram**, or as a **Statistics table**. Additionally, a **Text box** can be used to display additional information. Click the **'i'** button next to a plot to open the following properties window (refer to **Plot properties above**). Switch between the plot types by clicking on the respective button.

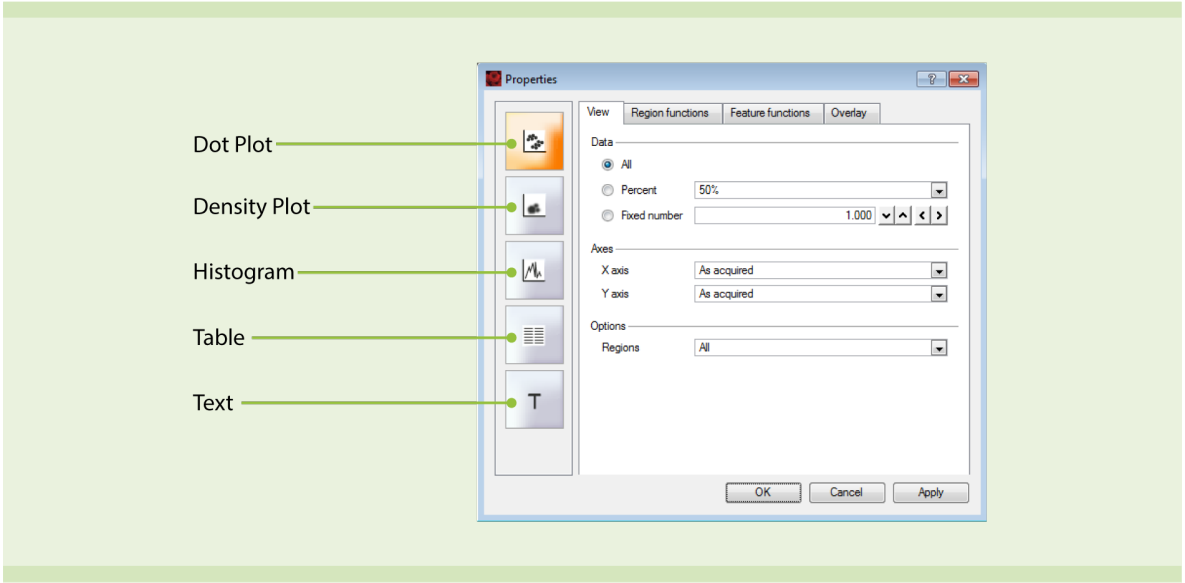
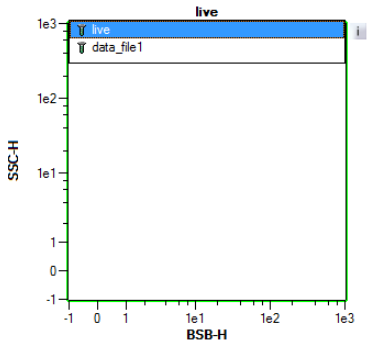


Figure 5.2: Switch between plot types or modify their appearance.

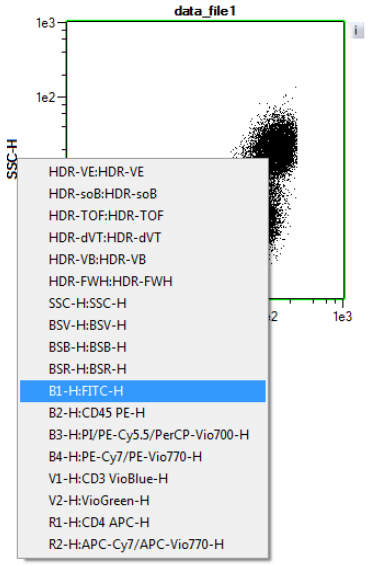
5.4 Displaying data

5.4.1 Displaying data using the plot header drop-down list

1 Click on the plot title and select a data file from the drop-down list (see also **Figure 5.1**).



2 The selected data file will be displayed in the plot. By default, the dot plot displays BSB-H (height of the backscatter blue laser signal) versus SSC-H (height of the side scatter signal). Click on the axis label and select one of the saved parameters in the drop-down list to modify the displayed parameters.



5.4.2 Displaying data by double-clicking on data file in sample list

1 Click on one plot to activate it. It will be highlighted with a green frame.

2 Double-click the file for analysis from the sample list in the side panel. It will populate the active plot in the layout.

3 By default, the plot displays BSB-H on the x-axis and SSC-H on the y-axis. Click on the axis label and select one of the saved parameters in the drop-down list to modify the displayed parameters.

5.4.3 Axis paramters

Select the desired axis parameter by clicking on the axis title. Besides the sidescatter, the backscatters, and the fluorescent channels, additional axis parameters can be displayed.

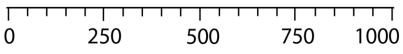
Parameter	Name	Description
HDR-VE	Valve event	Number of times the valve actuates.
HDR-soB	Sort bit	Event which is inside the sort gate. Sorting is enabled.
HDR-TOF	Time of flight	Time (µs) the event needs for crossing the blue and the violet laser.
HDR-dVT	Valve sync	Delta valve time : time since the last valve actuation.
HDR-VB	Valve bit	Event which is inside the sort gate. Sorting is enabled and the valve was actuated.
HDR-FWH	Full width half maximum	Pulse width on the violet laser.

Table 5.1: Axis parameters.

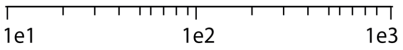
5.4.4 Adjusting data display scales

The MACSQuantify Tyto Software offers various options to scale and thereby properly visualize data. MACSQuant Tyto Cell Sorter can display data in a variety of formats. The side scatter (SSC), all backscatters (BSB: backscatter blue laser, BSV: backscatter violet laser, BSR: backscatter red laser), and all fluorescence channels (V1–2, B1–4 and R1–2) can be displayed in lin, log2, log3, log4, log5, and hlog scale.

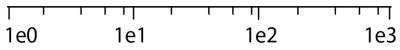
lin – Linear scale



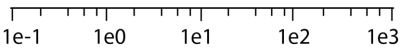
log2 – Logarithmic scale of two decades



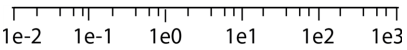
log3 – Logarithmic scale of three decades



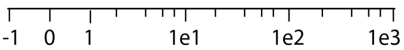
log4 – Logarithmic scale of four decades



log5 – Logarithmic scale of five decades



hlog – Biexponential scale

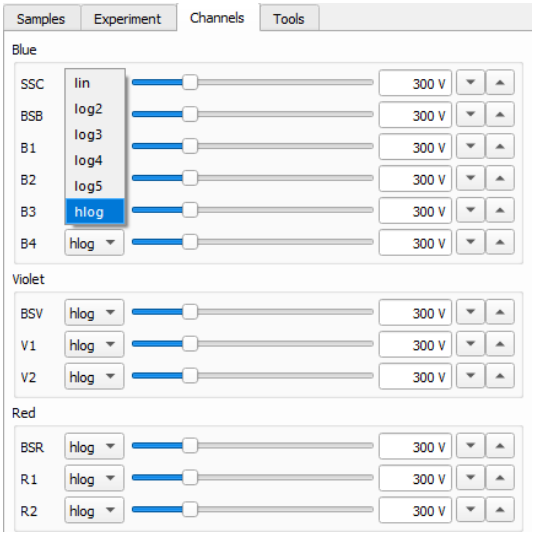


Standard dot plots showing side scatter (SSC) and backscatter (BSC) are typically displayed using linear scales. In some instances, a logarithmic scale is useful, for example, when analyzing cell lines or samples that include very large particles (e.g. CHO cells or compensation beads) as well as small particles (e.g. bacteria) that need to be analyzed simultaneously. The default scale of the MACSQuantify Tyto Software is hlog. Linear scaling is often not appropriate when displaying fluorescence intensity of different cell populations. Fluorescence signal intensities that extend over several orders of magnitude have to be displayed in a logarithmic scale.

The data display scales can be set prior to acquisition using the **Channels** tab of the side panel. To apply the desired scale during data acquisition, it has to be changed as described above. Changing the scale in the dot plot, properties will only change the display of the scale but not the scale for the data acquisition. If you want to collect and display data with the same channels, a default scale can be set.

1 Go to the **Channels** tab to change the data scaling for the specific channel.

2 Change the display scale by selecting the respective scale from the drop-down menu next to the specific channel.



Scales can also be adjusted after acquisition. For details, refer to **Plot properties on page 36**.

5.5 Modifying the data display

5.5.1 Dot plots and density plots

Dot plots and density plots are bivariate plots, as they both display two parameters. Density plots additionally depict the cell distribution as a color-coded gradient. Density plots are useful for large cell numbers.

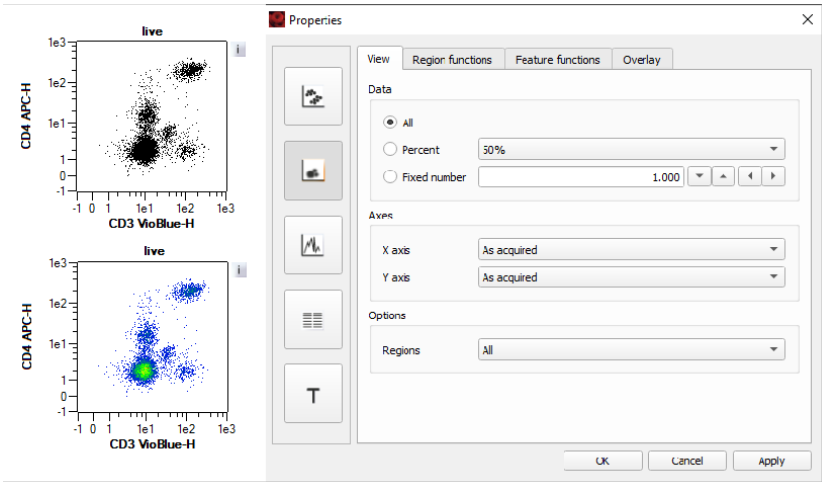


Figure 5.3: Dot plot (upper left) and density plot (lower left).

Number of events shown

If the number of events is very large, it may be useful to display only a fraction of all acquired events.

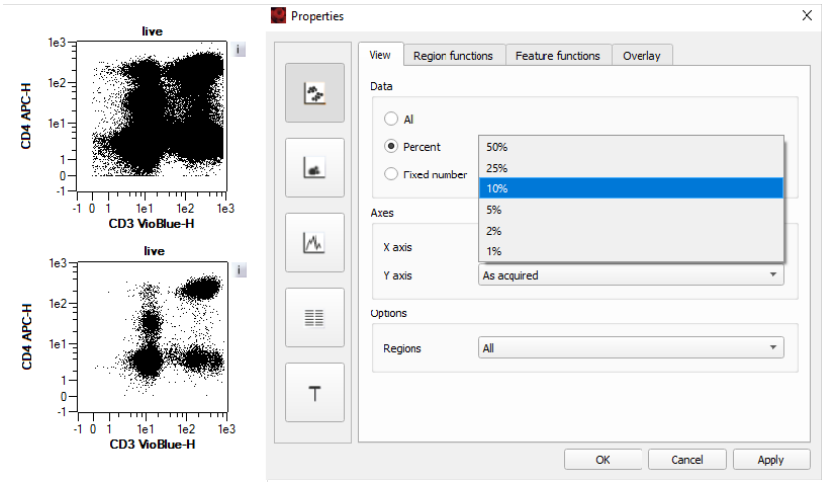


Figure 5.4: All events (upper left) versus a fraction (lower left) of all events in percent or as a fixed number.

1 To change the number of events that are displayed in a plot, click the **'i'** button next to the plot.



2 Go to the **View** tab.

3 From the **Data** section, select from the following:

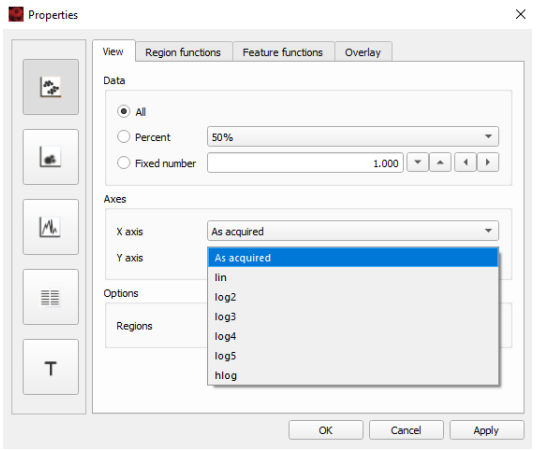
- **All** – All events are displayed on a dot plot or density plot.
- **Percent** – 50%, 25%, 5%, 2%, or 1% percentile values of the total events are displayed on a dot plot or density plot.
- **Fixed number** – A fixed number of events can be displayed on a dot plot or density plot.

Plot axes scales

1 To change the x- and y-axes, click the **'i'** button next to the plot.



2 Go to the **View** tab.



3 From the **Axis** section, select from the following:

- **As acquired** – In the same scale designated in instrument settings.
- **lin** – Linear scale.
- **log2-5** – Logarithmic scales from 2-5 decades.
- **hlog** – Bi-exponential scale.

Region options for dot plots and density plots

1 To define region options, click the **'i'** button next to the plot.

2 Go to the **View** tab.

3 From the **Options** section, select from the following:

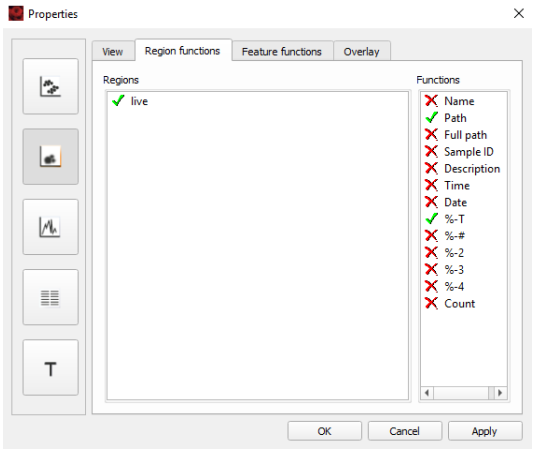
- **All** – All regions ("gates") are shown on the plot.
- **This** – Only the region ("gate") that was selected from the plot header drop-down is shown.
- **None** – No regions ("gates") are shown on the chart.

Region functions

To display specific parameters related to the current sample and experiment settings:

1 Click the **'i'** button next to the plot to open the **Properties** dialog box.

2 Go to the **Region functions** tab.



3 Select (✓) or deselect (✗) the function as required. By default, the region functions **Path** and **%-T** are shown.

4 Click **Apply** to confirm and **OK** to close.

Name	Description
Name	Sample\\P1\P2 -> P2
Path	Sample\\P1\P2 -> P1\P2
Full path	Sample\\P1\P2 ->Sample\\P1\P2
Sample ID	Sample ID as defined in the **Experiment** tab
Description	Description as defined in the **Experiment** tab
Time	Time in the format HH:MM
Date	Date in the format YYYY-MMM-DD
%-T	Percent total
%-#	Percent gated / percent parent
%-2	Percent grand parent
%-3	Percent great grand parent
%-4	Percent great-great grand parent
Count	Number of events

Table 5.2: Region functions.

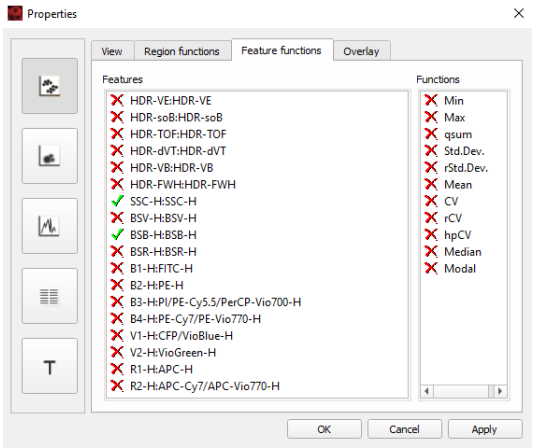
Feature functions

The current dot plot shows two different fluorescence channels.

1 To change the display of feature functions, click the **'i'** button next to the plot.



2 Go to the **Feature functions** tab.



3 Select (✓) or deselect (✗) the function as required.

4 Click **Apply** to confirm and **OK** to close.

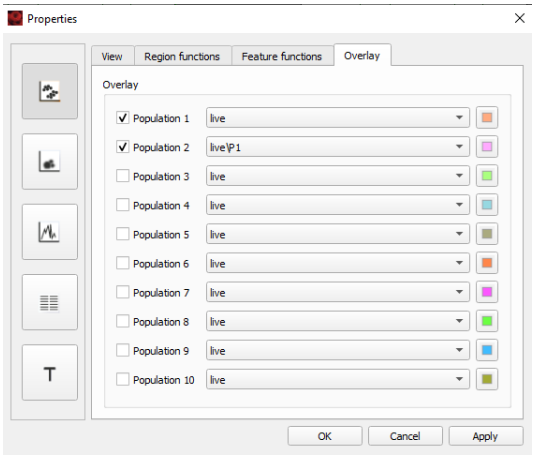
Name	Description
Min	Minimum value.
Max	Maximum value.
qsum	Sum of squares of the values.
Std.Dev.	Standard deviation of the values.
rStd.Dev.	Robust standard deviation calculated as 50 (Intensity at 84.13%ile - Intensity at 15.87%ile)/Median.
Mean	Mean value (sum of all intensity values divided by number of events) of events in the plot or within a gated population. Not suited for logarithmic data, as it is easily influenced by outliers.
CV	Coefficient of variation calculated as Std.Dev./Mean.
rCV	Robust coefficient of variation calculated as rStd.Dev./Median.
hpCV	Coefficient of variation calculated as full width at half maximum (FWHM) / Mean × 0.423 × 100%.
Median	Value where 50% of all event values lie above and 50% of all event values fall below. Median is a robust statistic and is best suited when analyzing logarithmic data.
Modal	Value that occurs most often among all events.

Table 5.3: Feature functions.

Overlay

1 To display several cell populations of different sort gates (e.g. live/P1/P2) or different data files in one density or dot plot, click the **'i'** button next to the plot.

2 Go to the **Overlay** tab.



3 Select the desired population from the drop-down list. Click on the box to the right to change the population color as desired.

4 Check the box to the left to mark the population as active. Only active populations are shown in the plot.

5.5.2 Histogram-related functions

Number of events shown

If the number of events is very large, it may be useful to display only a fraction of all acquired events.

1 To change the number of events that are displayed in a plot, click the **'i'** button next to the plot.

2 Go to the **View** tab.

3 From the **Data** section, select from the following:

- **All** – All events are displayed on the histogram.
- **Percent** – 50%, 25%, 5%, 2%, or 1% percentile values of the total events are displayed on the histogram.
- **Fixed number** – A fixed number of events can be displayed on the histogram. In the text box, type or select the desired number.

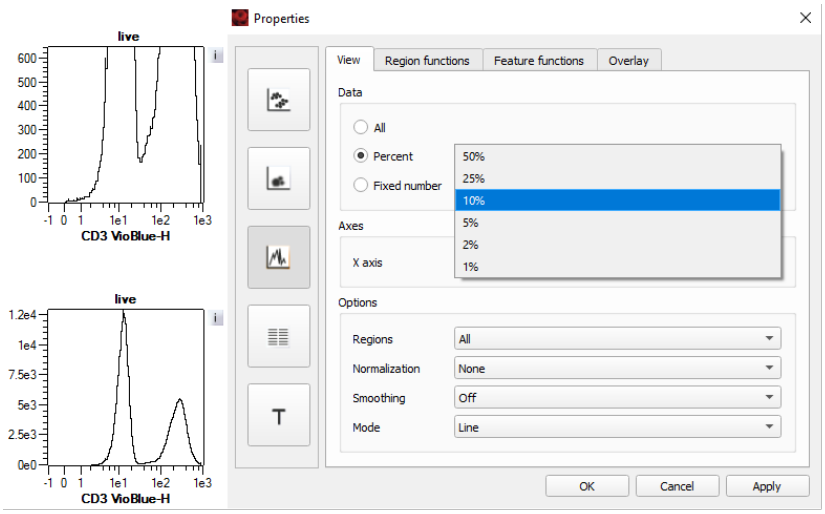


Figure 5.5: Histogram with all events (lower histogram) and a fraction of acquired events (upper histogram).

Plot axes scales

1 To change the x- and y-axes scales of a histogram, click the **'i'** button next to the plot.

2 Go to the **View** tab.

3 From the **Axis** section, select from the following:

- **As acquired** – In the same scale designated in instrument settings.
- **lin** – Linear scale.
- **log2-5** – Logarithmic scales from 2-5 decades.
- **hlog** – Bi-exponential scale.

Options for histograms

- **Regions**
  All – All regions ("gates") are shown on the chart.
  This – Only a selected region ("gate") is shown on the chart.
  None – No regions ("gates") are shown on the chart.
- **Normalization**
  The histogram graphically summarizes the distribution of a univariate data set. Data can be normalized by area (integral of total area under the curve) or by height.
- **Smoothing**
  Algorithms can be used to smooth the histogram. Light, medium, or strong smoothing is available.

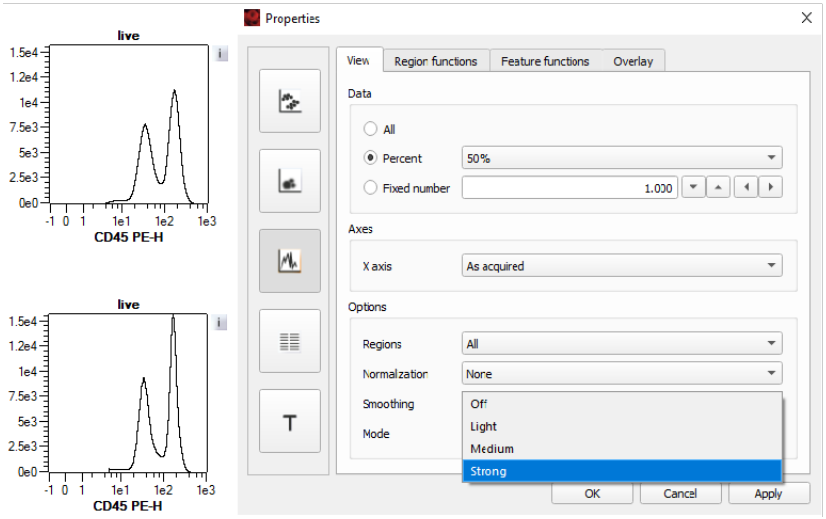


Figure 5.6: Original (lower chart) and smoothed histogram (upper chart).

- **Mode**
  Histograms can be displayed as a line chart or bar chart.

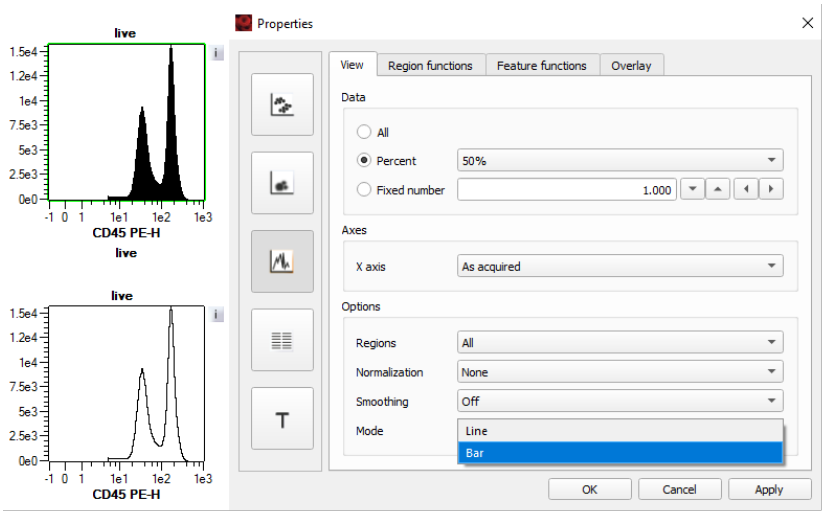


Figure 5.7: Histogram as a line chart (lower chart) and as a bar chart (upper chart).

Region and Feature functions

1 To display specific parameters of regions or features, click the **'i'** button next to the plot.

2 Go to the **Region functions** or the **Feature functions** tab.

3 Select (✓) or deselect (✗) the function as required.

Overlay

1 To display several cell populations of different data files in one histogram, click the **'i'** button next to the plot.

2 Go to the **Overlay** tab.

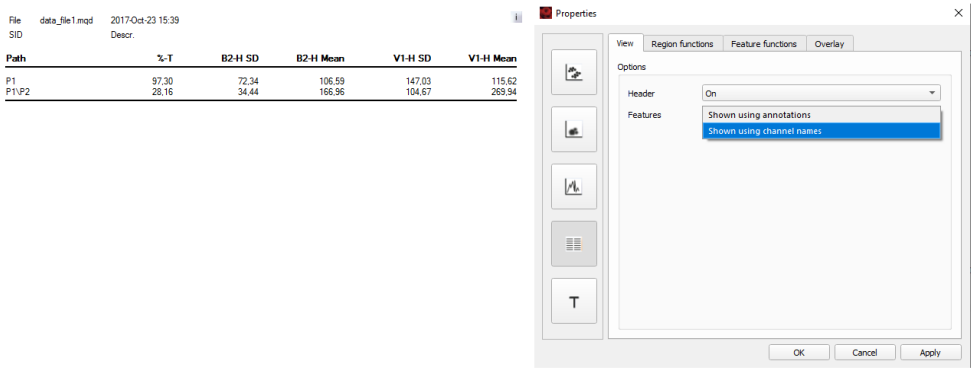
3 Select the desired population from the drop-down list.

4 Check the box to the left to mark the population as active. Only active populations are shown in the histogram.

5.5.3 Statistics tables

1 To change the format of the statistics table, click the **'i'** button next to the plot.

2 Go to the **View** tab.



3 From the **Options** section, select from the following:

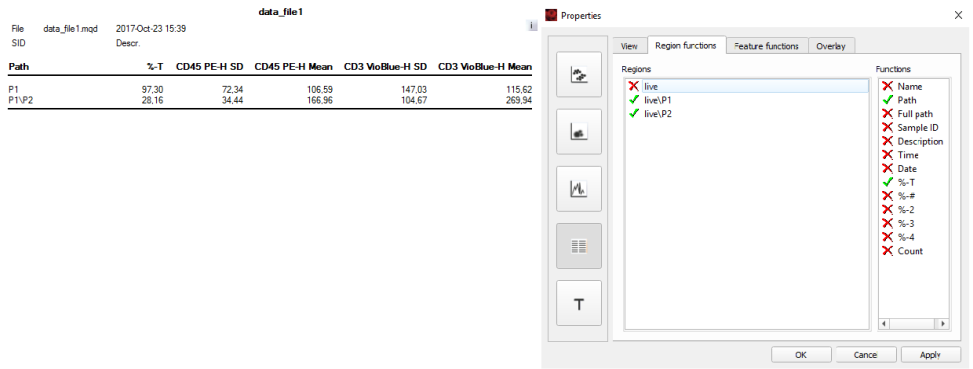
- **Header** – **On** or **off**
- **Features** – **Shown using annotations** or **shown using channel names**

Annotations are defined by the user in the **Experiment** tab.

Region and Feature functions

1 To change the display of region or feature functions, click the **'i'** button next to the plot.

2 Go to the **Region functions** or the **Feature functions** tab.



3 Select (✓) or deselect (✗) the function as required.

Overlay

1 To display several files in the statistics table, click the **'i'** button next to the plot.

2 Go to the **Overlay** tab.

3 Select the desired population from the drop-down list.

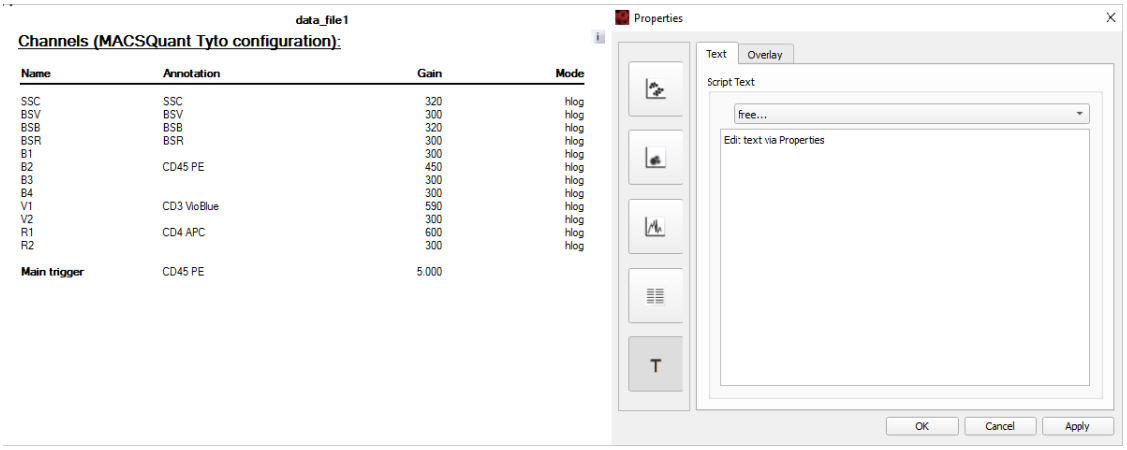
4 Check the box to the left to mark the population as active. Only active populations are shown in the statistics table.

5.5.4 Text boxes

Script text

1 To display additional information, click the **'i'** button next to the plot.

2 Go to the **Text** tab.



3 Enter free text or a MACSQuantify Tyto Software script into the **Script Text** box.
Scripts are based on HTML (hypertext mark-up language) and can be written to automatically display statistics about each region or gate. Specific regions can be removed from or added to a text table by using the **Overlay** tab. A script was used to create the text table.

Overlay

If a MACSQuantify Tyto Software script has been used in the **Text** tab, it is possible to add or remove regions (populations) by using the population check box.

5.6 Gating tools

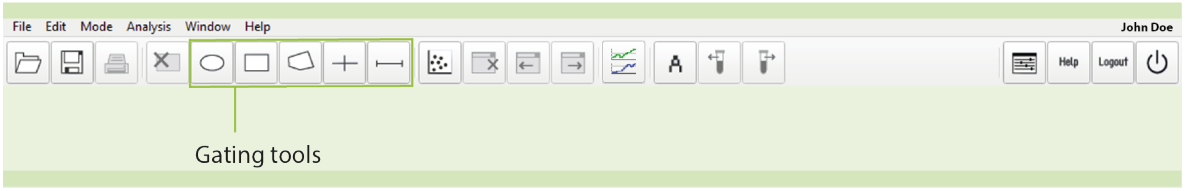


Figure 5.8: Position of the gating tool buttons in the toolbar.

1 Click on one of the gating tool buttons from the toolbar or select the tool from the **Edit** menu to create a gate or region.

2 If using ellipse, rectangle, quadrant, or interval, click on a plot and start dragging a gate. Release the left button to finish drawing.

3 If drawing a polygon, click once to start drawing the first point. Move the cursor and click again to draw the next point. Continue as desired. Double-click to draw the last point and finish the gate.

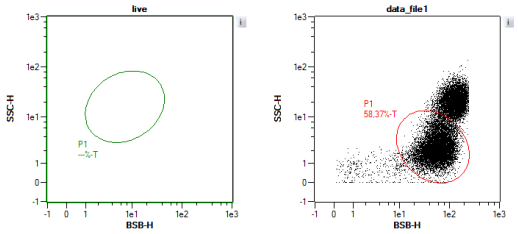
4 When the gate is selected, the edit points can then be used to adjust the size and shape.

5 Click and drag the gate to move it to a new position.

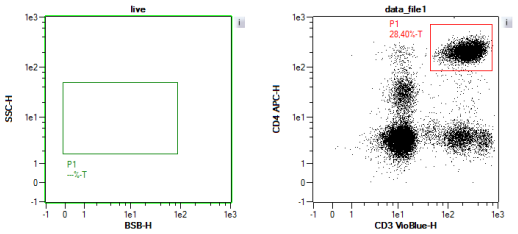
It is not possible to add additional edit points to a polygon once it is created.

5.6.1 Available gating tools

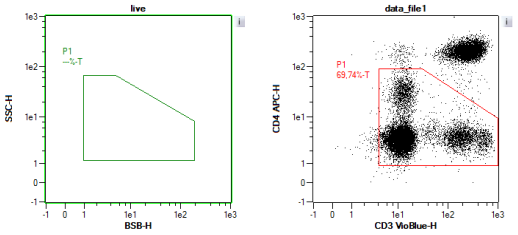
Ellipse – Click the **Ellipse** button in the toolbar or click **Edit > Ellipse**.



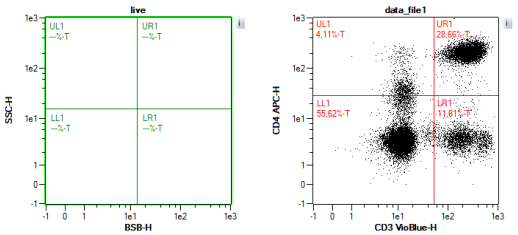
Rectangle – Click the **Rectangle** button in the toolbar or click **Edit > Rectangle**.



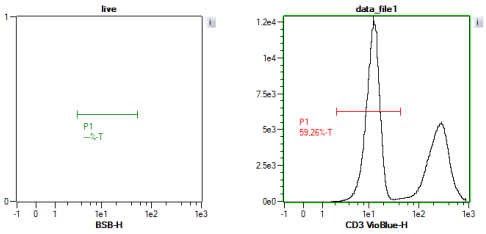
Polygon – Click the **Polygon** button in the toolbar or click **Edit > Polygon**.



Quadrant – Click the **Quadrant** button in the toolbar or click **Edit > Quadrant**.



Interval – Click the **Interval** button in the toolbar or click **Edit > Interval**.

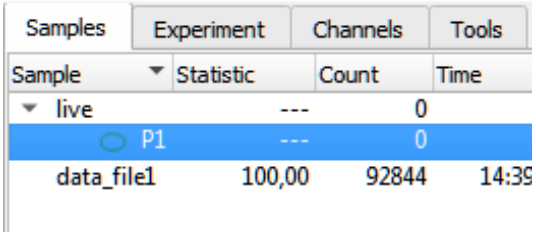


5.6.2 Copying gates

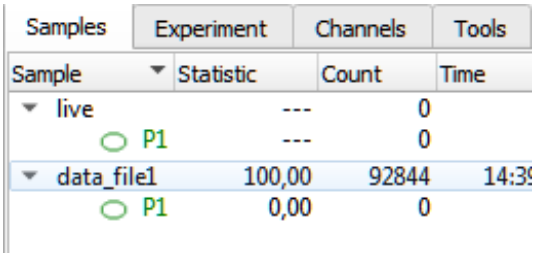
Copying gates via drag and drop

Regions or entire gating strategies can be copied to other files or moved to other areas of the gating strategy.

1 After establishing a gating strategy using one data file, click on the highest region within the hierarchy.



2 Drag it onto a file requiring the same gating strategy. The same hierarchy and any name or color changes will be applied to the new file.

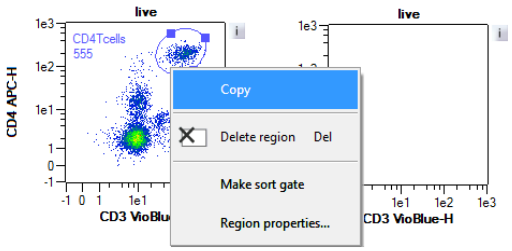


Copy/paste regions

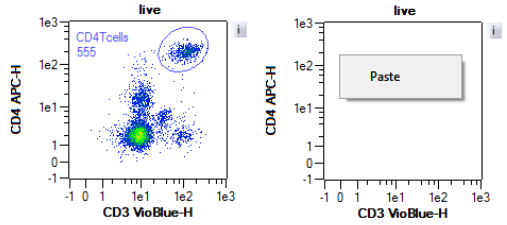
Copy a region from one plot and paste it to the same or compatible plot. A region from a dot plot can be pasted to a dot plot or density plot, but not to a histogram. The same physical coordinates are used for the region, even if the plot has different scales.

1 Click on a gating tool button and draw a region in the plot of interest. This region will be identified as P1.

2 Right-click on the plot and select **Copy**.



3 Right-click on a compatible plot and select **Paste**. This region will be identified as P2. This new region is completely modifiable using the edit points and **Region properties**.



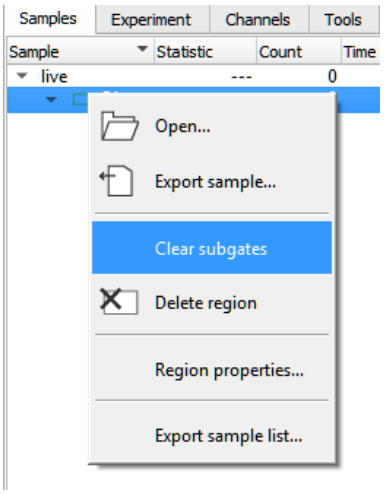
5.6.3 Deleting gates

To delete a gate, do one of the following:

- Select the gate and press the delete key.



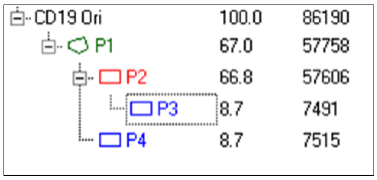
- Click the **Delete region** button in the toolbar.
- All regions under a file or under other regions can be deleted via right-click on the file or the top region in the hierarchy and click **Clear subgates**.

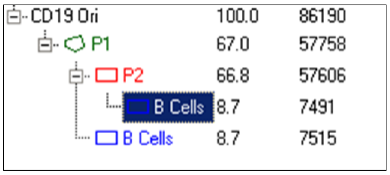


5.6.4 Moving a region within a gating hierarchy

After establishing a gating strategy within a data file, click on a region in the sample list and drag it under another region within the strategy. The region will become a new incremental region.

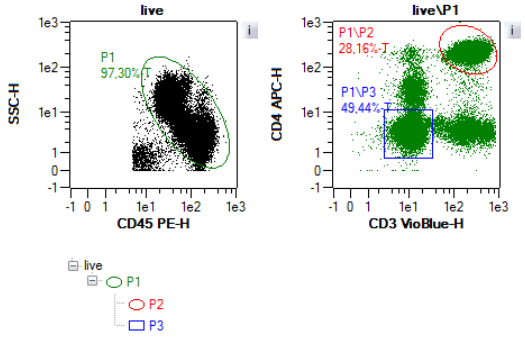
1. P1, P2, and P3 have already been drawn in a hierarchical strategy. It is necessary to have the P3 region drawn within P1 as well as P2.
2. Click on P3 and drag and drop it under P1.
3. The region under P1 will be renamed P4, but will have the same coordinates and plot association as P3, but different statistics.
4. If you change the name of region P3 or P4 under region properties, names of both regions will be changed.





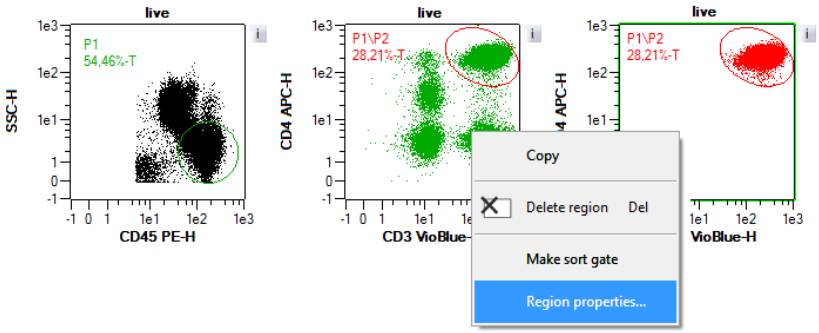
5.6.5 Dividing a parental gate in two regions with the same hierarchy

It is also possible to create multiple different regions from one parental gate. Regions drawn in a single plot will have the same hierarchy. In the example below, regions P2 and P3 (right) were both defined within region P1 (left) and therefore have the same hierarchy level.

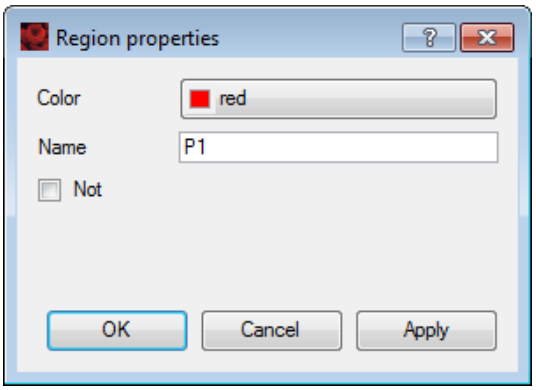


5.6.6 Changing region properties

1 To change color, region name, and/or define the region as a **Not gate** (refer to **Not gates on the next page**), right-click on the region name displayed in the sample list or on the activated gate in the dot plot.



2 Select **Region properties**. Adjust as desired and click **OK**.



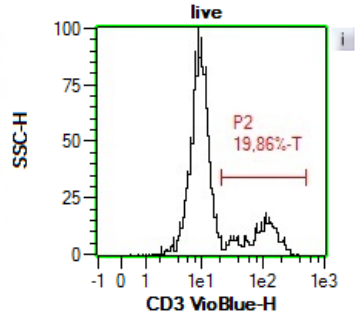
3 To change other region properties, click the **'i'** button next to the plot. Adjust as desired (refer to **Plot properties on page 36**).

5.7 Gating strategies

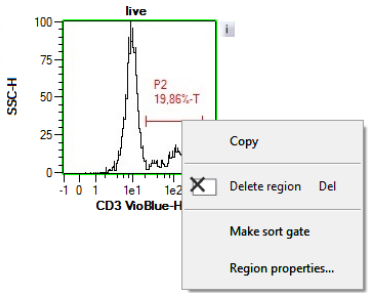
5.7.1 Not gates

Not gates are used to eliminate a cell population from analysis and are surrounded by a dashed line.

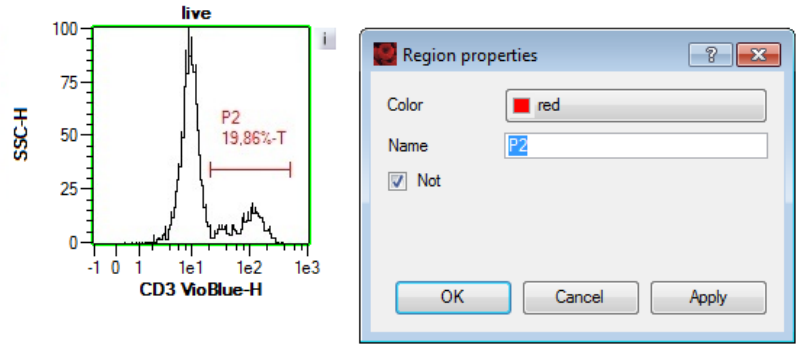
1 Draw a region or gate around the population to be excluded from analysis.



2 Within the plot, right-click on the region of interest.



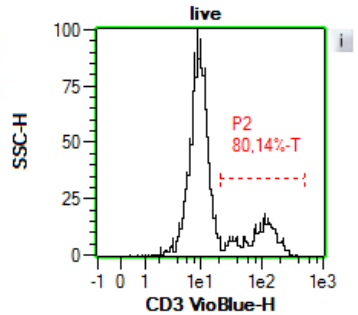
3 Select **Region properties** from the context menu.



4 Check the box **Not**. This region is now excluded from analysis. Optional: Select a color and/or name the **Not gate** as desired.

5 Click **Apply** and **OK**. The **Not gate** is now depicted as a dashed line.

6 In subsequent plots, gated cells are excluded.



5.7.2 Live Gate mode

The **Live Gate Mode** helps to set the sorting gate correctly when sorting rare cells. When the **Live Gate Mode** is activated, only events within the sort gate are shown in the plot. This increases the frequency of displayed target cells.

Follow the steps to use the **Live Gate Mode** to set a sort gate for a rare target cell population:

1. Set a sort gate at the expected position of the target cells. The sort gate should be bigger than the final sort gate.
2. Right-click on the sort gate and select **Make sort gate**.
3. Click on the **Live Gate** tool in the **Tools** tab to activate the **Live Gate Mode**. Now only events within the sort gate are shown in the plot (refer to **The Tools tab on page 20**).
4. Adapt the sort gate around the target cell population.
   Optional: Set a new sort gate around the target cell population and make this the new sort gate.
5. Click on the **Live Gate** tool in the **Tools** tab to activate the **Live Gate Mode**.

5.7.3 Multilayer mode display

Gated events within dot plots are displayed in their respective gate color. While establishing a gating strategy, each subsequent plot is formatted to display only events of the parent gate. Within the MACSQuantify Tyto Software it is possible to visualize the data of different grandparent or child levels within one dot plot, the so-called multilayer display. Gated events within dot plots are displayed in their respective gate color in this example. There are several options to activate the multilayer view.

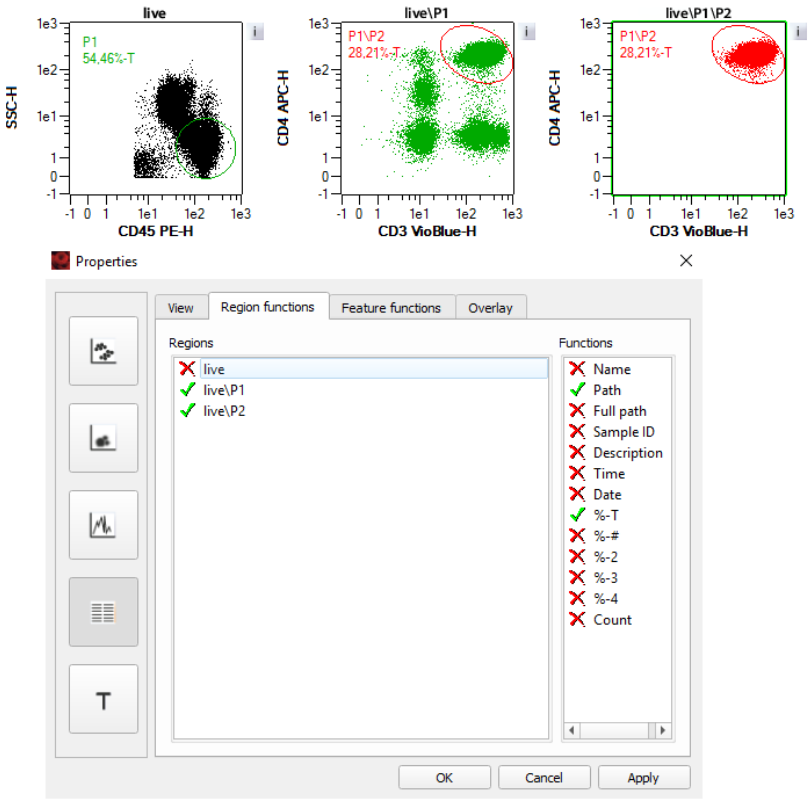


Figure 5.9: Standard view of a gating strategy.

In **Figure 5.9**, PBMCs were stained with CD45-PE, CD3-VioBlue, and CD4-APC. Cells were gated on $CD45^{++}$ leukocytes in the first plot (left plot) and for $CD3^{+}CD4^{+}$ T helper cells (middle plot). Visible in their specific region colors (black: all leukocytes; green: $CD45^{++}$ leukocytes; red: $CD3^{+}CD4^{+}$ T helper cells). Each subsequent plot is formatted to display only events of the parent gate. The properties window (below) shows the one region displayed in the P1/P2 dot plot (red).

Activate all parent and grandparent layers at once



1 Click the **New analysis window** button in the toolbar to open a new analysis window.

2 Define a hierarchical gating strategy, such as a $CD45^{+}$ cells (P1), $CD3^{+}CD4^{+}$ cells (P2). Also see the example in **Figure 5.10**.

3 Select **Multilayer mode** from the **Mode** menu.

4 The plot will now display all events with their gated region color.

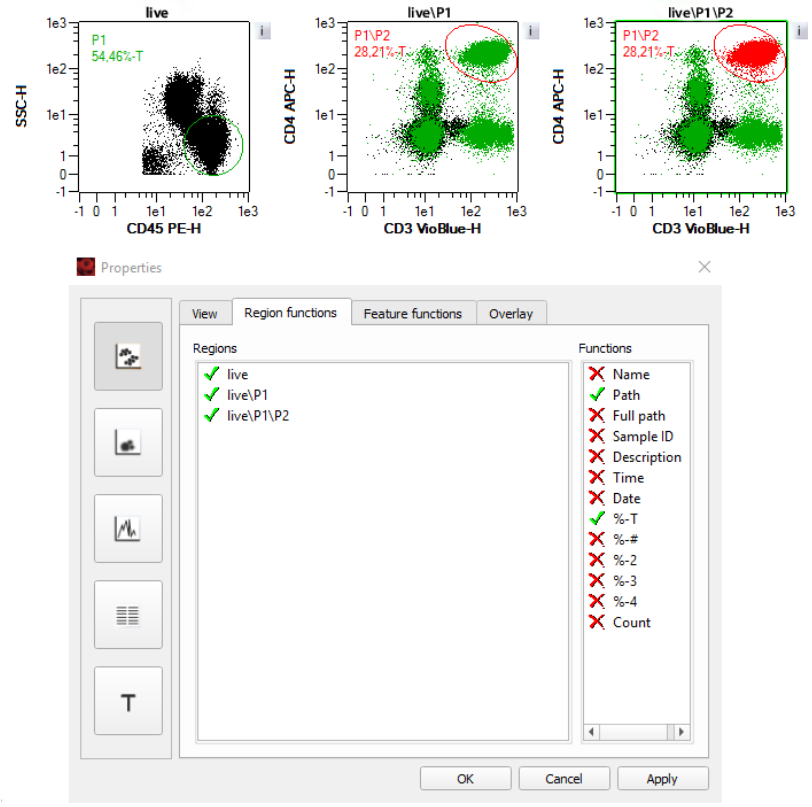


Figure 5.10: Multilayer mode example of a gating strategy.

In **Figure 5.10** PBMCs were stained with CD45-PE, CD3-VioBlue, and CD4-APC. Cells were gated on $CD45^{++}$ leukocytes in the first plot (left plot) and for $CD3^{+}CD4^{+}$ T helper cells (middle plot). Visible in their specific region colors (black: all leukocytes; green: $CD45^{++}$ leukocytes; red: $CD3^{+}CD4^{+}$ T helper cells). Each subsequent plot is formatted in multilayer mode and displays all events of the parent and grandparent gate. The properties window (below) shows the three regions displayed in the P1/P2 dot plot (red).

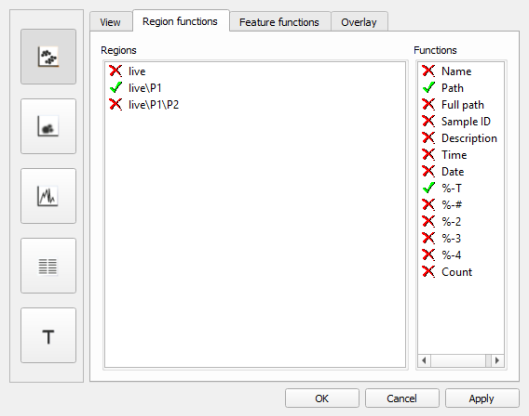
Select/Deselect different regions to display

Besides selecting to display the full gating strategy in multilayer mode it is possible to select or deselect different regions one by one. In addition to display the grandparent gates, the MACSQuantify Tyto Software also allows to display the child regions in a specific dot plot, so-called back gating. This can be useful for the user to identify within the previous plot where the cells of interest are displayed. Both methods are performed exactly the same way. To perform a backgating or to select/deselect different regions one by one, proceed as follows:

1 Identify the gated region to backgate onto the parent dot plot.

2 Click the **'i'** button next to the plot to open the **Properties** dialog box.

3 Go to the **Region functions** tab.



4 Select (✓) or deselect (✗) the region using the left mouse button.

5 Click **Apply** to confirm, then **OK** to close.

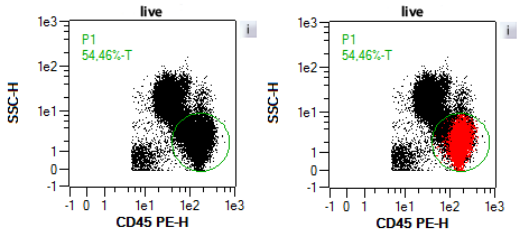


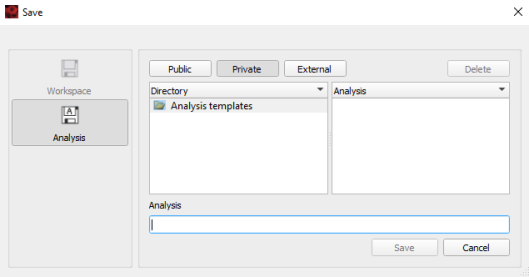
Figure 5.11: Dot plot before (left) and after (right) backgating of the P1/P2 gate. Populations are indicated in their designated gate color.

5.8 Managing data analysis templates

5.8.1 Saving analysis templates



1 Click the **Save** button in the toolbar and select **Analysis**.



2 Depending on where to save the template, select **Public**, **Private**, or **External**.

3 Enter a name for the analysis template file and click **Save**.

5.8.2 Opening analysis templates



1 Click the **Open** button in the toolbar and select **Analysis**.

2 Find the appropriate analysis template file in the **Public**, **Private**, or **External** directory.

3 Highlight the analysis template file in the box on the right and click **Open**.

5.8.3 Deleting analysis templates



1 Click the **Open** button or the **Save** button in the toolbar and select **Analysis**.

2 Find the appropriate analysis template file in the **Public**, **Private**, or **External** directory.

3 Highlight the analysis template file in the box on the right and click **Delete**. The file is permanently deleted.

5.9 Managing workspace settings



A workspace file is all encompassing and represents a master file including information for the analysis templates. It contains the following information:

- **Samples tab** – Samples currently displayed in the sample list.
- **Experiment tab** – All experiment parameters.
- **Instrument settings** – Current instrument settings including compensation.
- **Analysis template** – Current analysis view and template, if selected.

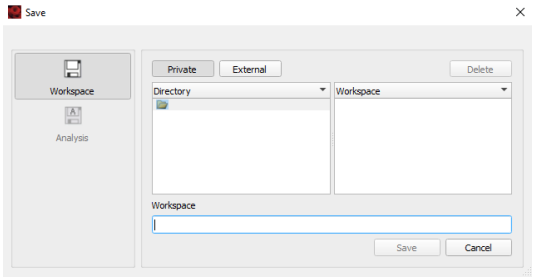
Loading a workspace file will reopen the MACSQuantify Tyto Software with the settings valid at the point of storing the workspace, i.e., it will enter the same parameters in the **Experiment** tab and instrument settings, and it will display the same analysis. Workspace files can be stored in the **Private** or **External** location but not in the **Public** location. Workspaces are saved in the **Private** or **External** location in the **prj** folder (refer to **Data storage on page 76**).

5.9.1 Saving workspace files



1 Click the **Save** button in the toolbar and select **Workspace**.

2 The **Private** folder is automatically selected.



3 Enter a name for the new workspace file and click **Save**.

The **Analysis mode** button must be deactivated (light grey) to save workspaces.

5.9.2 Opening workspace files



1 Click the **Open** button in the toolbar and select **Workspace**.

2 Find the appropriate workspace file in the **Private** or **External** directory.

3 Highlight the respective workspace file in the box on the right and click **Open**.

Depending on the selected **Cartridge** type in the **Experiment** tab, only workspace files for regular or high-speed settings can be opened.

When opening workspace files created with software versions before version 1.0 for the first time, a notification informs users about changed settings. It is recommended to review the settings for PMT gains and thresholds in the **Channels** tab before using the workspace (Refer to **The Channels tab on page 21**).

5.9.3 Deleting workspace files



1 Click the **Open** button or the **Save** button in the toolbar and select **Workspace**.

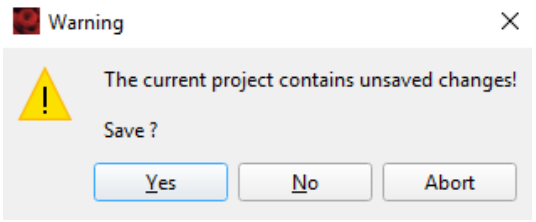
2 Select the file in either the **Private** or **External** directory.

3 Highlight the workspace file in the box on the right and click **Delete**. The file is permanently deleted.

5.9.4 Opening a new blank workspace

1 Go to **File** > **New Workspace**.

2 If the current workspace contains unsaved changes, a warning message appears. Click **Yes** to store current workspace settings in a workspace file or click **No** to discard.



3 A new, empty workspace will open.

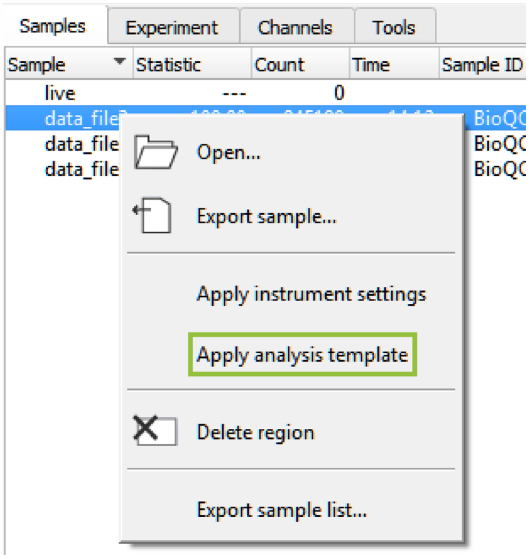
5.10 Post-acquisition data analysis

5.10.1 Applying analysis templates

An analysis template used in a previous experiment, including gating strategy and plots can be applied to data files.

1 Open the desired data file(s) and click on the **Samples** tab.

2 Right-click on the data file and select **Apply analysis template**.



3 The data file and corresponding analysis template will be loaded in analysis mode.

4 To view analyzed results of the previously defined analysis, right-click on the data file and select **View with analysis <name of analysis>**.

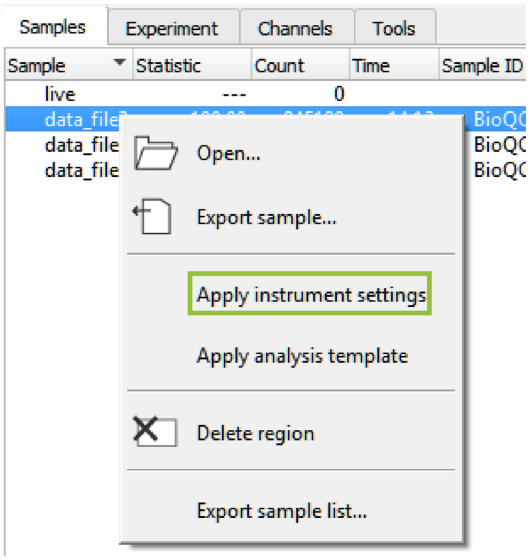
5 The analyzed results of a previously defined analysis are shown.

5.10.2 Applying PMT voltage and compensation settings

1 Open the desired data file(s). The files will appear in the **Samples** tab.

2 Click on the **Samples** tab.

3 Right-click on the data file and select **Apply instrument settings**.



6 Setting up the instrument

This chapter explains the steps in order to set up the instrument.

Before sorting a sample, adjust all necessary parameters to distinguish your population of interest and for an optimal outcome of your experiments:

- Adjust backscatter, side scatter, and fluorescence photomultipliert tube (PMT) gains.
- Set a noise threshold and optional trigger threshold to exclude unwanted signals.
- Compensate for spectral overlap.

All settings and adjustments are stored in the workspace file (Refer to **Workflow of a sort process on page 29**).

6.1 Adjusting the event detection settings

The event detection of the MACSQuant Tyto Cell Sorter is based on the backscatter signals. It is necessary to adjust the PMT gains and thresholds for the used cells or particles and the expected signal intensities of the fluorescent positive populations. It is recommended to use a sample that contains a negative and positive population for each of the utilized fluorescence channels for the experiment.

6.1.1 Adjusting backscatter (BSC) and side scatter (SSC) gains

If the desired population of events is unknown, it can be difficult to adjust BSC and SSC gains. Use a cell sample labeled with a fluorescent marker to identify the cells of interest. Use this marker for backgating to determine the proper BSC and SSC adjustment (refer to **Select/Deselect different regions to display on page 54**).





1. Click the **Open** button in the toolbar to open a pre-existing analysis template.
2. Place a cartridge containing the sample to be analyzed in the MACSQuant Tyto Cell Sorter. Click the **Start measurement** button in the status bar to start acquisition. As events begin to accumulate, open the **Channels** tab.
3. Depending on where the desired population of cells is located on the BSC versus SSC plot, adjust the voltage gains for BSC and (optional) SSC to bring the population of events into the dot plot. The signal intensities in the backscatter blue (BSB), backscatter violet (BSV), and backscatter red (BSR) channel should be correlated by adjusting the voltage gains for the backscatter channels. The correlation of the backscatter signals can be controlled in the two plots BSR versus BSB and BSV versus BSB in the **Channels** tab (**Figure 6.1**).
   Adjust gains by one of the following:

- Slide the scroll bar up or down to increase or decrease the gains, respectively.
- Activate the toggle bar by clicking on it and adjust the gains in increments of 10 V by clicking on the line above or below the scroll bar (recommended).
- Double-click the value box, type in a value, and press **Enter**.
- Use the up and down arrows to increase or decrease the value in small steps.

4 After an adjustment has been made, it is recommended to clear the previous events and only visualize events with the new settings. Click the **Clear** button in the status bar to refresh the events.



5 Continue to make adjustments until the population of interest is clearly defined.

6.1.2 Setting the noise threshold

The noise threshold is set in the three backscatter channels (BSB, BSV, and BSR) to cut off background signals, noise, and debris from the measurement. Signals below the threshold are ignored.

When setting the threshold take care not to cut off populations of interest in one of the backscatter channels (**Figure 6.1**). The threshold for all three backscatter channels is the same value in order to achieve good results. It is recommended to also correlate the signal intensities of the three backscatter channels (BSB, BSV, and BSR). The noise threshold in relation to the signal intensities of BSB, BSV, and BSR can be monitored in the two plots in the **Channels** tab. If necessary, adapt the backscatter gains to correlate the intensities.

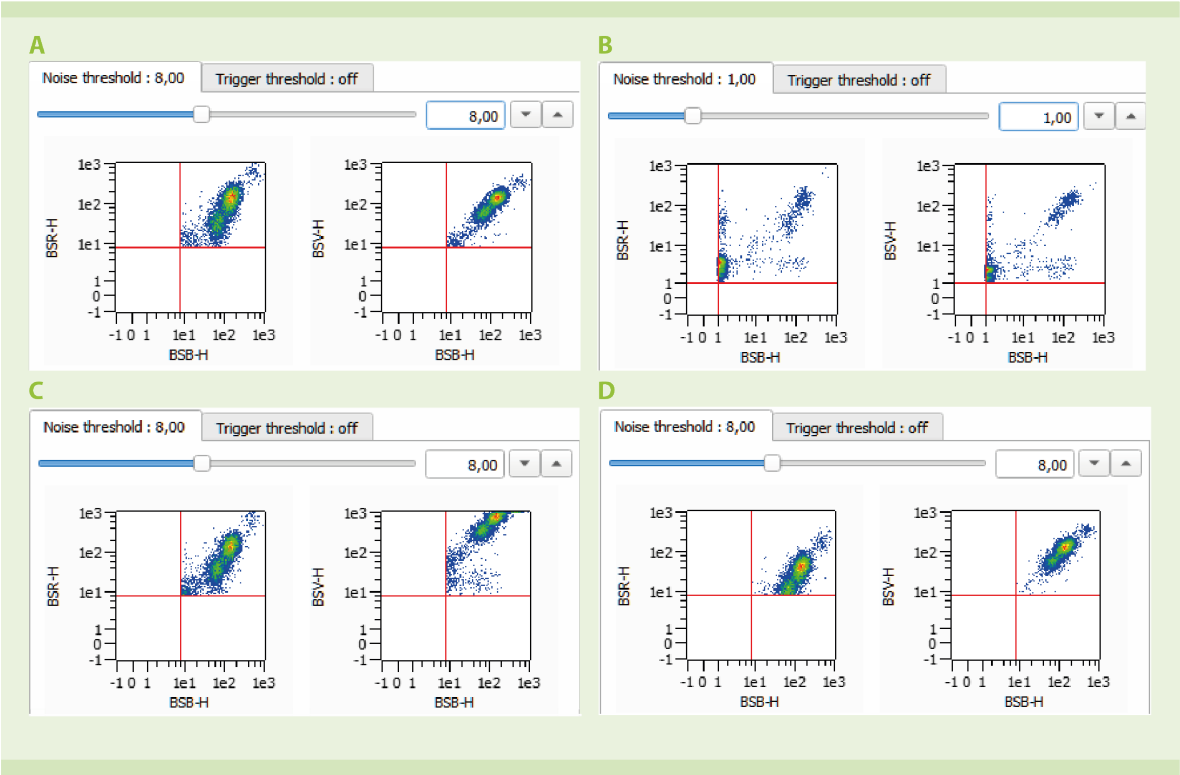


Figure 6.1: Example of different gain and threshold settings shown for white blood cells. (A) Good settings for noise threshold and gains. (B) Noise threshold is set too low and noise signals are disturbing the measurement. (C) BSV-H gain is set too high so that high BSV-H signals are clipped. (D) BSR-H gain is set too low so that the noise threshold cuts of events in the backscatter channels.

To set the noise threshold:

1 Go to the **Channels** tab.

2 Go to the **Noise threshold** tab.

3 The noise threshold can be adjusted by one of the following:

- Slide the trigger toggle bar right and left to increase or decrease the value.
- Click and hold the red threshold line in one of the BSC plots and move it to adjust the value.
- Click on either side of the toggle bar to adjust the value.
- Double-click inside the value box. Type in the desired value. Press **Enter** to apply.
- Use the up and down arrows to increase or decrease the value in small steps.

6.1.3 Setting fluorescence channel gains

It is recommended to use a sample that contains a negative and positive population for each of the utilized fluorescence channels for the experiment.

When setting the fluorescence channel gains, keep the respective negative and positive population on scale. Only make adjustments if one population is not on scale.

1 Format one of the dot plot displays to view the first fluorescent parameter.
2 Go to the **Channels** tab.
3 Determine the position of the positive and negative events. Optionally, draw a gate around the appropriate population in the BSC versus SSC dot plot and display only these events in the plot with the fluorescence display. Select the gate in the drop-down list of the plot header.
4 In the **Channels** tab, adjust the gains up or down for the specific fluorescence channel as needed to get both the positive and negative population on scale. To adjust gains, do one of the following:
   - Slide the scroll bar up or down to increase or decrease the gains, respectively.
   - Click the toggle bar. Adjust the gains in increments of 10 V by clicking on the line above or below the scroll bar (recommended).
   - Double-click the value box, type in a value, and press **Enter**.
5 It is not recommended to lower the PMT so that the negative population appear in the far left of the plot. This may decrease sensitivity for weakly expressed antigens.
6 Continue with other fluorescence channels if necessary.

Always adjust fluorescence channel PMTs appropiate for the used cells prior to applying any compensation.

6.1.4 Setting the trigger threshold (optional)

The trigger threshold can be used as an additional trigger to discard events that are not relevant. It can be set on a fluorescent or side scatter channel to only display events with intensities above the trigger threshold (**Figure 6.2**). Signals below the threshold are ignored.

To set the optional trigger threshold:

1 Go to the **Channels** tab.
2 Go to the **Trigger threshold** tab.
3 Select your channel.

4 Set the trigger threshold. The trigger threshold can be adjusted by one of the following:

- Slide the trigger toggle bar right and left to increase or decrease the value.
- Click and hold the red threshold line in one of the BSC plots and move it to adjust the value.
- Click on either side of the toggle bar to adjust the value.
- Double-click inside the value box. Type in the desired value. Press **Enter** to apply.
- Use the up and down arrows to increase or decrease the value in small steps.

5 Check the checkbox to activate the trigger threshold.

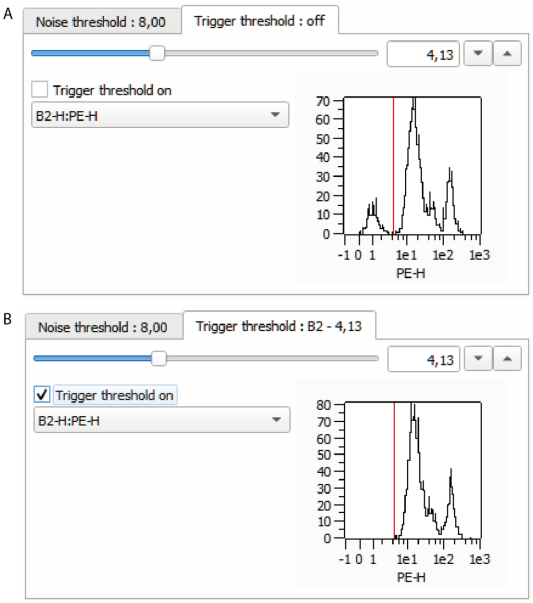


Figure 6.2: Optional trigger threshold deactivated (A) and activated (B).

The **Live Gate** mode is another tool to visiualize rare events, without ignoring events during the sort (refer to **Live Gate mode on page 53**).

6.2 Compensation for spectral overlap

Multiparameter cell analysis allows the examination of multiple cellular properties by simultaneously detecting different fluorochromes that identify these properties. Due to fluorescence spectral overlap or spillover, individual fluorochromes will be detected in more than one fluorescence channel. Thus, for multicolor analysis, compensation of spectral overlap is necessary to produce accurate and consistent results. Compensation can be done manually on the MACSQuant Tyto Cell Sorter.

If the combination of selected fluorochromes have emission spectra in overlapping wavelength ranges, it is possible that a certain percentage of detectable light from one fluorochrome spills over to an incorrect detection channel (**Figure 6.3**), e.g., FITC fluorescence should be detected in the B1 channel (525/50 nm), but is also detected in the B2 (PE) channel (585/40 nm).

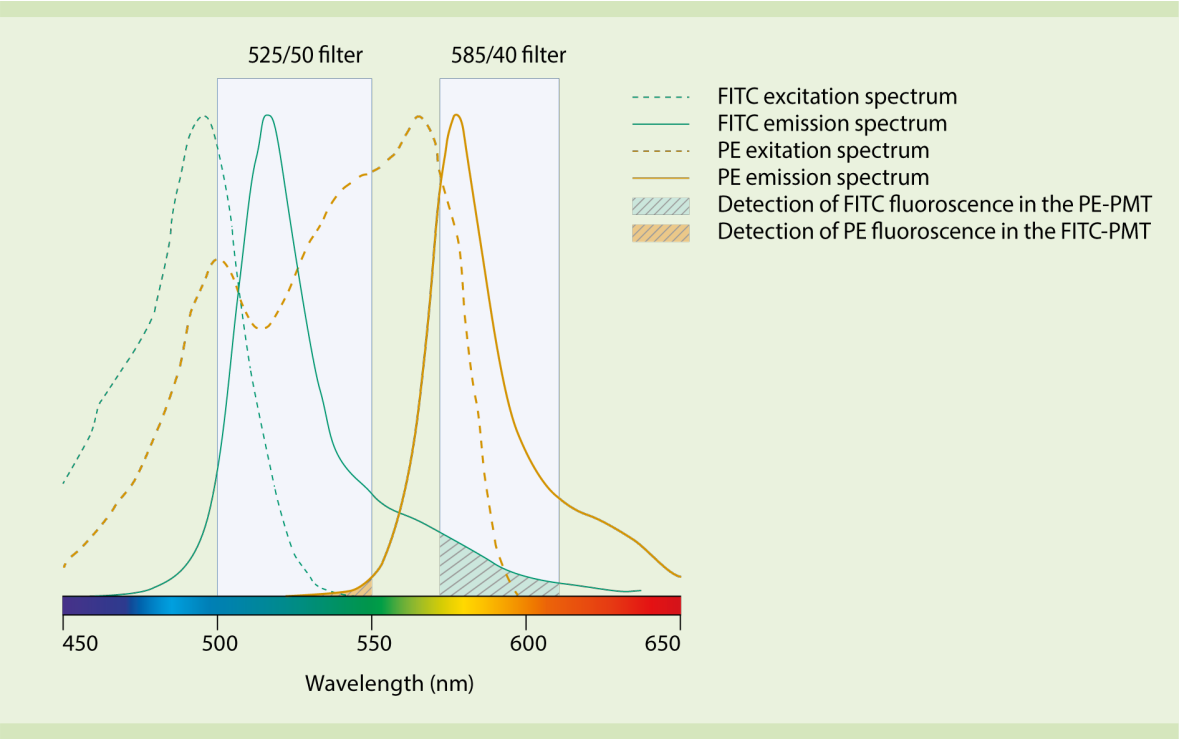


Figure 6.3: Emission spectra of FITC (green solid line) and PE (orange solid line). The green hashed area represents the amount of FITC signal detected in B2 (585/40 nm). The orange-hashed area represents the amount of PE signal detected in B1 (525/50 nm).

6.2.1 Example for over- and undercompensation

To compensate for spectral overlap, cells or antibody-capture compensation beads, e.g., MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900), that emit fluorescence signals from only one fluorochrome are used. The user can apply compensation adjustments to the instrument settings. Spillover is determined by acquiring single-stained samples and viewing the fluorescence signal in all adjacent detection channels.

With properly compensated instrument settings, the median fluorescence intensity detected in the spillover channel of the positive and negative population is equal. When the instrument settings are undercompensated, the median fluorescence intensity detected in the spillover channel of the positive population is higher than that of the negative population. When the instrument settings are overcompensated, the median fluorescence intensity detected in the spillover channel of the positive population is lower than that of the negative population (**Figure 6.4**).

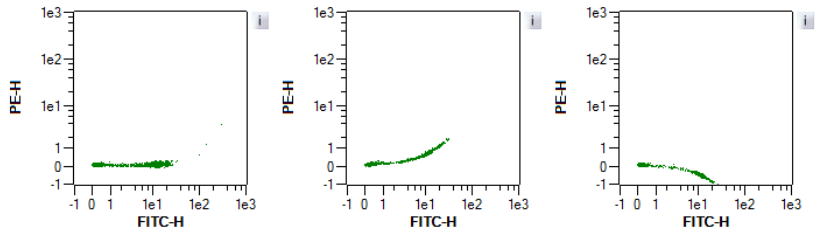


Figure 6.4: Compensated (left), undercompensated (middle), and overcompensated (right) dot plot.

6.2.2 Compensation guidelines

For proper compensation, the use of single-stained controls is absolutely necessary. On the MACSQuant Tyto Cell Sorter, single-stained cells are the preferred controls to set the PMT gains. Single-stained antibody-capture compensation beads, e.g., MACS Comp Bead Kit, anti-mouse igκ (# 130-097-900), are the preferred method to perform compensation.

When setting up and choosing single-stained controls for compensation, consider the following:

- For compensation, use the same fluorochrome that will be detected in the experimental panel. For example, if FITC is used in the experimental staining panel, FITC must be used to set the compensation. Do not substitute with other fluorochromes that are detected in the same channel, such as GFP or AlexaFluor 488.
- The positive and negative populations within the sample must have the same autofluorescence or level of background fluorescence in the spillover channel.
- If antibodies used in an experiment vary from antibodies used for compensation, consider that fluorochrome-conjugated antibodies used for setting the compensation adjustments should display fluorescence intensity levels equal or brighter than the antibody used in the experiment.
- All changes to voltages of fluorescence PMT detectors must be made prior to adding compensation adjustments. Changing PMT voltages after compensation will change the detected spillover and will require reevaluation of compensation values.
- The single-stained compensation controls must include a positive and negative population within the sample.
- It is best to aim for a positive population frequency of >10%. If using an antibody that detects a rare cell population, it might be possible to use compensation beads, a control cell line of similar autofluorescence (meaning positive and negative signal remain on scale), or another antibody coupled to the same fluorochrome that labels a higher frequency of cells in the sample.
- If using tandem fluorochromes, the exact same lot from the same manufacturer must be used to set compensation adjustments. In these instances, compensation beads, e.g., MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900), or control cell lines can be useful. Do not mix lots of tandem fluorochromes.
- Proper compensation should not rely on visual inspection of fluorescence intensity only. Use statistical analysis of the median fluorescence intensity for the spillover channel to set the adjustments.
- Ideally, only compensate for the channels used in the experimental panels.
- It is preferred to use hlog scaling for data display when visualizing compensated data.

6.2.3 Compensation matrix



The MACSQuantify Tyto Software uses a compensation matrix, which organizes the detection of individual fluorescence signals in columns. In order to display the compensation matrix (**Figure 6.5**), click the **Instrument settings** button in the toolbar and then check the **Matrix** checkbox. Column headers identify representative fluorochromes detected on the MACSQuant Tyto Cell Sorter. The rows indicate the fluorescence channels. The default value is set to 1.0 in the diagonal (column versus row). This position within the matrix indicates the primary detection channel for the respective fluorescence. The default value for all other positions is 0.000.

	FITC	PE	PI/PE-Cy5.5/PerCP-Vio700	PE-Cy7/PE-Vio770	CFP/VioBlue	VioGreen	APC	APC-Cy7/APC-Vio770
B1	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B2	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
B3	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
B4	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
V1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
V2	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
R1	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
R2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0

Reset

Figure 6.5: The 8×8 compensation matrix of the MACSQuant Tyto Cell Sorter displayed on the orange touchscreen.

If spillover is detected with single-stained samples, values between 0 and 1 are inserted into the spillover detection channel for compensation.

To adjust values in the compensation matrix, do one of the following:

- Click on the toggle bar and adjust the scroll bar up or down to increase or decrease the gains.
- Click on the line above or below the scroll bar to adjust the gains in increments of 0.01.
- Double-click the value box and type in a value. Click **Enter**.

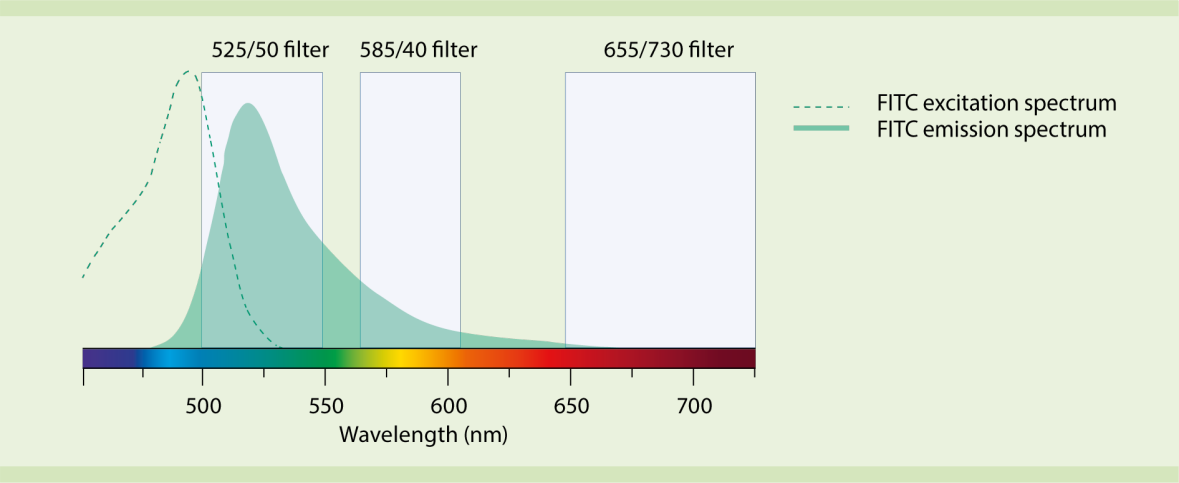


Figure 6.6: Spectrum of FITC single-stained cells. Channels: B1 (525/50), B2 (585/40), B3 (655–730). 15% of the detectable FITC signal is seen in the B2 (PE) channel. To compensate, add 0.150 to the FITC-B2 box of the compensation matrix. If additional spillover of the FITC signal is found in the B3 (PE-Cy5) channel, adjust values accordingly in box FITC-B3.

6.3 Flow control

The **Flow Control** section of the **Cartridge status** box shows and controls the parameters of the sample flow rate through the cartridge (**Figure 6.7**).

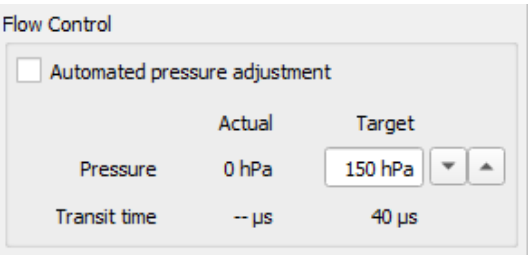


Figure 6.7: The Flow Control section of the Cartridge status box shows

It shows:

- the **Actual pressure** applied to the input chamber of the cartridge
- the set **Target pressure**
- the **Actual transit time** of events of interest
- set **Target transit time** depending on the cartridge type used (**Table 6.1**)

Cartridge type	Event transit time / Time-of-flight (TOF)	Pressure	Flow rate
Regular speed cartridge	40 µs	150 hPa	4 mL/h
High-speed cartridge	20 µs	1200 hPa	8 ml/h

Table 6.1: Default parameters for regular and high-speed cartridges.

After starting a measurement, the system applies constant pressure according to the cartridge type used. If needed, the pressure can be adjusted manually. This can be helpful while setting all parameters for the sort. To adjust the target pressure do one of the following:

- Double-click inside the value box. Type in the desired value. Press enter to apply.
- Use the up and down arrows to increase or decrease the value in small steps.

Per default, the **Automated pressure adjustment** is activated after starting a sort. The MACSQuant Tyto Cell Sorter now adjusts the pressure automatically to achieve the correct transit time for the events of interest and the cartridge type used during the whole sort process.

Optional, the pressure can be adjusted manually during the sort. Therefor, uncheck the **Automated pressure adjustment** checkbox. This is only recommended when no or unexpected events are observed in the **Real time sort statistics** window during a sort (refer to **in the MACSQuant Tyto Cell Sorter user manual**).

Achieving the correct transit time is important for an accurate signal assembly and an optimal outcome of the sort. It is recommended to use the **Automated pressure adjustment**. Different parameters, e.g., cell morphology (size, surface, structure) or flow resistance of the cartridge affect the transit time.

7 Reporting your data

The MACSQuantify Tyto Software offers several ways to create reports. Single plots or entire pages may be copied and pasted into external software, such as Microsoft® Word. If the instrument is connected to a printer, reports may be also printed directly or alternatively, saved as a PDF. Prior to printing or copying the results, you may change the color settings of all plot types and gates. In addition, the sample list may be exported to Microsoft® Excel for your records (refer to **Exporting the sample list to Microsoft® Excel on page 70**).

7.1 MACSQuant Tyto run report

A run report file summarizes data and statistics for a finished sort. It includes information about the project, cartridge and instrument used, instrument settings (set gains and thresholds), sort statistics, and a screenshot of the applied plots and gates. Run report files can be opened, copied (refer to **Data management on page 73**), and exported as PDF file.

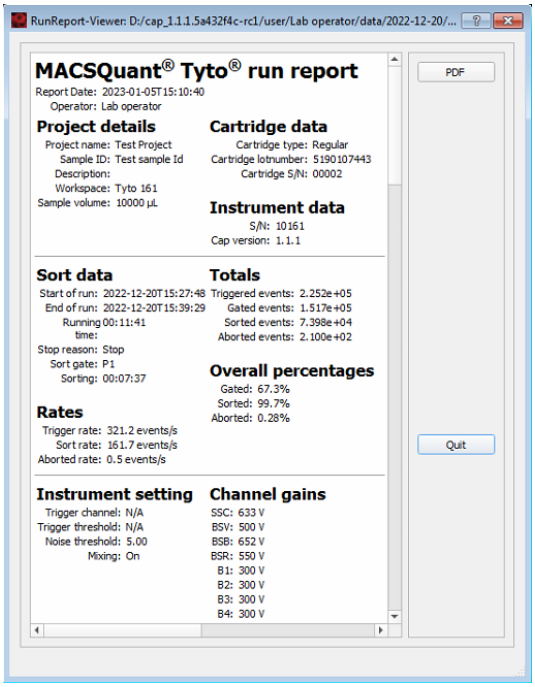


Figure 7.1: Example of a MACSQuant Tyto run report.

7.1.1 Opening run report files

1 To open run report files do one of the following:

- Click **Open** in the toolbar.
- Click **File > Open**.
- Right-click within the **Samples** tab and select **Open**.

2 Select **Report files** on the left-hand side of the dialog box.

3 Select the appropriate file location **Public**, **Private**, or **External**.

4 Select the desired run report file. Multiple files can be opened at once.

5 Confirm your selection with **Open**.

The run report file for the recent measurement and sort can also be opened via **Open Report** in the **Real time sort statistics** window.

7.1.2 Exporting a run report to PDF

1 Open the run report you want to export (refer to **Opening run report files above**).

2 The run report opens.

3 Click on **PDF** in the **Run Report** window to save it as a PDF file.

7.2 Copying pages or plots

7.2.1 Copying an entire page

1 Display and properly analyze data.

2 Click **Edit > Copy page**. The page is saved onto a clipboard.

3 Open a program for pasting, e.g., Microsoft® Word or PowerPoint.

4 Right-click and select **Paste** within this program.

5 The page including all the plots and/or statistic tables will be copied.

7.2.2 Copying a single plot

1 Display and properly analyze data.

2 Click on the specific plot or statistic table for copying. The plot is highlighted (light green rim).

3 Click **Edit > Copy plot**. The plot is saved onto a clipboard.

4 Open a program for pasting, e.g., Microsoft® Word or PowerPoint.

5 Right-click and select **Paste** within this program.

6 The plot including any gating and annotations will be copied.

7.3 Printing

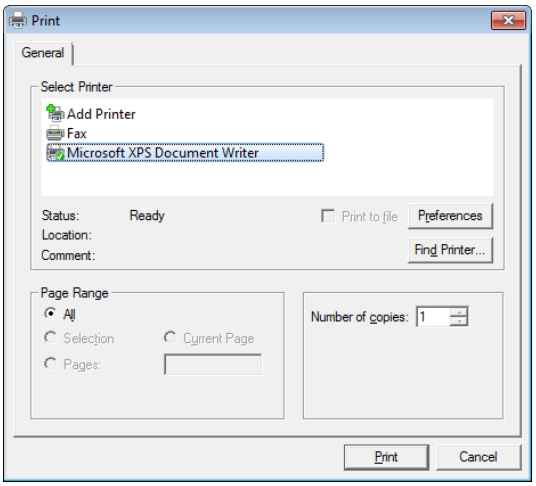
7.3.1 Printing data files



1 To print active workspaces, open the desired workspace or analysis window.

2 Click the **Print** button in the toolbar.

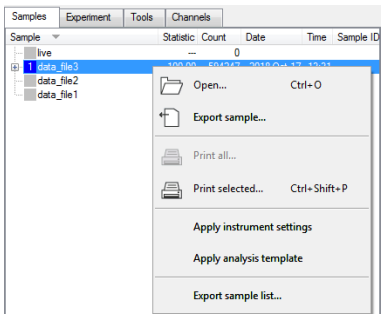
3 Select the desired printer. Click **Print**. The printer can be connected directly to the MACSQuant Tyto Cell Sorter or via a network connection.



7.3.2 Printing all windows

To print all samples in the sample list with the current analysis template:

1 Ensure that the current analysis is applied to all samples. Go to **File > Print all**. Alternatively, right-click on the **Sample list** and select **Print all**.



2 By default, the option button **All** is active and all analysis windows will be printed. Check **Pages** to select specific analysis windows. When performing multiple analyses in one analysis window, a number is assigned to the sample and its respective analysis and displayed in the sample list.

To use the **Print all** command, several parameters have to be accomplished. If a prerequisite for the **Print all** command is not fulfilled, the command will be disabled.

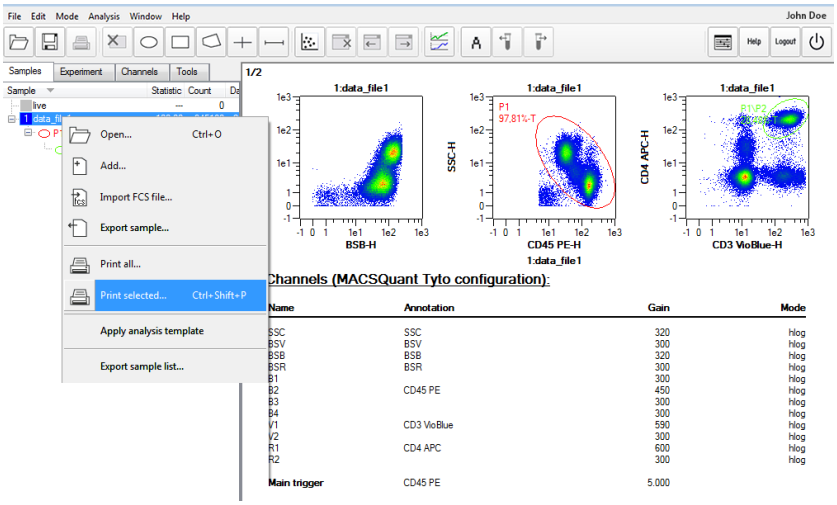
- The order of assigned numbers, referring to the corresponding analyses, will be moved along the samples in the sample list.
- The analyses, including the corresponding gating strategy, have to be applied to all samples in the sample list in the same order as the assigned numbers.
- All samples have to be included in the analyses.
- Empty live samples will be ignored for print.

7.3.3 Printing selected windows

The **Print selected** batch-print option is only accessible in analysis mode.

To print all selected samples with the current analysis template:

1. Select samples in the sample list. Ensure that the current analysis is applied to all selected samples.
2. Right-click on the sample list and select **Print selected**. By default, the option button next to **All** is checked and all analysis windows will be printed.



3. Check the option button next to **Pages** to select specific analysis windows for printing.
4. When performing multiple analyses in one analysis window, a number for the sample and its respective analysis is assigned. This number will be displayed in the sample list.

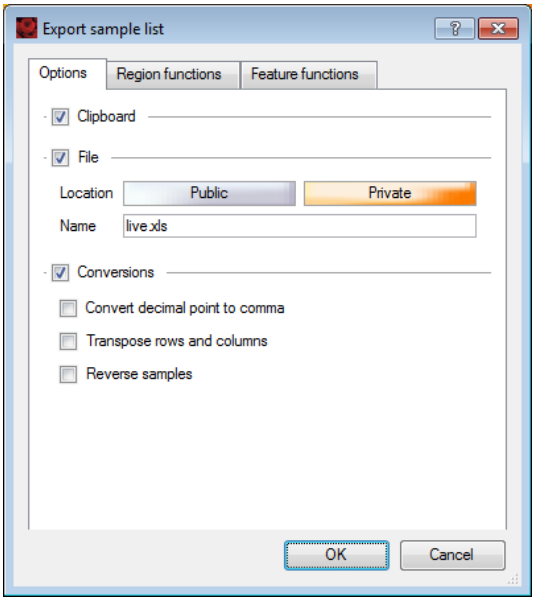
To use the **Print selected** command, several parameters have to be considered and accomplished. If a prerequisite for the **Print selected** command is not fulfilled, the command will be disabled.

- The order of assigned numbers, referring to the respective analyses, will be moved along the selected samples in the sample list.
- The analyses, including the corresponding gating strategies, have to be applied to all selected samples in the sample list in the same order as the assigned numbers, e.g., using the **Previous sample** or **Next sample** buttons in the toolbar.
- All selected samples have to be assigned to an analysis.
- Empty live samples will be ignored for print.

7.4 Exporting the sample list to Microsoft® Excel

1. Generate a gating strategy for the data files and apply it to all data files within the experiment.
2. Right-click within the **Samples** tab and select **Export sample list**.

3 A dialog box will open to select the parameters to export.



4 Select one or both of the following:

- Check the box **Clipboard** to save the data to the clipboard (saved data to be pasted into Excel).
- Check the box **File** to save the data as an Excel file. Depending on the selected location, the file will be saved within the cap folder structure of MACSQuantify Tyto Software either to **Public** or **Private** location.

5 Check the box next to **Conversions** and all desired parameters.

6 Go to the **Region functions** tab to select the region/gates for export and the percent and count statistics to be exported.

Several regions can be easily selected or deselected at once. Right-click on any region and select the desired region from the context menu.

7 Go to the **Feature functions** tab to select the optical parameters for export and which statistical information to be exported.

8 Click **OK**. If **Clipboard** was selected, open Excel and select **Paste**.

8 Data management

8.1 Files

8.1.1 Opening files



Access **Workspace**, **Analysis**, **Data** and **Report files** via the **Open** button in the toolbar.

1 To open files, do one of the following:



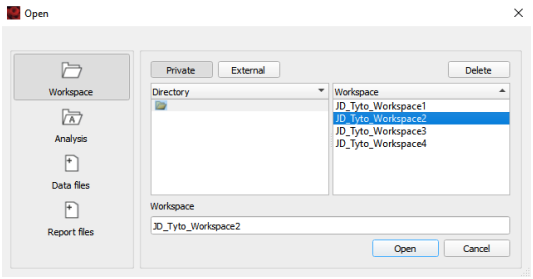
- Click **File > Open**.
- Click the **Open** button in the toolbar.
- Right-click within the **Samples** tab and select **Open**.

2 Select **Workspace**, **Analysis**, **Data** or **Report files** on the left-hand side of the dialog box.

3 Select the appropriate file location **Public**, **Private**, or **External**.

4 Select the desired data file. Multiple files can be opened at once.

5 Confirm your selection with **Open**.



Depending on the selected cartridge type in the **Experiment** tab, only workspace files for regular or high-speed settings can be opened.

8.1.2 Saving files

Workspaces and analysis files can be saved to the **Public** or **Private** locations as defined under **User settings**, or to **External** locations. Data files are saved automatically.



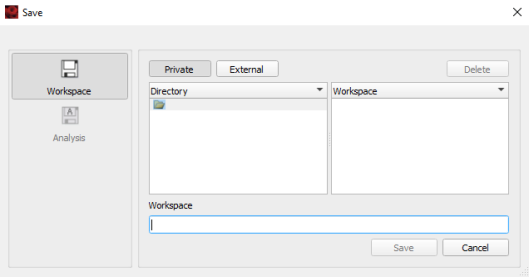
1 Click **File > Save** or click the **Save** button in the toolbar.

2 Select **Workspace** or **Analysis** to save the file type.

3 If necessary select the desired location (**Public, Private, External**).

4 Assign an appropriate file name.

5 Click **Save**.

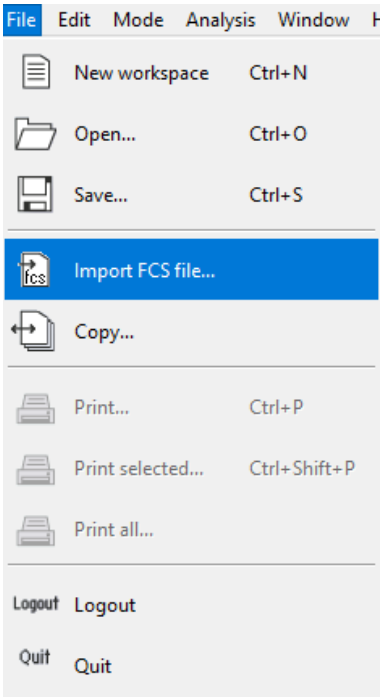


8.1.3 Importing FCS files

Import flow cytometry data generated with other instruments and analyze it with the MACSQuantify Tyto Software.

1 Click on the **File** menu in the menu bar.

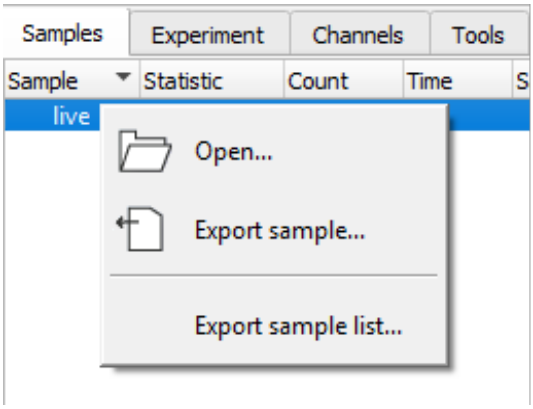
2 Select **Import FCS file** from the context menu.



8.1.4 Exporting FCS files

The MACSQuant Tyto Cell Sorter will store all acquisition data in the MQD file format.

1 To export MQD files as FCS or CSV files, right-click on opened or added data files in the **Samples** tab and select **Export sample**.



2 Select the desired file type (.fcs or .csv) from the drop-down list. If a gating strategy was applied to any of the data, MACSQuantify Tyto Software will create a separate FCS file for each gated population. If only one FCS file is required for the original data file, check the box next to **Skip subpopulations**. The FCS files will be stored to the same folder as the original MQD data files were opened or added from.

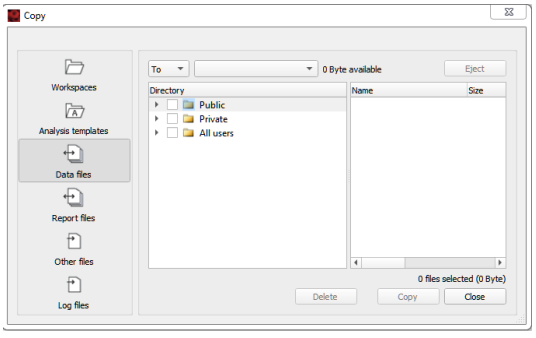
Go to **Edit > Options > Software > Acquire** to generate FCS files automatically after data acquisition (refer to **Acquisition on page 87**).

8.1.5 Copying files

An administrator can copy all files, including (private) files of all users, all files in **Public** locations, and administrator files in a **Private** location. By using the folder **All users** in the **Copy** dialog box, the administrator can clear up the hard drive after copying the files to a remote storage location (e.g. network folder, USB stick, hard drive). Custom mode users can only copy their own files (**Private** folder) and files in the **Public** folder (depending on their access rights).

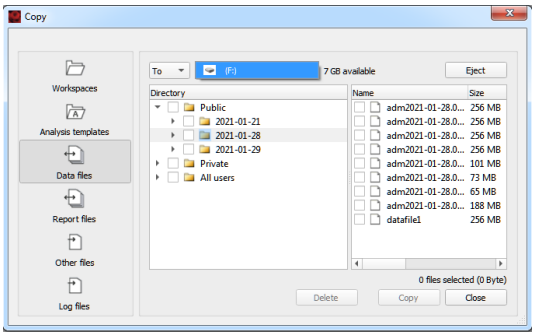
Go to **File > Copy** to access the folders **Other files** and **Log files**. In the folder **Other files**, screenshots in BMP format or the exported sample list as XLS files are saved. Generate screenshots using the print key of the MACSQuant Tyto Cell Sorter's keyboard. The folder **Log files** contains system files that include system relevant data that can be important for troubleshooting issues.

1 To copy files from the MACSQuant Tyto Cell Sorter to a remote storage location, select **File > Copy**.



To transfer data from the MACSQuant Tyto Cell Sorter to a USB stick, insert the stick into a USB port of the instrument. Wait for the device to be recognized.

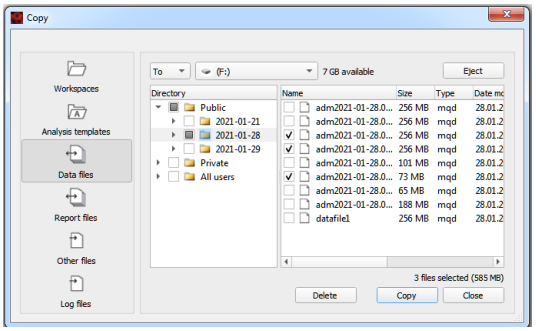
2 Select the destination from the available drop-down list in the dialog box. If you are transferring data to a network location, enter the password when prompted.



3 From the drop-down list, select:

- **To**: The selected files are copied to the selected destination. The source folder structure is also preserved at the destination location.
- **From**: Selected files will be imported. The files must be organized in the same folder structure as in the destination folder to be properly imported.

4 Check the box next to the desired files or folders.



5 Click **Copy**. When all files are copied, a report dialog box appears.

6 Close the box to execute another copy or deletion command.

7 When using a USB stick, click **eject and close** to safely remove the USB stick. Alternatively, you can select **Remove external media** from the **Tools** tab.

8.2 Data storage

Apart from flow cytometry data files, MACSQuantify Tyto Software also uses other files to store and reload user settings. Files can be stored and loaded from different locations. Within MACSQuantify Tyto Software, these locations are referenced as **Public**, **Private**, and **External**.

- **Public** files are located on the local hard drive of the MACSQuant Tyto Cell Sorter and can be accessed by any user.
- **Private** files are located on the local hard drive of the instrument and are only accessible by the logged-in user.
- **External** files that are located on an independent file storage device, e.g., a USB stick, can be accessed when the device is connected to the instrument.

Files are saved within the cap folder structure on the MACSQuant Tyto Cell Sorter hard drive (**Figure 8.1**):

- in a **Public** location in the folder global,
- in a **Private** location in folders bearing the name of the user (e.g. admin, John Doe, service).

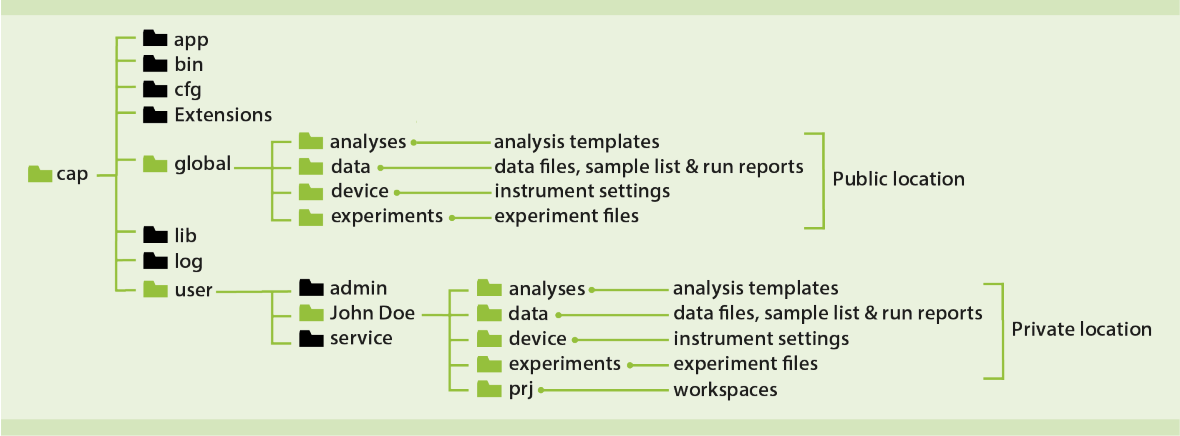


Figure 8.1: File structure of the cap folder on the hard drive of the MACSQuant Tyto Cell Sorter.

Access rights defined for a user determine the right to read and save files or to only read files from and to **Public** or **Private** locations. If an **External** location is available, any user can store and load files to and from this location.

The following file types are supported by the MACSQuantify Tyto Software and can be accessed using commands to open, save or copy files:

- The file type **Workspaces** can be stored only in the **Private** location with the **Save** dialog. **Workspaces** files can be loaded with the **Open** dialog.
- The file type **Analysis templates** can be stored and loaded with the **Save** and **Open** dialogs.
- Upon completion of a measurement, **Data files** are automatically stored in the location defined for the user. Click the **Open** button in the toolbar to load MQD data files into the sample list. In addition, MQD data files can be exported as FCS and CSV files.
- The file type **Report files** is stored automatically at the same location defined for the respective data files. **Report files** can be loaded with the **Open** dialog.
- All file types which apply to **Other files** are stored in the **data** folder in the **Public** location and can be copied to an external location with the **Copy** dialog.
- The file type **Log files** is saved automatically in the folder **log** and can be copied to an external location with the **Copy** dialog. This file type is only accessible for administrators.

9 The Administrator

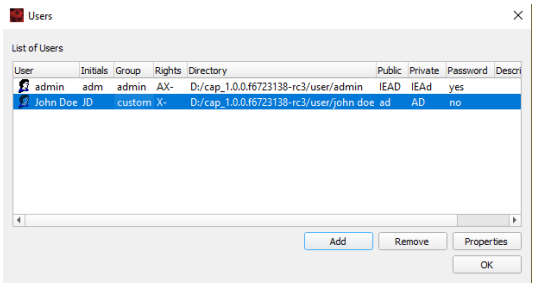
9.1 User management

This section describes user management for standard installation.

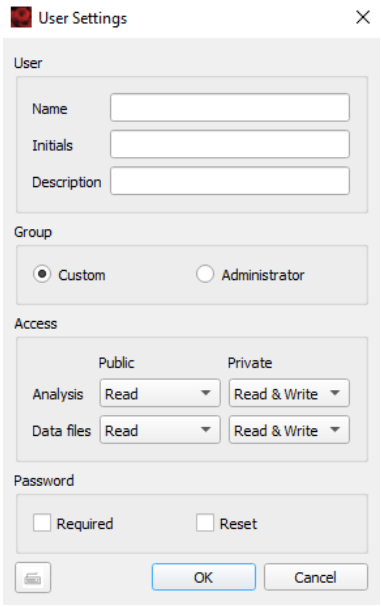
The MACSQuantify Tyto Software allows two types of user accounts with different permission levels: **Administrator** and **Custom mode user**. Administrators and custom mode users can both customize certain software and instrument settings, such as file name, experiment settings, instrument settings, and software settings. An administrator can manage different additional settings.

9.1.1 Creating new user accounts

1. To create a new user account, log in as an administrator.
2. Go to **Edit > User settings**
3. Click **Add** in the dialog box.



4. Enter a name and initials and designate the user to be **Custom** or **Administrator**.



- **Administrator** users can specify global settings for the instrument that apply to all users. Administrator users have the right to manage all user accounts (i.e. create and delete user accounts, set permission levels for each user, reset passwords). You should assign only one administrator per instrument.
- **Custom mode** users have access to software capabilities (except administrator privileges) such as establishing instrument settings.

5 Initials can be designated as part of the data file name and provide an easy identifier for user data files. Make sure that the initials for each user are unique.

6 Set up file access for **Analysis** and **Data files**, which can be saved either to **Public** or **Private**. The **Public** location is a shared location for every user. The **Private** location is individual for each user. In addition, users can save files to the **External** location which includes all external storage devices connected to the MACSQuant Tyto Cell Sorter.

- **Read** – The user can only open files from the specified location, but cannot save files to that location.
- **Read & Write** – The user can open and save files from the specified location.

7 **Analysis files** can be specified as **Read & Write** to both **Public** and **Private** locations at the same time. However, data files can only specify **Read & Write** for only one location, **Public** or **Private**, due to the fact they are saved automatically at the end of a sort process.

8 Click **OK** to complete the user setup.

9.1.2 Deleting user accounts

1 To create a new user account, log in as an administrator.

2 Go to **Edit > User settings**.

3 Select the user to delete from the list and click **Remove**.

4 A warning message will appear informing the administrator that the entire user's **Private** directory including all stored files will be removed.

5 Click **OK**.

9.1.3 Changing access rights of an existing user account

The access type and file access rights of each user can be modified. If you want to assign administrator rights to another user due to changes in your laboratory environment just modify the user.

1 Log in as user with administrator rights.

2 Go to **Edit > User settings** to open the user management tool.

3 Select the user to modify.

4 Click **Properties** or double click the line corresponding to the user.

5 Modify the user's type of access or the user's file access rights.

6 Click **OK** to save the modifications.

9.1.4 Resetting passwords

If a password is forgotten, the administrator can prompt the user to reset his or her password.

1 Log in as an administrator.

2 Go to **Edit > User settings**.

3 Select the user requiring a new password and then click **Properties**.

4 Click the box next to **Reset** at the bottom of the dialog box.

5 The user can log in without a password and set a new password.

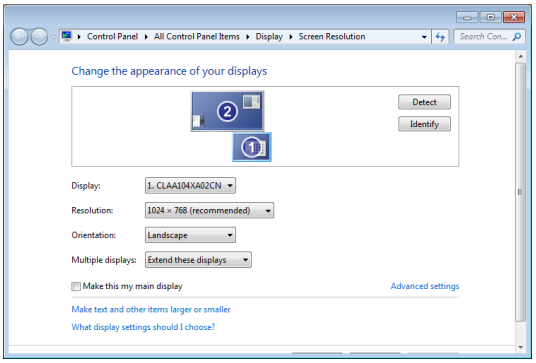
9.2 Hardware setup

In order to maintain a clean and untouched operating system, disconnect the MACSQuant Tyto Cell Sorter from the network connection and reboot the instrument each time before you do any of the following procedures:

- Install an external monitor.
- Calibrate the touchscreen.
- Change the time or date.

9.2.1 Installation of an external monitor

1. Disconnect the MACSQuant Tyto Cell Sorter from the network connection.
2. Connect the second monitor to the VGA or HDMI socket of the instrument.
3. Start the instrument and log in as an administrator.
4. Go to **Tools** > **Display settings** to adjust the display properties.



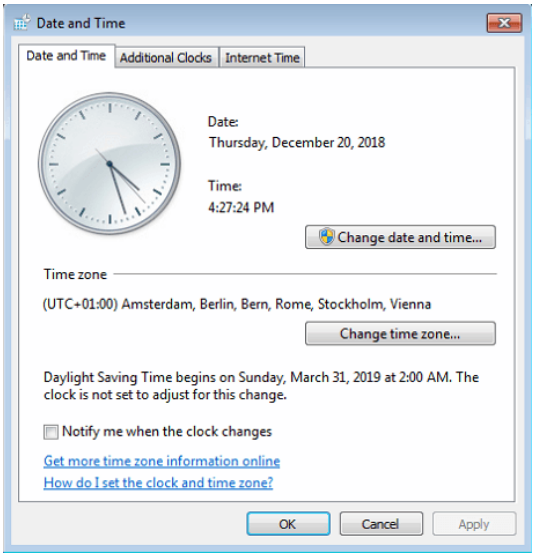
5. Select the 3rd (default) monitor, and check the **Make this my main display** checkbox.
6. Confirm with **Yes** that you would like to keep these settings.
7. Click on **Advanced settings** to configure the monitor.

9.2.2 Setting the time and date

On the instrument, time can only displayed in 24-hour format.

1. Disconnect the MACSQuant Tyto Cell Sorter from the network connection.
2. Start the instrument and log in as an administrator.

3 Click on **Tools > Time and Date** in the side panel of the MACSQuantify Tyto Software.



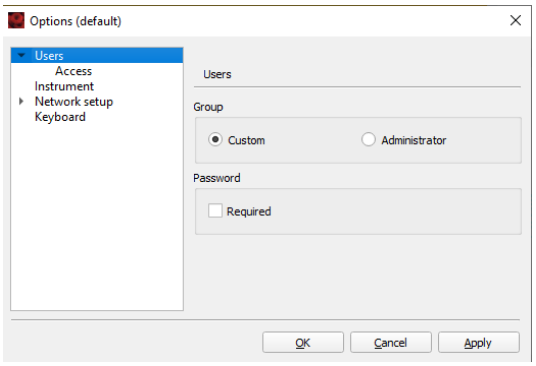
4 Adjust date, time, and time zone.

5 Click **Ok** to save the modifications.

9.3 Global customization options

9.3.1 Users

1 Click **Edit > Options (default) > Users** to adjust user settings for the complete group of either **Express**, **Custom**, or **Administrator** users.



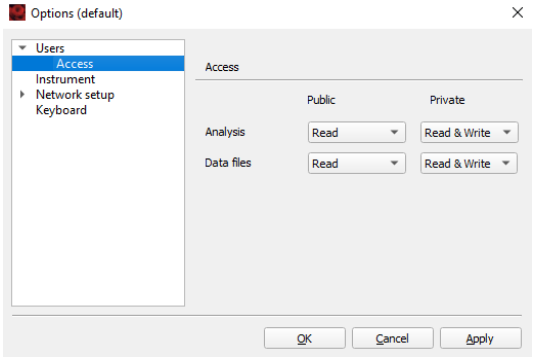
2 Assign a **Group** (**Express**, **Custom**, or **Administrator**) of users by checking the respective option button.

3 To set a password required for all user accounts, check the box next to **Required**.

4 Select **Apply** and click **OK**. Default values will apply for all newly created user accounts.

9.3.2 Access

1 Click **Edit > Options (default) > Users > Access** to assign default access permissions for user settings of your previously selected group of users.



These settings can be changed when creating a new user account.

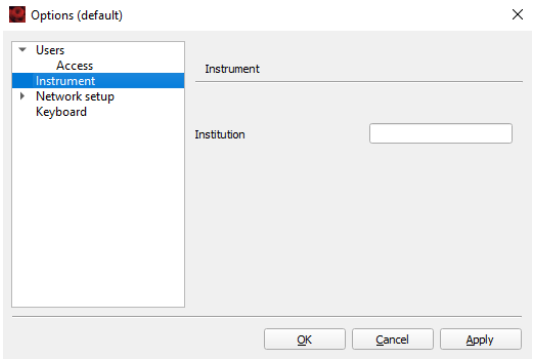
2 Define rights concerning the file management of **Analysis**, and **Data files**. Use the drop-down lists to select the access parameters as desired.

- **None** – Access is not available for the user.
- **Read** – User access is restricted to read files only.
- **Read & Write** – Full user access is available, i.e., read and write data to this folder.

Read & Write can only be selected for one location, either **Private** or **Public**.

9.3.3 Institution name

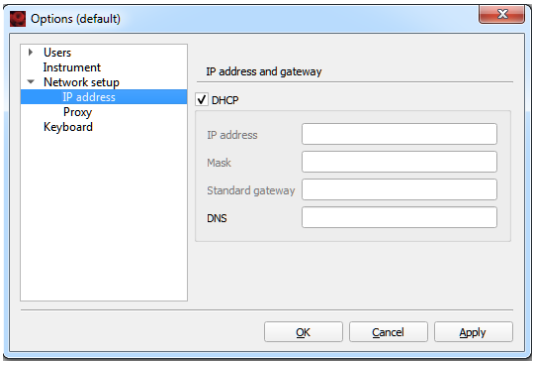
1 Go to **Edit > Options (default) > Instrument** to enter the institution name.



9.3.4 Network

The MACSQuant Tyto Cell Sorter is not intended or designed for integration into a domain infrastructure. The instrument locally manages user rights and permissions as part of the MACSQuantify Tyto Software in order to allow different user rights.

If your DHCP server does not provide the instrument with an IP address, set it manually under **Edit > Options (default) > Network setup > IP address**.



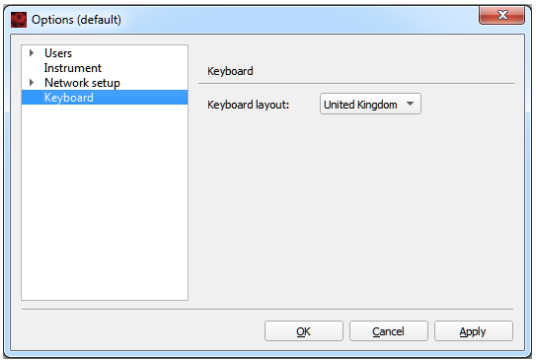
If the MACSQuantify Tyto Software will prompt for your credentials, specify a particular proxy server in order to access to the internet under **Network setup > Proxy**. For further information contact Miltenyi Biotec Technical Support.

The OSI firewall is enabled by default on the MACSQuant Tyto Cell Sorter and should not be disabled. The OSI firewall blocks any incoming network connections with the exception of network diagnostic by the OSI, remote assistance service, and UPnP framework (TCP 2869; UDP 1900). Outgoing network connections are not restricted by the OSI firewall.

It is necessary to set up a network connection if the remote monitoring function will be used.

9.3.5 Keyboard

1 Click **Edit > Options (default) > Keyboard** to access and assign the keyboard settings of the MACSQuant Tyto Cell Sorter and to adjust the keyboard layout.



2 Use the drop-down list to select an appropriate keyboard layout.

3 Click **Apply** to confirm. Click **OK** to close the window.

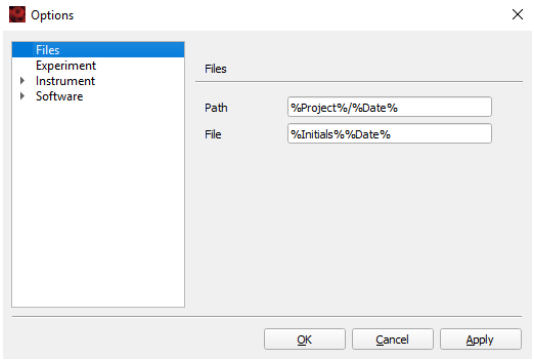
10 Changing default settings

This chapter gives an overview of all default settings and how to modify these.

10.1 Modifying the default file name

The default file name is composed of the initials of the logged-in user, the acquisition date, and an index that counts up beginning with 001. To change the file name, uncheck the box to the right of the file name and overwrite the name. Note that the counter will revert back to 001 as soon as you have changed the file name. The default file name structure can also be changed. Do not use the Windows characters **? / \ < > : * | "** and any character that can be typed using the ctrl key. The characters **. - , µl ( ) &** can be included in the file name. The folder structure under **Path** can also be specified. Take care to keep the forward slash **/** properly placed, i.e., **%Project%/%Date%**.

1 Click **Edit** > **Options** > **Files**.



2 Specify the automatic file name in the **File** text box, adhering to the identifiers listed below.

3 Click **Apply** to confirm your changes. Click **OK** to close the window.

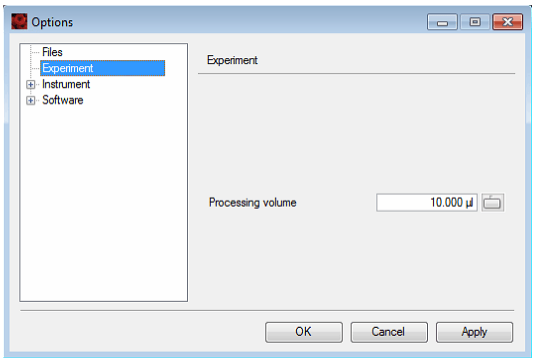
The **File** text box can handle free text and the following case-sensitive commands:

Command	Description
%Initials%	Initials of the currently logged-in user.
%User%	Name of the currently logged-in user.
%Date%	Acquisition date in the format YYYY-MM-DD.
%Description%	User description.
%Project%	Project name.
%Time%	Time in the format hh-mm-ss.
%SerialNo%	Serial number of the instrument.
%SampleID%	Text entered as the sample ID.

Table 10.1: Patterns for automatic file naming.

10.2 Assigning default experiment settings

1 Click **Edit** > **Options** > **Experiment** to access and assign default values for experiment settings. The settings will be displayed in the **Experiment** tab (refer to **The Experiment tab on page 19**).



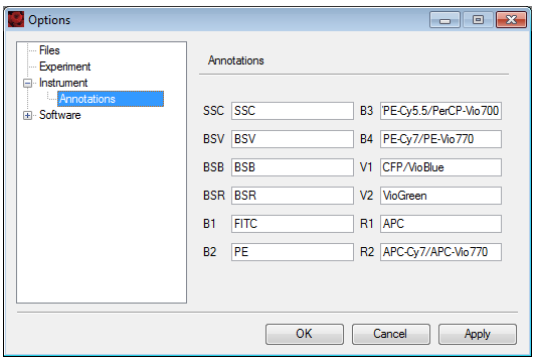
2 Modify the default values for the **Processing volume**.

3 Click **Apply** to confirm your changes. Click **OK** to close the window.

10.3 Optical channel annotations

Annotations provide descriptions of the twelve optical analysis channels that are available on the MACSQuant Tyto Cell Sorter. The default values for the annotations can be changed, which might be desired if other than the displayed fluorochromes are used.

1 Go to **Edit** > **Options** > **Instrument** > **Annotations**.



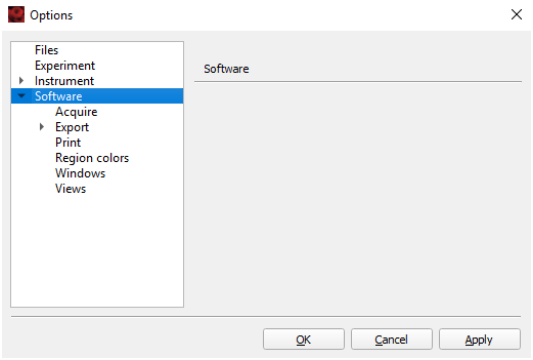
2 Highlight the default name and enter a new name.

3 Click **Apply** to confirm. Click **OK** to close the window. The saved values will be displayed in the **Experiment** tab.

10.4 Adjusting software settings

Under **Software**, you can set software-related properties.

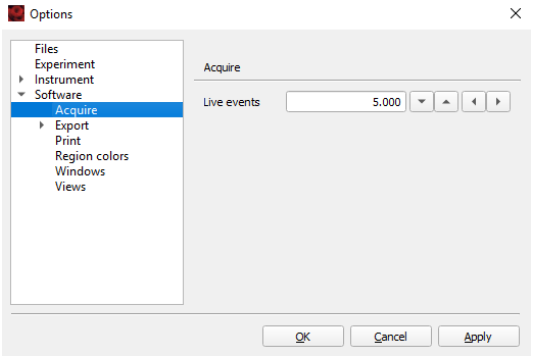
1 Go to **Edit** > **Options** > **Software**.



10.4.1 Acquisition

You can access and assign the settings for the data acquisition of the MACSQuant Tyto Cell Sorter.

1 Go to **Edit** > **Options** > **Software** > **Acquire**.



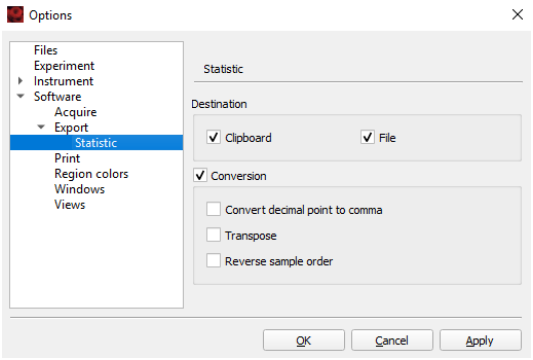
2 Check the according boxes to activate a feature that will be available during data acquisition (live mode).

3 Click **Apply** to confirm your changes. Click **OK** to close the window.

10.4.2 Exporting

Statistic

1 Go to **Edit** > **Options** > **Software** > **Export** > **Statistic** to change the default export settings for sample statistics.



2 Click **Apply** to confirm your changes. Click **OK** to close the window.

Options for data export can be selected by checking one or both boxes under **Destination:**

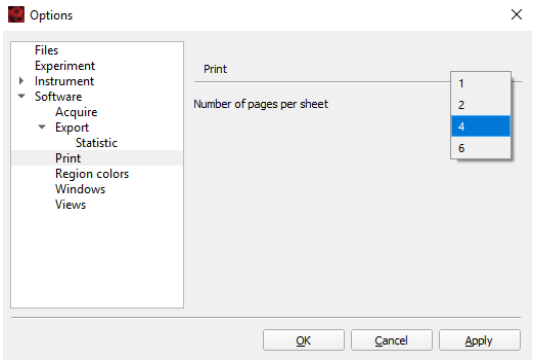
- **Clipboard** – Copy data to the clipboard. Data can be pasted into a spread sheet.
- **File** – Export data to an Excel file.

Options for **Conversion** of data can be selected by checking the according boxes:

- **Convert decimal point to comma** for compliance with your regional language settings.
- **Transpose** to invert rows and columns.
- **Reverse sample order** to sort samples in ascending instead of descending order.

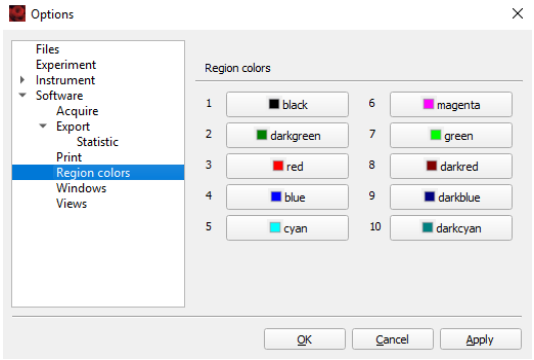
10.4.3 Printing

To change the numbers of analysis pages per sheet, go to **Edit > Options > Software > Print**.



10.4.4 Region colors

1 Default settings for region colors can be modified under **Edit > Options > Software > Regions > Colors**. Region numbers are assigned in ascending order from one to ten.



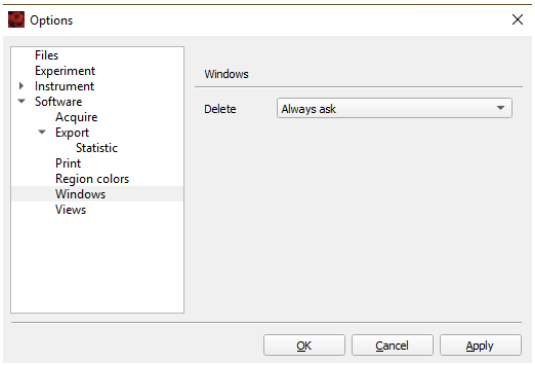
2 Click the **Color panel** button next to the according region number to open a dialog box from which colors can be defined by the user.

3 Click **Apply** to confirm your changes.

4 Click **OK** to close the window.

10.4.5 Windows

1 The default setting for closing dialog boxes (such as the analysis window) can be modified under **Edit > Options > Software > Windows**.



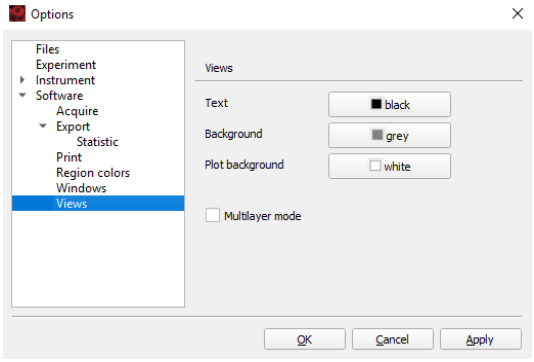
2 Select the desired option from the drop-down list:

- **Always ask** – You will be prompted to confirm closing an analysis window when selecting **Windows > Close** from the menu bar or clicking the **Close window** button from the toolbar. This is the default setting of the MACSQuantify Tyto Softwaree.
- **Never ask** – Analysis windows will be closed without displaying a dialog box.

When selecting **Windows > Close all** from the menu bar, windows will be closed immediately without displaying a dialog box for confirmation.

10.4.6 Views

1 To change default display settings for plots, histograms, and tables, go to **Edit > Options > Software > Views**.



2 Change the font color (**Text**), the workspace color (**Background**), and the **Plot background** color as desired.

3 Check or uncheck the **Multilayer mode** box to enable or disable multilayer mode.

4 Click **Apply** to confirm your changes.

5 Click **OK** to close the window.

11 Technical Support

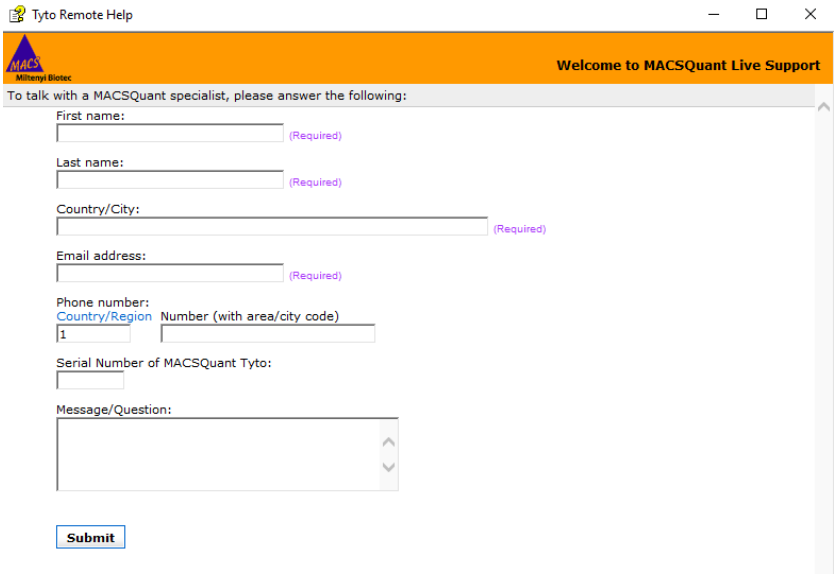
11.1 MACSQuant Live Support

MACSQuant Live Support is a real-time service provided by Miltenyi Biotec Technical Support. Highly trained MACSQuant specialists can be reached to assist with any queries you may have.

The MACSQuant Tyto Cell Sorter must be connected to the internet in order to use the MACSQuant Live Support.

1 Select **MACSQuant live support** from the **Tools** tab in the side panel of the MACSQuantify Tyto Software to access remote assistance.

2 Complete all text boxes and detail any queries you may have using the **Message/Question** box.



3 Click **Submit** to commence MACSQuant Live Support.

The folders **Log files** and **crashrpt** contain system files that include system relevant data that can be important for troubleshooting issues. Go to **File > Copy** to access the **Log files** folder. The **crashrpt** folder can be accessed via Windows® File Explorer and is located at **D > crashrpt**.

11.2 Contact

For technical support, contact your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support at Miltenyi Biotec headquarters:

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Visit **www.miltenyibiotec.com** for local Miltenyi Biotec Technical Support contact information.

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