Miltenyi Biotec

MACSQuantify™ Tyto® Software 3.2

User manual



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MACSQuantify™ Tyto® Software 3.2

User manual

Original instructions

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

⚠ WARNING

Before using the MACSQuantify Tyto Software, read the chapter **Important safety information** in the MACSQuant Tyto Instrument 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.

Supported instruments

This software guide applies to the MACSQuant Tyto Cell Sorter (# 130-103-931) and MACSQuant Tyto Lux (# 130-133-903) running MACSQuantify Tyto Software version 3.2.

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1 Software concept

1.1 User roles

The MACSQuantify Tyto Software defines access to functions by user roles.

To access MACSQuant Tyto Instrument (Tyto) related functions, following preconfigured user roles are available:

Role	Access/function
Tyto Administrator	User of the MACSQuant Tyto Instrument with access to the Tyto User interface and access to additional administrator specific functions
Tyto User	User of the MACSQuant Tyto Instrument with access to the Tyto User interface.

Table 1.1: Tyto related user roles

To access User management (UM) related functions, following preconfigured roles are available:

Role	Access/function
UM Administrator	Role with access to user management related functions
UM User	Role without access to the user management

Table 1.2: User management related user roles

A user with the UM Administrator role can create new user accounts and assign user roles. For an overview about available functions based on the user role, see **Table 10.1** and **Table 10.3**. See **Table 10.4** for setup of customized roles.

Access to functions for administrators, the different user roles, and how to manage user accounts are described in detail in the chapter **Administrator on page 97**.

1.2 File types

This chapter gives an overview about the different file types and their content. The MACSQuantify Tyto Software uses different file types to save acquired data and information about the experiment settings and analysis of samples. See **Table 1.3**. For detailed information on file management, see **Managing files on page 92** and **File storage on page 91**.

File type	Saving options	Saving folder	Access via
Workspace	**public** and **private**	**prj**	**File > Open** **File > Copy**
Analysis template	**public** and **private**	**analyses**	
Data files	public or private	**data**	
Report files	N/a	Same folder as the corresponding data file	
Other files	N/a	**data**	**File > Copy**
Log files	N/a	**log**	
Audit trail reports	N/a	**audit_trail**	
Crash reports	N/a	**coredump**	

Table 1.3: File types and saving options

1.2.1 Workspace file

A workspace represents a file containing information that is also found within other file types. It contains the following information:

- **Experiment** tab: currently shown experiment parameters
- Instrument setting: current instrument setting including compensation and channel settings
- Analysis template: current analyis view

Settings of the **Misc Controls** are not saved in a workspace file.

Opening a workspace reopens the parameters from the **Experiment** tab as well as the instrument settings and analysis template at the point of storing the workspace.

1.2.2 Analysis template file

An analysis template is a predefined layout for acquired data and can be composed of plots, statistics, or tables. It usually includes gating strategies, region names, and statistics. When an analysis template is saved and opened again, it does not contain any data.
Analysis templates can be created during data acquisition. They can also be set up in an analysis window prior to acquisition. The analysis template can be saved for later use. For details see **Setting up a sorting strategy on page 67**.

1.2.3 Data file

MACSQuant data files (.mqd) start automatically saving when a measurement is started. The MQD file contains the acquired data. Furthermore, it contains information about the instrument setting, experiment, and the analysis template which were applied during acquisition.

The analysis template can be retrieved from each MQD file to save the information separately if required. The number of files saved for one run can be set in the 21 CFR Part 11 compliant software setup. See **MQD file limit on page 115**.

1.2.4 Run report file

A run report file is created for each measurement and summarizes experimental data and statistics. 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 are automatically exported as PDF file. Run report files can be opened, copied, and reexported as PDF file. See **Reexporting a run report to PDF on page 87**. Additionally, the real time sort statistics are automatically exported as CSV file. See **Opening the Real time sort statistics on page 87**.

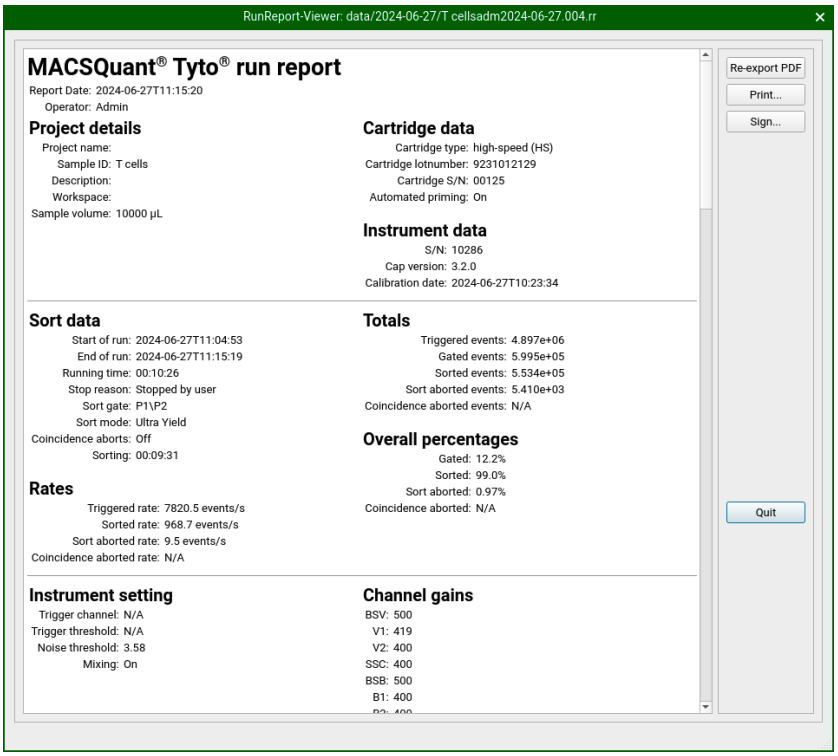


Figure 1.1: Example of a MACSQuant Tyto run report

Run reports of the initial calibration and the automated PMT calibration are automatically saved as calibration report in PDF format. The calibration report includes experiment and instrument data, as well as lot numbers of the used cartridge and calibration beads. It shows a screenshot of the plots and the calibration table.

In the 21 CFR part 11 compliant software setup, run reports can be electronically signed. See **Signing run reports on page 19**.

1.2.5 Other files

Other files contain exported sample lists and calibration table statistics, screenshots, and images made on the MACSQuant Tyto Instrument.

Exported sample lists are saved as XLS file. See **Exporting the sample list to Excel on page 88**.

The calibration table statistics are saved as HTML file. See **Opening the calibration table statistics on page 87**.

Screenshots generated using the print key of the MACSQuant Tyto Instrument's keyboard are saved in PNG format.

Images generated with the **Store page image** and **Store plot image** funciton are saved in PNG format. See **Edit menu on page 28**.

1.2.6 Log files

Log files contain information about actions executed by the MACSQuantify Tyto Software on the MACSQuant Tyto Instrument. Log files are created automatically by the software. There is one log file per day the instrument was started. The log file is named with the date the instrument was switched on.
Log files can be copied from the instrument to a USB flash drive or backup location in a Tyto Administrator account. They are important for troubleshooting and can be sent to Miltenyi Biotec Technical Support.

1.2.7 System audit trail

The system audit trail is available for both, the research use only version and the 21 CFR part 11 compliant software setup. More comprehensive events are tracked in the 21 CFR part 11 compliant software setup. See **Audit trail 21 CFR part 11 on the facing page** for a list of events which are tracked in the 21 CFR part 11 compliant software setup. See **Audit trail on page 121** for a list of events which are tracked in the research use only version. See **Exporting audit trail on page 122** for instructions about the audit trail export.

1.2.8 Crash reports

Crash reports are created if the MACSQuantify Tyto Software closes unexpectedly. Crash reports can be copied by a Tyto Administrator and can be provided to Miltenyi Biotec Technical Support for troubleshooting.

1.3 Compliance with 21 CFR Part 11

To enable compliance according to 21 CFR Part 11, the MACSQuant Tyto Instrument can be connected to the user management system of an institute, such as Microsoft Active Directory or LDAP (Lightweight Directory Access Protocol). Alternatively, 21 CFR Part 11 compliant accounts can be set up locally on the instrument.

Setup of the 21 CFR part 11 compliant software setup requires a service visit by Miltenyi Biotec Instrument Service.

For setup, authentication, and modification of LDAP user accounts, see **Adding a new LDAP user account on page 109**.
For setup, authentication, and modification of local user accounts, see **Adding a new local user account on page 105**.
For information on how to set up an LDAP connection, see **LDAP configuration on the next page**.
For information about user roles and permissions, see **User roles on page 9**.

If the MACSQuantify Tyto Software is installed in a 21 CFR part 11 compliant setup, **(Part 11)** is shown next to the software version in **Help > Info...**.

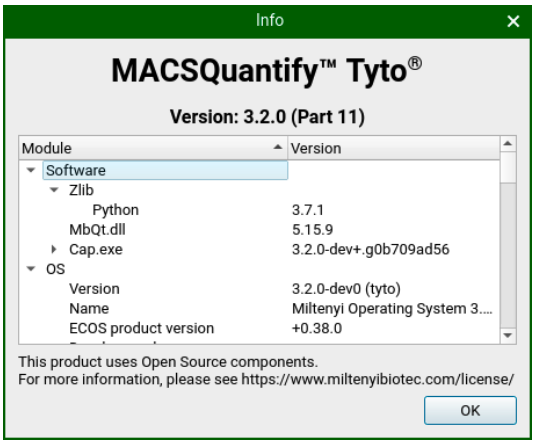


Figure 1.2: Help > Info window of a 21 CFR part 11 compliant setup

1.3.1 Audit trail 21 CFR part 11

The audit trail can be exported by a user with access to functions of the audit trail system, for example, a Tyto Administrator. See **Exporting audit trail on page 122**. The following user activities are tracked in the 21 CFR Part 11 compliant software setup:

Instrument specific activities	User management activities
User sign in (including failed sign ins)	Users added, role assignments
User sign out	Set user inactive/reactivate user
Lock/unlock screen (including failed unlocking)	Change user details
Unlock screen by UM Administrator	Roles added, changed or deleted
Create files	User sign in including failed sign ins/lock user
Errors	Change password
Software crash	Modify password policy including password expiration
Start/finish/pause/resume run	Create LDAP group including role assignments
Start/finish sort	Copy LDAP certificate from USB
Start/finish calibration/initial calibration	
Warmup before calibration	
Start/finish compensation	
Create/sign run report	
Start/stop ventilation	
Backup	
Export system audit trail	
Copy files	
Delete files	
Save files	
Sample export to CSV/ FCS	
Change date and time	
Import cartridge config files	
Move or rename gate during sort	
Automated priming on/off	
Sort mode	
Coincidence abort mode on/ off, coincidence abort rate	

Table 1.4: Tracked activities in the audit trail of the 21 CFR Part 11 compliant software setup

If time settings are changed due to summer and winter time, the events in the audit trail are listed based on the time stamp. This means, that if the time is set back to winter time, the corresponding event of the time change is listed in the audit trail after events that were actually done with the new winter time stamp.

1.3.2 LDAP configuration

Access to LDAP configuration is available in the 21 CFR Part 11 compliant software setup.

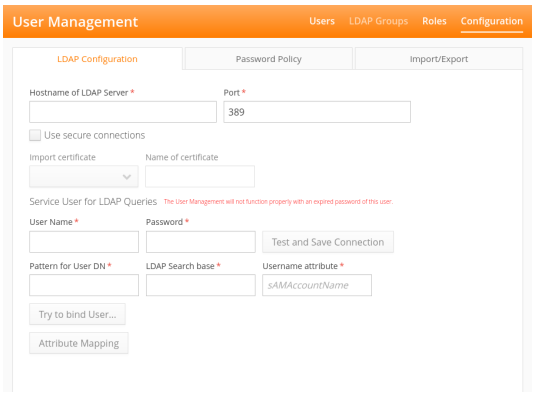
If a secure connection should be used, the certificate must be imported first before starting the LDAP configuration. For certificate import contact Miltenyi Biotec Technical Support

1 Sign in as UM Administrator.

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

3 Go to **Configuration**.

4 Select the **LDAP Configuration** tab.



5 Enter the hostname or IP addess of the LDAP server.

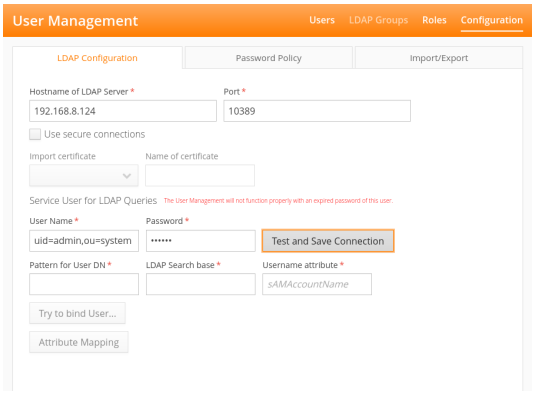
6 Select the checkbox **Use secure connection** to use a secure connection via a LDAPS protocol (recommended). Clear the checkbox to use an LDAP protocol.

7 Enter the port number. Typically, use 636 for secure connections or 389 for insecure connections.

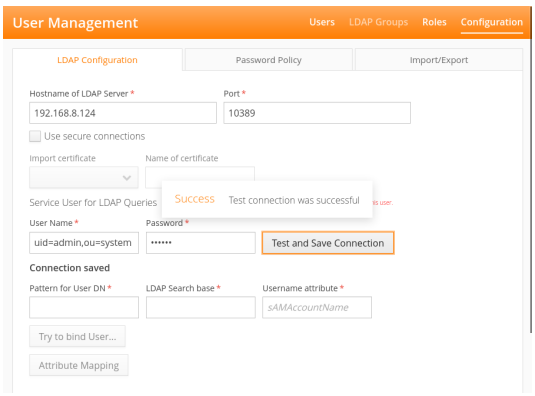
8 Select the certificate for import from the dropdown list.

9 Select **Import**.

10 Enter a user name and password that can be used to send queries to the server. The user name should include the correct domain, for example, Domain_Name\LDAP_TestUser.



11 Select **Test and Save Connection**. If the test was successful, a message appears and **Connection saved** is shown.



12 Go to the **Pattern for User DN** field.

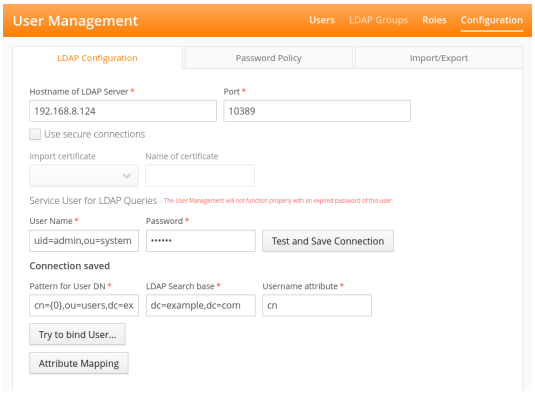
13 Enter a pattern to construct a distinguished name from a user name (i.e. user id) to send the correct credentials as request for authentication to the LDAP system. The sequence '{0}' within the pattern is replaced with the user id for authentication requests.

- For Microsoft Active Directory it is **Domain_Name\{0}**.
- For standard LDAP systems it is **uid={0}, ou=People**.

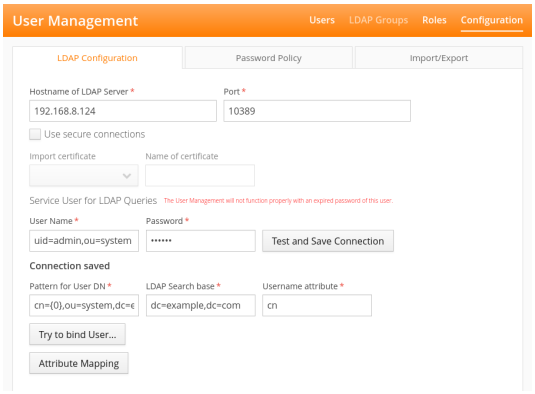
14 Go to the **LDAP Search base** field.

15 Enter the root distinguished name (DN) to use when running queries against the LDAP server. Examples:

- o=example,c=com
- ou=organisation_name,dc=ad,dc=example,dc=com
- For Microsoft Active Directory, specify the base DN in the following format: dc=domain1,dc=local. Replace the domain1 and local for your specific configuration. Microsoft Server provides a tool called ldp.exe which is useful for finding out and configuring the LDAP structure of the server.

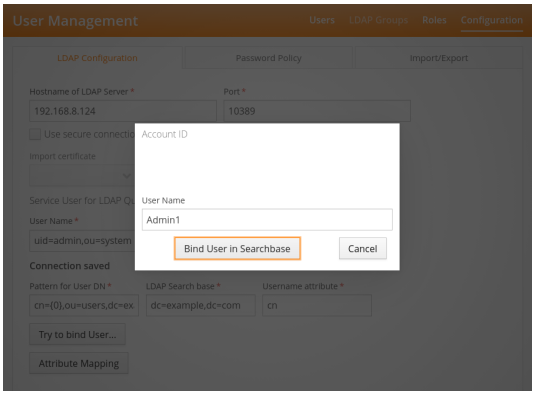


16 Select **Try to bind User...**.

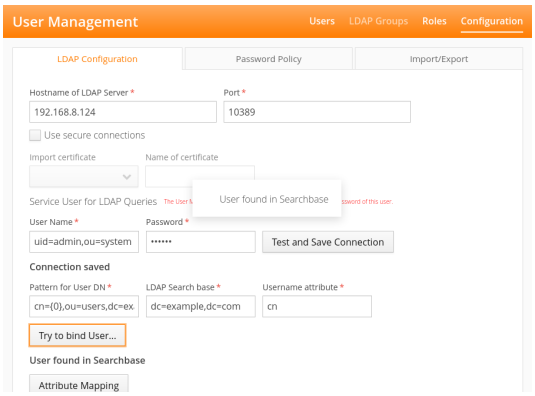


17 Enter the user name of a regular account to test the configuration.

18 Select **Bind User in Searchbase**.



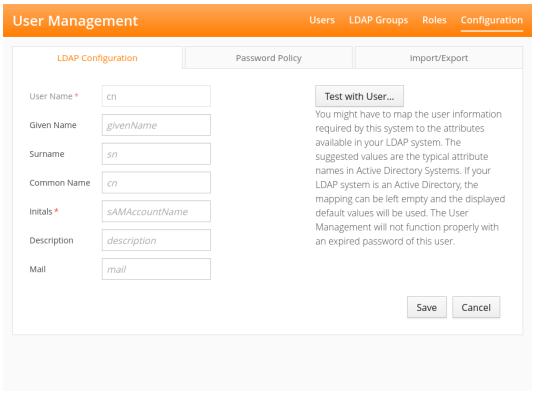
If the user is found in the searchbase, a confirmation message appears.



19 Go to **Username attribute**.

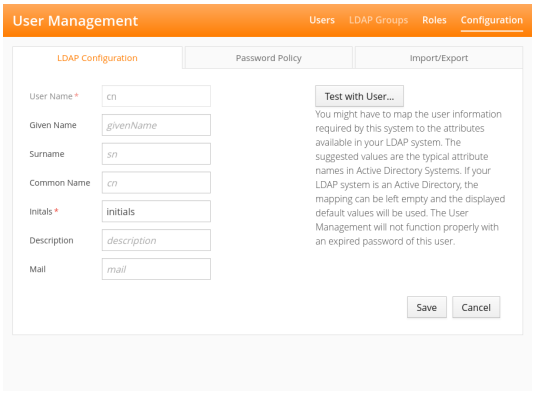
20 Enter the username attribute used by the LDAP system.

21 Select **Attribute Mapping**. A window opens.



22 Enter the information in the fields as shown in the screenshot below, for example, "cn" in the **User name** field.

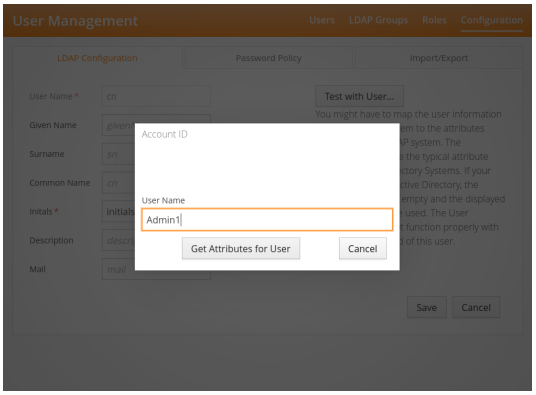
23 Enter "Initials" in the **Initials** field. For a Microsoft Active Directory setup, this must be "sAMAccountName".



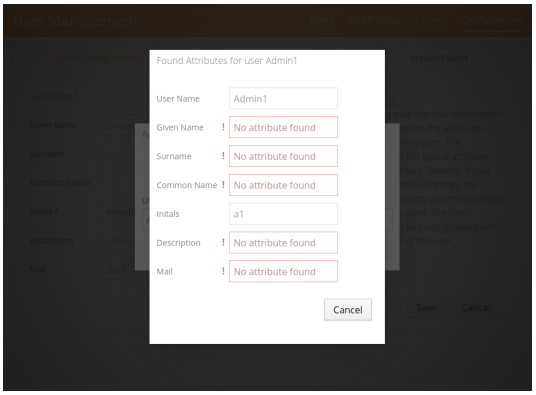
24 Test the configuration with an LDAP user account.

25 Select **Test with User...**.

26 Enter a user name.

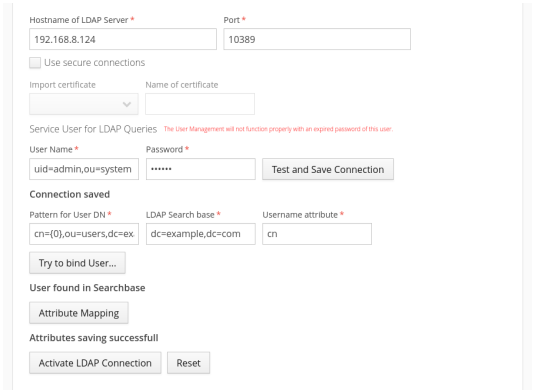


27 Select **Get Attributes for User**. A window opens to show the attributes found for the user.

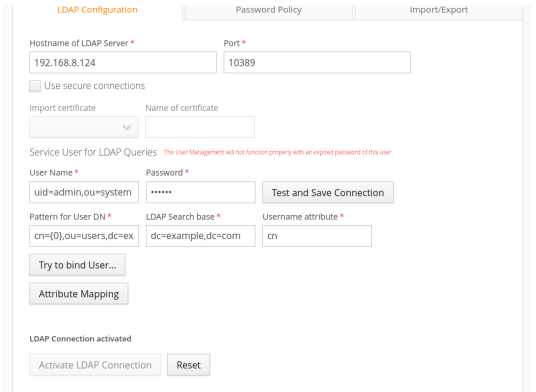


28 Select **Cancel**. The window closes.

29 Click **Save**. Successful attribute mapping is indicated by **Attributes saving successful**.

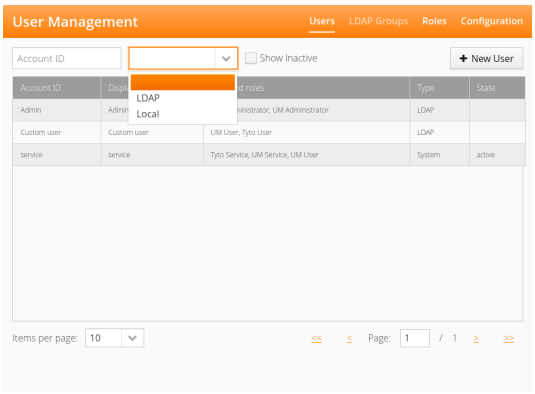


30 Select **Activate LDAP Connection** to save the LDAP configuration.



31 Optional: Select **Reset** to inactivate the LDAP connection.

After activating the LDAP connection, restart the MACSQuant Tyto Instrument. When setting up new users, it is possible to select **LDAP** from the dropdown list. Additionally, the option **LDAP Groups** becomes available. See **Assigning user roles via an LDAP group on page 103**.



The fields **State** and **Display name** remain empty for LDAP users.

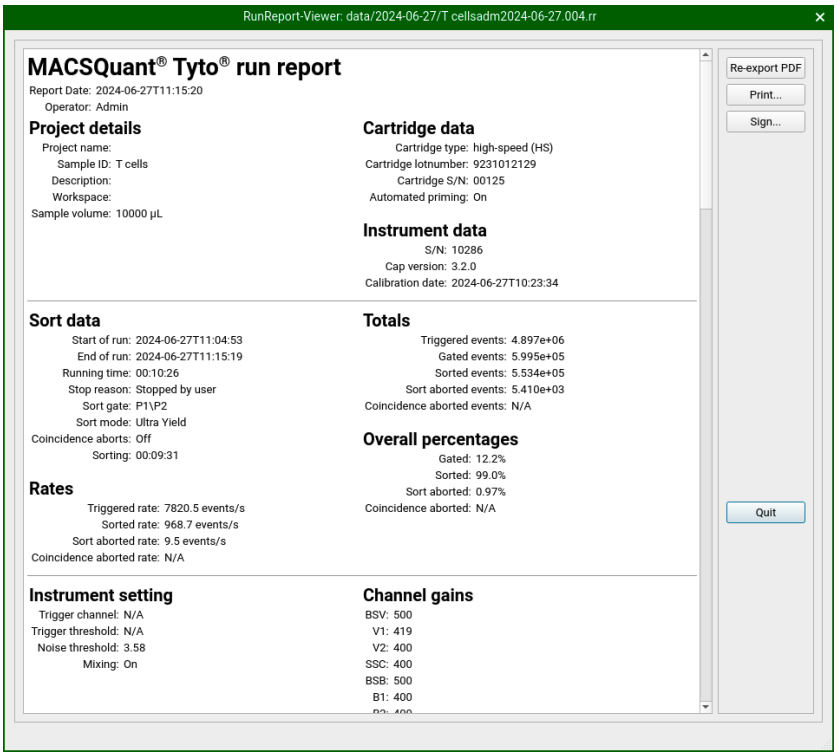
1.3.3 Signing run reports

Run reports can be signed electronically by Tyto Administrators and Custom users in the 21 CFR part 11 compliant software setup. See **Run report file on page 11**.

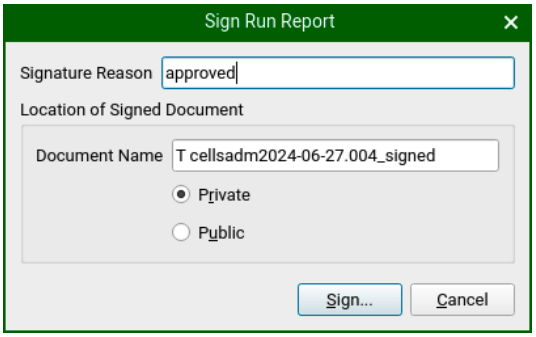
To sign a run report:

1 Open the run report.

2 Click **Sign**.



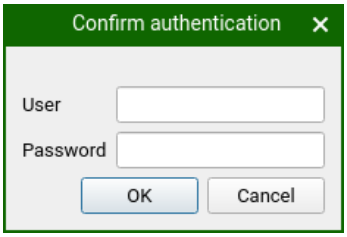
3 Enter a signature reason.



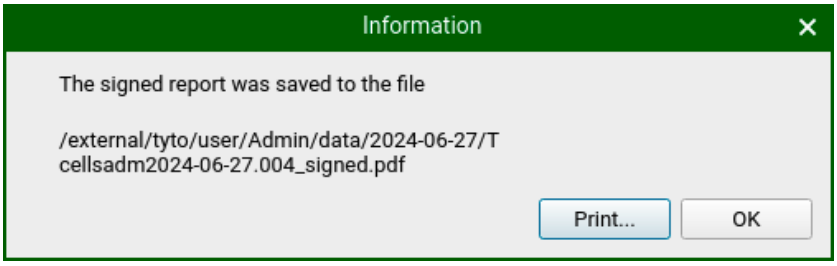
4 Keep the default document name or enter a new name.

5 Click **Sign** to proceed or **Cancel** to abort.

6 Enter your name and password to electronically sign the report.



7 Click **OK** to generate the report. A report in PDF/A format is generated. A pop-up window indicates the location where the run report is saved.



The report includes:

- a front page including the electronic signature



- the current analysis
- a section with meta information about the acquired sample

8 Go to **File > Copy** to copy the signed run report. See **Copying files on page 93**.

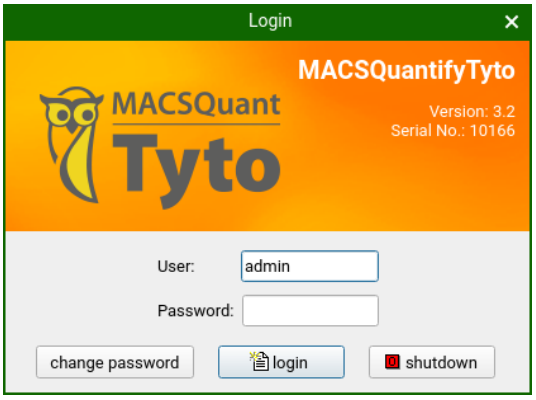
2 Basic functions

2.1 Switching on the instrument

1. Switch on the power switch on the rear side of the instrument to turn the instrument into stand-by mode. A red LED on the touchscreen confirms stand-by mode.
2. Tap the touchscreen to start the MACSQuantify Tyto Software. This can take 1-2 minutes. The **Login** window appears.

2.2 Signing in

1. Enter your user name.
2. Enter your password.
3. Click **login**.

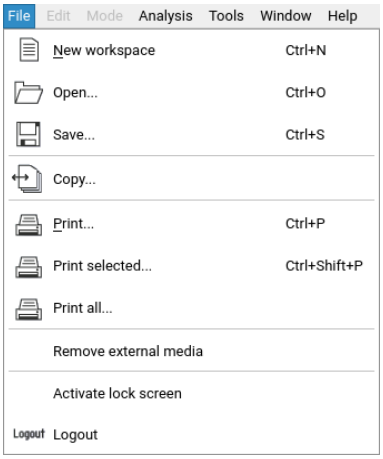


2.3 Locking the screen

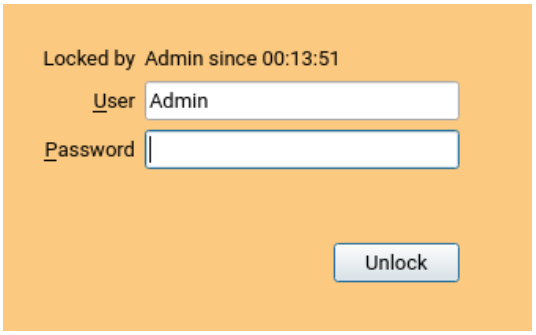
This function is mandatory in the 21 CFR part 11 compliant software setup and optional in the research use only version. The current user can manually lock the screen to prevent other users from accessing the instrument. See **Timers on page 119** to define the idle time before the screen is locked automatically or to deactivate this feature.

1 Go to **File** and select **Activate lock screen**.

The screen is locked.



2 To unlock the screen, enter the password of the user account.



3 Click **Unlock**.

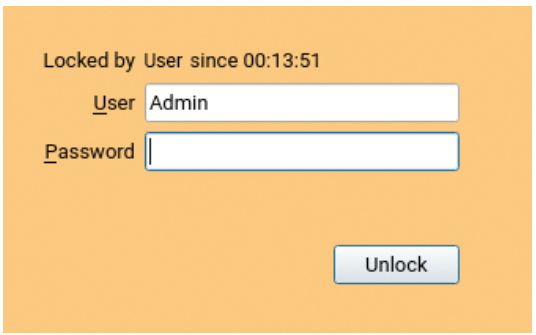
The MACSQuantify Tyto Software is accessible again.

If the password is entered incorrectly, the account is locked depending on the **Number of login attempts** allowed in the user management. To unlock an account, contact the UM Administrator.

2.4 Unlocking the screen by a UM Administrator

If the currently signed-in user is not available for unlocking the screen, the UM Administrator can unlock the screen and access the currently signed-in user account.

1 To unlock the screen, sign in with a UM Administrator account.



2 Click **Unlock**.

2.5 Signing out



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

2.6 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 right side of the toolbar. The MACSQuantify Tyto Software and all processes on the MACSQuant Tyto Instrument shut down. This can take 1-2 minutes. The instrument is now in standby.

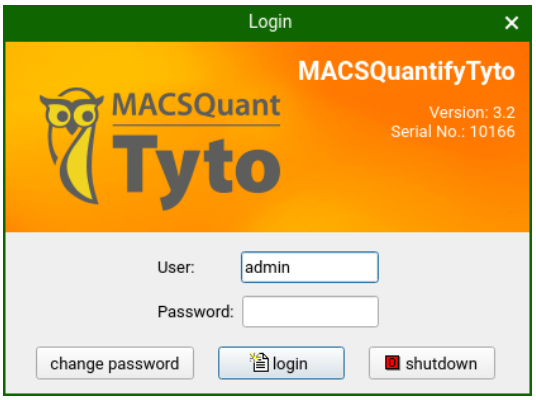
2 Ensure that the MACSQuantify Tyto Software has shut down. Switch off the instrument. The power switch is on the rear side of the instrument.

2.7 Changing the password

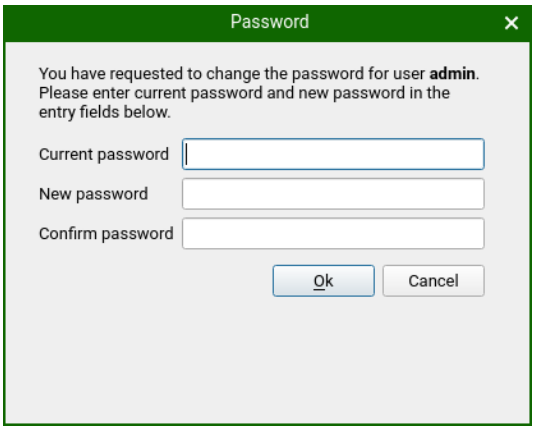
If your instrument is connected to an LDAP system, see **Authentication of LDAP users on page 110**.

1 Enter the user name.

2 Click **change password** on the **Login** screen.



3 A pop-up window opens.



4 Enter the current password.

5 Enter the new password.

6 Confirm the new password.

7 Click **Ok**.

3
User interface

Most functions can be accessed via the menu bar under **File**, **Edit**, **Mode**, **Analysis**, **Tools**, **Window**, and **Help**. See **Menu bar on the next page**.

Frequently used functions can also be accessed directly from the toolbar. See **Toolbar on page 31**.

The currently logged-in user is shown at the far right side of the menu bar. Experiment and instrument settings can be set in the side pane.

The **Real time sort statistics** window opens on the right side of the user interface when the software is started. See **Real time sort statistics window on page 38**.

Via the progress bar, access the **Clear**, **Sort**, **Start/Stop measurement**, and **Pause** buttons. See **Progress bar on page 41**.

The touchscreen shows the **Cartridge** and **Compensation** tab to monitor and set cartridge settings and access and activate the spillover matrix. See **Cartridge tab on page 42** and **Compensation tab on page 46**. The **Cartridge** tab is open per default and shows the **Camera live view** and **Misc controls**.

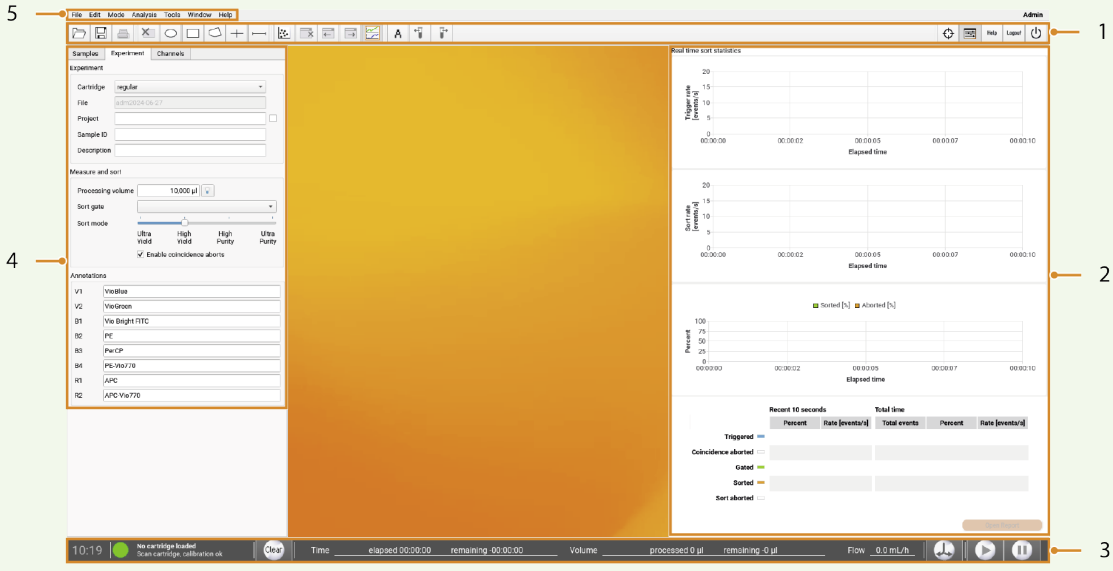


Figure 3.1: The MACSQuantify Tyto Software user interface on the external monitor

1	Toolbar	**4**	Side pane
2	Real time sort statistics window	**5**	Menu bar
3	Progress bar		

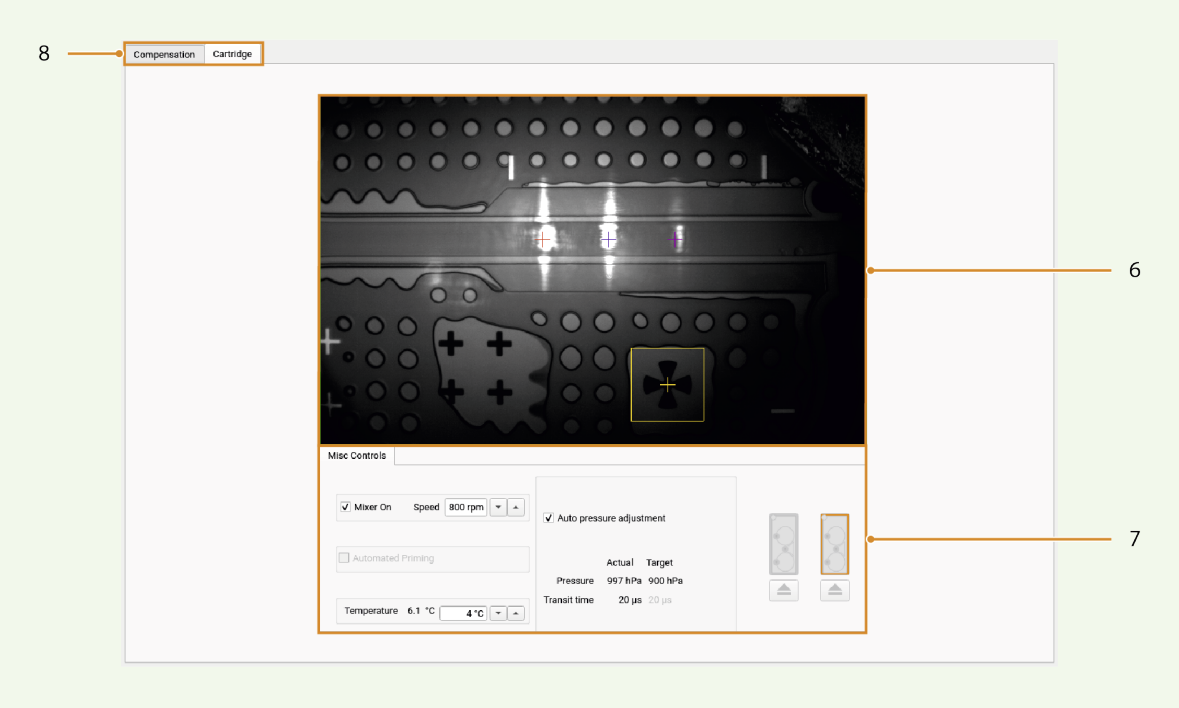


Figure 3.2: The MACSQuantify Tyto Software user interface on the touchscreen during sort

6 Camera live view

7 Misc controls

8 Compensation/Cartridge tab

3.1 Menu bar

The menu bar provides access to the **File**, **Edit**, **Mode**, **Analysis**, **Tools**, **Window**, and **Help** menu. Available options in the menu differ depending on the user role.

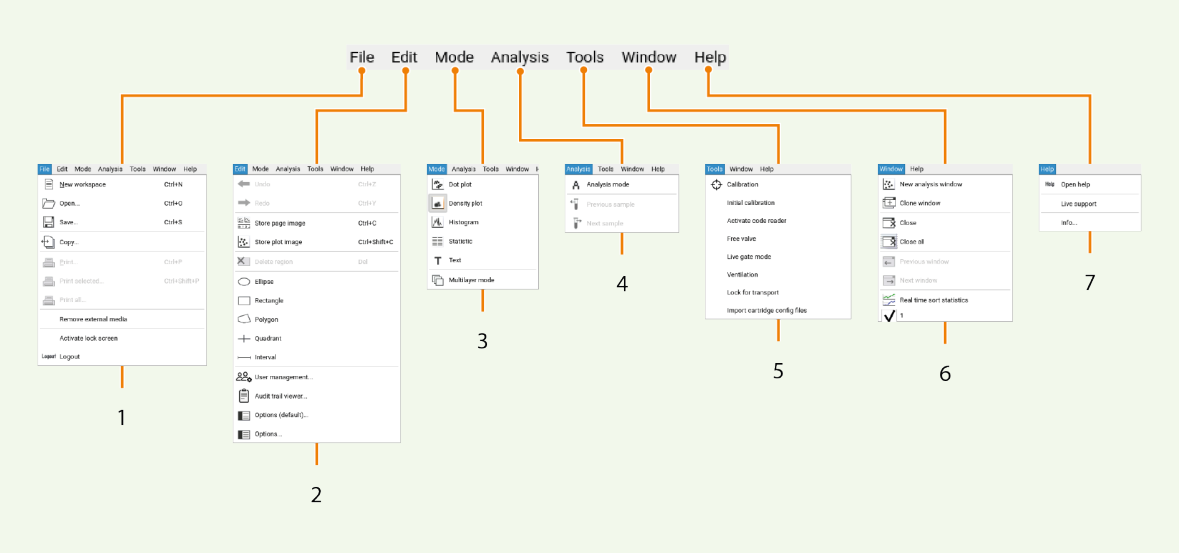


Figure 3.3: The menu bar.

1 File menu

2 Edit menu

3 Mode menu

4 Analysis menu

5 Tools menu

6 Window menu

7 Help menu

3.1.1 File menu

	**New workspace**	Creates a new workspace. The new workspace is initialized with the default instrument settings.
	**Open**	Opens workspaces, analysis templates, data files, or report files.
	**Save**	Saves workspaces or analysis templates.
	**Copy**	Transfers workspaces, analysis templates, data files (MQD or FCS files), report files, other files (PNG or excel files), log files, or crash reports to and from the instrument.
	**Print**	Prints active analysis windows. See **Printing a single data file on page 89**. See **Print on page 128** to change the default number of analysis pages printed per sheet.
	**Print selected**	Prints selected samples in the sample list. See **Printing selected data files on page 89**.
	**Print all**	Prints all samples in the sample list. See **Printing all data files on page 89**.
	Remove external media	Safely ejects removable media from a MACSQuant Tyto Instrument to prevent data loss or corrupted data after writing to a USB flash drive.
	Activate lock screen	Locks the screen manually.
	**Logout**	Logs out of the current user without shutting down the MACSQuantify Tyto Software.

Table 3.1: Functions of the **File** menu

3.1.2 Edit menu

 **Undo**	Cancels the last action.
 **Redo**	Repeats the last action.
 **Store page image**	Save the entire content of the selected analysis page as image in PNG format. Copy the images via the folder **Other files**.
 **Store plot image**	Save a single selected plot as image in PNG format. Copy the images via the folder **Other files**.
 **Delete region**	Deletes a selected region.
 **Ellipse**	Draws a gate of the respective shape. Interval is only available for histograms.See **Gating tools on page 77**.
 **Rectangle**	
 **Polygon**	
 **Quadrant**	
 **Interval**	
 **User management**	Opens the **User management** window. Only available for UM Administrators. See **UM Administrator on page 97**.
 **Audit trail viewer**	Opens the **Audit trail viewer**. See **Audit trail 21 CFR part 11 on page 13**. The complete audit trail is only available in the 21 CFR part 11 compliant software setup. **Audit Trail Management** rights are required to open the audit trail. See **Table 10.4**.
 **Options (default)**	Customizes global settings that apply to all users. Only accessible for Tyto Administrators. See **Editing global software options on page 114**.
 **Options**	Customizes user-specific settings. See **Tyto User on page 125**.

Table 3.2: Functions of the **Edit** menu

3.1.3 Mode menu

Dot plot	Shows data in the selected plot as a dot plot. See **Dot plots and density plots on page 68**.
Density plot	Shows data in the selected plot as a density plot (color-gradient). See **Dot plots and density plots on page 68**.
Histogram	Shows data in the selected plot as a histogram. See **Histograms on page 69**.
Statistic	Shows data in the selected plot in a statistics table. See **Statistics tables on page 69**
Text	Opens a text box. See **Text box on page 70**.
Multilayer mode	Shows data in the selected plot in a multilayer format. See **Multilayer mode display on page 84**.

Table 3.3: Functions of the **Mode** menu

3.1.4 Analysis menu

Analysis mode	Activates (light gray) and deactivates (dark gray) the analysis mode. Deactivate to modify analysis templates and plots. Activated analysis mode automatically deactivates **Edit** and **Mode** in the menu bar.
Previous sample/ Next sample	Browses through the files in the sample list if several sample files are open.

Table 3.4: Functions of the **Analysis** menu

3.1.5 Tools menu

Calibration	Starts the PMT calibration process. See **Photomultiplier tube calibration on page 47**.
Initial calibration	Starts the initial PMT calibration process. See **Initial calibration on page 119**.
Activate code reader	Activates the 2D barcode reader to scan MACSQuant Tyto Calibration Beads.
Free valve	Activates valve movement to unclog the sort valve.
Live gate mode	Activates the **Live gate mode**. Only events within the sort gate are shown. See **Live Gate mode on page 83**.
Ventilation	Starts and stops ventilation of the internal hardware reservoirs during decontamination of the MACSQuant Tyto Instrument by gas. Refer to the chapter Decontamination by vaporous hydrogen peroxide in the **MACSQuant Tyto Instrument 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.
Import cartridge config files	Imports cartridge configuration files from a USB flash drive. See **Importing cartridge configuration files on page 121**.

Table 3.5: Functions of the **Tools** menu

3.1.6 Window menu

 **New analysis window**	Opens a new analysis window using predefined templates. The total number of opened windows and the active window are indicated in the upper left corner of the screen.
 **Clone window**	Creates an exact copy of the analysis window.
 **Close**	Closes an analysis window.
 **Close all**	Closes all currently opened analysis windows.
 **Previous window/Next window**	Browses through open analysis windows.
 **Real time sort statistics window**	Opens and closes the **Real time sort statistics** window. See **Real time sort statistics window on page 38** for more information about the statistics shown.

Table 3.6: Functions of the **Window** menu

3.1.7 Help menu

 **Open help**	Opens the MACSQuantify Tyto Software user manual.
Live support	Opens a dialog box to start a MACSQuant Live Support session. See **MACSQuant Live Support on page 131**.
Info...	Shows information about the currently installed MACSQuantify Tyto Software version.

Table 3.7: Functions of the **Help** menu

3.2 Toolbar

Frequently used functions can be accessed in the toolbar. Select an icon to access a function.

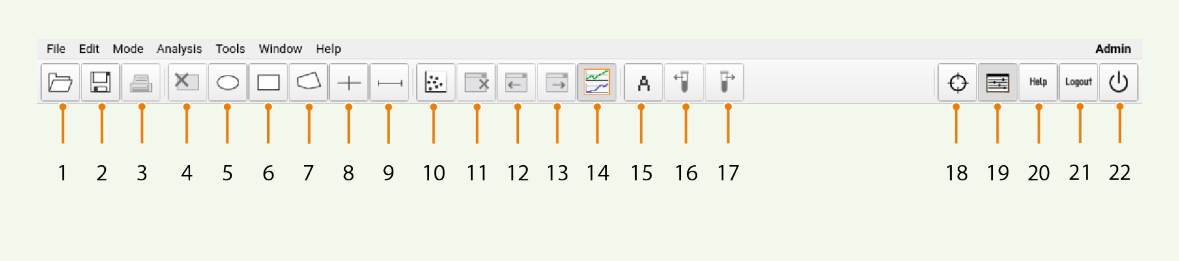


Figure 3.4: Icons in the toolbar.

1	Open	**12**	Previous window
2	Save	**13**	Next window
3	Print	**14**	Real time sort statistics
4	Delete	**15**	Analysis mode
5	Ellipse	**16**	Previous sample
6	Rectangle	**17**	Next sample
7	Polygon	**18**	Calibration
8	Quadrant	**19**	Instrument settings
9	Interval	**20**	Help
10	New analysis window	**21**	Logout
11	Close	**22**	Shutdown

3.2.1 Toolbar functions

Open	Opens workspaces, analysis templates, data files, or report files. See **File storage on page 91** for more information about file types and storage.
Save	Saves workspaces or analysis templates. See **File storage on page 91** for more information about file types and storage.
Print	Prints active analysis windows. See **Printing a single data file on page 89**. See **Print on page 128** to change the default number of analysis pages printed per sheet.
Delete	Deletes a gate. Select an existing gate (edit points will be visible) and click the **Delete** button.
Ellipse **Rectangle** **Polygon** **Quadrant** **Interval**	Draws a gate of the respective shape. Interval is only available for histograms. See **Gating tools on page 77**.

New analysis window	Opens a new analysis window.
Close	Closes an analysis window.
Previous and Next window	Browses through open analysis window pages.
Real time sort statistics window	Opens and closes the **Real time sort statistics** window. See **Real time sort statistics window on page 38** for more information about the statistics shown.
Analysis mode	Activates (light gray) and deactivates (dark gray) the analysis mode. Deactivate to modify analysis templates and plots. Activated analysis mode automatically deactivates **Edit** and **Mode** in the menu bar.
Previous and Next sample	Browses through the files in the sample list if several sample files are open.
Calibration	Starts the PMT calibration process. See **Photomultiplier tube calibration on page 47**.
Instrument settings	Opens the instrument settings.
Help	Opens the MACSQuantify Tyto Software user manual.
Logout	Logs out of the current user without shutting down the MACSQuantify Tyto Software.
Shutdown	Shuts down the software and all processes on the instrument.

Table 3.8: Functions of the toolbar

3.3 Side pane

The side pane has three tabs: **Samples**, **Experiment**, and **Channels**.

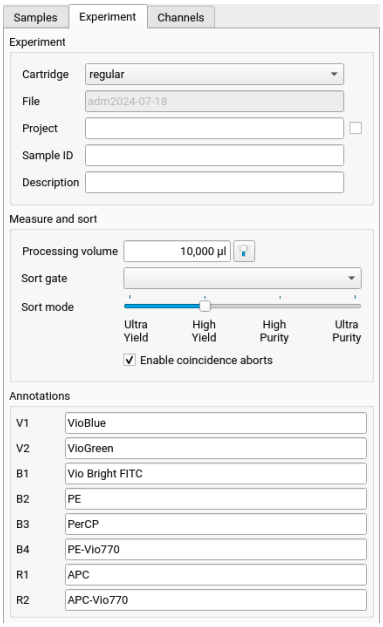


Figure 3.5: The side pane

3.3.1 Samples tab

Go to the **Samples** tab to view a list of opened data files. If a gating hierarchy was applied, this is indicated by a small arrow in front of the sample. Click the arrow to show the gating hierarchy. Further functions can be accessed by right-clicking a sample. See **Sample context menu on the next page**.

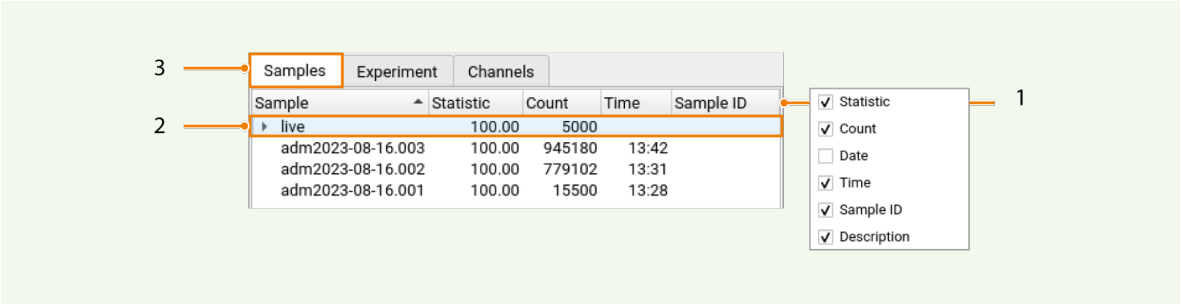


Figure 3.6: The side pane: **Samples** tab options

1 Sample list column parameters

2 Live sample and loaded samples

3 Samples tab

Sample list columns

The sample list contains columns that can be changed from the default appearance. Move the scrollbar at the bottom of the sample list to view all columns. Hover the pointer over a sample to open a pop-up window containing the information of all columns.

Column title	Description
Sample	Name of the data file
Statistic	%-T (percent of total)
Count	Number of total 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

Table 3.9: Columns of the sample list

Customizing the sample list columns

The columns of the sample list can be customized.

Column	Customizing options
Sample order	Click on any column header to use this column for sorting in ascending or descending order.
Column width	Click and hold the line right of a column. Drag the line to the width of choice to change the width.
Column order	Click and hold the column header. Drag the column header to the desired position to change its position. It is recommended that the **Sample** column remains the left most column.
Columns displayed	Right-click a column and select or clear checkboxes in the context menu. Only selected columns are shown.

Table 3.10: Customizing options of the columns of the sample list

Sample context menu

Right-click a sample in the sample list to access the sample context menu. See **Table 3.11** for the **Samples** context menu functions.

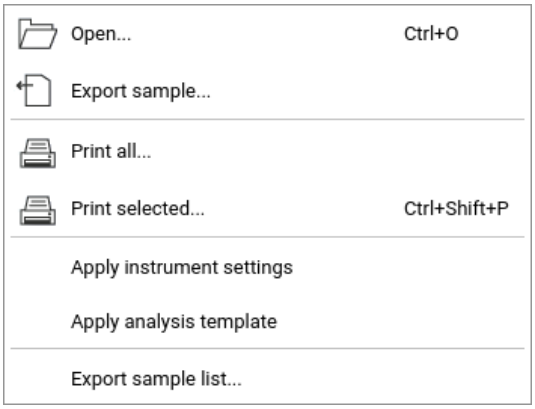


Figure 3.7: Sample context menu after right-clicking a sample

Option	Description
Open…	Opens files such as workspaces, instrument settings, experiments, analysis templates, or data files (MQD). See **Opening files on page 92**.
Export sample …	Exports samples in .csv (comma-separated values) or .fcs (flow cytometry standard) format. See **Exporting FCS files on page 95**.
Print all...	Prints the current analysis for all samples in the sample list. The current analysis template must be applied to all samples in the sample list prior to print (**Analysis Mode** active). See **Printing all data files on page 89**. A printer must be configured to enable printing.
Print selected...	Prints the current analysis for the selected samples in the sample list. The current analysis template must be applied to all selected samples in the sample list prior to print (**Analysis Mode** active). See **Printing selected data files on page 89**. A printer must be configured to enable printing.
Apply instrument settings	Applies the instrument settings that were run with a selected data file. This includes compensation, channel settings, sort mode, automated priming, and coincidence aborts settings. For the MACSQuant Tyto Lux, the laser mode is included as well.
Apply analysis template	Applies the analysis template, which was open during acquisition to the selected sample.
Export sample list ...	Exports the sample list and associated statistics to Microsoft Excel. See **Exporting the sample list to Excel on page 88**.

Table 3.11: Options of the sample context menu

3.3.2 Experiment tab

On this tab, set parameters specific for the experiment.

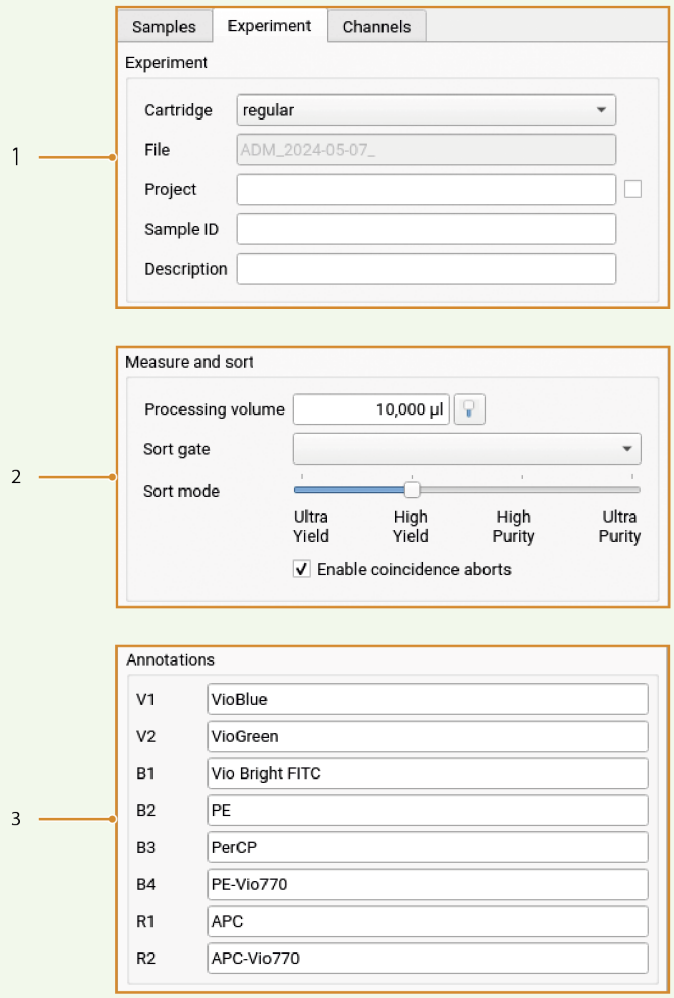


Figure 3.8: The side pane: **Experiment** tab options

1 Experiment

2 Measure and sort

3 Annotations

Experiment

Option	Description
Cartridge	Shows the cartridge type. It is recommended to scan the cartridge to automatically enter information in this text box. Alternatively, select the cartridge type from the dropdown list. If it is unclear which cartridge type is used and whether it is suitable for, e.g., high-speed sorting, see the data sheet of the respective cartridge.
File	Name of the current file. See **Identifiers for file naming on page 125** for file naming options.
Project	Enter the name for a project folder in which files should be saved. Select the checkbox to the right to select an existing project folder from the dropdown list.
Sample ID	Enter an alphanumeric name for the sample. See **Using special characters on page 126** for more information about accepted characters.
Description	Add additional information about the sample.
Laser mode	Only available for the MACSQuant Tyto Lux. Allows to switch on or off the yellow laser. See **Laser mode on page 63**.

Table 3.12: Options in **Experiment**

Measure and sort

Option	Description
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 dropdown list. Events within the sort gate are sorted into the positive selection chamber of the cartridge during the sorting process.
Sort mode	Select a sort mode. See **Sort mode on page 64**.
Enable coincidence aborts	Select the checkbox to enable coincidence aborts. Statistics for coincidence aborted events are shown in the table of the **Real time sort statistics window on the next page**.

Table 3.13: Options in **Measure and sort**

Annotations tab

Annotations of an experiment can be changed in the **Annotations** tab of the **Experiment** tab. See **Annotations on page 65**.

Go to **Edit > Options > Instrument > Annotations** to set the default annotations for the different channels. The detector names in the **Channels** tab are renamed according to the annotations.

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

3.3.3 Channels tab

Open the **Channels** tab to view and modify optical channel settings. See **Adjusting the event detection settings on page 51**.

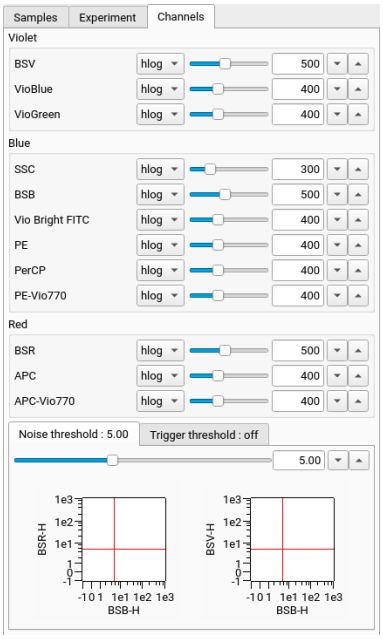


Figure 3.9: The **Channels** tab

Violet/Blue/Red – Adjust scale and gain 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 show events with intensities above the trigger threshold.

When using the **High Purity** or **Ultra Purity** mode keep the trigger values at the minimum. Increasing the trigger values to exclude noise can hide non-target cells from the sorting algorithm. This leads to lower purity.

3.4 Real time sort statistics window

The **Real time sort statistics** window shows different statistics over the course of a measurement after the acquisition was started. Statistics are shown as graphs and tables.



The **Real time sort statistics** window opens automatically 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. Statistics are recorded, even if the window is closed.

The graphs and statistics for a measurement are summarized in a run report file after the measurement is finished. See **Run report file on page 11**. Click **Open Report** at the bottom of the window to open the **Run Report** for the finished measurement.
The **Real time sort statistics** are automatically exported as a CSV file. See **Opening the Real time sort statistics on page 87**.

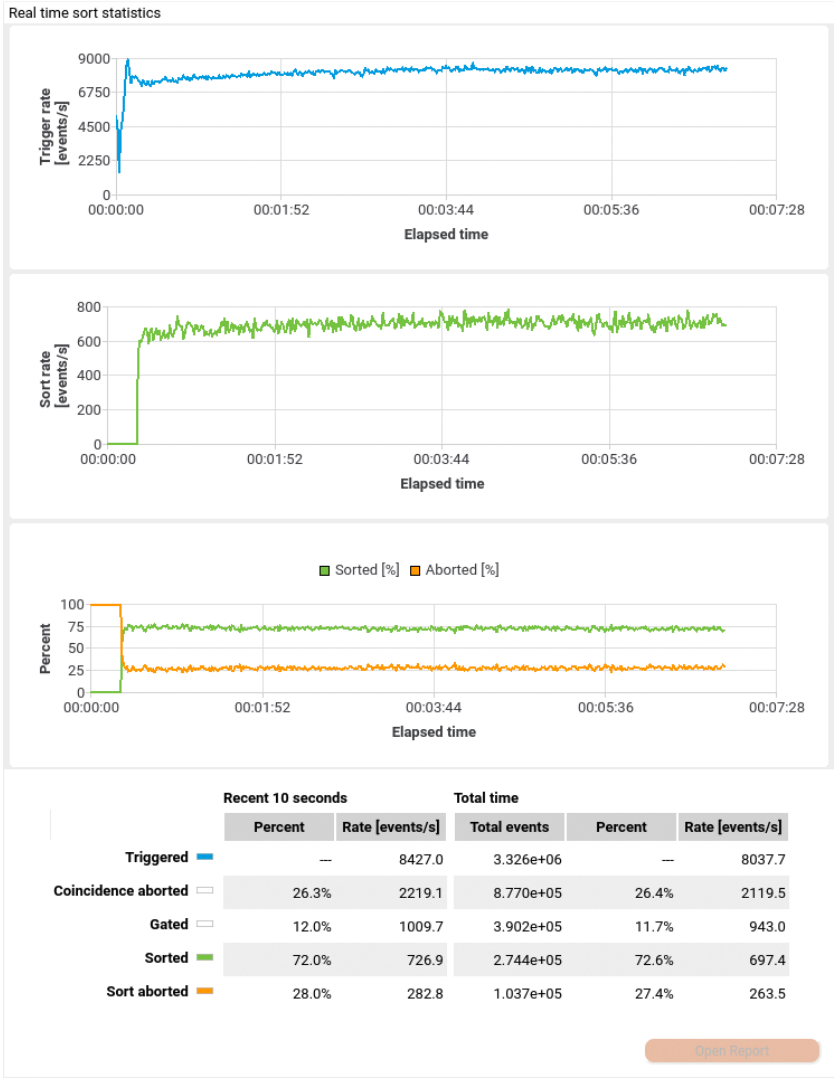


Figure 3.10: Example of **Real time sort statistics** for a sort. Statistics of **Triggered**, **Sorted** and **Aborted** events are shown in graphs and tables. Statistics of **Gated** and **Coincidence aborted** events are only shown in tables.

Graphs

The three graphs depend on the elapsed measurement and sort time on the x-axis.

The first two graphs show event rates per second for the following statistic parameters:

Parameter	Description
Trigger rate	Rate of all triggered events (first graph, blue line)
Sort rate	Rate of target events inside the sort gate which trigger a sort pulse (second graph, green line)

Table 3.14: Statistic parameters shown in the first two graphs of the **Real time sort statistics**

The third graph relates only to events inside the sort gate and shows the following statistic parameters:

Parameter	Description
Sorted	Percentage of events which trigger a sort pulse (green line)
Sort aborted	Percentage of sort aborted events (orange line)

Table 3.15: Statistic parameters shown in the third graph of the **Real time sort statistics**

To zoom in the graphs, draw a square into the respective graph or right-click and select **Rectangular Zoom**. To zoom out, double-click the graph. During the measurement, right-click and select **Auto Scrolling** to zoom out.

Tables

The statistic paramters in the tables are shown for events measured or sorted during the last 10 seconds (table **Recent 10 seconds**, **Figure 3.10**) and for the total measurement and sort duration (table **Total time**, **Figure 3.10**).
Statistics for **Triggered**, **Gated**, and **Coincidence aborted** events are calculated for each measurement and sort. Statistics for **Sorted** and **Sort aborted** events are only available for the sort periods.

The tables show total event numbers, percentages, and event rates for the following statistic parameters:

Parameter	Description
Triggered	All triggered events
Coincidence aborted	Percentage of coincident events out of all triggered events. Coincident events are not shown in the plot. Coincident events in the sort gate are aborted. The coincidence detection algorithm is sort mode dependent.
Gated	Events in the sort gate
Sorted	Events in the sort gate, which trigger a sort pulse
Sort aborted	Events in the sort gate, which are aborted, including coincidence aborted events

Table 3.16: Statistic parameters shown in the table of the **Real time sort statistics**

Events count as **Sort aborted** in case:

- the maximum valve opening time is reached,
- the event is too fast,
- the valve is in recovery time,
- the sort mode algorithm defines an event as abort, e.g., if a non-target and a target event are too close together.

Events count as **Coincidence aborted** in case:

- two events are too close together to be discriminated as single events or
- events appear as doublets.

3.5 Progress bar

The progress 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.

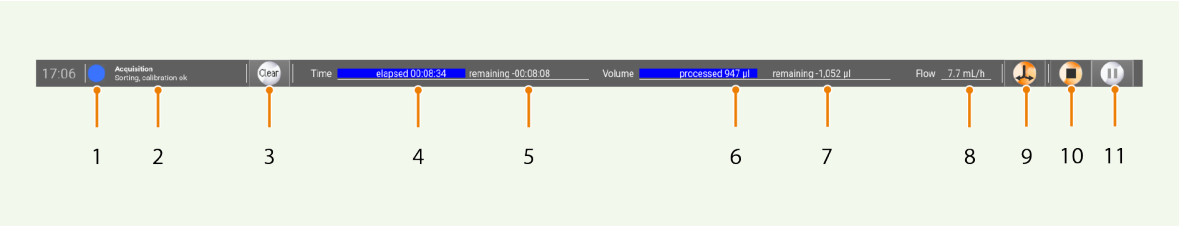


Figure 3.11: Elements of the progress bar

1	Status indicator	**7**	Remaining volume
2	Instrument mode	**8**	Flow rate
3	Clear current measurement	**9**	Sort
4	Elapsed time	**10**	Start/stop measurement
5	Remaining time	**11**	Pause
6	Processed volume		

Option	Description
Status indicator	Shows the color code of the current status of the instrument and changes its color depending on the current status. See **Status indicator on the next page**.
Instrument mode	Shows the current status of the instrument in text format including the calibration state. Calibration state can be ok, due (> 24 h), expired (> 48 h), or not calibrated.
Clear current measurement	Deletes the current data from the dot plots.
Time – elapsed	Shows the elapsed time since start of sorting.
Time – remaining	Shows 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	Shows the estimated remaining volume based on the processing volume entered in the **Experiment** tab.
Flow rate	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. The button may be inactive during certain processes.
Start/stop measurement	Initiates or stops the complete alignment routine and data acquisition of the cartridge and starts or stops the flow of the sample through the microchip.
Pause	Pauses the acquisition. Stops the flow and turns off the lasers. Unlocks the door, but the cartrige is locked in the cartridge holder. The button is inactive during sorting and stays inactive until stopping the measurement.

Table 3.17: Options in **Progress bar**

3.5.1 Status indicator

Status indicator	Status
Green	No cartridge is inserted. Instrument is ready for insertion of a cartridge.
Blue	Cartridge is inserted and recognized. The instrument is processing a sample. The instrument is sorting a sample.
Orange	Measurement paused. Measurement is finished but cartridge is still installed.
Red	Error alert. See the chapter **Troubleshooting** in the **MACSQuant Tyto Instrument user manual** or contact Miltenyi Biotec Technical Support.

Table 3.18: Overview of statuses

3.6 Cartridge tab

The **Cartridge** tab is located on the touchscreen and open per default.

3.6.1 Camera live view

The **Cartridge** tab on the touchscreen shows the camera live view of the sorting mechanism in the cartridge. The camera images are evaluated and coordinates for cartridge alignment are calculated. An overlay image is transfered to the alignment tab. Correct alignment is ensured if the yellow crosshair is centered on the fiducial mark (**Figure 3.12**).

The alignment process is automatic and continously maintained during the sort. If no overlay image is available, a plain gray image is shown. The status of the built-in camera is visualized by a colored frame around the outer camera image:

- Green frame: Camera is connected and overlay images are shown. Cartridge alignment is working.
- Yellow frame: Camera is connected but transfer of the overlay images to the alignment tab is not working. Cartridge alignment is working.
- Red frame: Camera failed to connect and overlay images are not transfered. Cartridge alignment is not working. See the chapter **Troubleshooting** in the **MACSQuant Tyto Instrument user manual**.

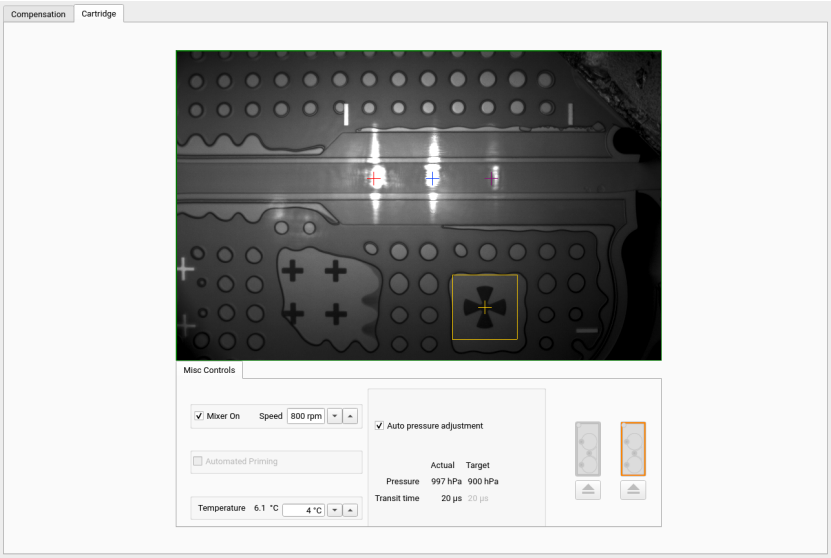


Figure 3.12: Overlay image showing alignment of the MACSQuant Tyto Cartridge.

3.6.2 Misc Controls

The **Misc Controls** tab shows the current mixer speed in the cartridge, automated priming, the chamber temperature, flow control parameters, and the Cartridge status box.

Mixer

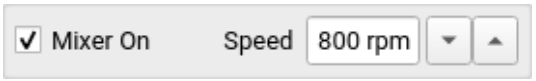
The mixer of the cartridge keeps the cells in the sample in suspension. If needed, adjust the propeller parameters of the cartridge in the mixer section of the side pane.

The mixer settings do not revert to default upon opening a new workspace. Set the mixer settings before each run.

1 In the mixer section of the **Misc Controls** tab, enter a number between 100 and 800 rpm (step size of 10)

2 Press enter to apply.

3 To initiate or stop rotation of the propeller, select or clear the **Mixer On** checkbox.

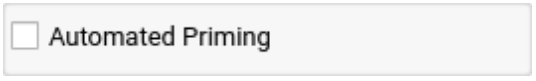


The preset mixer speed of 800 rpm is recommended for most cell types.

Automated priming

The automated priming feature enables automated cartridge priming on the instrument. During the priming process sample buffer and target cells are sorted to the positive collection chamber. Sort counts during the priming process are taken into account in the sort statistics. The automated priming can be activated as default. See **Experiment on page 126**.

1 To activate automated priming, select the checkbox.



2 Insert the cartridge containing the sample and click the **Start measurement** button in the progress bar.

3 To start the priming process, Click the **Sort** button .

The priming process takes approximately one minute and is indicated in the instrument mode section of the progress bar. See **Progress bar on page 41**.

The automated priming can be deactivated during the priming process. Upon reactivating the automated priming, the instrument will only prime for the remaining time of the initial priming process.

4 After priming the instrument automatically proceeds with sample sorting.

Flow control

The flow control section of the **Misc Controls** tab shows and controls the parameters of the sample flow rate through the cartridge (**Figure 3.13**).

The flow control settings do not revert to default upon opening a new workspace. Set the flow control settings before each run.

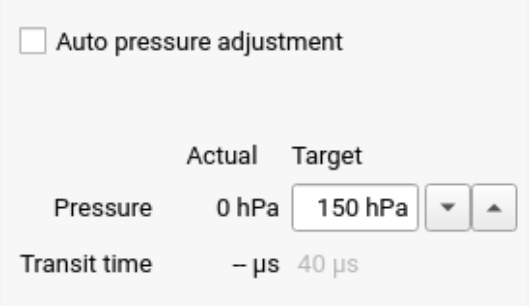


Figure 3.13: The flow control section of the **Misc Controls** tab

The flow control section shows:

- the **Actual pressure** applied to the input chamber of the cartridge,
- the set **Target pressure**,
- the **Actual transit time** of triggered events, and
- the **Target transit time** depending on the cartridge type used.

After starting a measurement, the system applies constant pressure according to the cartridge type used. If needed, the pressure can be adjusted manually, for example in case of clogging. To adjust the target pressure, perform one of the following:

- Double-click inside the value box. Enter 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 **Auto pressure adjustment** is activated after starting a sort. The MACSQuant Tyto Instrument 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. Clear the **Auto pressure adjustment** checkbox and adjust the value as desired. This is only recommended when no or unexpected events are observed in the **Real time sort statistics** window during a sort.

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 **Auto pressure adjustment**. Different parameters, for example, cell morphology (size, surface, structure) or flow resistance of the cartridge, affect the transit time.

Chamber temperature

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) in the chamber section of the side pane.



Figure 3.14: The chamber section in the **Experiment** tab

1 Double-click inside the value box. Enter the desired value.
2 Press enter to apply.

The target temperature does not revert to default upon opening a new workspace. Set the target temperature before each run.

Cartridge status box

The **Cartridge status box** shows the cartridge status and the **Eject** button. The cartridge is ejected automatically after an acquisition or sort was stopped manually or automatically.

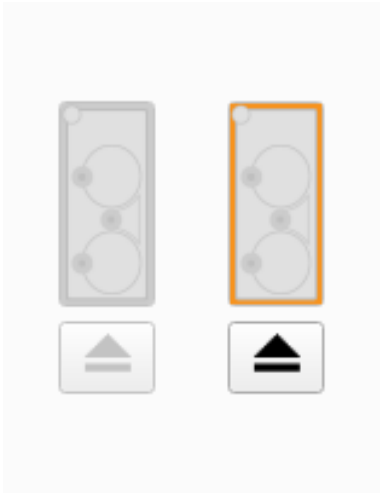
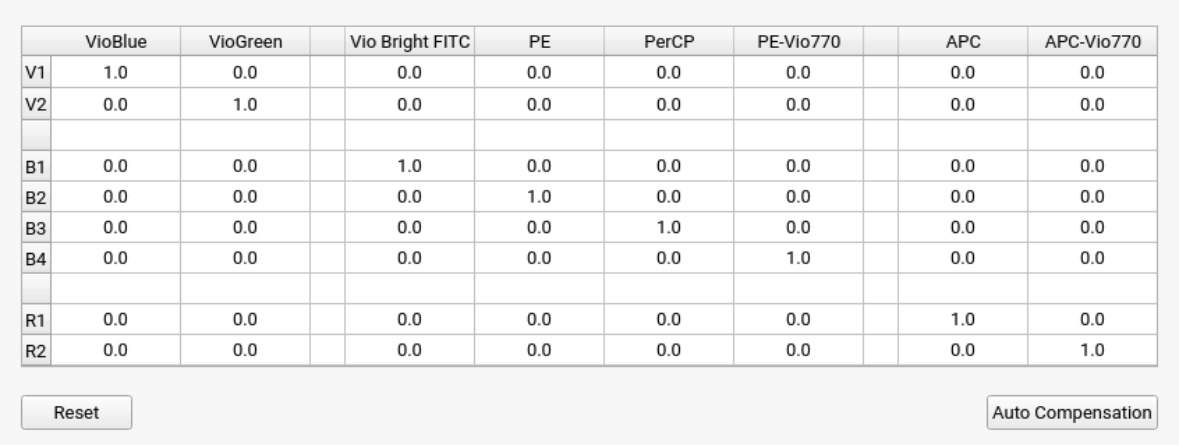


Figure 3.15: The **Cartridge status box**

3.7 Compensation tab

The **Compensation** tab is located on the touchscreen. It shows the spillover matrix, which organizes the detection of individual fluorescence signals in columns. The matrix values can be adjusted to compensate the specific channel. See **Spillover matrix on page 59**. To show the spillover matrix (**Figure 3.16**), click the **Compensation** tab on the touchscreen and then select the **Activate compensation** checkbox.



	VioBlue	VioGreen		Vio Bright FITC	PE	PerCP	PE-Vio770		APC	APC-Vio770
V1	1.0	0.0		0.0	0.0	0.0	0.0		0.0	0.0
V2	0.0	1.0		0.0	0.0	0.0	0.0		0.0	0.0
B1	0.0	0.0		1.0	0.0	0.0	0.0		0.0	0.0
B2	0.0	0.0		0.0	1.0	0.0	0.0		0.0	0.0
B3	0.0	0.0		0.0	0.0	1.0	0.0		0.0	0.0
B4	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

Figure 3.16: Compensation matrix on the touchscreen

4 Setting up the instrument

This chapter explains the steps 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:

- Calibrate the photomultiplier tubes (PMTs).
- Adjust backscatter, side scatter, and fluorescence PMT gains.
- Set a noise threshold and optional trigger threshold to exclude unwanted signals. Consider the sort mode when settings the thresholds. See **Sort mode on page 64**.
- Perform automated or manual compensation

All settings and adjustments are stored in the workspace file. See **Creating a workspace on page 85**.

4.1 Photomultiplier tube calibration

The reproducibility and stability of the fluorescence signal over time is of vital importance. To ensure a stable measurement that is independent of time, the instrument needs to be calibrated. Calibrate the MACSQuant Tyto Instrument using the MACSQuant Tyto Calibration Beads (# 130-122-730). A daily calibration compensates for potential differences in laser performance or PMT sensitivity by adjusting the gains accordingly.
The MACSQuant Tyto Calibration Beads contain blank beads, bright beads, and three populations of single-stained fluorescent beads yielding intermediate signals in the target channels. The beads serve as a reference to establish predetermined, lot-specific median fluorescence intensity (MFI) values for each channel. This is achieved by adjusting the voltages on each of the PMTs for the appropriate channel during the automated PMT calibration process.

It is recommended to calibrate the instrument at the beginning of every day the instrument is used.

For information about initial calibration, see **Initial calibration on page 119**.

4.1.1 Smart Gain technology

The MACSQuant Tyto Instrument uses PMTs to detect scatter and fluorescent signals.
The external photoelectric effect is the underlying principle of PMT signal detection. When external light hits a photocathode material in a vacuum tube, photons produce electrons that are accelerated to hit a downstream array of dynodes. The dynodes serve as a high–grade amplifier. Finally, the secondary electrons are detected by the PMT anode. An output signal with improved sensitivity and resolution is produced.
Typically, PMTs are powered by high voltage that determines the signal amplification factor. To achieve

optimal signal detection in each channel, application-dependent and user adjustable instrument settings need to be defined. This ensures that too low or too high PMT voltages are avoided. Low voltages can result in dim signals or in signals outside the linear detection range. High voltages can lead to bright signals or in signals outside the linear detection range.

The MACSQuantify Tyto Software version 3.0 or higher provides the Smart Gain technology. Smart Gains allow intra- and inter-instrument standardization of multicolor cell sorting. Based on known median fluorescence intensity of calibration beads, the gain of each detection channel is normalized. The Smart Gain technology assigns a gain value of 400 to each fluorescent channel during calibration once the known median fluorescence intensity of the calibration beads is reached. This standardized gain value is saved in the default instrument setting shown in the **Channels** tab. See **Automated PMT calibration on the facing page**. The Smart Gain technology is not applied to the scatter channels (backscatter and side scatter).
The default Smart Gains can be adjusted for each application and saved in a new workspace. See **Adjusting the event detection settings on page 51**. For multicolor applications, compensation of spectral overlap must be created and saved as part of an application specific workspace. See **Compensating spectral overlap on page 55**.

Smart Gains enable

- performance over time for longitudinal experiments
- data reproducibility of intra- and inter-instrument assays

Smart Gains allow the extended use of workspaces on the same MACSQuant Tyto Instrument. It also allows to share instrument settings between MACSQuant Tyto Instruments with the same optical bench configuration.

Each time the automated calibration process is performed and the actual PMT voltages are adjusted to maintain the predefined median values, the Smart Gains of the fluorescent channels are normalized to 400. After the automated PMT calibration, the gains can be adjusted for a specific application. If the application specific workspace is transferred to another MACSQuant Tyto Instrument with the same optical bench configuration, then the desired data display for the fluorescent channels is maintained and results can be compared between instruments. The scatter channels need to be set individually on the instrument.
Refer **Table 4.1** for implementation of Smart Gains.

On the same MACSQuant Tyto Instrument	Across MACSQuant Tyto Instrument
Perform PMT calibration	Perform PMT calibration on MACSQuant Tyto Instrument A and B
Optimize gains of required channels for application	Optimize gains of required channels for application on MACSQuant Tyto Instrument A
Perform compensation	Save workspace
Save workspace	Transfer workspace to MACSQuant Tyto Instrument B
Re-use workspace	Set the scatter gains and perform compensation on MACSQuant Tyto Instrument A and B
	Save workspace
	Re-use workspace

Table 4.1: Implementation of Smart Gains for longitudinal studies

4.1.2 Automated PMT calibration

1 Switch on the MACSQuant Tyto Instrument. Wait at least 30 minutes to warm-up the optical bench before performing the automated PMT calibration.

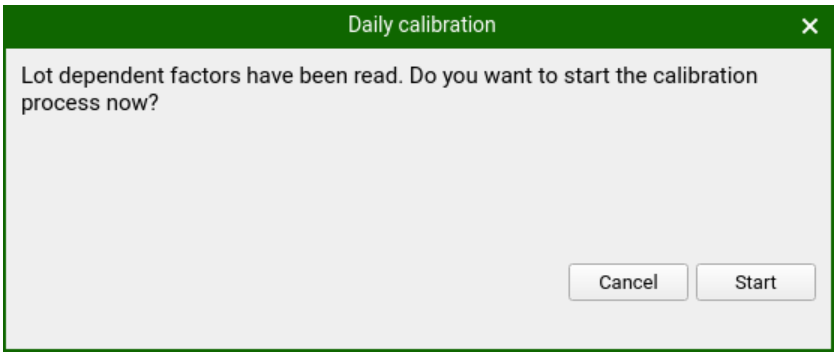
The warm-up time is tracked by the MACSQuant Tyto Instrument. If calibration is started before a period of 30 minutes, a pop-up window indicates the warm-up time. It is documented in the **MACSQuant Tyto calibration report** and in the audit trail if the PMT calibration was performed before a warm-up period of 30 minutes.

2 Vortex the MACSQuant Tyto Calibration Beads for 10 seconds to break up aggregates.

3 Add 3 drops of MACSQuant Tyto Calibration Beads to 1.5 mL MACSQuant Tyto Running Buffer and vortex. Fill the diluted MACSQuant Tyto Calibration Beads into the cartridge.

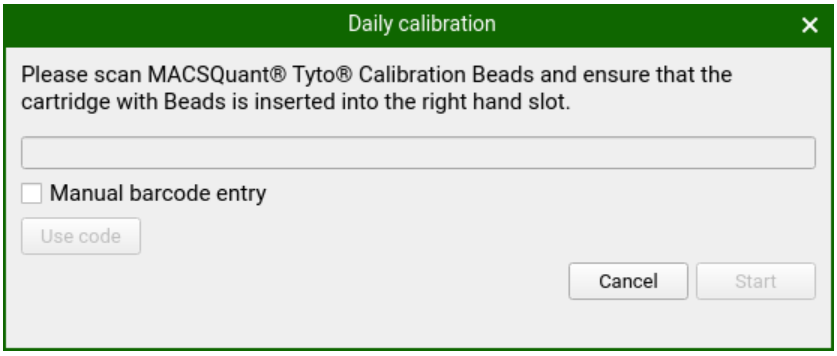
4 Scan and insert the cartridge.

5 Scan the barcode of the MACSQuant Tyto Calibration Beads.





6 Optional: If scanning of MACSQuant Tyto Calibration Beads is not possible, click the **Calibration** button in the toolbar. Select **Manual barcode entry** to enter the barcode of the MACSQuant Tyto Calibration Beads manually.



7 Click **Start** to start the calibration. The gain for each channel is automatically adjusted during calibration.

8 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary. See **Figure 4.1** as an example of a successful calibration.

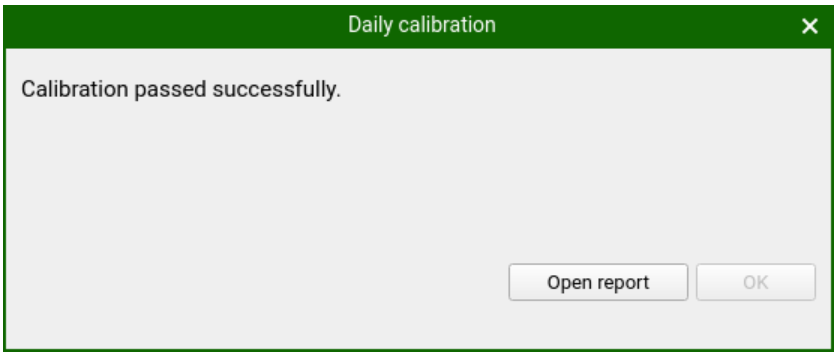
9 Successful calibration is indicated for each fluorescent channel by the column **Info** giving the status **passed**. Furthermore, the table header of the calibration analysis page shows **Calibration: passed** and the instrument progress bar reports **calibration ok**. See **Table 4.2** for details about the shown parameter. The calibration table is automatically saved as HTML file. To export the calibration table, see **Opening the calibration table statistics on page 87**.

2024-08-23 Calibration: passed

Procedure Version: 1.1.0

Channel	Gain	V	ΔV (last)	ΔV (init)	Stain Index	CV	Info
BSV	500	500	—	—	—	—	n/a
V1	400	388	-4	-4	271.9	14	passed
V2	400	438	-7	-7	792.0	15	passed
SSC	300	300	—	—	—	—	n/a
BSB	500	500	—	—	—	—	n/a
B1	400	482	-5	-5	710.8	9	passed
B2	400	538	-6	-6	1638.7	9	passed
B3	400	565	-6	-6	357.1	9	passed
B4	400	544	-6	-6	23.6	11	passed
BSR	500	500	—	—	—	—	n/a
R1	400	496	-2	-2	2050.0	15	passed
R2	400	542	-8	-8	215.1	16	passed

10 Click **Open report** to open the **MACSQuant Tyto calibration report**. The calibration report is automatically saved as PDF file.



Gains and threshold of the PMT calibration are loaded as a default instrument setting when a new workspace is generated via **File > New workspace**.

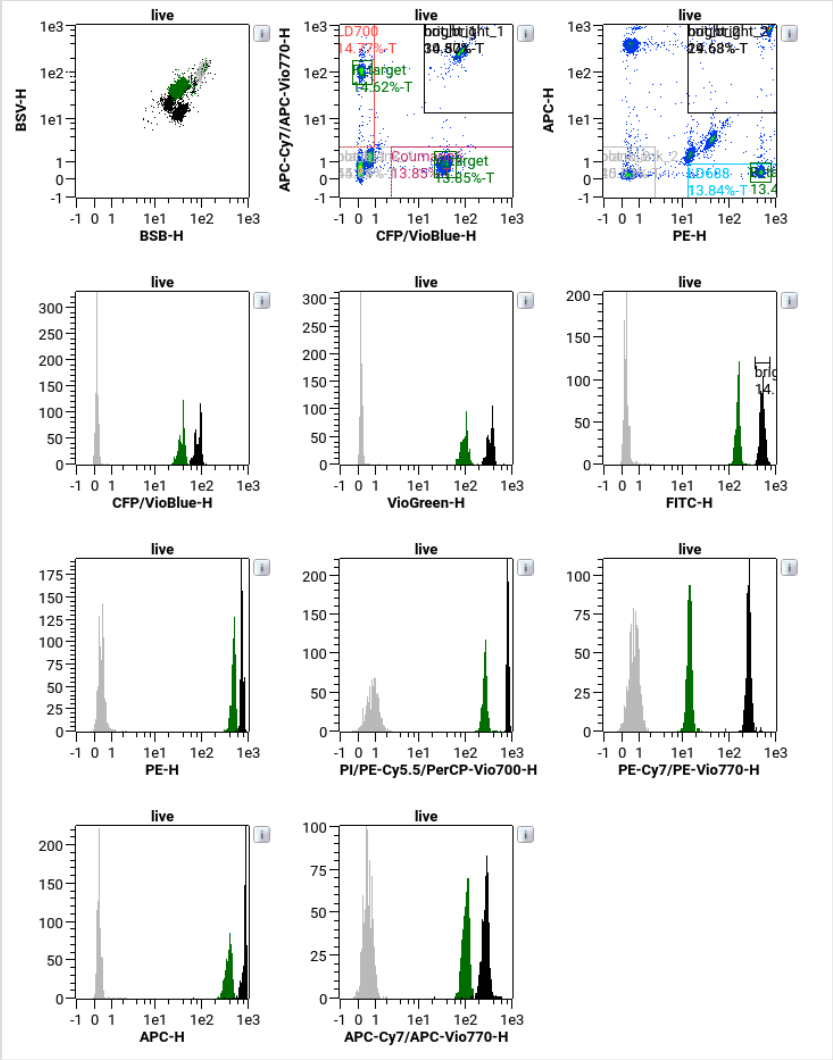


Figure 4.1: Calibration results of a MACSQuant Tyto Cell Sorter (gray: blank beads; black: bright beads;green: fluorescenct beads for the respective target channels)

Parameter	Explanation
Gain	Normalized Smart Gain. See **Smart Gain technology on page 47**.
V	Voltage on PMT
ΔV (last)	Difference in voltage on PMT compared to last successful calibration
ΔV (init)	Difference in voltage on PMT compared to last successful initial calibration
Stain Index	$(MFI_{pos} - MFI_{neg})/2 \times SD_{neg}$
CV	Coefficient of variation based on MFI
Info	Info on success of calibration

Table 4.2: Parameters of the PMT calibration shown in the analysis template, MFI: Median fluorescence intensity

4.2 Adjusting the event detection settings

The event detection of the MACSQuant Tyto Instrument is based on the backscatter signals. It may be necessary to adjust the PMT voltages and thresholds for the used cells or particles as well as the signal intensities of the fluorescent positive populations. It is recommended to use the sort sample. It should contain a negative and a positive population for each of the used flourochromes of the sort panel. Avoid increasing PMT voltages above 700 V.

4.2.1 Adjusting backscatter and side scatter gains

If the location of the target events in the plot is unknown, it can be difficult to adjust backscatter (BSC) and side scatter (SSC) gains. Use a fluorescent marker to label and identify the target events. Use this marker for backgating to determine the proper BSC and SSC adjustment. See **Back gating on page 83**.



1 Click the **Open** button in the toolbar to open a preexisting analysis template or create a suitable analysis template. See **Setting up a sorting strategy on page 67**.

2 Scan and insert a cartridge containing the sample to be analyzed in the MACSQuant Tyto Instrument.



3 Click the **Start measurement** button in the progress bar to start acquisition. As events begin to accumulate, open the **Channels** tab.

4 Adjust the gains for BSC and (optional) SSC to visualize the population of events in the dot plots appropriately. 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 BSC channels. The correlation of the BSC signals can be controlled in the two plots BSR versus BSB and BSV versus BSB in the **Channels** tab (**Figure 4.2**).

Adjust gains by one of the following:

- Click inside the value box. Enter the desired value. Press enter to apply.
- Click on the line right or left from the slider to adjust the gains in increments of 10.
- Use the up and down arrows to increase or decrease the value in small steps.
- Slide the scroll bar right or left to increase or decrease the gains, respectively.



5 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 progress bar to refresh the events.

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

7 Save the workspace.

4.2.2 Setting the noise threshold

The noise threshold setting is based on BSB, BSR, and BSV signals. Signals below the threshold are ignored and not saved or considered for sort decisions. Adjust this setting to minimize noise and debris while maintaining detection of events of interest (**Figure 4.2**). Make sure to include non-target events as well, when using the **High Purity** and **Ultra Purity** sort mode. Noise threshold adjustments affect all BSC channels equally, therefore it is recommended to correlate the signal intensities of the three BSC 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 BSC gains to correlate the intensities. Events in the sort gate are automatically backgated in the noise threshold plots. The backgated events have the color of the corresponding sort gate.

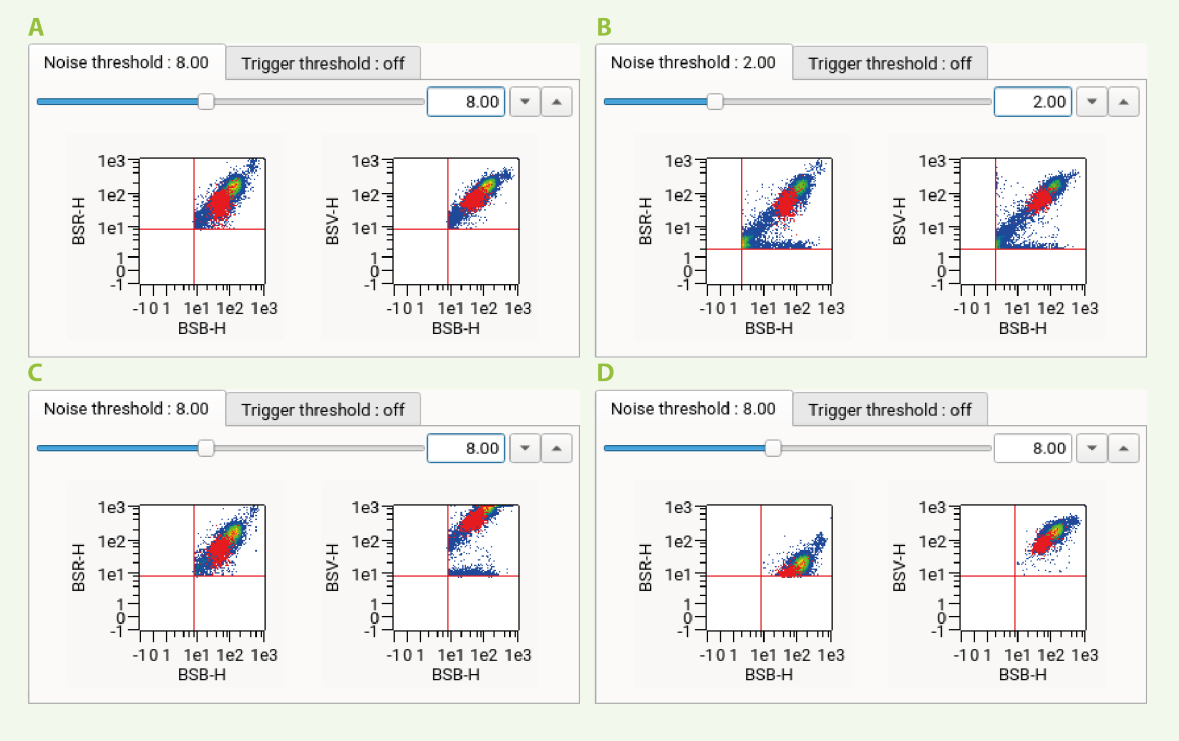


Figure 4.2: Example of different gain and threshold settings shown for white blood cells. Cells in the sort gate are automatically backgated (red). (A) Good settings for noise threshold and gains. (B) Noise threshold is set too low and noise signals are disturbing the measurement. (C) BSV-H gain is set 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 BSC 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 doing one of the following:

- Click and hold the red threshold line in one of the BSC plots and move it to adjust the value.
- Double-click inside the value box. Enter the desired value. Press enter to apply.
- Click on the line right or left from the slider
- Double-click inside the value box. Enter the desired value. Press enter to apply.
- Use the up and down arrows to increase or decrease the value in small steps.
- Slide the scroll bar right or left to increase or decrease the gains, respectively.

4.2.3 Setting fluorescence channel gains

It is recommended to use the sort sample that contains a negative and positive population for each of the used flourochromes of the sort panel.

When setting the fluorescence channel gains, consider following recommendations:

- Adjust the gain if the negative or the positive population is not on scale.
- Adjust the gain to optimize the signal-to-noise ratio.
- Adjust the gain to maintain good signal resolution
- Adjust the gain so that the fluorescence intensity is brightest in its primary detection channel.

- Avoid gains above 700.
- Avoid adjustments that increase the background variability.

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 show only these events in the plot with the fluorescence display. Select the gate in the dropdown list of the plot header.
4 In the **Channels** tab, adjust the gains for the specific fluorescence channel as needed to get both the positive and negative population on scale. To adjust gains, perform one of the following:
   - Double-click inside the value box. Enter the desired value. Press enter to apply.
   - Click on the line right or left from the slider to adjust the gains in increments of 10.
   - Slide the scroll bar right or left to increase or decrease the gains, respectively.
   - Use the up and down arrows to increase or decrease the value in small steps.
5 Adjust the gains according to the recommendations above.

Always adjust fluorescence channel gains prior to applying compensation.

4.2.4 Setting the trigger threshold (optional)

The trigger threshold can be used as an additional trigger to discard irrelevant events. It can be set on a fluorescent or SSC channel to only show events with intensities above the trigger threshold (**Figure 4.3**). Signals below the threshold are not recorded or considered for sort decisions.

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:
   - Click and hold the red threshold line in one of the histograms and move it to adjust the value.
   - Double-click inside the value box. Enter the desired value. Press enter to apply.
   - Click on either side of the toggle bar to adjust the value.
   - Use the up and down arrows to increase or decrease the value in small steps.
   - Slide the trigger toggle bar right and left to increase or decrease the value.
5 Select the checkbox to activate the trigger threshold.

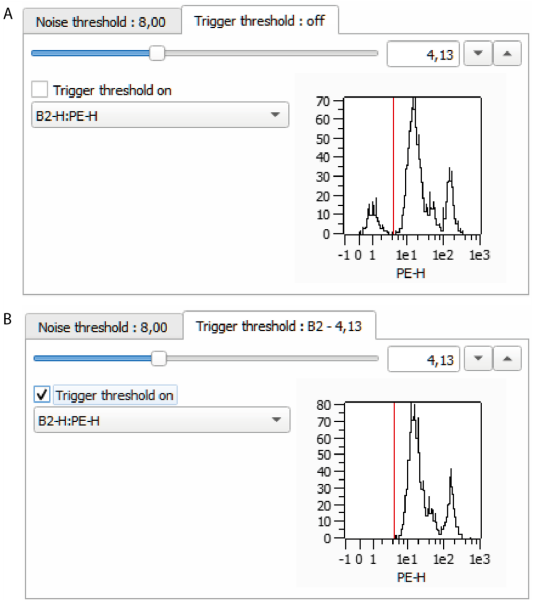


Figure 4.3: Optional trigger threshold deactivated (A) and activated (B)

The **Live Gate mode** is another tool to visualize rare events. When the **Live gate mode** is activated, only events within the sort gate are shown in the plot. See **Live Gate mode on page 83**.

4.3 Compensating 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.

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 4.4**), 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).

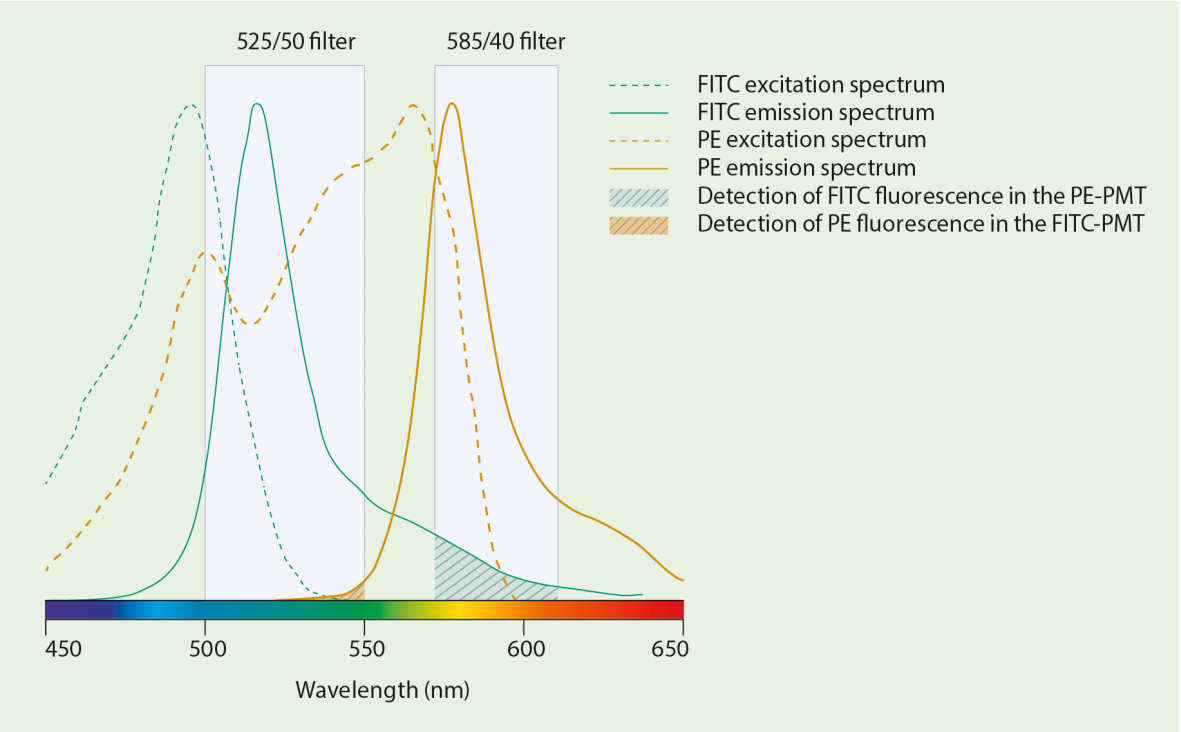


Figure 4.4: Emission spectra of FITC (green solid line) and PE (orange solid line). The green-hashed area represents the amount of FITC signal detected in B2 (585/40 nm). The orange-hashed area represents the amount of PE signal detected in B1 (525/50 nm).

4.3.1 Compensation guidelines

It is mandatory to use single stained samples for compensation.

Compensation controls can be cells, prestained compensation beads, or antibody-capture compensation beads, for example the MACS Comp Bead Kit, anti-REA (# 130-104-693). When setting up and selecting single-stained controls for compensation, consider the following:

- For compensation, use the same fluorochrome that is used in the experiment. For example, if FITC is used in the 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 VioBright FITC.
- The positive and negative populations within the sample must each have the same autofluorescence and level of background fluorescence in the spillover channel.
- Compensation control samples must have fluorescence intensity levels equal or brighter than the samples used in the experiment.
- Each fluorescent signal must be the brightest signal in its designated channel.
- All changes to voltages of fluorescence PMT detectors must be made prior to compensation. Changing PMT voltages after compensation changes the spillover detected and requires reevaluation of compensation values.
- Do not change the laser mode after compensation. This applies only to the MACSQuant Tyto Lux.
- The negative and positive population should have a frequency of at least 10% in the compensation sample. If using an antibody that detects a rare cell population, it is possible to use compensation beads, a control cell line of similar autofluorescence, or another antibody coupled to the same fluorochrome that labels a higher frequency of cells in the sample. An exception is the use of tandem fluorochromes. See the next point.
- If using tandem fluorochromes, use the same reagent for the compensation and experiment. Do not mix different lots of tandem fluorochromes.

- If all single stained samples are of the same material, it is possible to use one blank sample as reference for the negative population for all of them.
- If different sample material is used for the single stained controls, for example, cells and compensation beads, it is recommended to include the blank population into the same tube as the stained sample. Use the same blank sample material as for the stained sample to ensure the same autofluorescence.
- 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 compensation.
- Ideally, only compensate for the channels used in the experimental panels.
- Hlog scaling for data display is preferred when visualizing compensated data.

4.3.2 Example for over- and undercompensation

To compensate for spectral overlap, cells or antibody-capture compensation beads, e.g., MACS Comp Bead Kit, anti-REA (# 130-104-693), which 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 proper compensation, the median fluorescence intensity detected in the spillover channel of the positive and negative population is equal. When the sample is undercompensated, the median fluorescence intensity detected in the spillover channel of the positive population is higher than that of the negative population. When the sample is overcompensated, the median fluorescence intensity detected in the spillover channel of the positive population is lower than that of the negative population (**Figure 4.5**).

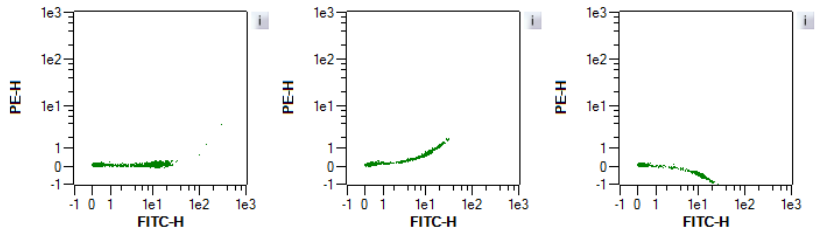


Figure 4.5: Compensated (left), undercompensated (middle), and overcompensated (right) dot plot.

4.3.3 Automated compensation

The automated compensation corrects spillover for every channel.

Materials required

- MACSQuant Tyto Cartridge
- MACSQuant Tyto Running Buffer
- 1.5 mL sample tubes
- Centrifuge
- Fluorochrome-conjugated antibodies used in the experiment
- MACS Comp Beads suitable for the used fluorochrome-conjugated antibodies (for example, MACS Comp Bead Kit, anti-REA (# 130-104-693)

Preparation of MACS Comp Beads

1 Label a separate sample tube for each fluorochrome-conjugated antibody used in the experiment.

These are the single stain controls.

2 Label one sample tube for the blank beads.

3 Add 150 µL of the MACSQuant Running Buffer to each single stain control sample tube.

4 Vortex the MACS Comp Beads and the MACS Comp Beads – blank thoroughly before use.

5 Add one full drop of MACS Comp Beads per single stain control.

6 Add the appropriate amount of fluorochrome-conjugated antibodies to the appropriate sample tube. For an antibody dilution of 1:50 use 4 µL antibody.

7 Mix well and incubate for 5–10 minutes in the dark at room temperature. Shake sample during incubation.

8 Prepare the blank sample during incubation. Add 250 µL of the MACSQuant Running Buffer to the blank sample tube and add one full drop of the MACS Comp Beads – blank.

The blank sample does not require washing.

9 To wash the single stain controls, add 1 mL of MACSQuant Running Buffer to each single stain control sample and mix well.

10 Centrifuge the single stain control sample tubes at 300×g for 5 minutes at room temperature.

11 Carefully aspirate or decant the supernatant.

12 Resuspend each pellet in 300 µL MACSQuant Running Buffer.

13 Ensure that the MACSQuant Tyto Instrument is calibrated and the workspace is set up. See **Protocol for automated compensation below** steps **1**–**5**.

14 Load the single stain control samples and the blank sample into the cartridge. Load equal volumes of the single stain control samples.

15 Proceed immediately with the autocompensation. See **Protocol for automated compensation below** steps **6**–**13**. Do not store the mixed compensation control sample.

Prerequisites for automated compensation

Follow the general compensation guidelines. See **Compensation guidelines on page 56**. Additionally, consider following prerequisites for successfull automated compensation.

- Fill the mixed control into the cartridge and start the automated compensation immediately. Do not store the mixed control before starting the compensation.
- The MFI of the positive population must be at least 8.
- If using other material than MACS Comp Beads, prepare a sample containing at least $3x10^5$ events/mL in total.
- The region of interest (ROI) must comprise at least 500 positive events in the respective target channels.

Protocol for automated compensation

1 Ensure that the MACSQuant Tyto Instrument is calibrated.

2 Go to **File > New workspace** to open an empty workspace.

3 Optional: Open an existing workspace containing the correct PMT channel values.

4 Adjust all PMT channels. See **Setting fluorescence channel gains on page 53**.

The MFI of the positive population must be at least 8.

5 Adjust the sample volume in the **Experiment** tab.

6 Scan and insert the cartridge containing the compensation control samples.

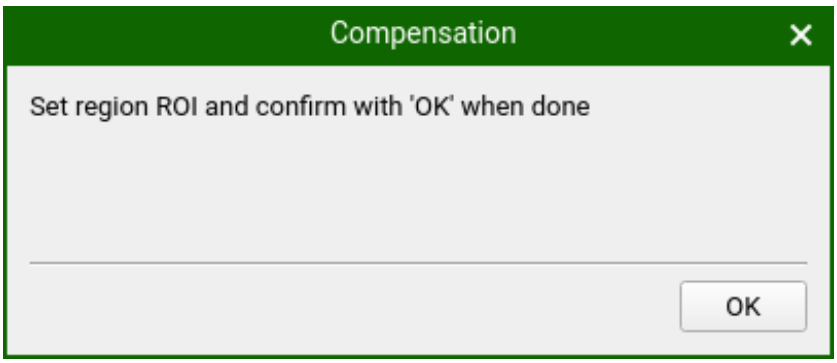
7 Go to the **Compensation** tab on the touchscreen.

8 Select the **Activate compensation** checkbox.

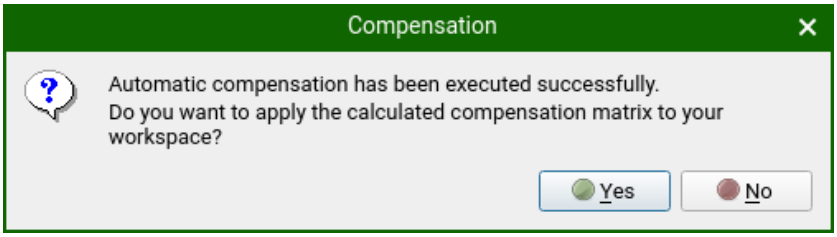
9 Click the **Auto Compensation** button.

10 Follow the dialog box instructions.

11 When prompted, adjust the region around the scatter population in the BSV-H/BSB-H plot that defines the cells or beads used for compensation. If working with cells, include only cells of interest in the ROI.



12 When compensation is completed, a dialog box opens to apply the calculated spillover matrix to your workspace. Click **Yes**.



13 A dialog box opens. Click **Yes** to save the workspace.

4.3.4 Spillover matrix

The MACSQuantify Tyto Software uses a spillover matrix, that organizes the detection of individual fluorescence signals in columns. Column headers identify representative fluorochromes detected on the MACSQuant Tyto Instrument. 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 fluorochrome.

	VioBlue	VioGreen		Vio Bright FITC	PE	PerCP	PE-Vio770		APC	APC-Vio770
V1	1.0	0.0		0.0	0.0	0.0	0.0		0.0	0.0
V2	0.0	1.0		0.0	0.0	0.0	0.0		0.0	0.0
B1	0.0	0.0		1.0	0.0	0.0	0.0		0.0	0.0
B2	0.0	0.0		0.0	1.0	0.0	0.0		0.0	0.0
B3	0.0	0.0		0.0	0.0	1.0	0.0		0.0	0.0
B4	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 Auto Compensation

Figure 4.6: The spillover matrix of the MACSQuant Tyto Instrument on the touchscreen

The default value for all other positions in a column is 0.000. While the value in the primary detection channel stays always 1, the values of the other positions change depending on the spillover.
The values in the spillover matrix columns represent the relative intensity of the signal in the spillover channels (rows) relative to the primary detection channel. To adjust values in the spillover matrix select the field to be changed. For details about adjustment, see **Manual compensation on the next page**.

If the samples are overcompensated, decrease the spillover matrix value of the fluorochrome (column) against the spillover channel (row). If the samples are undercompensated, increase the value in the spillover matrix.

4.3.5 Manual compensation

Materials required

- MACSQuant Tyto Cartridge
- MACSQuant Tyto Running Buffer.
- Single-stained MACS Comp Beads representing all fluorochromes used in the experimental staining panel combined into one MACSQuant Tyto Cartridge. Ensure that there is a comparable positive and negative population for setting compensation. See **Preparation of MACS Comp Beads on page 57**.

Protocol for manual compensation

1 Ensure that the MACSQuant Tyto Instrument is calibrated.

2 Go to **File > New workspace** to open an empty workspace.

3 Optional: Open an existing workspace containing the correct PMT channel values.



4 Select an analysis template by clicking the **New analysis window** button in the toolbar. Select the respective channels of the stained sample on the axis of the plots.

5 Scan and insert the cartridge containing the sort sample.

6 Adjust all PMT voltage channels. Each fluorescent signal must be the brightest signal in its designated channel.

7 Click the **'i'** button of a plot.

8 Change the plot into a histogram.

9 Select the respective channel of the stained sample on the x–axis, for example use B1 for FITC.

10 Click the **Compensation** tab on the touchscreen to open the spillover matrix. Select the **Activate compensation** checkbox.

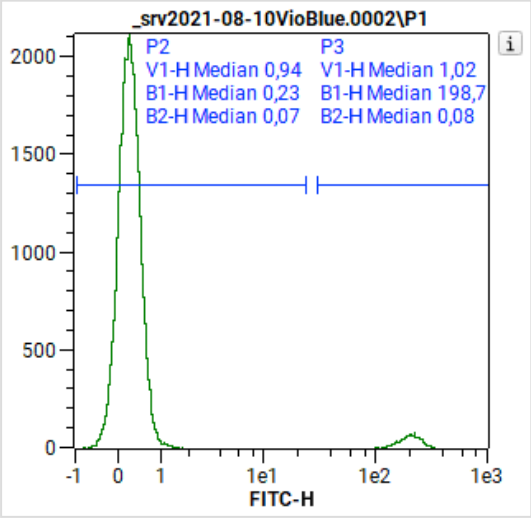
11 Scan and insert the cartridge containing the single stained controls.

12 Click the **Start measurement** button.

13 When events start to appear on the plots, draw a region around the population of interest within the scatter dot plot. This is P1.

14 Select P1 from the plot header of the histogram plot to show only events in P1.

15 Draw an interval region around the negative (P2) and the brightest positive population (P3).



16 Optional: Rename P2 and P3 regions to negative and positive, respectively. Right-click the region and select **Region properties**.

17 Click the **'i'** button of the histogram.

18 Go to the **Feature Functions** tab to simultaneously show the medians for all channels used in the experiment. Select the respective channels and select **Median**.

19 Click **OK**.

20 To adjust compensation, select the appropriate cell in the spillover matrix (**Figure 4.6**). The columns represent the measured fluorochrome, the rows represent the detection channels, from which spillover fluorescence is substracted. For example, to correct the spillover of a FITC-stained sample into the PE channel, go to the cell FITC/B2.

21 Adjust the values until the median fluorescence in the spillover channel for the positive and negative populations are equal.

22 Adjust the matrix values doing one of the following:

- Double-click the value box and enter a value. Press enter to apply.
- Click the toggle bar to adjust the value in increments of 0.01.
- Use the up and down arrows to change the values in increments of 0.001.
- Click the toggle bar and move the slider up and down to change the value.

23 Once the compensation is adjusted for one fluorochrome, continue with compensation for all other fluorochromes.

24 When finished, save as a **Workspace** file.

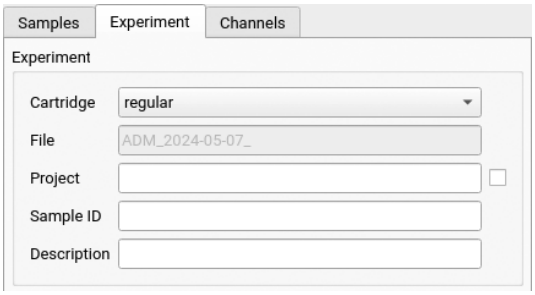
5 Setting up an experiment

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.

5.1 General experiment settings

To set up an experiment, define the parameters in the **Experiment** tab.

1 Go to the **Experiment** tab.



2 To set the cartridge type, scan the 2D barcode of the cartridge by presenting it to the 2D barcode reader window. Alternatively, select the cartridge type using the **Cartridge** dropdown list.

3 Enter alphanumeric text for the **Project**, **Sample ID**, and **Description** if desired. Avoid special characters. See **Using special characters on page 126**. See **Changing path or file naming on page 125** for automated file naming.

5.2 Laser mode

This feature is only available on the MACSQuant Tyto Lux. The MACSQuant Tyto Lux has four Continuous Wave Lasers: violet (405 nm), blue (488 nm), yellow (552nm), and red (638 nm). The blue and the yellow laser are collinear. The laser mode for the yellow laser can be set.

The following modes are available from the **Laser mode** dropdown list:

Laser mode	405 nm	488 nm	552 nm	638 nm
yellow off	On	On	Off	On
yellow only	On	minimal power for scatter detection only	On	On
yellow/blue balanced	On	On, reduced power	On, reduced power	On

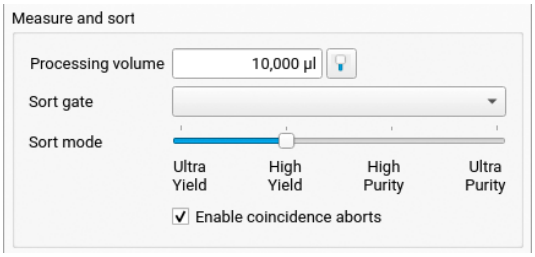
Table 5.1: Laser modes on the MACSQuant Tyto Lux

5.3 Measure and sort

The section **Measure and sort** shows the volume to be processed by the instrument, a dropdown list to select the sort gate, and the sort modes. For more information about the sort modes, see **Sort mode below**.

1 Enter the volume to be processed by the instrument in the **Processing volume** box. Press enter. Once this volume has been processed, the acquisition is stopped, data files are finalized, and the cartridge is ejected automatically.

2 Select the desired gate as sort gate from the **Sort gate** dropdown list. By default, no sort gate is selected.



5.3.1 Sort mode

The MACSQuant Tyto Instrument offers four sort modes. Use the slider to set the sort mode. The default mode is **High Yield**. Set the sort mode before the sort. It is not possible to change it during sorting. Following sort modes are available:

Sort mode	Description
Ultra Yield	Obtain a maximal yield of the events of interest at the expense of purity. There are no sort mode based aborts in this mode.
High Yield	Default mode. Obtain a high yield. Only target cells are considered for sorting, therefore two cells which are close together will be sorted, as long as one of them is a target cell.
High Purity	Obtain high purity. Non-target cells are avoided for sorting. The sort pulse is adjusted to try and miss the non-target cell. However, if the non-target cell and a target cell are close together, those cells are aborted. It is recommended to enable coincidence aborting. See **Enable coincidence aborts on page 37**.
Ultra Purity	Obtain a maximal purity of the events of interest. If a non-target cell and a target cell are close together, those cells are aborted. It is recommended to enable coincidence aborting. See **Enable coincidence aborts on page 37**.

Table 5.2: Sort modes

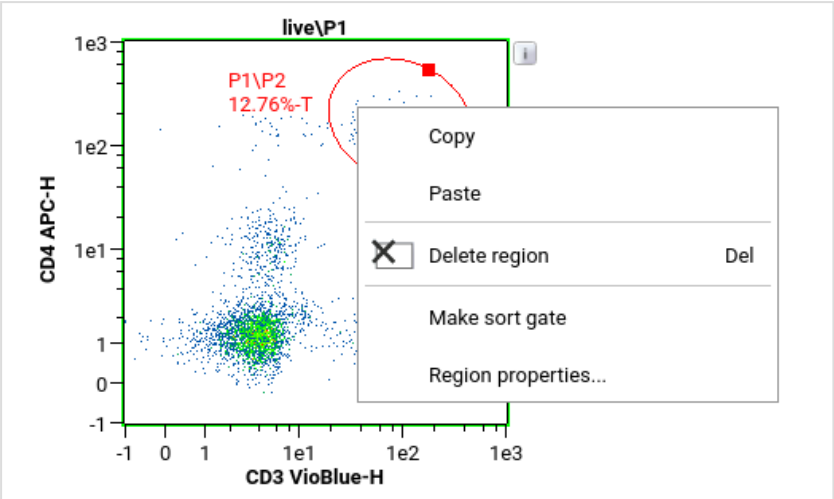
When using the **High Purity** or **Ultra Purity** mode keep the trigger values at the minimum. Increasing the trigger values to exclude noise can hide non-target cells from the sorting algorithm. This leads to lower purity.

5.4 Make sort gate

Select the sort gate appropiate for your target cells and your gating strategy. See **Setting up a sorting strategy on page 67**. Events within the sort gate are sorted into the positive selection chamber of the cartridge during the sorting process.

1 Right-click a gate within a dot plot.

2 Select **Make sort gate**.

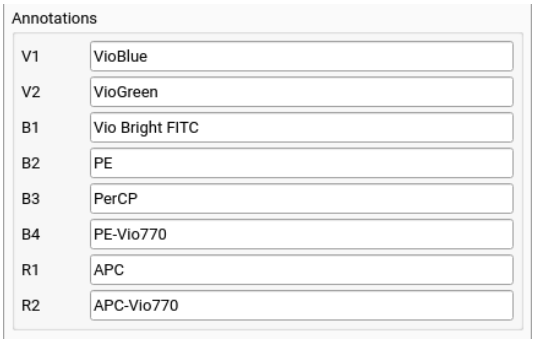


5.5 Annotations

The annotations of the fluorochrome channels can be changed in the **Annotations** section of the **Experiment** tab. The detector names in the **Channels** tab are renamed according to the annotations.

1 Go to the **Experiment** tab.

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



6
Setting up a sorting strategy

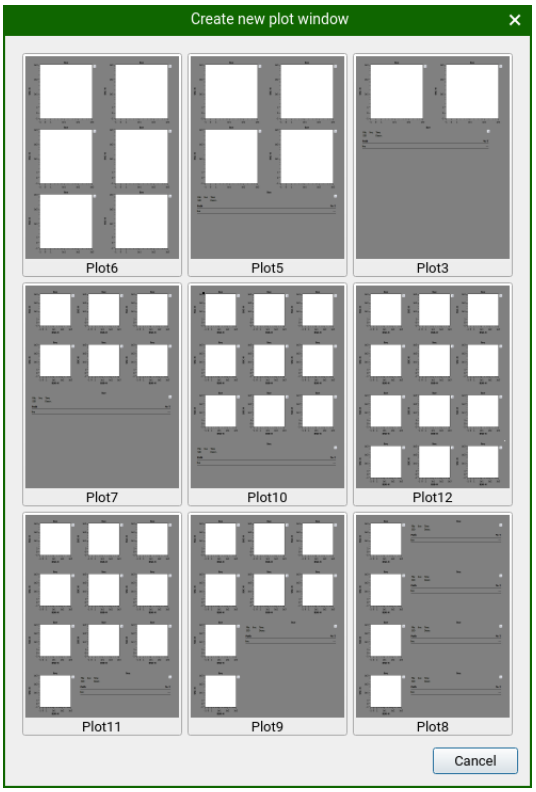
This chapter explains how to create, modify, and save analysis templates. An analysis template is a file type that contains all information related to the visible dot plots. In addition, all functions related to modify dot plots are explained in this chapter.

6.1 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 window options. Multiple pages can be chosen to match the plot layout necessary for the analysis template.





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

Acquired data are shown 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 density plots and statistics tables. Several analysis windows can be opened at one time. Gating strategies can be created during sample acquisition (live gating) and saved for future use, or they can be created before or 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. Analysis template can be saved.

6.2 Plot types

Flow cytometry data can be shown in different formats: as a dot plot, density plot, histogram, or as a statistics table. Additionally, a text box can be used to show additional information. Click the **'i'** button next to a plot to open the following properties window. See **Plot properties on page 70**. Switch between the plot types by clicking the respective button.

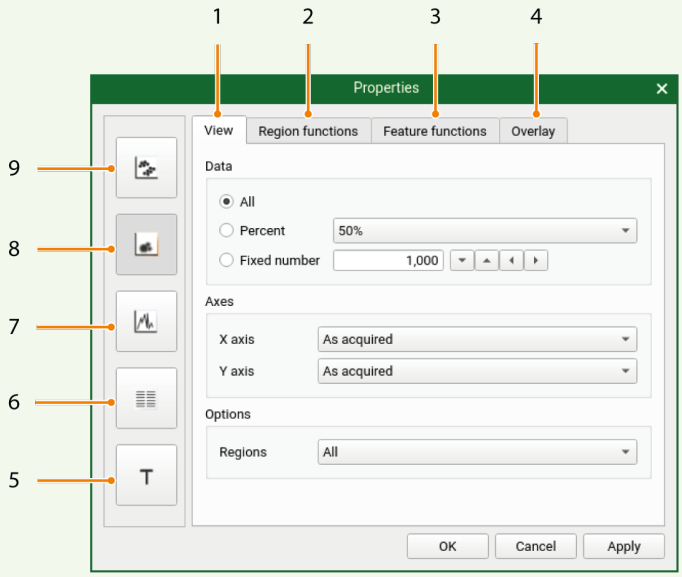


Figure 6.1: Switch between plot types or modify their appearance

1	Change general plot options	**6**	Select statistics and show statistical parameters
2	Select region functions	**7**	Select and adjust histogram as plot type
3	Select feature functions	**8**	Select and adjust density plot as plot type
4	Adjust overlay options	**9**	Select and adjust dot plot as plot type
5	Select script text and add information about a sample		

6.2.1 Dot plots and density plots

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

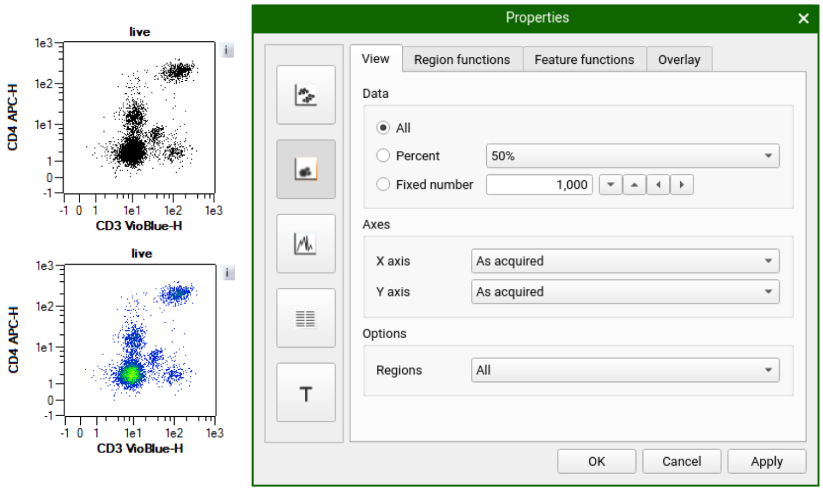


Figure 6.2: Dot plot (upper left) and density plot (lower left)

6.2.2 Histograms

Histograms are univariate plots that show one parameter.

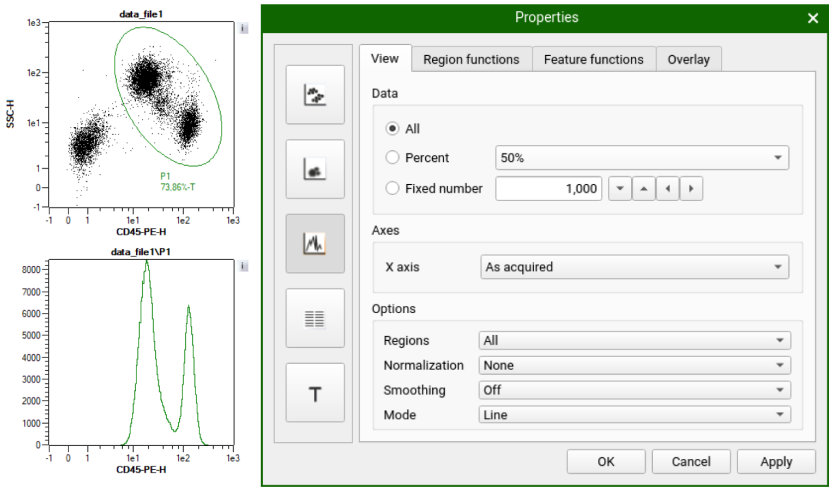


Figure 6.3: Dot plot (upper left) and histogram (lower left, gated cells only)

6.2.3 Statistics tables

Statistic tables show statistic features of one or more samples in a table format.

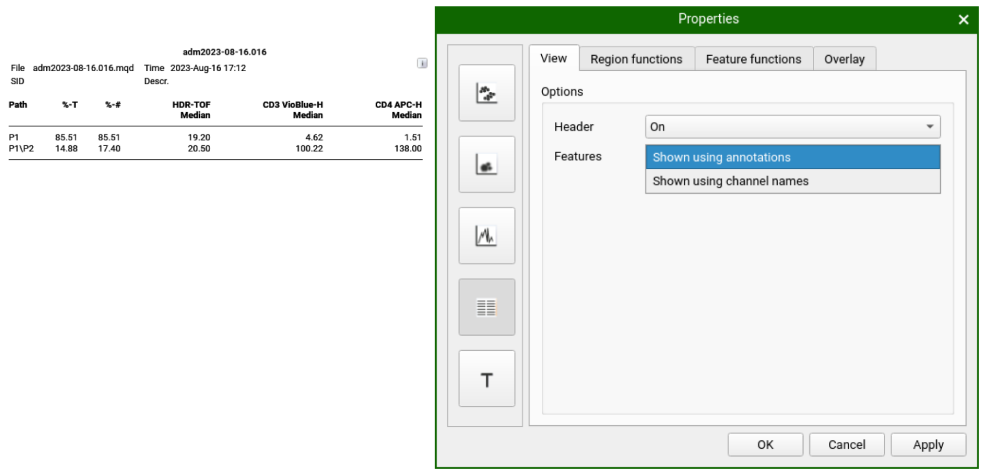


Figure 6.4: Statistics table

6.2.4 Text box

The **Script Text** box can be used to enter free text.

free...– Enter free text into the empty text field. Users experienced in script text can write their own scripts using HTML (hypertext mark-up language). Experienced users can for example automatically show statistics about a region or gate. Specific regions can be removed from or added to a text table by using the overlay tab.

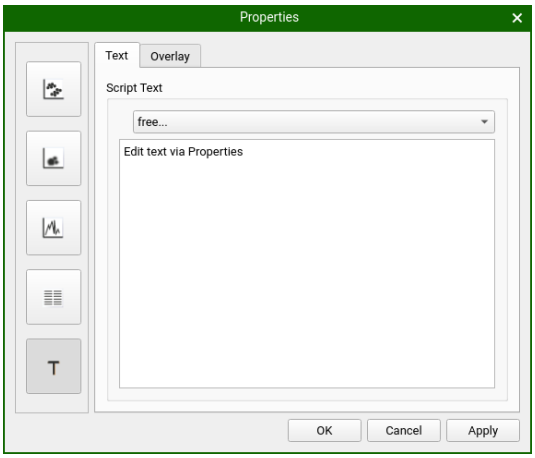


Figure 6.5: Script Text properties

6.3 Plot properties

Click the **'i'** button to change plot type and properties. Double-click a plot to view it full screen. Double-click again to resize. Click the axis label to modify the shown parameters.

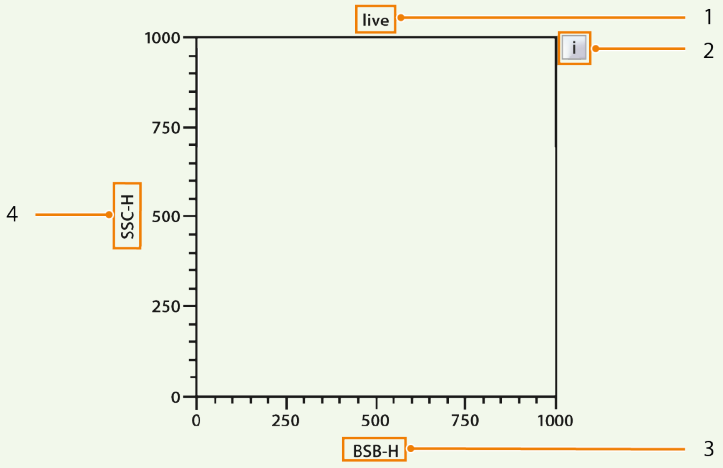


Figure 6.6: Features of a plot

1	Plot header	**3**	Shown parameter for x-axis
2	**'i'** button	**4**	Shown parameter for y-axis

6.3.1 View

Data

In case of large event numbers, only a fraction of all acquired events can be shown. The number of events can be adjusted for dot plots, density plots, and histograms.



1 To change the number of events that are shown 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 shown.
- **Percent** – 50%, 25%, 10%, 5%, 2%, or 1% percentile values of the total events are shown.
- **Fixed number** – A fixed number of events is shown. Enter the desired value and press enter to apply, or change the default value using the arrows.

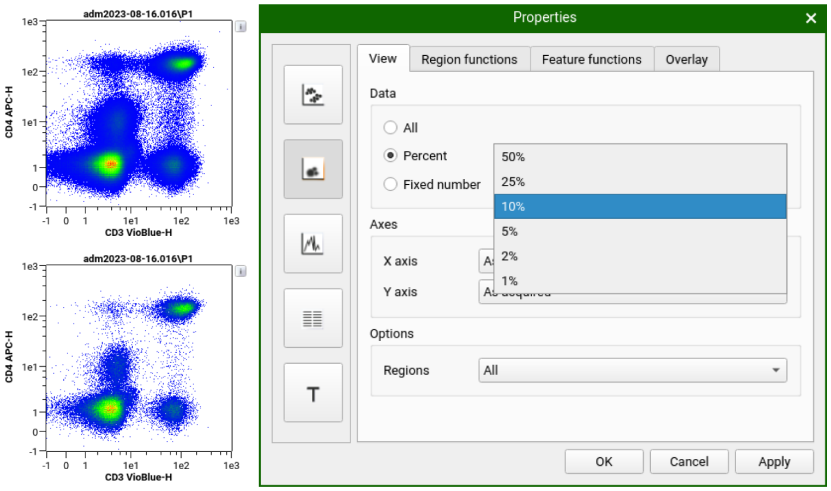


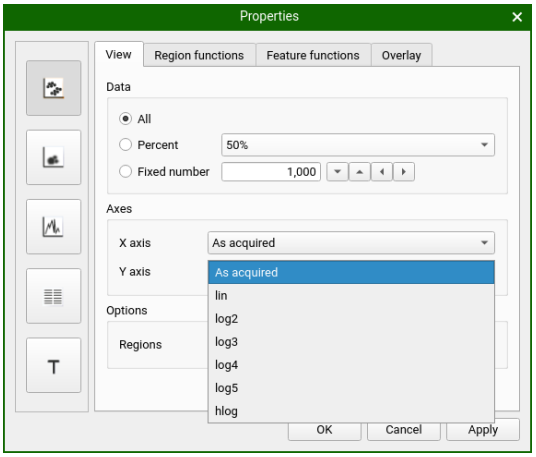
Figure 6.7: Density plot with 100% of events (upper left) and 10% of events (lower left)

Axes

Axes of a plot can be changed after data acquisition. Changing the axes will only change the view of the shown events. The data file remains unchanged.
To change the scale for data acquistion, change the scale in the **Channels** tab. See **Adjusting data display scales on page 77**.

1 To change the axes scales of a dot plot, density plot, or histogram click the 'i' button next to the plot.
2 Go to the **View** tab.
3 From the **Axes** section, select from the following:

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



Options

The **Options** section differs between the plot types.

The properties in the **Options** section of a plot can be changed. Change the shown regions, select settings for the appearance of histograms, or define the layout of a table. See the respective section in this chapter. To change the properties of the **Options** section proceed as follows:

1 Click on the **'i'** button next to the plot.
2 Go to the **View** tab.
3 From the **Options** section, select the available options based on the plot type:

Options for dot plots, density plots, and histograms

It is possible to define, which gates are shown in a dot plot or density plot. This is useful if two plots have the same axes label but the parental gate is on different hierarchy levels.

All – All regions ("gates") drawn in either of the plots is shown.

This – Only the region ("gate") drawn in the selected plot is shown.

None – No regions ("gates") are shown in the plot.

Options specific for histograms

For histograms **Normalization**, **Smoothing**, and **Mode** can be adapted.

Normalization: Data can be normalized by area (integral of total area under the curve) or by height. Select **None**, **Area**, or **Height** to deactivate or activate normalization.

Smoothing: Algorithms can be used to smooth the histogram. Select **Off**, **Light**, **Medium**, or **Strong** to adapt smoothing of the curve.

Mode: Histograms can be shown as a line chart or bar chart. Select **Line** for histogram drawn with a single line or select **Bar** to color the area under the curve.

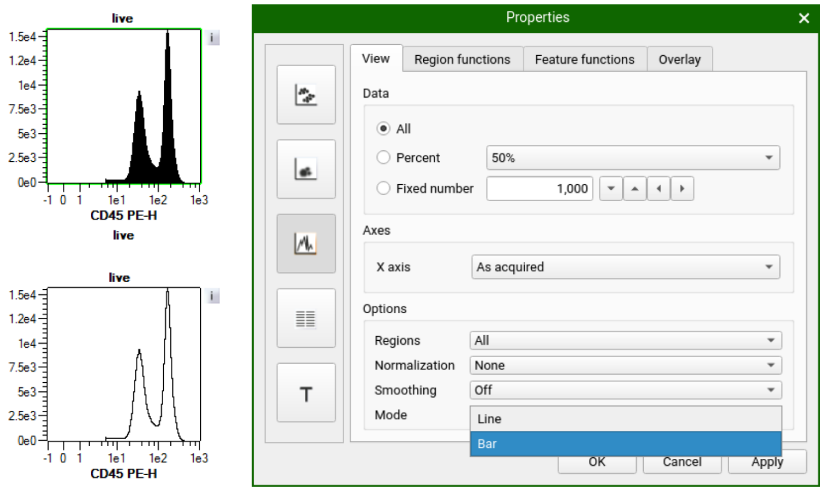


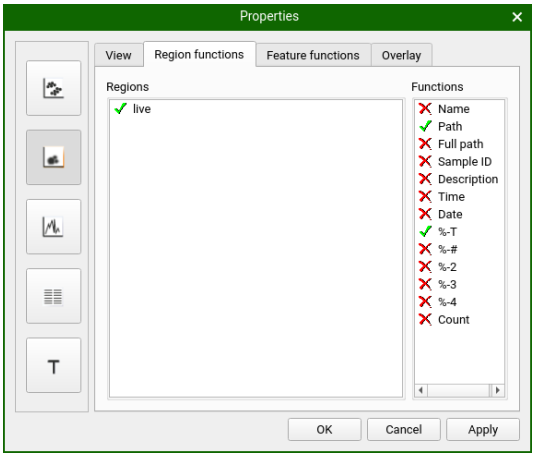
Figure 6.8: Histogram specific options

6.3.2 Region functions

To show 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 clear 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	Name of the gate
Path	Path of the gating hierarchy
Full path	Full path of the gating hierarchy
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 of the gated population
%-#	Percent gated / percent parent of the gated population
%-2	Percent grandparent of the gated population
%-3	Percent great grandparent of the gated population
%-4	Percent great-great grandparent of the gated population
Count	Number of triggered events

Table 6.1: Region functions

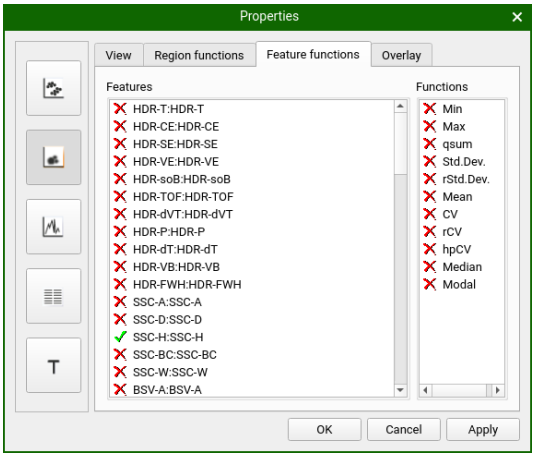
6.3.3 Feature functions

The current dot plot shows two different optical 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 clear 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 related to the mean
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 data across a broad value range, e.g. logarithmic data.
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. Recommended for data values over a broad numerical range, e.g. logarithmic data
Modal	Value that occurs most often among all events

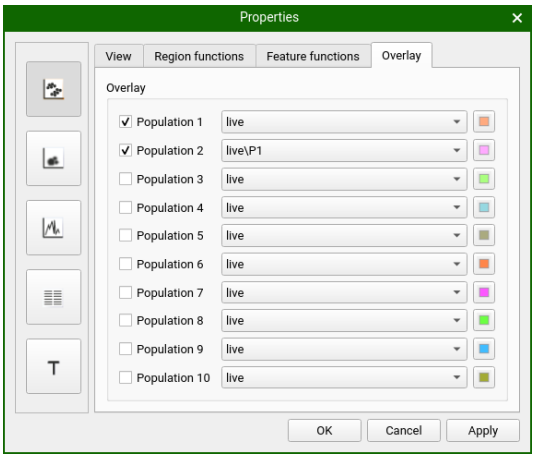
Table 6.2: Feature functions

6.3.4 Overlay



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

2 Go to the **Overlay** tab.



3 Select the desired population from the dropdown list.

4 Optional: Click the box to the right to change the population color as desired.

5 Select the checkbox to the left to mark the population as active. Only active populations are shown in the plot.

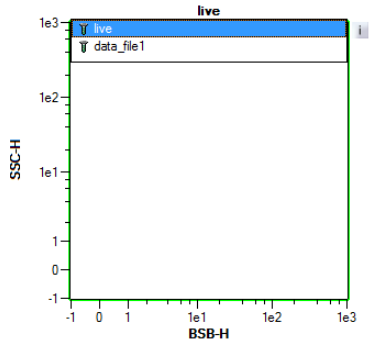
6.4 Displaying data

6.4.1 Displaying data in a plot

1 Load a data file to the sample list. See **Opening files on page 92**.

2 To display data in a plot perform one of the following:

- Click the plot title and select a data file from the dropdown list.
- Select a plot and double-click a file in the sample list.



3 Click the axis label and select one of the saved parameters in the dropdown list to change the displayed parameters. See **Axis parameters below**. The dot plot displays BSB-H versus SSC-H per default.

6.4.2 Axis parameters

You can select the desired axis parameter by clicking the axis title. Besides the SSC, the BSC, and the fluorescent channels, additional axis parameters can be shown.

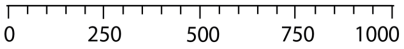
Parameter	Name	Description
HDR-T	Time	Elapsed time
HDR-CE	Created events	Trigger count
HDR-soB	Sort bit	Valve actuations to sort a cell
HDR-TOF	Time of flight	Time (µs) the event needs for crossing the blue and the violet laser
HDR-dVT	Delta valve time	Time since the last valve actuation
HDR-FWH	Full width half maximum (FWHM)	Width of the voltage pulse at 6.25% signal intensity from BSC-V. The FWHM from the violet laser signal is important for signal assembly and transit time determination.
A	Area	Area of the voltage pulse
H	Height	Height of the voltage pulse

Table 6.3: Axis parameters including header (HDR) and pulse processing parameters

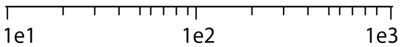
6.4.3 Adjusting data display scales

The MACSQuantify Tyto Software offers various options to scale and thereby properly visualize data. The MACSQuant Tyto Instrument can show data in a variety of formats. The SSC, all BSC (BSB, BSV, BSR), and all fluorescence channels (MACSQuant Tyto Cell Sorter: V1–2, B1–4, and R1–2; MACSQuant Tyto Lux: V1–V4, B1, B+Y2–B+Y5; R1–R3) can be shown in lin, log2, log3, log4, log5, and hlog scale.

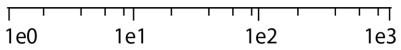
lin – Linear scale



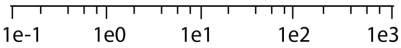
log2 – Logarithmic scale of two decades



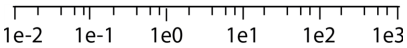
log3 – Logarithmic scale of three decades



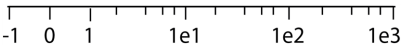
log4 – Logarithmic scale of four decades



log5 – Logarithmic scale of five decades



hlog – Biexponential scale

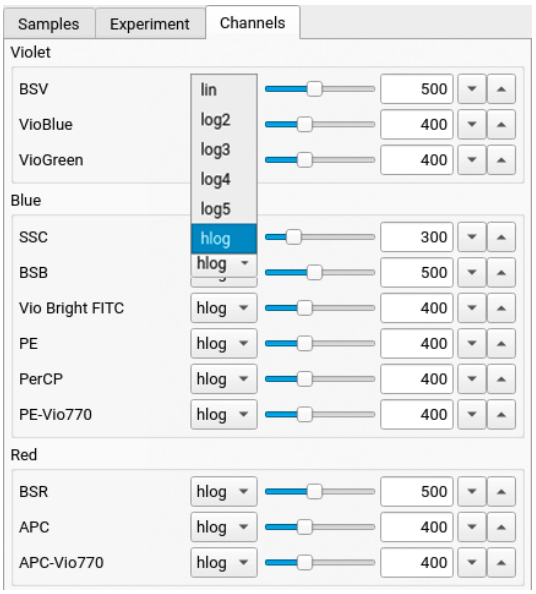


The default scale of the MACSQuantify Tyto Software is hlog. In case of analyzing cell lines or samples comprising both, large and small particles, a logarithmic scale is useful. Also fluorescence signal intensities that extend over several orders of magnitude must be shown in a logarithmic scale.

Set the data display scales in the **Channels** tab of the side pane before acquisition. Select the desired scale for data acquisition as described below. Changing the scale in the dot plot properties only changes the display of the scale but not the scale for data acquisition.

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

2 Select the respective scale from the dropdown list next to the specific channel.



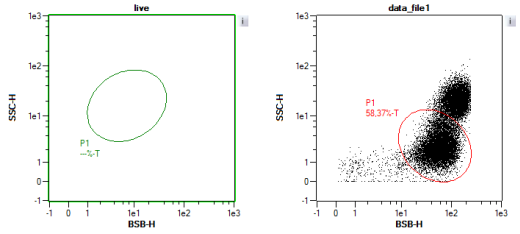
6.5 Gating tools

Gating tools can be selected in the toolbar to create a region around a population. Created regions in a hierarchical gating can be selected to show only events of interest.

1 Select a gating tool in the toolbar.

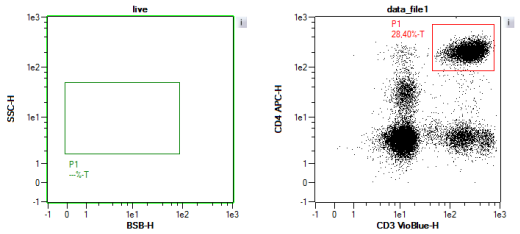


- **Ellipse**



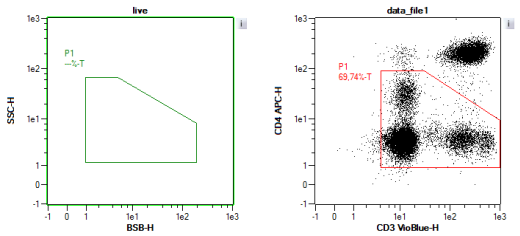


- **Rectangle**



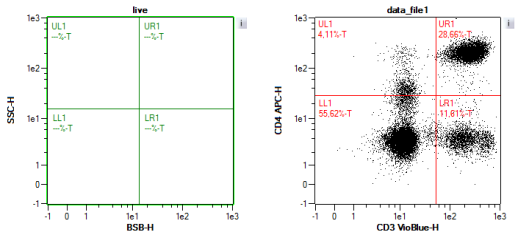


- **Polygon**



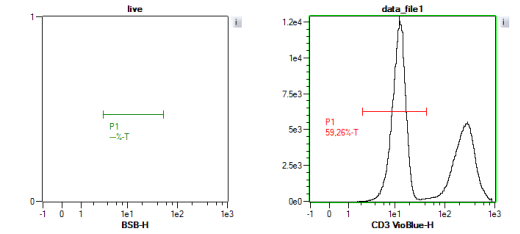


- **Quadrant**





- **Interval**



2 Draw a gate of the respective shape.

- Ellipse, rectangle, quadrant, or interval: Click and hold the mouse. Drag the cursor as desired. Release the mouse to finish drawing.
- 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.

3 To change the appearance of a gate, highlight the gate and select from the options below:

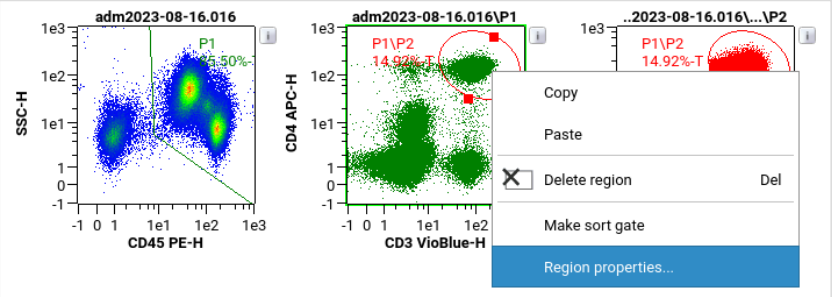
- Click the size handle in the corners and drag to adjust the size of the gate.
- Click and hold to drag the gate and move it to a new position.

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

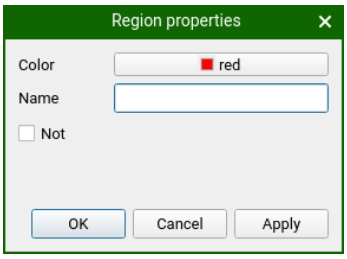
6.5.1 Changing region properties

1 Right-click the region name or right-click into the selected gate in the plot.

2 Select **Region properties**.



3 Change the color of a region, region name, or set a Not gate. See **Setting a Not gate below**.



4 Click **Apply** to apply the changes or **OK** to apply and close the window.

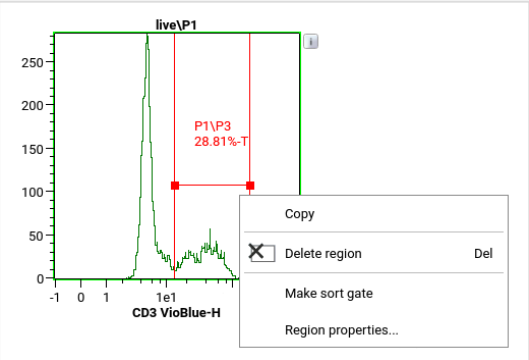
6.5.2 Setting a Not gate

Not gates are used to exclude a cell population from analysis and are indicated by a dotted line.

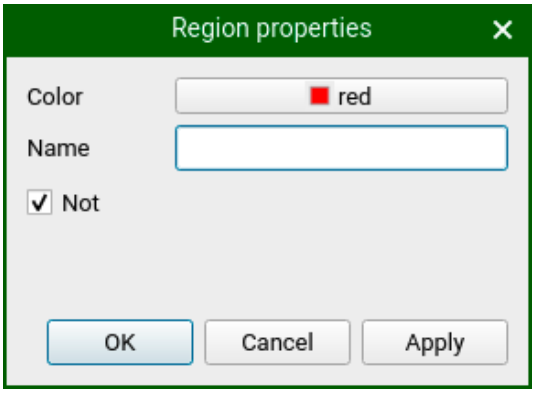
1 Draw a region around the population to be excluded from analysis.

2 Right-click the region.

3 Select **Region properties** from the dropdown list.

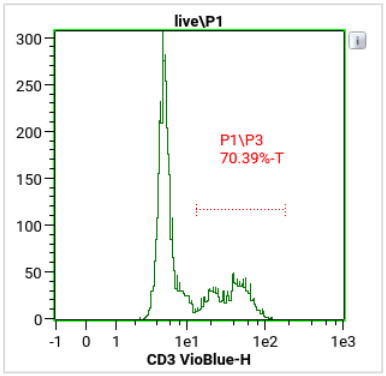


4 Select the checkbox **Not**.



5 Optional: Select a color and/or name the Not gate.

6 Click **Apply** to implement changes and **OK** to close the window. The Not gate is shown as a dotted line.



6.5.3 Copying regions and gating strategies

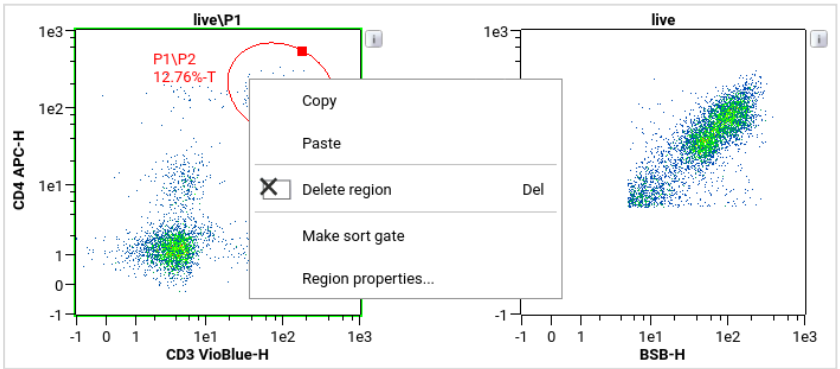
Regions can be copied and pasted from one plot to another plot. Regions or entire gating strategies can be also copied to other files via drag and drop within the sample list.

Copying and pasting regions

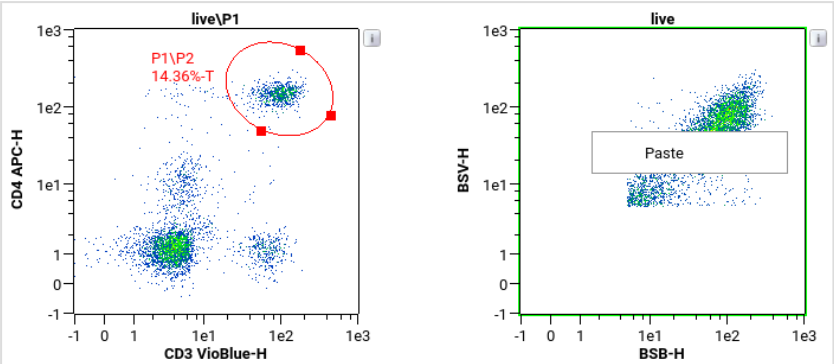
Copy a region from one plot and paste it to the same or a compatible plot. A region from a dot plot can be pasted to a dot plot or density plot, but not to a histogram. The same physical coordinates are used for the region, even if the plot has different scales.

1 Click a region button and draw a region in the plot of interest.

2 Right-click the region and select **Copy**.



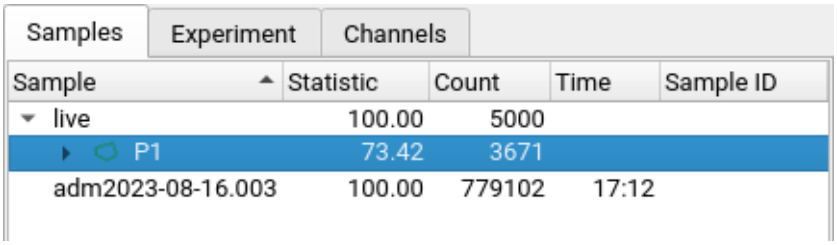
3 Right-click in a compatible plot type and select **Paste**. The new region is modifiable using the edit points and **Region** properties.



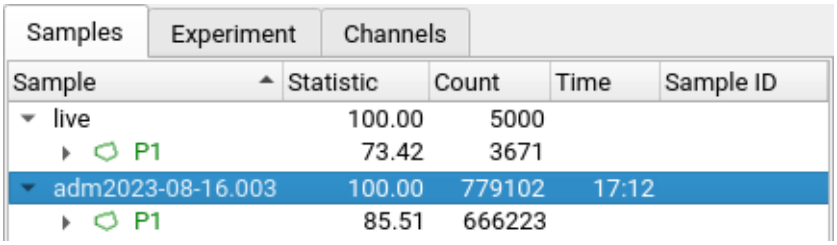
Copying regions or gating strategies via drag and drop

Regions or entire gating strategies can be copied to other files in the sample list or moved to other hierarchy levels in the gating strategy.

1 After establishing a gating strategy on a data file in the sample list, click 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 are applied to the new file.



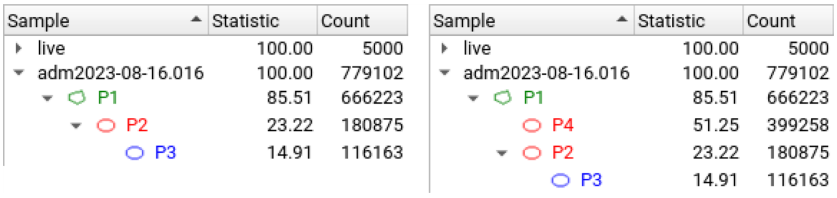
Moving a region within a gating hierarchy

After establishing a gating strategy within a data file, click a region in the sample list and drag it under another region within the strategy. The region becomes 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.



Dividing a parental gate in two regions with the same hierarchy

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

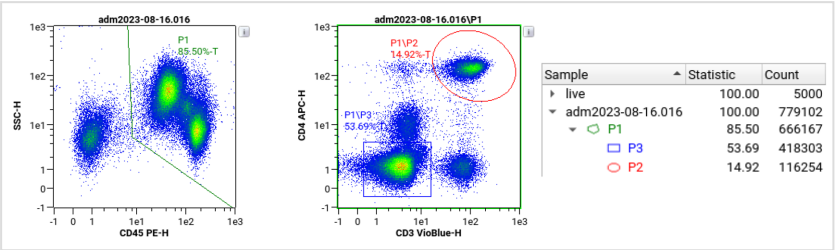


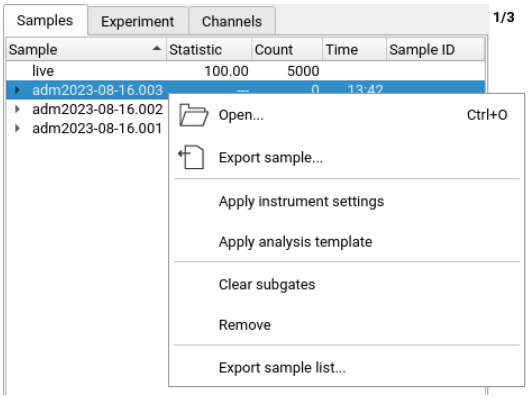
Figure 6.9: Regions P2 and P3 have the same hierarchy level. P1 is the parental gate of both

6.5.4 Deleting gates

1 Deleting a gate also deletes all subgates. To delete a gate, select the gate in the plot and perform one of the following:

- Press **Delete** on the keyboard.
- Select the **Delete region** button in the toolbar.
- Right-click the gate and select **Delete**.
- Select the gate in the sample list, right-click, and select **Remove**.

2 All regions under a file or under other regions can be deleted at once. Right-click on the file or the top region in the hierarchy and click **Clear subgates**.



6.6 Gating strategies

6.6.1 Hierarchical gating

Create a hierarchical gating strategy.

1 Click the plot title of the next plot and select P1. Only events of P1 are shown.

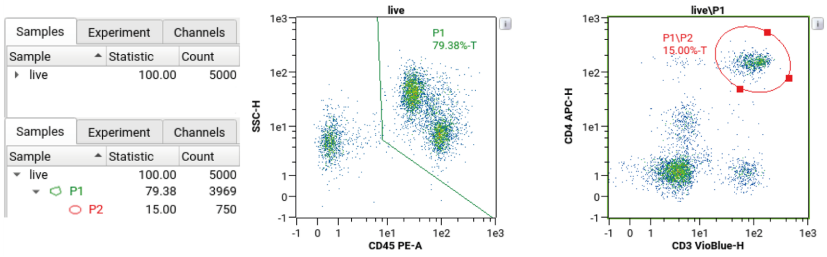
2 Select the axis parameters.

3 Draw a gate (P1) in the plot.

> The gating hierarchy will not change if the plot title is changed after the gate was drawn.

4 Draw a gate (P2) within the plot showing the events from P1.

5 Click the **Arrow** icon next to the sample in the **Samples** tab to open the gating hierarchy. The hierarchy level, gate type, name, and color are shown in the hierarchy tree.



6.6.2 Live Gate mode

The **Live gate mode** helps to set the sorting gate correctly. This mode is especially helpful for 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 shown target cells.

To activate the live gate mode:

1 Set an initial sort gate at the expected position of the target cells.

> The initial sort gate must be larger than the final sort gate to avoid cutting off target cells.

2 Right-click the initial sort gate and select **Make sort gate**.

3 Click **Live gate mode** in the **Tools** menu. Only events within the sort gate are shown in the plot. See **Tools menu on page 29**.

4 Adjust the sort gate around the target cell population to create the final sort gate.

5 Optional: Set a new sort gate around the target cell population and make this the new sort gate.

6 Click **Live gate mode** in the **Tools** menu to deactivate the live gate mode.

6.6.3 Back gating

Back gating is a technique to re-analyze a subset of cells that have already been gated, by visulaizing them on different plots with other parameters.



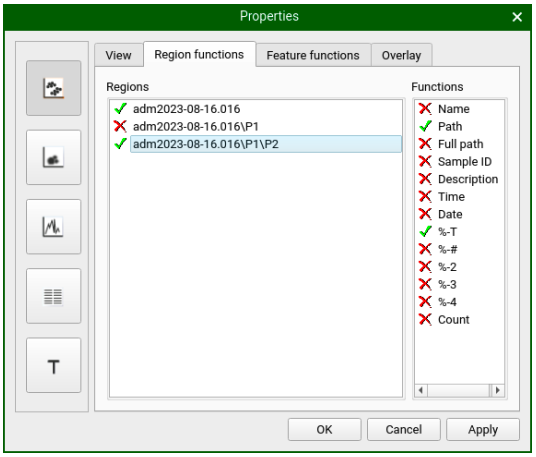
1 Click the **New analysis window** button in the toolbar to open a new analysis window.

2 Define a hierarchical gating strategy.



3 Click the **'i'** button of the plot in which the cell subset of interest should be shown.

4 Go to the **Region functions** tab and select the region for back gating.



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

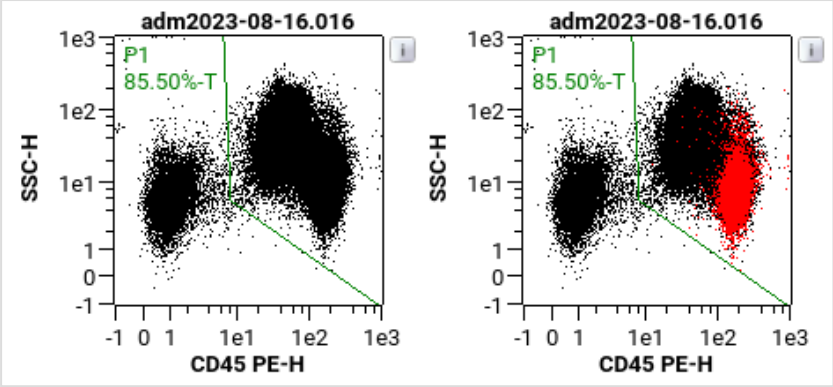


Figure 6.10: Dot plot before (left) and after (right) backgating of the P1/P2 gate. Populations are indicated in their designated gate color.

6.6.4 Multilayer mode display

Gated events within dot plots are shown in their respective gate color. While establishing a gating strategy, each subsequent plot is formatted to show only events of the parent gate. By using the multilayer mode, all events are displayed in one plot and are identified using the color of their analysis hierarchy.



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

2 Define a hierarchical gating strategy.

3 Right-click the **i** button of the plot to be modified.

4 Select **Multilayer mode**.

5 The respective plot shows all events of the entire gating strategy with their gated region color.

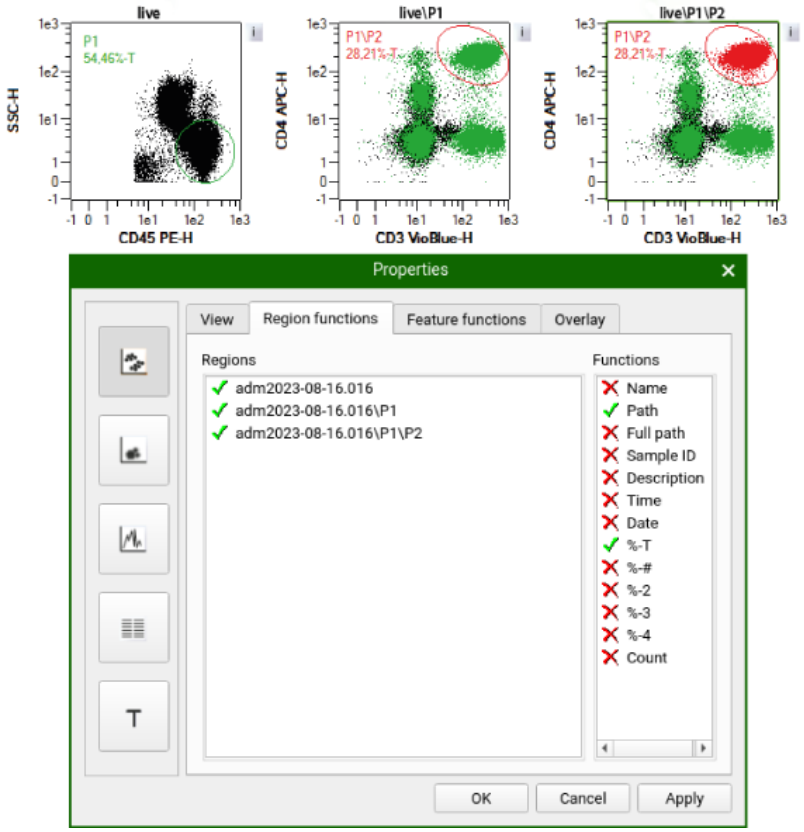


Figure 6.11: Multilayer mode gating strategy and display. PBMCs are stained with CD45-PE, CD3-VioBlue, and CD4-APC. In the left plot, cells are gated on $CD45^{++}$ leukocytes (P1). The middle plot shows only cells from P1. In this plot, $CD3^{+}CD4^{+}$ T helper cells are gated (P2). The right plot shows only cells from P2. The Multilayer mode is active for the middle and left plot. The cells are shown in the color of the parental gate. Depending on the frequency, some colors might not be visible in multilayer mode.

7 Workflow of a sort process

The MACSQuant Tyto Instrument workspace manages all aspects of the cell sort. It comprises the analysis template, experiment parameters for a particular sort, instrument settings, and allows monitoring of the sort. The sort process can be based on a preestablished workspace. Thus, the MACSQuant Tyto Instrument allows for cell sorting without the need for specialized technical expertise for daily operation. Laser alignment, flow control, and speed detection are fully automated. The following instructions describe the main setup parameters for a sort workspace.

7.1 Creating a workspace

1. Ensure that the MACSQuant Tyto Instrument is calibrated. See **Automated PMT calibration on page 49**.
2. Select an analysis template. See **Opening an analysis window on page 67**.
3. Annotate the fluorescence channels. See **Annotations on page 65**.
4. Select the plot axis parameters from the axis dropdown list. See **Plot properties on page 70**.
5. Set up a gating strategy. See **Gating tools on page 77** and **Gating strategies on page 82**.
6. Define a sort gate. See **Make sort gate on page 64**.
7. Start measurement. See **Progress bar on page 41**.
8. Set the photomultiplier tube (PMT) voltages. See **Adjusting the event detection settings on page 51**.
9. Set the noise threshold. See **Setting the noise threshold on page 52**.
10. Optional: Set the trigger threshold. See **Setting the trigger threshold (optional) on page 54**.
11. Optional: Set up a compensation (dependent on fluorochrome panel). See **Compensating spectral overlap on page 55**.
12. Save the workspace. See **Saving files on page 92**.

7.2 Setting up a sort

1. Ensure that the MACSQuant Tyto Instrument is calibrated. See **Automated PMT calibration on page 49**.
2. Load a workspace. See **Opening files on page 92**.
3. Optional: Adjust the sample volume in the **Experiment** tab according to the particular sample. See **Measure and sort on page 64**.
4. Check and adjust the misc controls in the cartridge tab. See **Mixer on page 43**, **Flow control on page 44**, and **Chamber temperature on page 45**.

   Optional: Activate the automated priming. See **Automated priming on page 43**
5. Load a cartridge containing the sample into the instrument. See the **MACSQuant Tyto Instrument user manual**.
6. Click the **Start measurement** button in the progress bar to initialize data acquisition.



7 Click the **Sort** button in the progress bar to start the sort process. The **Real time sort statistics** window shows the statistics for the current sort. See **Real time sort statistics window on page 38**.

8 The measurement stops automatically after the set sample volume is sorted or the input chamber of the cartridge is empty.



9 Optional: Click the **Stop measurement** button in the progress bar to stop the measurement manually.

10 Ensure that the measurement has stopped before taking out the cartridge. See the **MACSQuant Tyto Instrument user manual**.

8 Reporting data

8.1 Reexporting a run report to PDF

Run reports are automatically saved as PDF file. Run reports from an initial calibration or an automated PMT calibration are automatically saved as calibration report in PDF format. To reexport the run report PDF file do the following:

1. Open the run report you want to reexport. See **Opening files on page 92**. Alternatively, click **Open Report** in the **Real time sort statistics** window.
2. Click on **Re-export PDF** in the **Run Report** window to save it again as a PDF file.

Run reports can be signed electronically by Tyto Administrators and Custom users in the 21 CFR part 11 compliant software setup. To sign a run report see **Signing run reports on page 19**.

8.2 Opening the Real time sort statistics

The **Real time sort statistics** are automatically saved as a CSV file in the same location as the data files.

1. To open the CSV file, copy it to a remote storage location or a USB flash drive. The CSV file can be copied from the location **Data files**, **Report files** or **Other files**.
2. Open the CSV file on a PC.
3. To optimize the view of the data, convert text to columns. Use tab as delimiter and **Text** as data format.

8.3 Opening the calibration table statistics

The calibration table statistics from the initial calibration and automated PMT calibration are automatically saved as a HTML file.

1. To open the HTML file, copy it to a remote storage location or a USB flash drive. The HTML file can be copied from the location **Other files**.
2. Open the HTML file with a suitable program, e.g. Excel, on a PC.

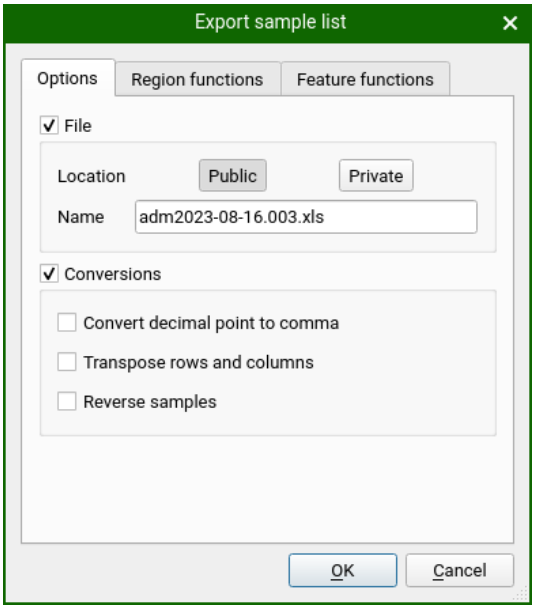
8.4 Exporting the sample list to Excel

Statistics of samples in the sample list can be exported.

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 opens to select the parameters to export.



4 Select the checkbox **File** to save the data as an Microsoft Excel file. Depending on the selected location, the file is saved within the MACSQuantify Tyto Software folder structure either to **Public** or **Private** location.

5 Optional: Select the checkbox **Conversions** and all desired parameters below.

6 Go to the **Region functions** tab to select the regions for export and the % and count statistics for export.

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

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

8 Click **OK**.

9 Go to **File > Copy > Other files** to copy the Microsoft Excel file.

8.5 Printing

The analysis of data can be printed including plots, tables, and the gating hierarchy. It is possible to print selected data files or all data files within the sample list with an applied analysis template. The printer can be connected directly to the MACSQuant Tyto Instrument or via a network connection.

8.5.1 Printing a single data file

To print a singlel data file in the sample list with the current analysis template:



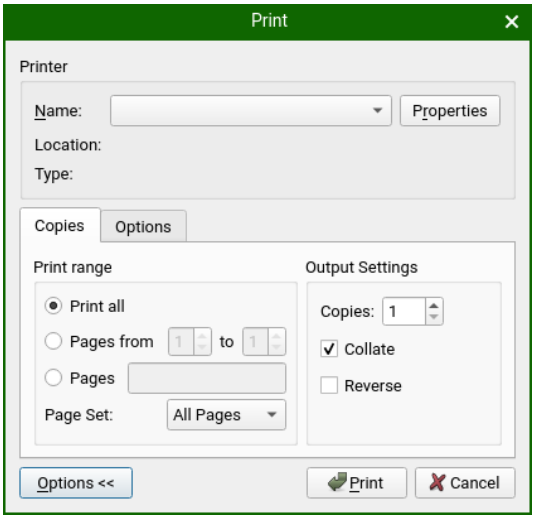
1 Ensure that the current analysis is applied to thel sample.

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

3 Select the desired printer.

4 Click **Options** to access and change the printing options.

5 Click **Print**.



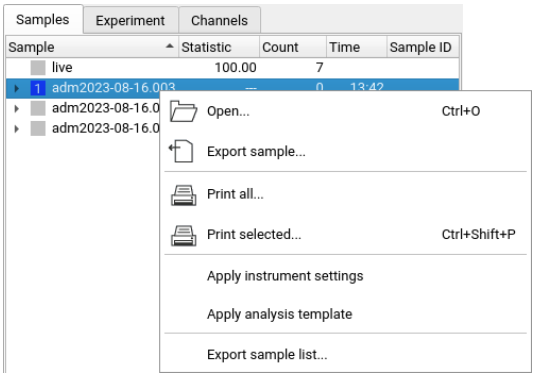
8.5.2 Printing all data files

The **Print all...** option is only accessible in **Analysis mode**. To print all data files in the sample list with the current analysis template:

1 Ensure that the current analysis is applied to all samples.

2 If not activated yet, click the **Analysis** button to activate the analysis mode.

3 Right-click the **Sample list** and select **Print all**.



8.5.3 Printing selected data files

Selected samples can be printed if the same analysis template is applied to the samples. The **Print selected...** option is only accessible in **Analysis mode**.

1 Ensure that the current analysis is applied to the samples.

2 If not activated yet, click the **Analysis** button to activate the analysis mode.

3 To select the samples, press and hold the **Ctrl** key and click on the samples.

4 Right-click the **Sample list** and select **Print selected**.

9 File management

9.1 File storage

Apart from acquired data files, the MACSQuantify Tyto Software also uses other files to save and reload user settings. Files can be saved, opened, and copied from different locations. Within the 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 Instrument and can be accessed by any user.
- Private files are located on the local hard drive of the MACSQuant Tyto Instrument and are only accessible by the logged in user.
- External files are located on an independent file storage device, e.g., a USB flash drive. They can be accessed when the device is connected to the MACSQuant Tyto Instrument.

Files are saved within the **tyto** folder structure on the MACSQuant Tyto Instrument hard drive:

- in a public location in the folder global
- in a private location in folders labeled with the name of the user

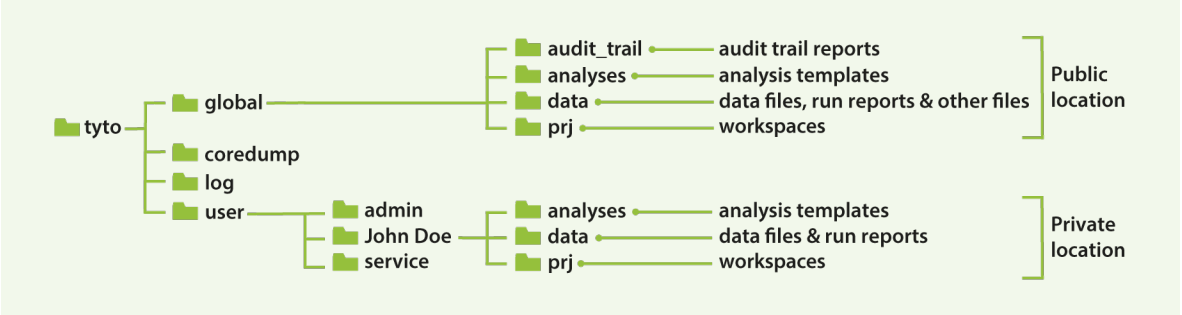


Figure 9.1: Folder structure of the tyto folder on the hard drive of the MACSQuant Tyto Instrument

See **File types on page 9** for an overview of the different file types and their content.

9.2 Managing files

9.2.1 Opening files

Access **Workspace**, **Analysis**, **Data files**, and **Report files**.

1 To open files, perform one of the following:



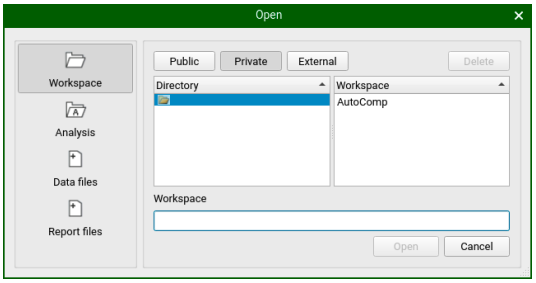
- Click **File > Open**.
- Click the **Open** button in the toolbar.
- Right-click within the **Samples** tab and select **Open**.

2 Select **Workspace**, **Analysis**, **Data files**, 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. For data files, multiple files can be opened at once.

5 Confirm your selection with **Open**.



9.2.2 Saving files

Workspaces and analysis files can be saved in both public or private locations as defined under **UM Administrator on page 97**. Files can also be saved to **External** locations. Data files are saved automatically in **Public** or **Private** depending on user access rights and saving decision after signing in. See **File access rights on page 98**.



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

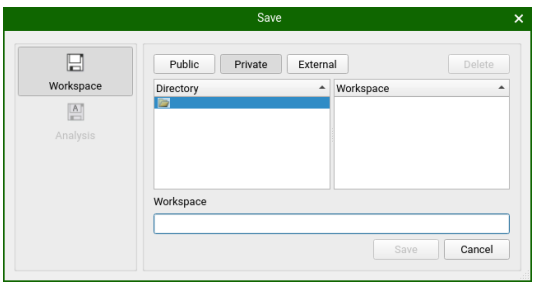
2 Select **Workspace** or **Analysis** to save the file type.

Saving analysis files requires an activated analysis mode.

3 Select the desired saving location (**Public, Private, External**).

4 Assign an appropriate file name.

5 Click **Save**.



9.2.3 Copying files

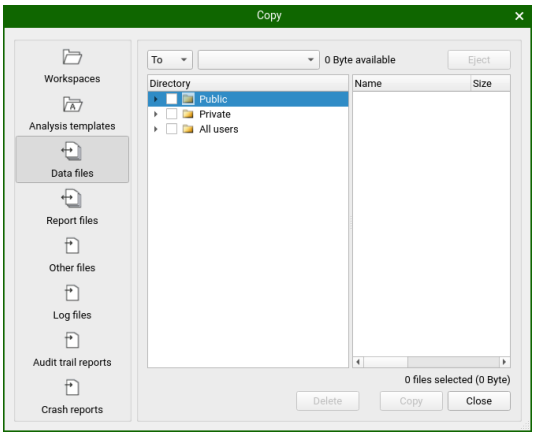
The Tyto Administrator can copy public and private files of all users, except of data files and run reports. See **Table 9.1** for an overview of files which can be copied and deleted by the preconfigured Tyto Administrator role. In case additional copy and deletion rights are required, create a new user role. See **Adding a new user role on page 100**.

Tyto Administrator rights allow access to additional files: **Other files**, **Log files**, and **Crash reports**. See **File types on page 9** for a description of the different file types.

File type	Private location	Public location	All users
Analysis template	Copy, delete	Copy, delete	Copy, delete
Data	Copy, delete	Copy	No access
Run report	Copy, delete	Copy	No access
Workspace	Copy, delete	Copy, delete	Copy, delete
Other files	Copy, delete	Copy, delete	Copy, delete

Table 9.1: File copying and deletion rights of the preconfigured Tyto Adminisrator role.

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



2 To transfer data from the MACSQuant Tyto Instrument to a USB flash drive, insert the stick into a USB port of the instrument. Wait for the device to be recognized.

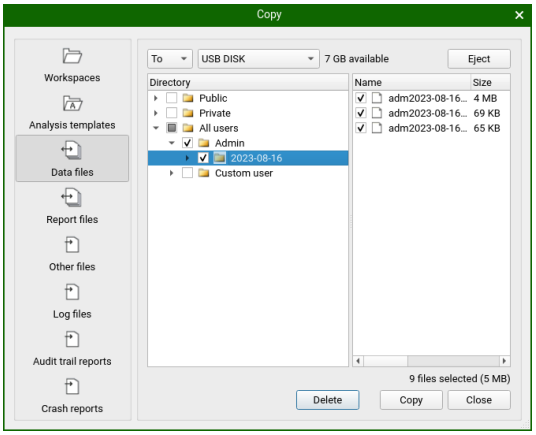
Make sure that the USB flash drive is formatted as exFAT for compatibility with the operating system. If required, change the format on a PC.

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

4 From the dropdown list, select:

- **To**: The selected files are copied to the selected destination. The source folder structure is also preserved at the destination location. See **File storage on page 91**.
- **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.

5 Select the checkbox next to the desired files or folders.



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

7 Close the dialog box to execute another copy command.

8 When using a USB flash drive, click **Eject and close** to safely remove the USB flash drive. Alternatively, select **Remove external media** from the **File** menu.

9.2.4 Deleting files

Files can be permanently deleted from the hard drive of the instrument by a Tyto Administrator. See **Table 9.1** for an overview of files which can be copied and deleted by the preconfigured Tyto Administrator role. In case additional copy and deletion rights are required, create a new user role. See **Adding a new user role on page 100**.

To avoid data loss make sure to copy or backup the files before deletion.

1 To delete files from the hard drive of the MACSQuant Tyto Instrument, select **File > Copy**.

2 Select a file type from the column on the left-hand side of the dialog box.

3 Optional: Select the directory **All users** to delete the selected file type from all user accounts.

4 Click the arrow in front of the directory to show the entire content.

5 Select the checkbox in front of a folder to select all files in a folder or select single files within a folder.

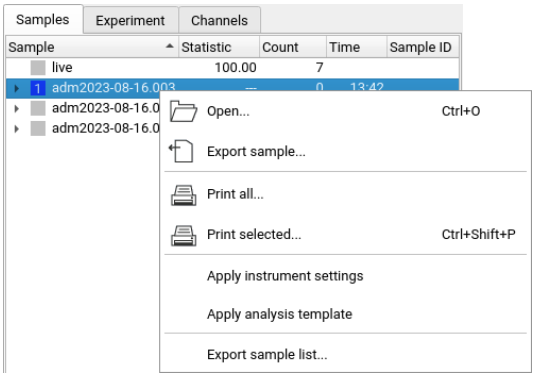
6 Click **Delete**.

7 Selected files are permanently deleted.

9.2.5 Exporting FCS files

The MACSQuant Tyto Instrument will store all acquisition data in the MQD file format. Data files can be exported as CSV file or FCS 3.1 file.

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



2 Select the desired file type (.fcs or .csv) from the dropdown list. If a gating strategy was applied to any of the data, the 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, select the checkbox 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.

10 Administrator

10.1 UM Administrator

The UM Administrator role is required to create user accounts and new user roles and to assign roles to the account. The assigned roles define access to functions and file access rights. See **Table 10.3** for an overview about UM Administrator specific functions.

10.1.1 User roles

The MACSQuantify Tyto Software defines access to functions by assigned user roles to each account. The user roles are assigned by a UM Administrator during setup of a new user account. The roles of an account can be changed by a UM Administrator after setup of the account. The MACSQuantify Tyto Software offers the possibility to use preconfigured roles. A UM Administrator can create new user roles.

User rights define access to functions within the MACSQuantify Tyto Software, access permissions for file types, and access to the user management system. The user rights are defined within a user role. See **Table 10.2** and **Table 10.3** for an overview of user rights based on the different user roles.

System and Standard user roles cannot be changed.

For the 21 CFR part 11 setup, user roles can be assigned via LDAP groups. See **Assigning user roles via an LDAP group on page 103**.

Tyto User role

Tyto Users can plan and run their own experiments. They can define their personal software settings that apply to the private user. Additionally, they can perform a calibration and run a multicolor compensation. They can create analysis templates, data files, and workspaces.

Tyto Administrator role

The Tyto Administrator role includes the same functions of a Tyto User. The user interface is the same. The Tyto Administrator can define personal software settings and additionally global software settings that apply to all users. The Tyto Administrator has access to additional file types. Furthermore, he has the rights to delete certain file types from the hard drive of the MACSQuant Tyto Instrument. In the 21 CFR Part 11 compliant software setup, the Tyto Administrator role allows the export of audit trail events.

Function	Description	Preconfigured role	
		Tyto Administrator	Tyto User
Access to **Edit > Options**	To define user specific software settings	Yes	Yes
Access to **Edit > Options (default)**	To define global software settings for all users	Yes	No
Export audit trail events	To export a report about user activities that is saved as public file	Yes	No
Delete audit trail events	See **Table 10.4**	No	No
Copy and delete analysis templates, workspaces, and other files of other users	To copy and delete analysis templates, workspaces, and other files of other users	Yes	No
Copy and delete data files and run reports of other users	To copy and delete data files and run reports of other users	No	No
Run a calibration	To calibrate the instrument	Yes	Yes
Perform automated compensation	To perform a multicolor compensation	Yes	Yes
Run initial calibration	To run an initial calibration. Required after first installation of the instrument or if requested by technical support for troubleshooting reasons.	Yes	No
Import cartridge config files	To import configuration files of new cartridge types.	Yes	No
Sign run reports (21 CFR part 11 only)	To create a PDF/A report of acquired samples	Yes	Yes
Copy log files	To copy log files to a remote storage location	Yes	No
Access to crash reports	To copy crash reports to a remote storage location	Yes	No

Table 10.1: Access to functions of the preconfigured Tyto roles

File access rights

File type	Preconfigured role			
	Tyto Administrator		Tyto User	
	Private location	Public location	Private location	Public location
Analysis template	Write, delete	Write, delete	Write	Write
Data	Write, delete	Write	Write	Write
Run report	Write, delete	Write	Write	Write
Workspace	Write, delete	Write, delete	Write	Write
Other files	Write, delete	Write, delete	Write	Write

Table 10.2: File access rights of the preconfigured Tyto roles

Data files are stored in either private or public location. They cannot be stored at both locations at the same time. The preconfigured roles have data file write rights for both locations. When logging in to a user account created with a preconfigured role, a pop-up window appears.

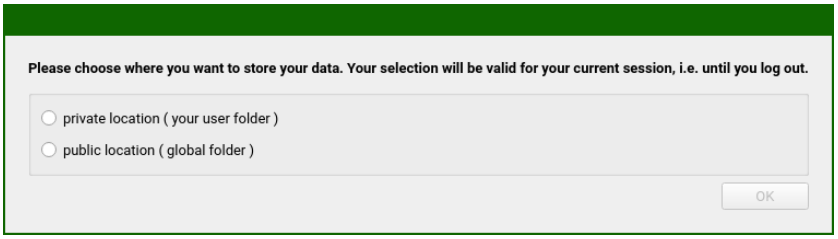


Figure 10.1: Options for the data storage location

UM Administrator role

The UM Administrator role is required to get access to the user management of the MACSQuantify Tyto Software. See **User management rights below** for an overview of UM Administrator functions.

UM user role

The UM user role does not have access to the user management. However, the UM user role provides the rights to read details and rights of the own account. These rights are mandatory to enable a user to sign in to the own account.

User management rights

Table 10.3 lists user management rights of the preconfigured UM roles. The rights can be assigned by a UM Administrator in **Edit > User Management > Roles**.

Function	Description	Preconfigured roles	
		UM Administrator	UM User
Assign role	To assign a role to a user or group	Yes	No
Configure LDAP connection	To configure a connection to an external LDAP server	Yes	No
Create role	To create a new user role	Yes	No
Create user	To create a new user account	Yes	No
Export	To export roles and role assignments of users and groups	Yes	No
Import	To import roles and role assignments of users and groups	Yes	No
Read Password Policy	To read the current password policy	Yes	No
Read details of foreign account	To read the details of all accounts including the own account	Yes	No
Read details of own account	To read the details of own account	Yes	Yes
Read own user details	To read details of own user profile	Yes	No
Read rights of foreign account	To read rights of all accounts including the own account	Yes	No
Read rights of own account	To read rights of own account	Yes	Yes

Function	Description	Preconfigured roles	
		UM Administrator	UM User
Read role	To read the role definition	Yes	No
Read user details	To read detailed information of users	Yes	No
Set user active	To set inactive users active	Yes	No
Set user inactive	To set active users inactive	Yes	No
Unlock user	To unlock user accounts	Yes	No
Update Password Policy	To change the current **Password Policy**	Yes	No
Update own user details	To change details of own user account	Yes	No
Update role	To change a user role	Yes	No
Administer user accounts	To create, change, delete, or deactivate user accounts and roles	Yes	No
Import and export user settings	To import or export user settings from another MACSQuant Instrument	Yes	No
Reset password	To reset a password of a user account	Yes	No
Unlock user accounts	To unlock a user account	Yes	No

Table 10.3: Access to user management functions of preconfigured UM roles

10.1.2 Adding a new user role

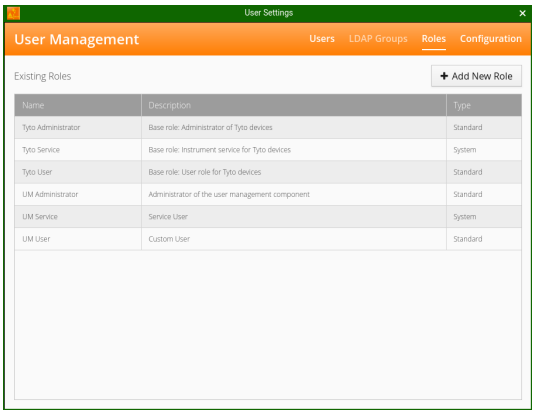
A UM Administrator can add new user roles besides the preconfigured user roles. See **Table 10.3** for a description of user management rights.

1 Sign in as UM Administrator.

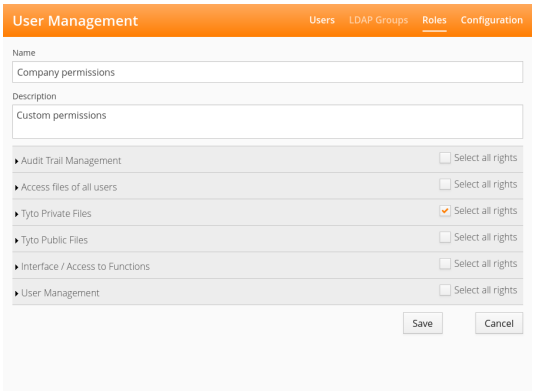
2 Go to **Edit > User management**.

3 Go to the **Roles** tab. The existing user roles are listed.

4 Click the **+ Add New Role** button to add a user-defined role.



5 Enter a new role name and description.



6 Click the arrows in front of the sections to show and change rights.

7 To select all rights at once, select the checkbox **Select all rights** on the right-hand side.

8 Assign the rights as needed. See **Table 10.4**. The following options are available:

- write: enables the user role to save and open the selected file type
- read: enables the user role to open the selected file type
- delete: enables the user role to delete the selected file type from the hard drive of the instrument
- copy: enables the user role to copy the selected file type of other users
- export: enables the user role to create audit trail reports.

9 Click **Save**.

Section of user role	Right options
Audit Trail Management	**Delete** **Export** **Read** These rights are only recommended for Tyto Administrator.
Access files of all users	**Copy** **Delete** These rights are only recommended for Tyto Administrator.
Tyto Private Files	**Write** **Read** **Delete** (only recommended for Tyto Administrator).
Tyto Public Files	**Write** **Read** **Delete** (only recommended for Tyto Administrator).
Interface / Access to Functions	Select **Additional Administrator functions** to enable Tyto Administrator functions. See **Tyto Administrator on page 114**.
User Management	The rights **Read details of own account** and **Read rights of own account** are mandatory to enable a user to sign in to the own account.

Table 10.4: Rights selection for new user roles

10.1.3 Changing a user role

1 Sign in as UM Administrator.

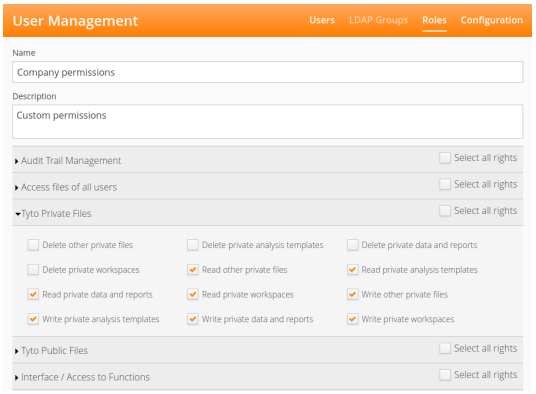
2 Go to **Edit > User management**.

3 Select the **Roles** tab. The user roles are listed.

4 Select the role to be changed.

> System and Standard user roles cannot be changed.

5 Click the arrows to view and change rights. See **Table 10.3** and **Table 10.2**.



6 Click **Save**.

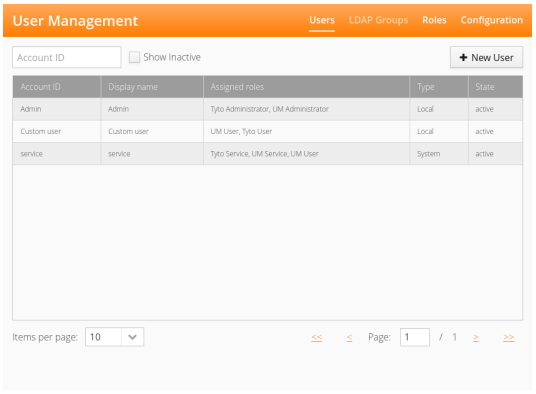
10.1.4 Assigning or changing user roles of a user account

1 Sign in as UM Administrator.

2 Go to **Edit > User management**. The **Users** tab is shown per default.

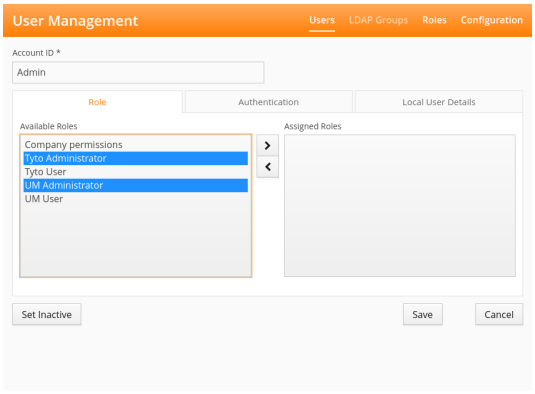
> Users that have roles assigned only via a group assignment are not shown here. See **Assigning user roles via an LDAP group on the facing page**.

3 Select the user to be changed.



4 To add a role, select the desired role from the left panel **Available Roles**. Selected roles are highlighted in blue.

5 Double-click to move it from **Available Roles** to **Assigned Roles**.



The rights to read details and rights of the own account are mandatory to sign in to the own account. The UM User role provides these rights.

6 To remove a role, select the desired role from the right panel **Assigned Roles** and double-click.

7 Click **Save**.

10.1.5 Assigning user roles via an LDAP group

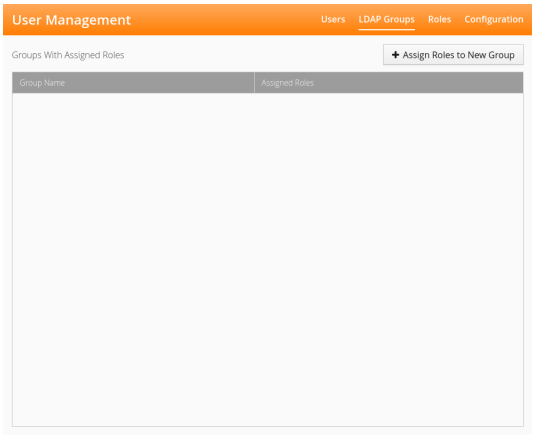
User accounts can be connected to an existing user management system, for example, a Microsoft Active Directory or LDAP.

1 Sign in as UM Administrator.

2 Go to **Edit > User management**. The **Users** tab is shown per default.

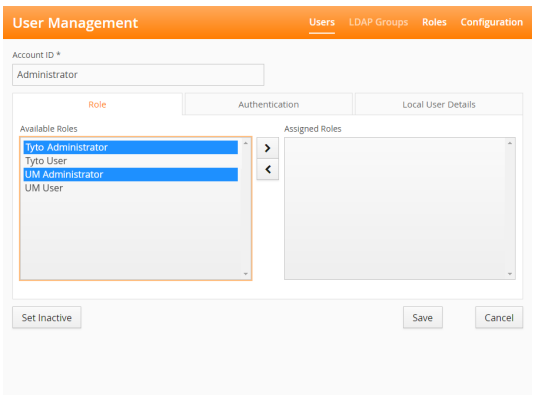
3 Select **LDAP Groups**.

4 Select **+ Assign Roles to New Group** to create a new group and to assign roles to the group.



5 Enter a group name.

6 To add a role, select the desired role from the left panel **Available Roles**. Selected roles are highlighted in blue.



7 Click the **right arrow** button to move the selected role(s) to the panel **Assigned Roles**.

8 To remove a role, select the desired role from the right panel **Assigned Roles**.

9 Click the **left arrow** button to move the selected role(s) to the panel **Available Roles**.

10 Click **Save**.

10.1.6 User accounts

User accounts in the MACSQuantify Tyto Software can be set up locally or connected to an existing user management system, for example, a Microsoft Active Directory or LDAP system. Connection to an LDAP system requires the 21 CFR part 11 compliant option of the MACSQuantify Tyto Software. Creating local user accounts is also possible in the research use only version of the software.

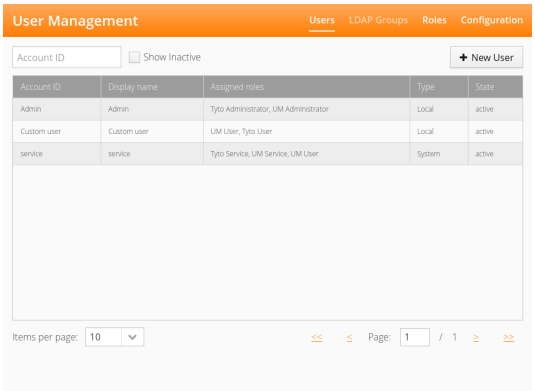
See **Adding a new LDAP user account on page 109** for setup, authentication, and modification of LDAP user accounts.
See **Adding a new local user account on the facing page** for setup, authentication, and modification of local user accounts.
See **LDAP configuration on page 14** for information on how to set up an LDAP connection.

Displaying user accounts

1 Sign in as UM Administrator.

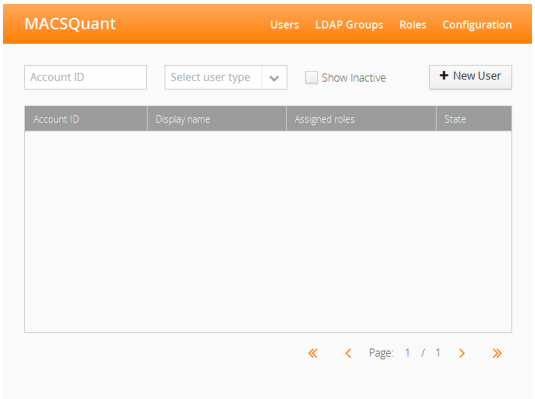
2 Go to **Edit > User management**.

3 Select the **Users** tab.



4 Account ID, Display name, assigned roles, and the current state of the user accounts are shown. The state of a user can be LDAP, active/inactive (local accounts only), or locked (both LDAP and local accounts).

5 Optional (LDAP only): Select **LDAP** or **Local** from the **Select user type** dropdown list to show only one account type.



6 Optional: Select the checkbox **Show inactive** to show deactivated users only. By default, only active user accounts are listed.

Managing local user accounts

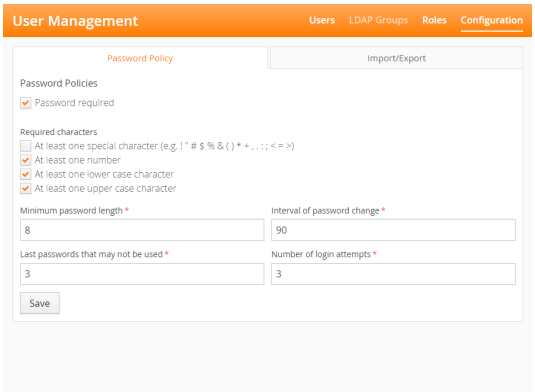
The MACSQuantify Tyto Software offers the possibility to create local user accounts without connection to an LDAP system. If 21 CFR part 11 compliance is required with local user accounts, password settings and user information must be set up in a specific way.

Password configuration

1 Sign in as UM Administrator.

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

3 Select **Configuration**. The **Password Policy** tab is shown per default.



4 If the checkbox **Password required** is selected, every local user account requires a password. If the checkbox is cleared, the user can optionally set a password for the individual account.

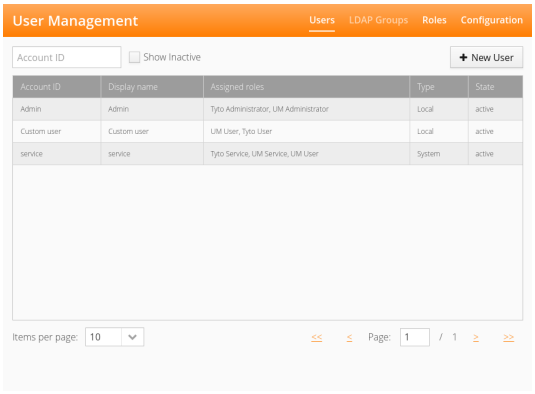
5 Determine the stringency of the password. Adjust password length, required characters, etc. as needed.

6 Click **Save**.

Adding a new local user account

1 Sign in as UM Administrator.

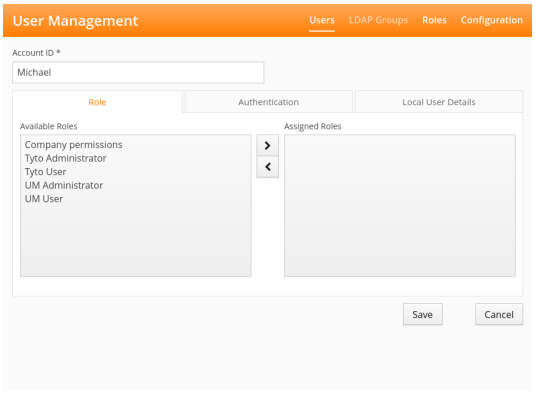
2 Go to **Edit > User management**. Select the **Users** tab.



3 Click **+ New User** to add a user.

4 Enter a user name. Click outside the **Account ID** field. The account ID is not case sensitive.

The user name must not contain special characters or spaces.



5 Assign user roles as described in **Assigning or changing user roles of a user account on page 102**.

6 Proceed with **Authentication of local users below**.

Authentication of local users

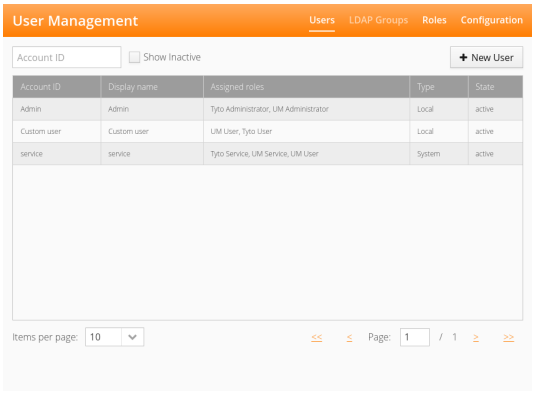
Depending on the selected password configuration, the fields in the **Authentication** tab need to be filled out accordingly. See **Password configuration on the previous page**.

1 Sign in as UM Administrator.

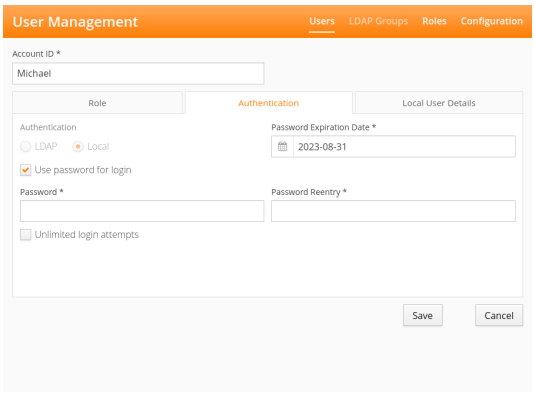
2 Go to **Edit > User management**.

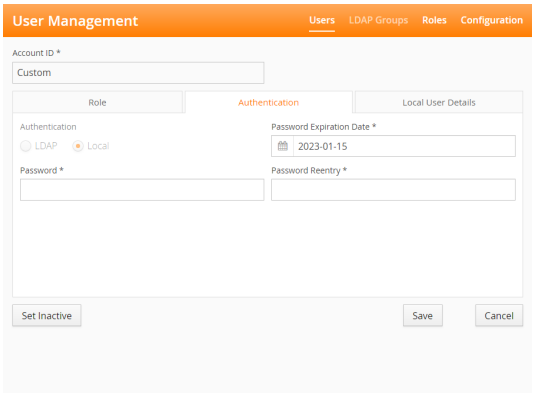
3 Select the **Users** tab. All users with assigned roles are listed. Users that have roles assigned only via a group assignment are not shown here.

4 Select a user.



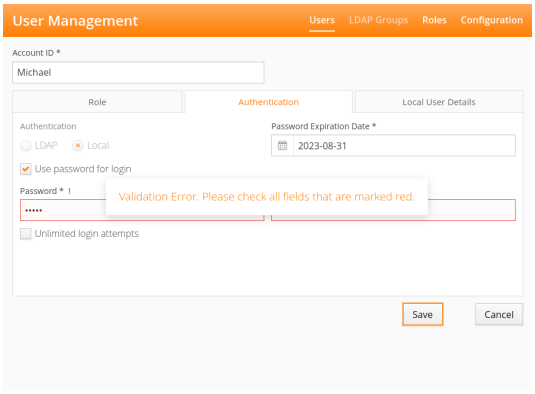
5 Go to the **Authentication** tab. If a password is required, the left screenshot is shown. If no password is required, the right screenshot is shown. The user has the possibility to select the checkbox **Use password for login** to set a password.





6 Optional: Set an expiration date of the password. An expiration date is required for 21 CFR Part 11 compliance. By default, the password expiration date is automatically generated as defined in the password policy. See **Password configuration on page 105**.

7 Optional: Enter a password. If a password is selected that does not meet the requirements, an error message appears.



8 Re-enter the password.

9 Click **Save**.

Adding user details to a local account

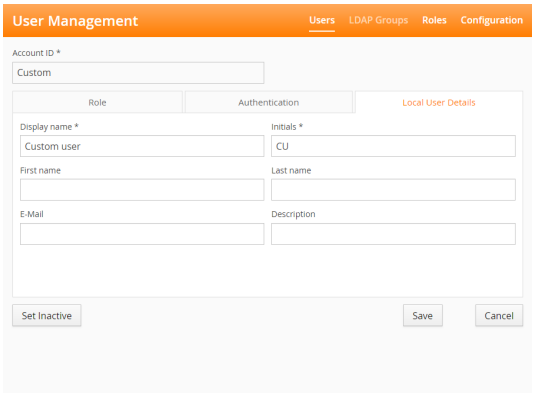
1 Sign in as UM Administrator.

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

3 Select the **Users** tab.

4 Select a user.

5 Select the **Local User Details** tab.



6 Enter information as required. Fields marked with a red asterisk are mandatory. The account ID is not case sensitive.

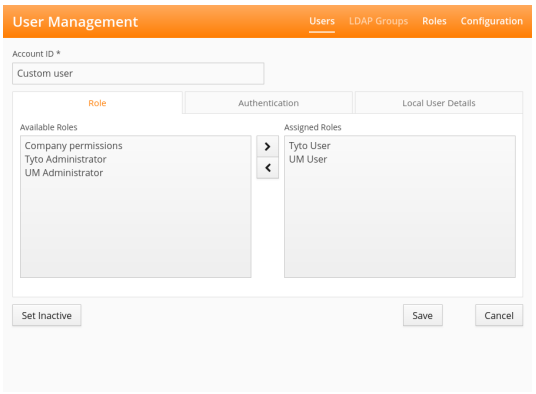
It is recommended to avoid the same display name and initials for different users.

7 Click **Save**.

Inactivating a local user account

User accounts that were set up locally on the instrument cannot be deleted, only inactivated. Inactivated user accounts can be activated again.

1 Sign in as UM Administrator.
2 Go to **Edit > User management**.
3 Select the **Users** tab. All active users are listed.
4 Select the user to be inactivated.
5 Click the **Set Inactive** button.



6 Click **Save**.

Managing LDAP user accounts

To enable compliance according to 21 CFR Part 11, it is possible to connect the MACSQuant Tyto Instrument to an existing user management system, for example, a Microsoft Active Directory or LDAP system. The connection to the user management of the institute needs to be configured during the setup of the MACSQuant Tyto Instrument. This is done by Miltenyi Biotec's instrument service together with the local IT department.

If the instrument is connected to a company's LDAP system, the MACSQuantify Tyto Software passes requests to the user management system to validate the user name and password. When creating user accounts on the MACSQuant Tyto Instrument, account settings such as password policy already existing in the company's LDAP system are used. Users sign into their MACSQuantify Tyto accounts with the user name and password as defined in the company’s user management system.

Adding a new LDAP user account

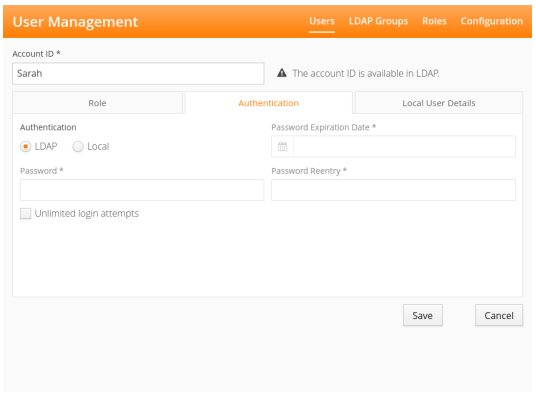
Connect the instrument to your LDAP system before setting up LDAP user accounts.
See **LDAP configuration on page 14**.

Once the MACSQuant Tyto Instrument has been connected to the LDAP system, existing LDAP users can be linked to a new MACSQuant user.
Users can be added as individual LDAP accounts in the **Users** tab via **Edit > User management** or can be added as part of an LDAP user group.

Do not add users simultaneously via LDAP groups and as individual user.

If LDAP groups are used, all users in the LDAP group have the same user roles assigned. New users can be added to the group via the LDAP system of your IT. See **Assigning user roles via an LDAP group on page 103**.
If user accounts are added individually in the MACSQuantify Tyto Software, it is possible to assign roles individually to each account.
Users that have roles assigned via an LDAP group cannot be deleted individually.

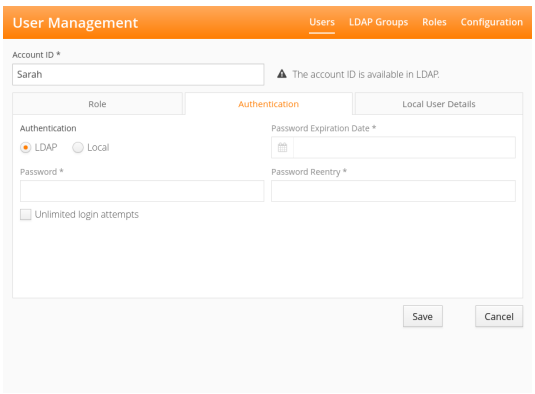
1. Sign in as UM Administrator.
2. Go to **Edit > User management**.
3. Select the **Users** tab.
4. Click the **+ New User** button to add a new user.
5. Enter the user name used in your LDAP system into the field **Account ID**. The account ID is not case sensitive.
6. Click outside the **Account ID** field. If the user account name is entered correctly and located in the directory, a confirmation message appears.



7. Optional: Add local user details. See **Adding local user details to an LDAP account on the next page**.
8. Click **Save**.

Authentication of LDAP users

If an LDAP system is used, the password configuration is determined by the LDAP system to which the instrument is connected. Modification via the **Authentication** tab is not possible.



Adding local user details to an LDAP account

Add local details to an LDAP account if the information provided by the LDAP system is not sufficient for 21 CFR Part 11 compliance.

1 Sign in as UM Administrator.
2 Go to **Edit > User management**.
3 Select the **Users** tab.
4 Select a user.
5 Select the **Local User Details** tab.
6 Select the checkbox **Add Local User Details to LDAP User**.
7 Enter information as desired. Mandatory fields are marked with a red asterisk.
8 Click **Save**.

Deleting an LDAP user account

Individual user accounts that are set up with connection to an LDAP system can be deleted from the instrument. Users can have individual accounts or can be part of an LDAP user group. Users that have roles assigned only via a group cannot be deleted individually.

If users have roles assigned via a group, the user still has access to the instrument, even if the individual account was deleted.

1 Sign in as UM Administrator.
2 Go to **Edit > User management**.
3 Select the **Users** tab. All active user accounts are listed.

Users that have roles assigned only via a group are not shown.

4 Optional: Select the checkbox **Show inactive** to show deactivated users only.
5 Select the user to be deleted.
6 Click **Delete**.
7 Click **Save**.

Unlocking a user account

User accounts are locked if a user attempts to sign in with wrong sign in credentials. This applies also to the lock screen in the 21 CFR part 11 compliant setup.

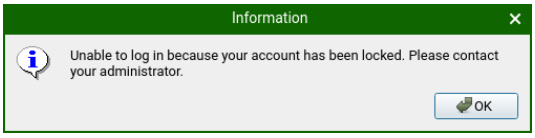
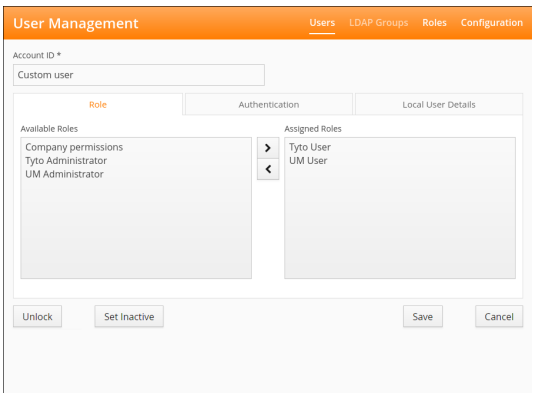


Figure 10.2: Message about locked user account

The number of attempts before an account is locked can be set during password configuration. The default value are three attempts before locking the account. See **Password configuration on page 105**.

1 Sign in as UM Administrator.
2 Go to **Edit > User management**.
3 Select the **Users** tab.
4 Select the locked user account.
5 Click the **Unlock** button to unlock the user account.



Resetting passwords

If a password is forgotten and the user account is locked, see **Unlocking a user account above**.

To change a forgotten password, follow the instructions below.

1 Sign in as UM Administrator.
2 Go to **Edit > User management**.
3 Go to the **Users** tab.
4 Select the user.
5 Go to the **Authentication** tab.
6 Enter a password for the user. The checkbox **Use password for login** must be selected.
7 Enter the password again in the field **Password Reentry**.

Note that the new password must differ from previously used passwords. See the field **Last passwords that may not be used** in the **Configuration** tab.

8 Click **Save**.
9 Share the password with the user.

10 Ask the user to change the password immediately after signing in. See **Changing the password on page 23**.

10.1.7 Importing and exporting user settings

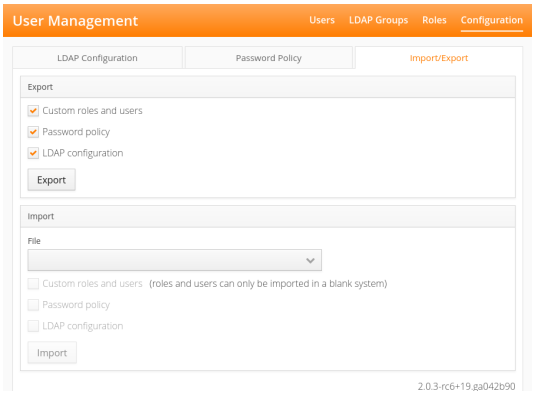
User accounts can be transferred between MACSQuant Tyto Instruments running MACSQuantify Tyto Software version 3.0. The accounts and their settings can be exported from a MACSQuant Tyto Instrument and imported into another MACSQuant Tyto Instrument.

Exporting user accounts

Export of user accounts is only available between instruments with MACSQuantify Tyto Software version 3.0 or higher. For earlier software versions the user accounts have to be set up manually.

1 Sign in as UM Administrator.
2 Format a USB flash drive as exFAT.
3 Connect the USB flash drive to the USB port of the instrument.
4 Go to **Edit > User management**.
5 Go to the **Configuration** tab.
6 Select the **Import/Export** tab.
7 Select the checkboxes **Custom roles and users**, **Password policy**, and/or **LDAP configuration** for export.

Export of the LDAP configuration is only available with a 21 CFR part 11 compliant setup.



8 To start the export, click **Export**.
9 Go to **File > Copy**.
10 Select **Other files**.
11 Copy the Export file to the USB flash drive.
12 Select the USB flash drive from the dropdown list.
13 Click **Eject**.

Importing user accounts

Export of user accounts is only available between instruments with MACSQuantify Tyto Software version 3.0 or higher. For earlier software versions, the user accounts have to be set up manually.

Import of user accounts is only possible into a clean system without any changes to the user management. No users must be created before import.

1 Sign in as UM Administrator.

2 Connect a USB flash drive that contains the settings previously exported from a system.

3 Go to **File > Copy**.

4 Select **From** from the dropdown list.

5 Select the USB flash drive from the dropdown list.

6 Select **Other files**.

7 Select the exported file.

8 Click **Copy**. The file is copied to other files.

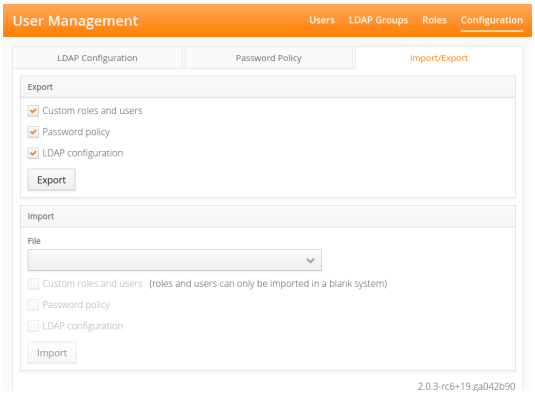
9 Go to **Edit > User management**.

10 Go to the **Configuration** tab.

11 Select the **Import/Export** tab.

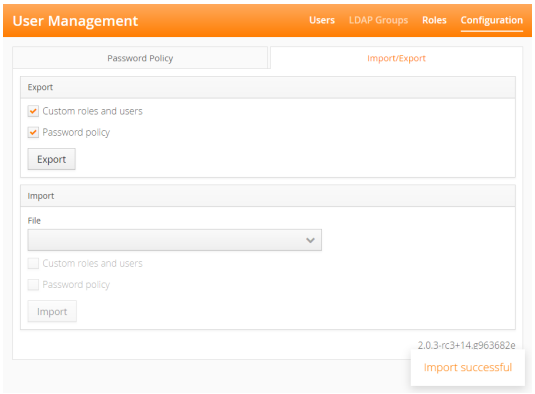
12 Go to the section **Import**.

13 Select the file from the **File** dropdown list to import previously exported user accounts.



14 Select the checkboxes **Custom roles and users**, **Password policy**, and/or **LDAP configuration** for import.

15 Click **Import** to start the import. If the import is successful, a message is shown.



16 Go to **File > Remove external media** to eject the USB flash drive.

17 Select the USB flash drive from the dropdown list.

18 Click **Eject**.

Next time the **Login** screen is shown, all imported users can sign in. The assigned roles are the same as on the instrument the user accounts were exported from.

10.2 Tyto Administrator

The Tyto Administrator can define MACSQuantify Tyto Software related global settings that apply to all users. The Tyto Administrator has also access to further functions compared to the Tyto User of the MACSQuantify Tyto Software. See **Table 10.1** for an overview of all Tyto Administrator functions.

10.2.1 Editing global software options

This chapter describes the **Edit > Options (default)** menu that is accessible by a Tyto Administrator. Software settings in this menu apply to all users of the instrument.

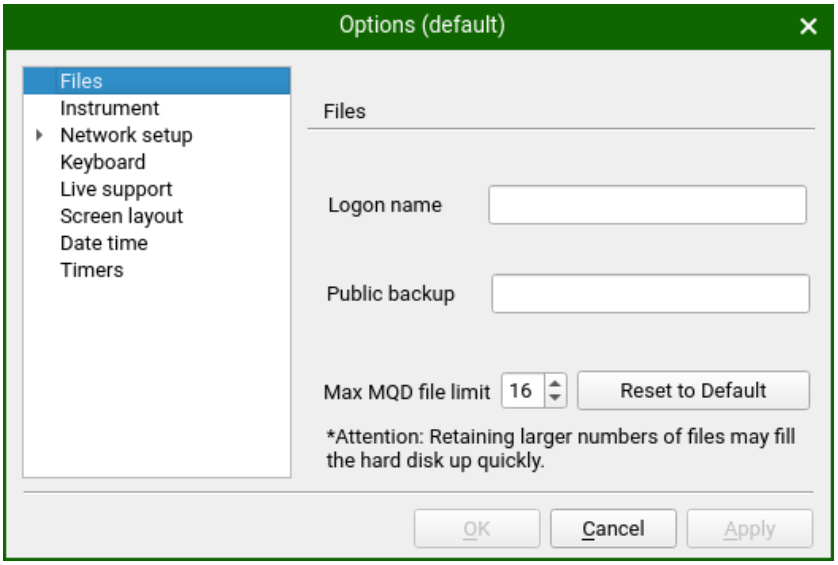


Figure 10.3: The **Edit** > **Options (default)** window

Some changes in **Edit > Options (default)** are only applied after signing out and signing in again.

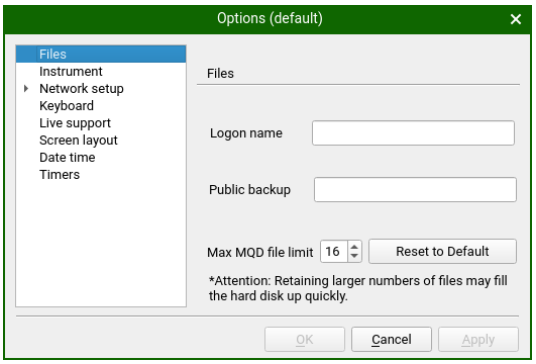
Files

The Tyto Administrator can define a public backup path.

1 Assign a folder for **Public backup** on a network.

2 Sign in as Tyto Administrator.

3 Go to **Edit > Options (default) > Files**.



4 Optional: Enter a **Logon name**. Depending on the network structure, access of the backup folder might require a user name and a password, which can differ from the user sign in credentials for the MACSQuant Tyto Instrument.

5 Click in the field **Public backup** to enter the path of the network folder. Enter a location on the network that can be accessed by all users of the MACSQuant Tyto Instrument. The following path formats are allowed:

- nfs:
  //RemoteServerIP/RemoteFolder
- smb:
  //RemoteServerIP/RemoteFolder
  \\RemoteServerIP\RemoteFolder

6 Click **Apply** to implement the changes and **OK** to close the window.

MQD file limit

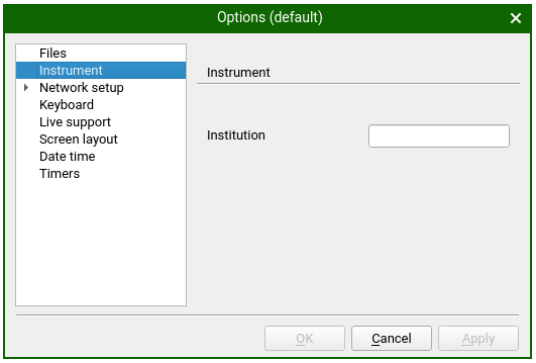
This feature is only available in the 21 CFR Part 11 compliant software setup. Setting the **Max MQD file limit** defines the number of MQD files saved for one run. The default **Max MQD file limit** is set to 16. The file size of one MQD file is 256 megabytes (MB), corresponding to approximately 15 million event counts. After reaching this limit, a new MQD file is generated. When the maximum MQD file limit is reached, the already generated MQD files are overwritten, starting from file number 1.

1 Click **Edit** > **Options (default)** > **Files** to define the **Max MQD file limit**.
2 Enter the number for the **Max MQD file limit**. The maximum number which can be set is 99.
3 Click **Apply** to implement changes and **OK** to close the window.

Setting the **Max MQD file limit** to a high number may fill the hard drive up quickly.

Instrument

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



2 Click **Apply** to implement changes and **OK** to close the window.

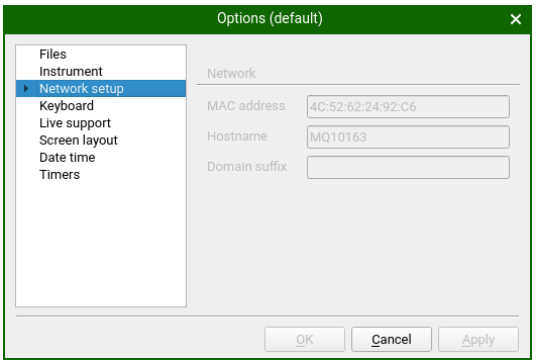
Network setup

Connect the MACSQuant Tyto Instrument to a network if the MACSQuant Live Support is used.

The MACSQuant Tyto Instrument is not intended or designed for integration into a domain infrastructure. Usually, domain infrastructures allow network administrators to maintain all computers on the domain. However, there is no need for any administrative maintenance of the MACSQuant Tyto Instrument. The MACSQuant Tyto Instrument manages user rights and permissions locally as part of the MACSQuantify Tyto Software to allow different user rights.

1 Go to **Edit > Options (default) > Network setup** to access the network settings.

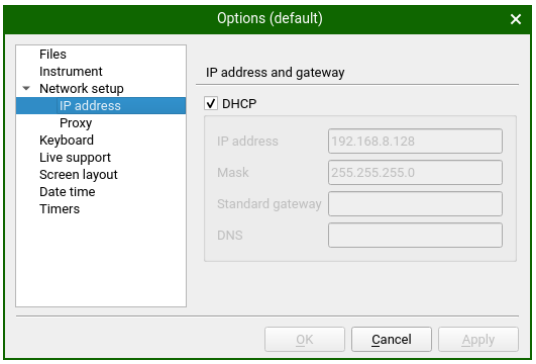
2 Click the arrow to access options for **IP address** and **Proxy**.



IP Address

1 Go to **Edit > Options (default) > Network setup** to access the network settings.

2 Click the arrow to access options for **IP address**.



3 Select the checkbox **DHCP** if IP addresses are assigned automatically.

- Entries for **IP address**, **Mask**, and **Standard gateway** are provided by the network.
- Enter a **DNS** server address. Contact your local IT for details.

4 Clear the checkbox **DHCP** if static IP addresses are used.

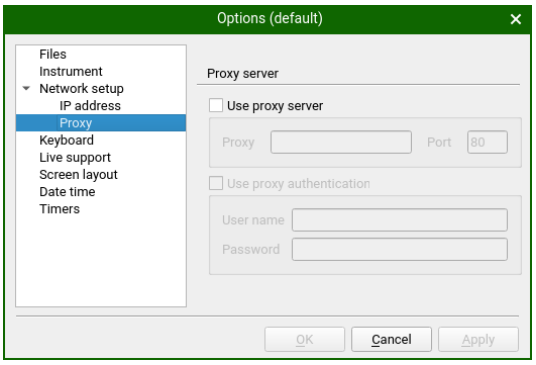
- Enter **IP address**, **Mask**, **Standard gateway**, and **DNS** server address. Contact your local IT for details.

5 Click **Apply** to implement the changes and **OK** to close the window.

Proxy

1 Go to **Edit > Options (default) > Network setup** to access the network settings.

2 Click the arrow to access options for **Proxy**.



3 Select the checkbox **Use proxy server** if the MACSQuant should not have direct access to the internet.

4 Ask your local IT for details about the proxy server and port.

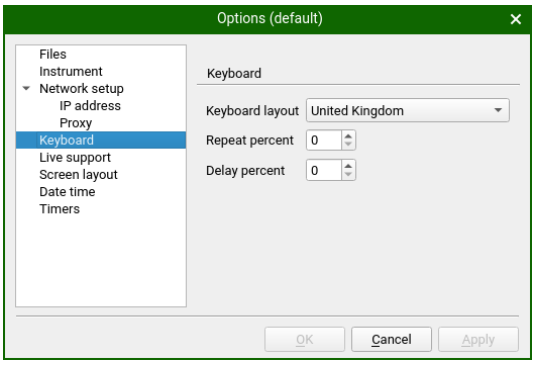
Use port 5938 for MACSQuant Live Support. Alternatively, ports 443 and 80 can be used.

5 Optional: Clear the checkbox **Use proxy authentication**. If user credentials are needed to access the proxy server, the system asks for it.

6 Click **Apply** to implement the changes and **OK** to close the window.

Keyboard

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



2 Use the dropdown list to select an appropriate keyboard layout.

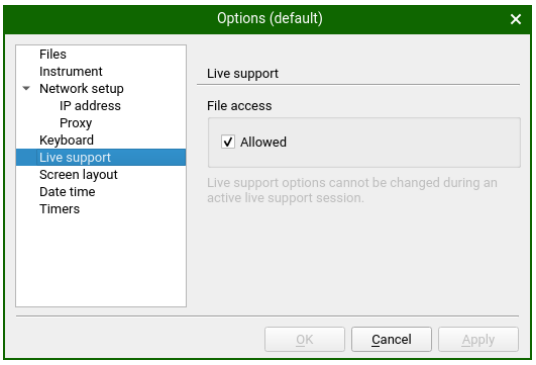
3 If desired, adjust the character repeat rate (**Repeat percent**) and repeat delay (**Delay percent**).

4 Click **Apply** to implement the changes and **OK** to close the window.

Live support

This option enables the Miltenyi Biotec Technical Support to transfer files for troubleshooting during an active live support session. File transfer is only possible if the option **Remote control allowed** or **Operating system access allowed** is granted additionally. If the checkbox for file access is not selected, file transfer is not possilby. For information about the live support connection control settings, see **MACSQuant Live Support on page 131**.

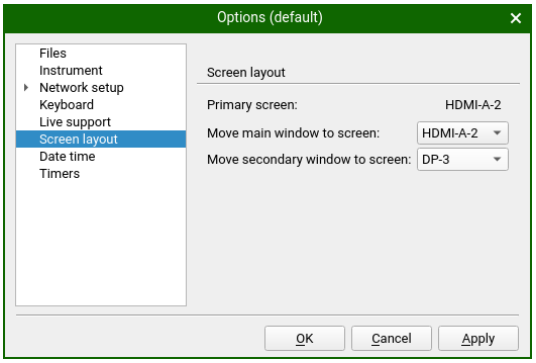
1 Go to **Edit** > **Options (default)** > **Live support** to allow file access during a live support session.



2 Click **Apply** to implement the changes and **OK** to close the window.

Screen layout

1 Go to **Edit > Options (default) > Screen layout** to access, and assign which window is shown on the primary screen, and which window is shown on the touchscreen.

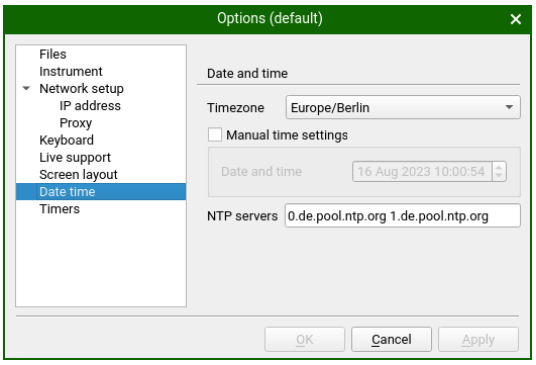


2 Select the desired screen for the main window and the secondary window from the dropdown list.

3 Click **Apply** to implement the changes and **OK** to close the window.

Setting date and time

1 Go to **Edit > Options (default) > Date time** to access and assign time and date settings of the MACSQuant Tyto Instrument.



2 Use the dropdown list to select the desired timezone.

3 Optional: Select the checkbox **Manual time settings** to enter the date and time manually.

4 Click **Apply** to implement the changes and **OK** to close the window.

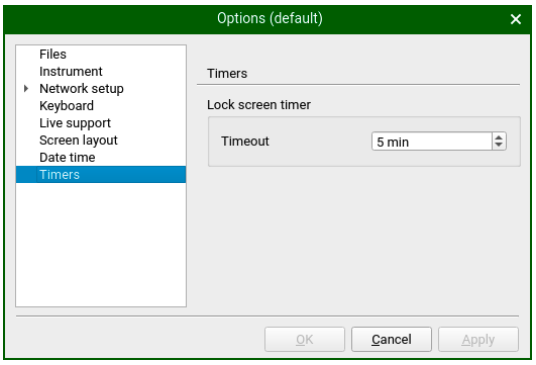
5 Restart the MACSQuant Tyto Instrument.

Restarting the MACSQuant Tyto Instrument is mandatory to ensure correct creation of run reports.

Timers

The lock screen timer defines the idle time (no user input) before the screen is locked automatically. This feature is mandatory in the 21 CFR part 11 compliant software setup.

1 Go to **Edit** > **Options (default)** > **Timers**.



2 Change **Timeout** value as needed.

3 Optional for the research use only version: To deactivate the lock screen timer, clear the checkbox.

4 Click **Apply** to implement the changes and **OK** to close the window.

10.2.2 Initial calibration

The initial calibration is performed by Miltenyi Biotec service personnel during first setup of the instrument or after a service and maintenance visit. The initial calibration is required to ensure proper normalization for Smart Gain technology. The initial calibration should be performed by a Tyto Administrator only upon request by Miltenyi Biotec Technical Support.

1 Switch on the MACSQuant Tyto Instrument. Wait at least 30 minutes for the warm-up of the optical bench before performing the initial calibration.

2 Sign in as Tyto Administrator.

3 Go to the **Tools** tab.

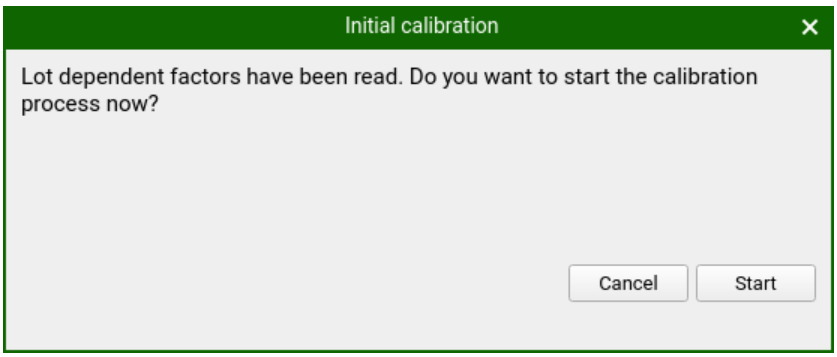
4 Select **Initial calibration**.

5 Vortex the MACSQuant Tyto Calibration Beads for 10 seconds to break up aggregates.

6 Add 18 drops of MACSQuant Tyto Calibration Beads to 9 mL MACSQuant Tyto Running Buffer and vortex. Fill the diluted MACSQuant Tyto Calibration Beads into the cartridge.

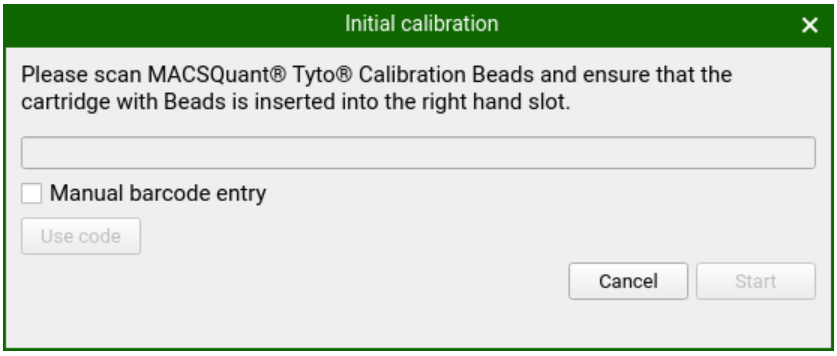
7 Scan and insert the cartridge.

8 Scan the barcode of the MACSQuant Tyto Calibration Beads.





9 Optional: If scanning of MACSQuant Tyto Calibration Beads is not possible, click the **Calibration** button in the toolbar. Select **Manual barcode entry** to enter the barcode of the MACSQuant Tyto Calibration Beads manually.



10 Click **Start** to start the calibration. The gain for each channel is automatically adjusted during calibration.

11 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary.

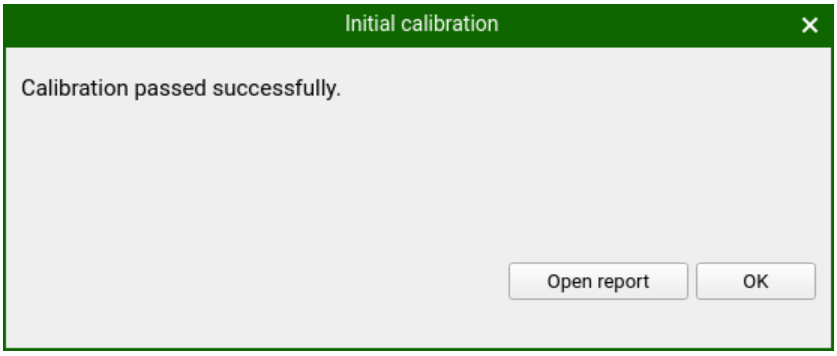
12 A successful calibration is indicated for each fluorescent channel by the column **Info** giving the status **passed**. Furthermore, the table header of the calibration analysis page shows **Calibration: passed** and the instrument progress bar reports **calibration ok**. See **Table 10.5** for details about the shown parameter. The calibration table is automatically saved as HTML file. To export the calibration table, see **Opening the calibration table statistics on page 87**.

2024-06-27 Initial Calibration: passed

Procedure Version: 1.1.0

Channel	Gain Voltage		Stain Index		CV		Info.
BSV	500	500	—	—	—	—	n/a
V1	400	428	141.5	A	24	A	passed
V2	400	480	453.9	A	24	A	passed
SSC	300	300	—	—	—	—	n/a
BSB	500	500	—	—	—	—	n/a
B1	400	522	485.9	A	17	A	passed
B2	400	553	1130.9	A	16	A	passed
B3	400	582	251.2	A	16	A	passed
B4	400	591	16.5	A	17	A	passed
BSR	500	500	—	—	—	—	n/a
R1	400	505	1352.1	A	15	A	passed
R2	400	558	190.0	A	16	A	passed

13 Click **Open report** to open the **MACSQuant Tyto calibration report**. The calibration report is automatically saved as a PDF file.



Parameter	Explanation
Gain	Normalized Smart Gain. See **Smart Gain technology on page 47**.
Voltage	Voltage on PMT
Stain Index	$(MFI_{pos} - MFI_{neg})/2 \times SD_{neg}$
CV	Coefficient of variation based on MFI
Info	Info on success of calibration

Table 10.5: Parameters of the initial calibration shown in the analysis template, MFI: Median fluorescence intensity

10.2.3 Importing cartridge configuration files

The MACSQuant Tyto Instrument has preconfigured cartridge types which are availvable for sorting in the **Experiment** tab. If other cartridge types are used, the configuration file must be imported to the instrument. After the import, the new cartridge type will be available in the **Cartridge** dropdown list in the **Experiment** tab, additionally to the preconfigured cartridge types. Contact the Miltenyi Biotec Technical Support to receive the correct cartridge configuration file.

To import new cartridge configuration files, perform the following steps:

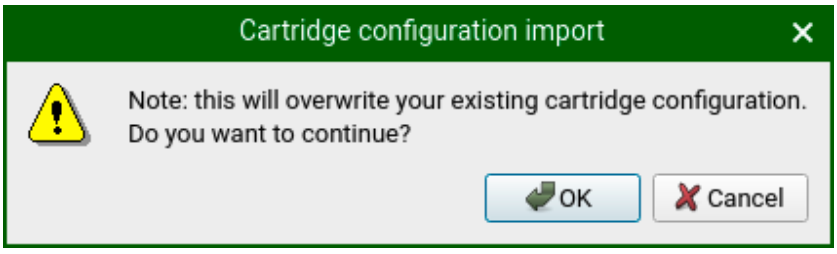
1 Sign in as Tyto Administrator.

2 Connect a USB flash drive containing the cartridge configuration file to the USB port of the instrument.

Make sure that the USB flash drive is formatted as exFAT for compatibility with the operating system. If required, change the format on a PC.

3 Go to **Tools > Import cartridge config files**.

4 A pop-up window appears.



5 Click **OK**.

6 To safely remove the USB flash drive, select **Remove external media** from the **File** menu.

The cartridge type can be selected from the **Cartridge** dropdown list.

10.2.4 Audit trail

The audit trail viewer can be opened and exported by a Tyto Administrator. For the details about tracked events in the audit trail viewer of the 21 CFR part 11 compliant setup, see **Audit trail 21 CFR part 11 on page 13**.

The following user activities are tracked in the research use only version:

Instrument specific activities	User management activities
User sign in (including failed sign ins)	Users added, role assignments
User sign out	Roles added, changed or deleted
Stop run/sort	Change password
Stop calibration	
Stop calibration	
Export system audit trail	

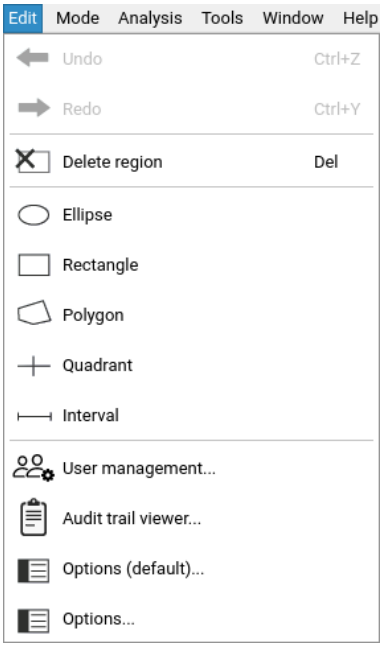
Table 10.6: Tracked activities in the audit trail of the research use only software version

For instructions on exporting the audit trail, see **Exporting audit trail below**.

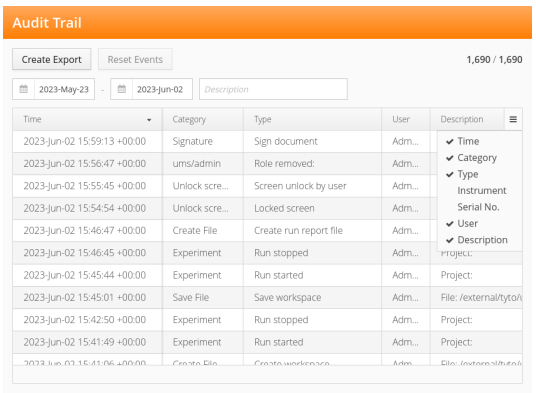
Exporting audit trail

1 Sign in as Tyto Administrator.

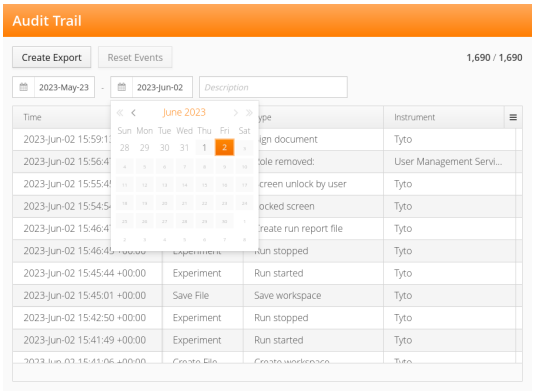
2 Go to **Edit > Audit trail viewer**.



3 Select the symbol in the right-hand corner to customize the shown columns. Select the checkboxes to show the content of a column.

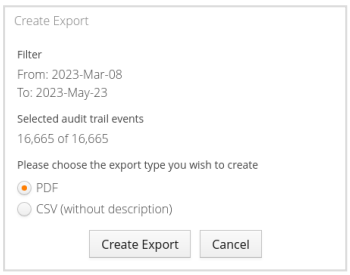


4 Define the time span in the **Audit trail** window.



5 Optional: Enter search criteria in the **Description** field to limit the number of events.

6 Click **Create Export.**



7 Select the file type.

The PDF file export is only available in the 21 CFR part 11 compliant software setup.

8 Go to **File > Copy** and select **Audit trail reports**.

9 Select the file.

10 Click **Copy**.

See **Copying files on page 93** for details how to copy files between instruments or a PC.

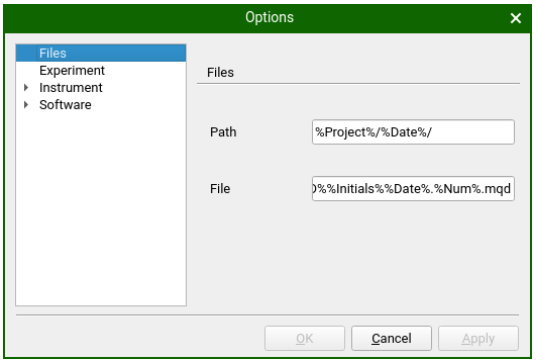
11 Tyto User

This chapter describes functions of the MACSQuantify Tyto Software accessible by Tyto Users. Tyto Users can define their personal software settings via **Edit > Options**. The functions described in this chapter are also accessible by a Tyto Administrator but do not require the Tyto Administrator role.

11.1 Changing path or file naming

The default file name is composed of the sample ID, the initials of the logged-in user, the acquisition date, and an index that counts up beginning with 001. To change the file name, adjust the commands in the **File** text box. Note that the counter will revert back to 001 as soon as you have changed 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 Go to **Edit > Options > Files**.



2 Specify the automatic file name in the **File** text box. See **Identifiers for file naming below**.

> The file name must contain **%Num%** and must end with **.mqd**.

3 Click **Apply** to implement changes and **OK** to close the window.

11.1.1 Identifiers for file naming

To define a path or automatic file name enter free text or the commands listed in **Table 11.1**. The default file naming is **%SampleID%%Initials%%Date%.%Num%.mqd**.

> Use the commands exactly as they are listed below to ensure a correct file naming. The commands are case-sensitive.

Command	Description
%Date%	Acquisition date in the format YYYY-MM-DD
%Description%	Description set by user
%Initials%	Initials of the currently logged in user
%Num%	Adds a number automatically counting up
%Project%	Project name
%SampleID%	Text entered as the sample ID
%SerialNo%	Serial number of the instrument
%Time%	Time of acquisition start in the format hh-mm-ss
%User%	Name of the currently logged-in user

Table 11.1: Identifiers for automatic file naming and folder structure for data storage

11.1.2 Using special characters

Use the ASCII characters A-Z, a-z, 0-9 for any entries. Do not use any special characters except full stop, en dash and underscore (.-_) for file naming, sample ID, or description.

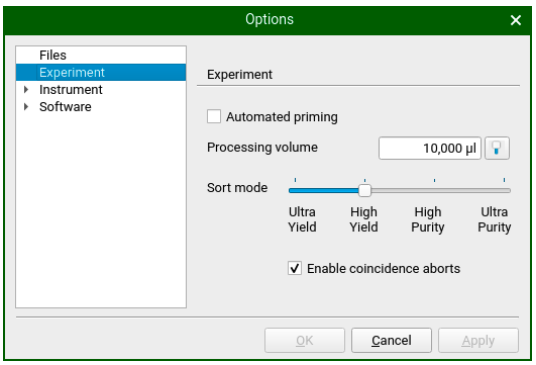
Special characters to avoid
? / \ < > : * | "
CON, PRN, AUX, NUL
COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9
LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9
special characters such as ö, µ, ü, ä, ♦,➢,♥, ☻↕
any characters typed using the ctrl and alt key

Table 11.2: Special characters and reserved names to avoid in the MACSQuantify Tyto Software

11.2 Experiment

Set default values for automated priming, processing volume, and sort mode in the **Experiment** section of the **Options** dialog. The processing volume and sort mode are shown in the **Measure and sort section** of the **Experiment** tab. See **Experiment tab on page 36**. The automated priming feature is shown in the **Misc Controls** tab. See **Automated priming on page 43**.

1 To access the experiment settings, go to **Edit > Options > Experiment**.



2 Assign default values for the respective experiment setting:

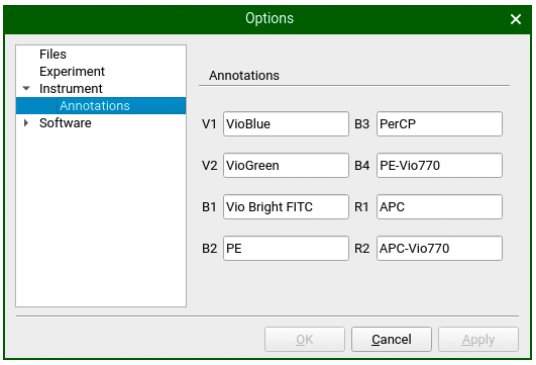
- Modify the default value for the processing volume. Press enter.
- Select the checkbox to activate automated priming as default.
- Select a default sort mode. For information about the sort modes see **Sort mode on page 64**.
- Select the checkbox to enable coincidence aborts as default. Aborting coincidence events can be set for each sort mode individually.

3 Click **Apply** to implement changes and **OK** to close the window.

11.3 Annotations

Annotations provide descriptions of the optical analysis channels that are available on the MACSQuant Tyto Instrument. The default values for the annotations can be changed if other than the shown fluorochromes are used.

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



2 Highlight the default name and enter a new name.

3 Click **Apply** to implement changes and **OK** to close the window. The saved values are shown in the **Experiment** and **Channels** tab.

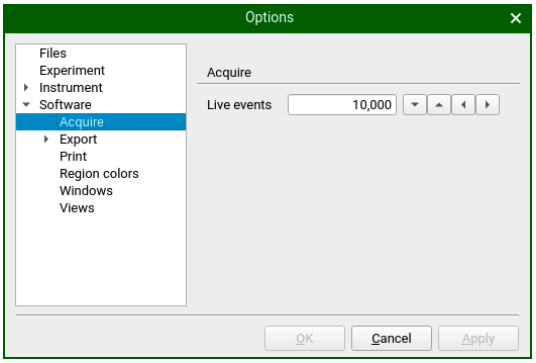
11.4 Software

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

11.4.1 Acquire

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

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



2 Define the number of live events which are shown during acquisition.

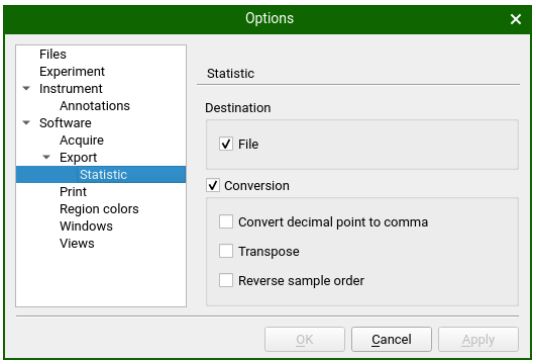
The maximum number of live events is 10.000.

3 Press enter.

4 Click **Apply** to implement changes and **OK** to close the window.

11.4.2 Export statistic

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



2 Select the checkbox **File** in the section **Destination** to export data to an Excel file.

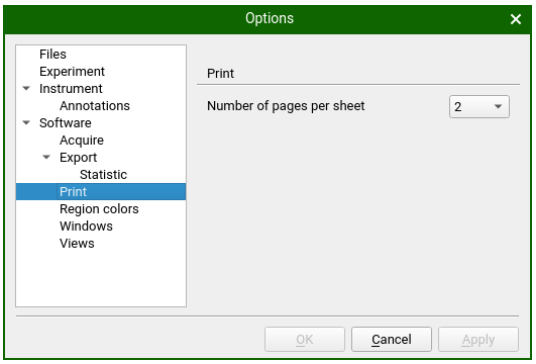
3 Select options for **Conversion** of data by selecting the according checkboxes:

- **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.

4 Click **Apply** to implement changes and **OK** to close the window.

11.4.3 Print

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

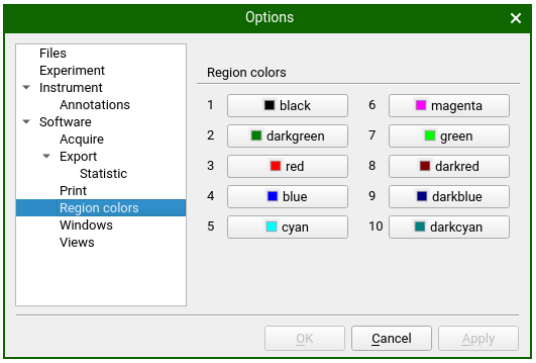


2 Click **Apply** to implement changes and **OK** to close the window.

11.4.4 Region colors

Default settings for region colors can be modified.

1 Go to **Edit** > **Options** > **Software** > **Regions** > **Colors**. Region numbers are assigned in ascending order from one to ten.



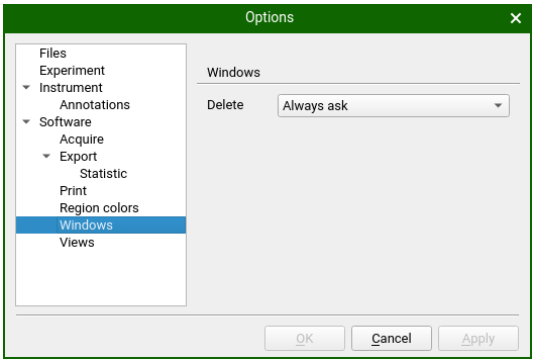
2 Optional: To define custom colors, click the **Color panel** button next to the according region number.

3 Click **Apply** to implement changes and **OK** to close the window.

11.4.5 Windows

Define the default behavior when analysis windows are closed.

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



2 Select the desired option from the dropdown list:

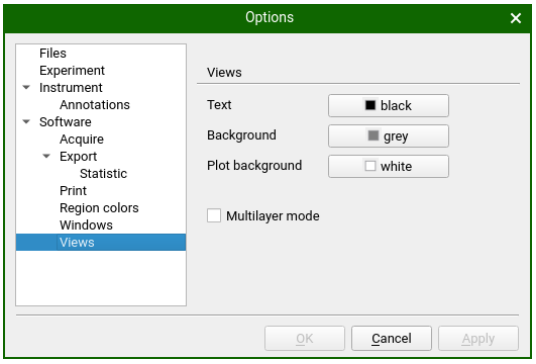
- **Always ask** – The software asks to confirm closing an analysis window (default setting).
- **Never ask** – Analysis windows are closed without asking.

3 Click **Apply** to implement changes and **OK** to close the window.

11.4.6 Views

Define the default display settings for plots, histograms, and tables.

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



2 Select one of the boxes next to **Text**, **Background**, or **Plot background** to change the font color, the background color of the analysis window, or the background color of plots.

3 Define the desired color in the **Select color** window.

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

5 To activate the multilayer mode for every plot, select the checkbox **Multilayer Mode**. See **Multilayer mode display on page 84**.

6 Click **Apply** to implement changes and **OK** to close the window.

12 Technical support

12.1 Technical support

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

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone: +49 2204 8306 3803
technicalsupport@miltenyi.com

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

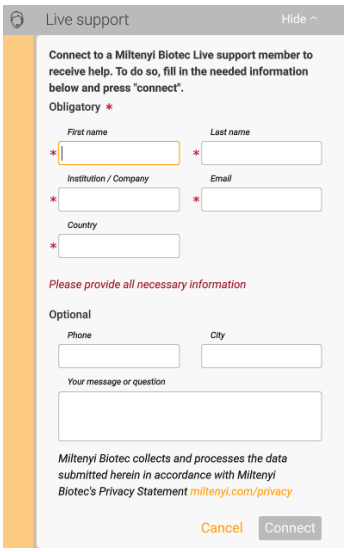
12.2 MACSQuant Live Support

MACSQuant Live Support is a real-time diagnostic service provided by Miltenyi Biotec Technical Support. The Live support is available for all users without any costs 24 h a day from Monday through Friday. Highly trained MACSQuant specialists can be reached to assist with any queries. If required, the MACSQuant Tyto Instrument can be remotely controlled for diagnosis or file transfer. A webcam can be connected to a free USB port at the rear side of the instrument and is automatically detected by the MACSQuantify Tyto Software. Disconnect the webcam during normal operation. The MACSQuant Tyto Instrument must have network access to the internet to use the MACSQuant Live Support. See **Network setup on page 115**.

Use port 5938 for MACSQuant Live Support. Alternatively, ports 443 and 80 can be used. See **Proxy on page 116**.

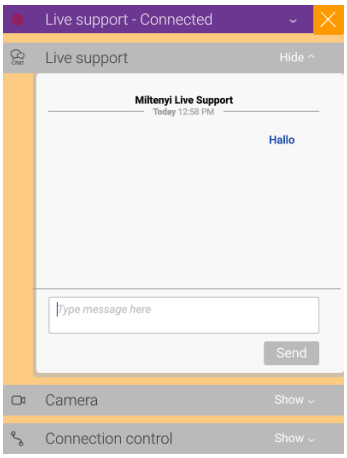
1 Go to **Help** > **Live Support**.

2 Complete all obligatory fields.



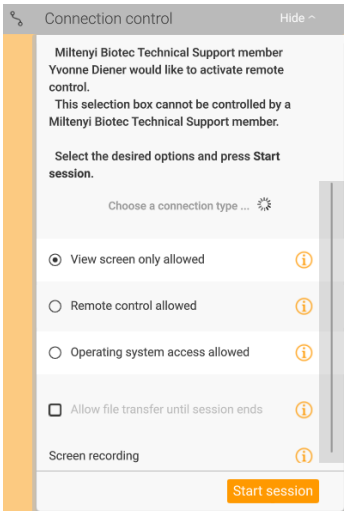
3 Select **Connect** to start the MACSQuant Live Support. A chat window opens.

4 Type a message and click **Send**.



At this time, Miltenyi Biotec Technical Support can only communicate via chat. If remote control is required, it is necessary to enable remote control.

5 Go to **Connection Control** and select the options for remote control by Miltenyi Biotec Technical Support. Click the **'i'** button to view details about the different options.

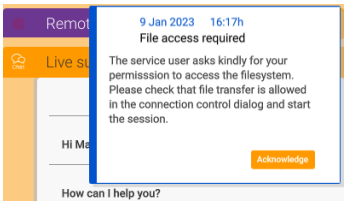


6 Select one of the available options. See **Table 12.1** for further details.

- **View screen only allowed**: Allows the Miltenyi Biotec Technical Support member to see the screen of the instrument but not to interact with it.
- **Remote control allowed**: Allows the Miltenyi Biotec Technical Support member to interact with the instrument like you do.
- **Operating system access allowed**: Allows the Miltenyi Biotec Technical Support member to interact with the instrument like you do and to interact with the operating system.
- **Allow file transfer until session ends**: Allows the Miltenyi Biotec Technical Support member to transfer files to or from the instrument. For example, it allows the download of log files to check for recent software events.
- **Screen recording**: Miltenyi Biotec may use recording to improve its products and services and/or to fix errors in accordance with Miltenyi Biotec's Privacy Statement. Visit **www.miltenyibiotec.com/privacy**.

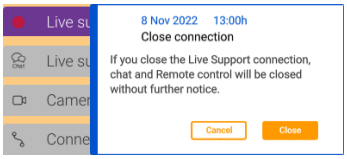
7 Click **Start session**. Miltenyi Biotec Technical Support is now able to remotely control the MACSQuant Tyto Instrument and record the screen based on the selection of connection controls.

8 If file transfer has not been selected before starting the session, a Miltenyi Biotec Technical Support may ask for permission.



9 Click the cross in the upper right corner to end the session.

10 Select **Close** to close the session.



Option	View screen	Remote control	Operating system access	File transfer	Screen recording
View screen only allowed	Yes	No	No	No	Yes
Remote control allowed	Yes	Yes	No	Optional	Yes
Operating system access allowed	Yes	Yes	Yes	Optional	Yes

Table 12.1: Connection control options during MACSQuant Live Support

13 Legal notes

13.1 Limited warranty

Except as stated in a specific warranty statement which may accompany this product or as otherwise agreed in writing by an authorized representative of Miltenyi Biotec, Miltenyi Biotec's warranty to you, the original purchaser and end user ("you" or "your"), with respect to the product accompanied by this limited warranty shall be subject to the following provisions and the general terms and conditions of sale of the company within the Miltenyi Biotec group which supplied the product in effect at the date of purchase. Those terms and conditions of sale may vary by country and region. Nothing in this document should be construed as constituting an additional warranty.

Miltenyi Biotec warrants that this product will operate or perform substantially in conformance with Miltenyi Biotec's published specifications and be free from material defects in material and workmanship, when subjected to normal, proper, and intended usage by properly trained personnel, for the period of time set forth in the product documentation or package inserts accompanying the product (the "Warranty Period").

Miltenyi Biotec agrees, during the Warranty Period, to repair or replace, at Miltenyi Biotec's option, the defective product so as to cause the same to operate in substantial conformance with said published specifications; provided that you shall (a) promptly notify Miltenyi Biotec in writing upon the discovery of any nonconformity or defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim; and (b) return the nonconforming or defective product to Miltenyi Biotec, freight prepaid, only after receipt of a Return Material Authorization ("RMA") from Miltenyi Biotec, which may include biohazard decontamination procedures and other product-specific handling instructions, if applicable.

Miltenyi Biotec shall have no obligation to make repairs, replacements, or corrections to the product or any component thereof required, in whole or in part, as the result of (i) normal wear and tear, (ii) improper handling, installation, operation, storage, service, maintenance, or repair, (iii) failure to follow the instructions, cautions, warnings, and notes set forth in the product documentation provided with the product or provided by Miltenyi Biotec from time to time, (iv) abnormal use, misuse, neglect, abuse, mishandling, misapplication, modification, or alteration of the product, (v) use of the product in a manner for which it was not designed, (vi) causes external to the product such as, but not limited to, power failure or electrical power surges, (vii) use of the product in combination with equipment, accessories, consumables, or software not supplied or approved by Miltenyi Biotec, or (viii) accident, disaster, or acts of God. ANY INSTALLATION, MAINTENANCE, REPAIR, SERVICE, OR ALTERATION TO OR OF, OR OTHER TAMPERING WITH, THE PRODUCT PERFORMED BY ANY PERSON OR ENTITY OTHER THAN MILTENYI BIOTEC AUTHORIZED PERSONNEL WITHOUT MILTENYI BIOTEC'S PRIOR WRITTEN APPROVAL, OR ANY USE OF REPLACEMENT PARTS NOT SUPPLIED BY MILTENYI BIOTEC, SHALL IMMEDIATELY VOID AND CANCEL ALL WARRANTIES WITH RESPECT TO THE AFFECTED PRODUCT.

Miltenyi Biotec's warranty does not cover products sold AS IS or WITH ALL FAULTS, or which had its serial number defaced, altered, or removed, or any consumables or parts identified as being supplied by a third party.

Miltenyi Biotec must be informed promptly if a claim is made under this limited warranty. If a material or manufacturing defect occurs within the Warranty Period, Miltenyi Biotec will take the appropriate steps, at Miltenyi Biotec's option, to make repairs, replacements, or corrections to the product or any component thereof as may be required to restore the full usability of your product. If Miltenyi Biotec determines that a product for which you have requested warranty services is not covered by the warranty hereunder, you shall pay or reimburse Miltenyi Biotec for all costs of investigating and responding to such request at Miltenyi Biotec's then prevailing time and materials rates. If Miltenyi Biotec provides repair services or replacement parts that are not covered by this warranty, you shall pay Miltenyi Biotec therefor at Miltenyi Biotec's then prevailing time and materials rates.

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13.2 Trademarks

MACS, MACSQuant, MACSQuantify, the Miltenyi Biotec logo, Tyto, the Tyto logo, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide.

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