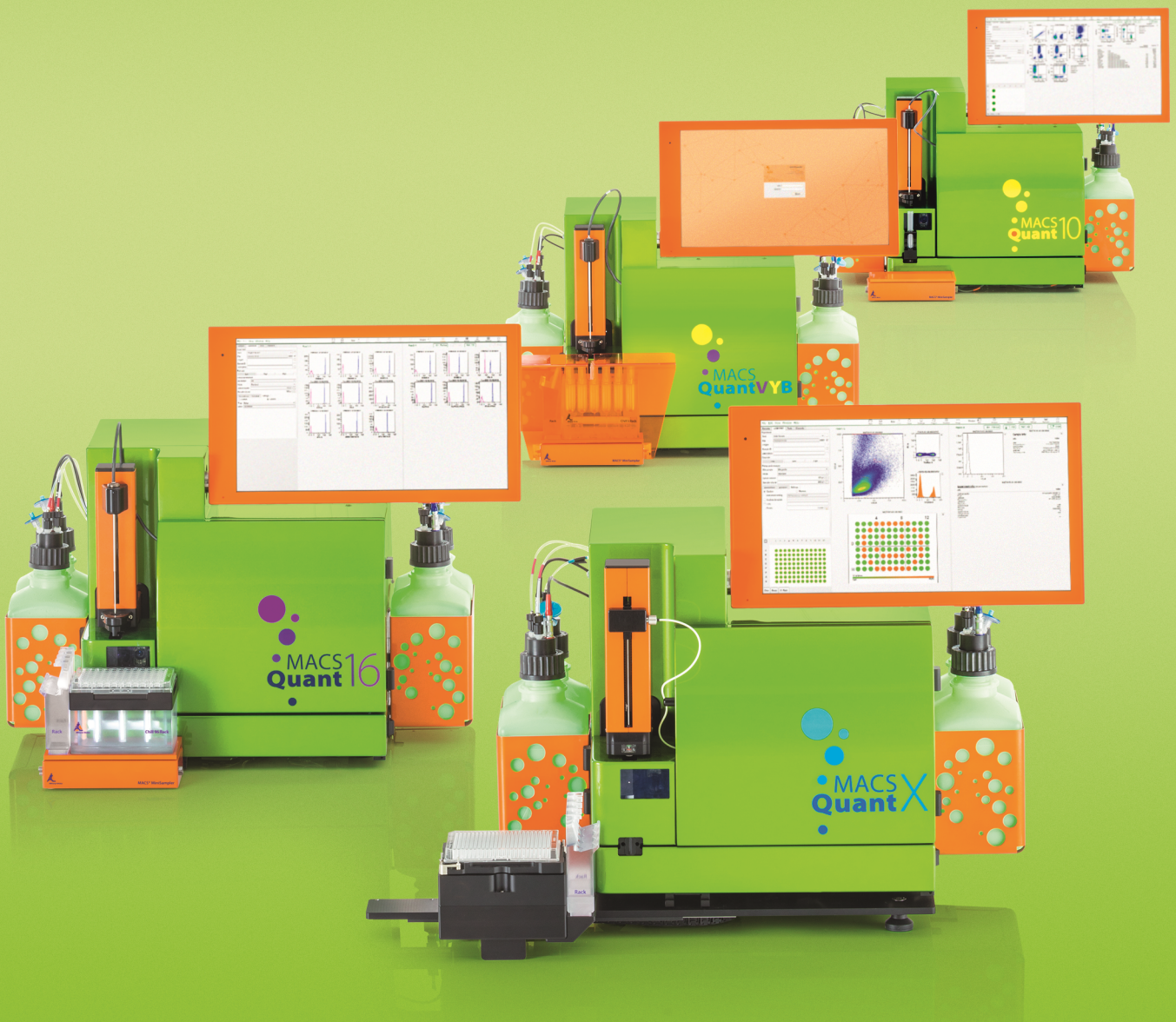
Miltenyi Biotec

MACSQuantify® Software 3.1

User manual



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MACSQuantify™ Software 3.1

User manual

Original instructions

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

⚠ WARNING

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

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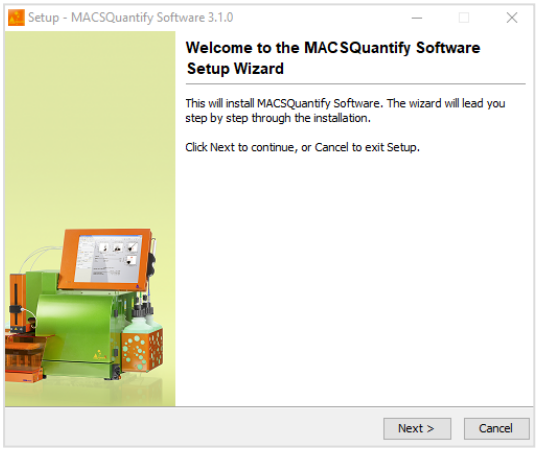
1 Software concept

1.1 Installing the software on a PC

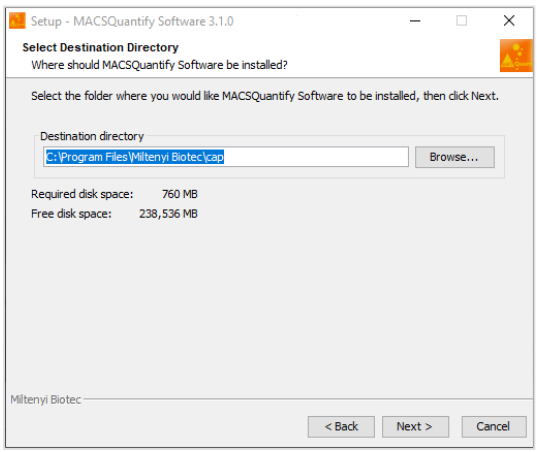
To analyze data from an instrument, the MACSQuantify Software can be installed on a PC. Availability of functions differ depending on whether the software is installed on a PC or on the MACSQuant Instrument. The MACSQuantify Software can be installed on a PC running Windows® 11 operating system.
Download recent software versions from the Miltenyi Biotec website.

1 Start the software installer on the PC.

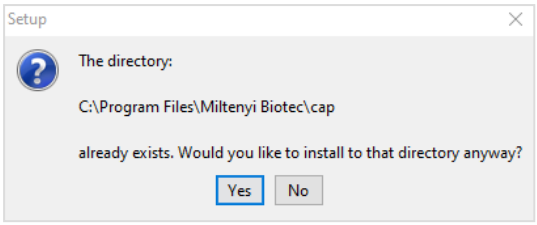
2 Follow the instructions of the installation wizard.



3 Select the destination for the software.

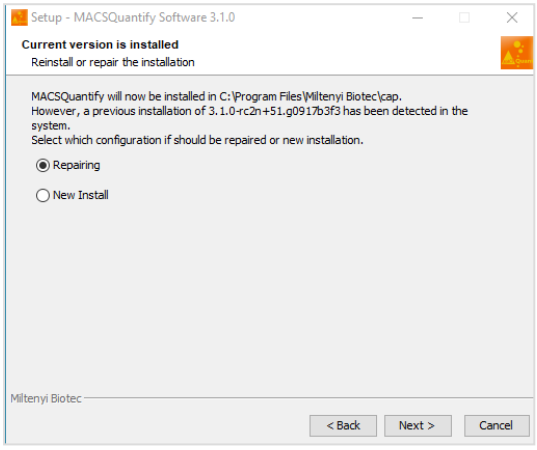


4 If a previous MACSQuantify Software version is installed, a window opens.



5 Click **Yes**.

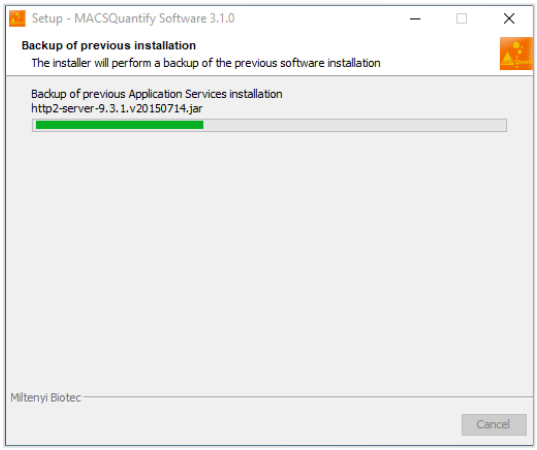
6 To replace the older version with the curent one, click **Repairing**.



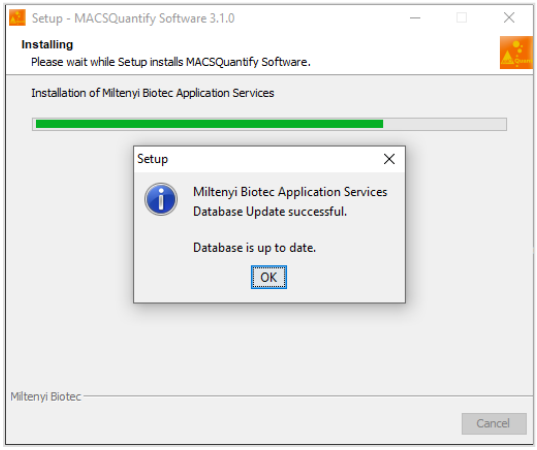
7 To install the new version and also keep the old, click **New Install**.

8 Click **Next**.

9 Wait until files are transferred.

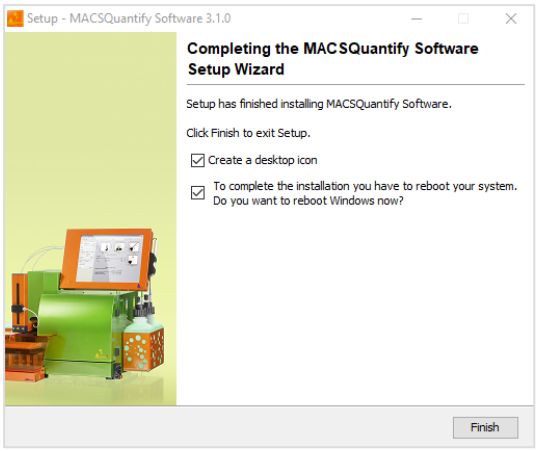


10 Wait until the Application Services are installed. This happens also if no previous version is installed. Click **OK**.



11 Select the box **Create a desktop icon** to create a desktop icon.

12 Select or clear the option to reboot the system after installation. If the box is cleared, restart the PC before using the MACSQuantify Software.

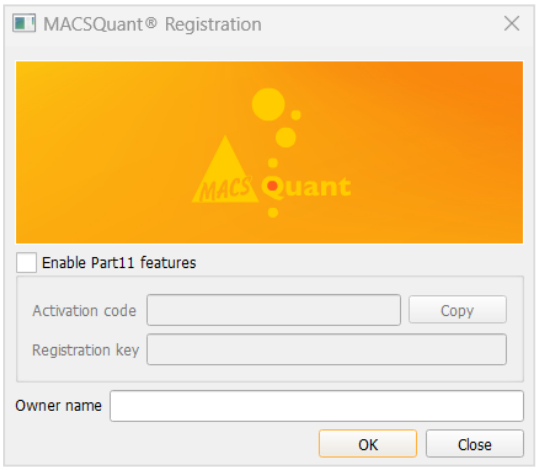


13 Click **Finish**.

14 Right-click the MACSQuantify Software desktop icon. Select the option to run the software as administrator.

15 The sign in window of the MACSQuantify Software opens.

16 Enter an **Owner name**. This name is shown in the upper left corner of the software independent on the signed in user.



17 If a 21 CFR part 11 compliant setup is required, continue with **Software registration 21 CFR part 11 below**.

18 If the software should run as research use only (RUO) version, click **OK**.

19 Optional: If files were transferred, it is required to create the same users with the same user roles as in the previous version. Otherwise, the files are not visible in the MACSQuantify Software.

20 Continue with **First sign-in on the next page**.

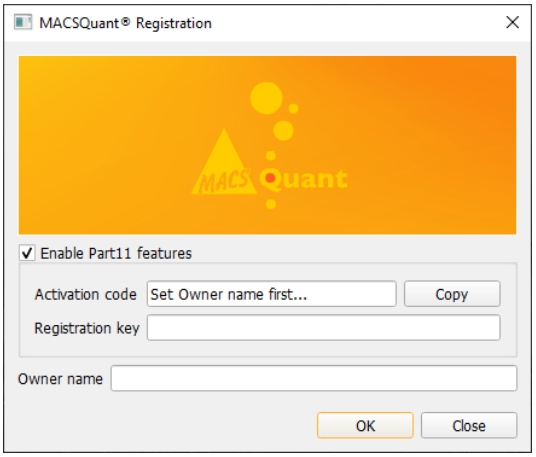
It is recommended to use scaling of 100% on your PC system to avoid problems with data display and resolution.

1.2 Software registration 21 CFR part 11

To use the MACSQuantify Software with a 21 CFR part 11 compliant setup on a PC , it is necessary to register the software. The RUO setup on the PC does not require a registration key.

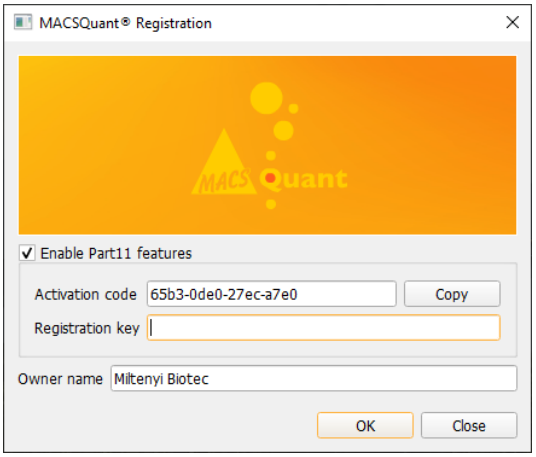
1 Right-click the MACSQuantify Software desktop icon. Select the option to run the software as administrator. The sign in window of the MACSQuantify Software opens.

2 Select the box **Enable Part11 features** to activate the 21 CFR part 11 compliant setup.



3 Enter an **Owner name**. This name is shown in the upper left corner of the software independent of the signed in user.

4 An **Activation code** is shown in the window.



5 Use the activation code to request a registration key from Miltenyi Biotec Technical Support. Send also the name of the complete software version to Miltenyi Biotec Technical Support.

6 Enter the registration key.

7 Click **OK**.

8 Continue with **First sign-in below**.

1.3 First sign-in

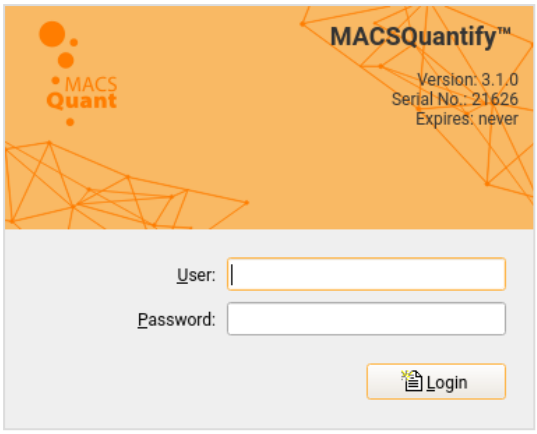
Use the system user **first_setup_account** for the first signing and creation of new users.

> Change the password of the first setup account after a new user with MQ Administrator and/or UM Administrator role has been created. See **User roles on page 20**.

1 Start the MACSQuantify Software.

2 Enter **first_setup_account** as user.

3 Enter **first_setup_account** as password.



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

5 Add a new user with the UM Administrator and MQ Administrator role. See **Adding a new local user account on page 173**.

6 Add new users as required in the new user account or import user settings. See **Importing user accounts on page 180**.

7 Click the username in the upper left corner.

8 Select **Change password**.

9 Change the password of the account.

1.4 Copying files from 3.0 to 3.1

Files from MACSQuantify Software version 3.0 can be copied into the MACSQuantify Software version 3.1. This can be automatically done during installation. See **Installing the software on a PC on page 13**. Alternatively, files can be transferred manually.

1 Start the MACSQuantify Software 3.1.

2 Sign in as UM Administrator.

3 Create the same users with the same user roles as in the MACSQuantify Software 3.0. See **User accounts on page 172** and **User roles on page 20**.

4 Copy the required files from MACSQuantify Software 3.0 to MACSQuantify Software 3.1. See **Copying files to an external location on page 150**.

1.5 File types

This chapter gives an overview about the different file types and their content.
The MACSQuantify Software uses different file types to save acquired data and information about the experiment settings and analysis of samples. See **Table 1.1**. For detailed information on file management, see **Data storage on page 147**.

File type	Saving options	Access via
Workspace	private	**File > Open** **File > Copy**
Instrument setting	public and private	
Experiment	public and private	
Analysis template	public and private	
Data file	public or private	
Reports	public or private	**File > Copy**
Other files	N/a	
Log files	N/a	
System audit trail	N/a	
Crash reports	N/a	

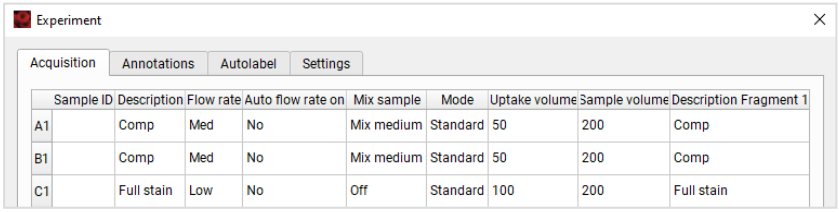
Table 1.1: File types and saving options on the MACSQuant Instrument

To copy log files, system audit trails, and crash reports requires the MQ Administrator role.

When starting a measurement, a MACSQuant data (.mqd) file is automatically saved. Information about the instrument setting, experiment, and analysis template are saved within the data file.
It is possible to retrieve the instrument setting and analysis template from the data file and to store it separately. This can be used if an instrument setting, experiment, or analysis template should be used for another acquisition or data analysis.

1.5.1 Experiment file

Experiment files can be used if similar assays and samples are acquired repeatedly. An experiment file contains all information defined in the **Experiment** tab, for example, flow rate, mixing, uptake volume, and sample ID for a particular sample or a set of samples. The experiment file also contains information about the used instrument settings and analysis templates if applied. See **Figure 1.1** for an overview of the loaded experiment setting.



	Sample ID	Description	Flow rate	Auto flow rate on	Mix sample	Mode	Uptake volume	Sample volume	Description Fragment 1
A1		Comp	Med	No	Mix medium	Standard	50	200	Comp
B1		Comp	Med	No	Mix medium	Standard	50	200	Comp
C1		Full stain	Low	No	Off	Standard	100	200	Full stain

Figure 1.1: The experiment table

Experiment files can also be generated in the PC version of the MACSQuantify Software and can be copied to the MACSQuant Instrument.

1.5.2 Instrument setting file

Instrument setting files contain photomultiplier tube (PMT) gains, channel scales, compensation matrix, height, width, area, threshold selection, annotations, and the doublets processing algorithm. The instrument settings can be saved and reloaded to use it with other samples.

Instrument settings can be saved as a separate instrument setting file, for example, after running a compensation. Alternatively, the instrument setting can be retrieved from any MACSQuant data file. For details how to use instrument settings see **Using an instrument setting on page 77**. Default instrument settings from the PMT calibration are automatically saved as public files. Manually created instrument settings can be saved either as private and public files in the folder **device** depending on the access permissions of the user. See **Data storage on page 147**.

1.5.3 Analysis template file



An analysis template is a predefined layout for acquired data and can consist of plots, statistics, tables, and a gating strategy. When an analysis template is saved and opened again, it does not contain any data.
Analysis templates can be created during analysis of an acquired data file. 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 **Creating an analysis template on page 105**.
An existing analysis template can be assigned to a sample prior to acquisition. The analysis template is then saved as part of the .mqd data file. For further details see **Applying analysis templates on page 135**.
If required, the analysis template file can be retrieved from the MACSQuant data file to be saved separately. Saved analysis templates can be applied to acquired data. See **Applying analysis templates on page 135**.

1.5.4 Data file



MACSQuant data files (.mqd) are automatically saved when a measurement is started. The MQD file not only contains acquired data, but instrument settings, analysis template, and experiment settings used to acquire it. The instrument setting file and analysis template can be retrieved from each MQD file to save the information separately to use them for another acquisition or data analysis.
Data files can only be saved as public or private files depending on the access permissions of the user. The MACSQuantify Software prevents overwriting an existing data file when a measurement is started.

1.5.5 Workspace file



A workspace file combines file types including a link to the data files. It contains the following information:

- Sample list: samples currently shown in the sample list
- **Experiment** tab: currently shown experiment parameters
- Instrument setting: current instrument setting including compensation and channel settings
- Analysis template: current analysis view and template if selected

Loading a workspace re-opens the data files with the settings used at the point of storing the workspace. Parameters from the **Experiment** tab are loaded together with the applied instrument setting and analysis template.

If data files saved in a workspace are moved or deleted, then the workspace cannot be used anymore. The workspace only contains the link to the data file.

1.5.6 Reports



Reports contain all the report types that can be exported by MACSQuant Instrument. It contains the following files:

- Analysis reports: export of electronically signed documentation of data analysis. See **Analysis reports on page 250**.
- Performance QC & tracking reports: PDF export of the generated Performance QC & tracking charts. See **Generating Performance QC & tracking charts on page 231**.
- Printout of analysis pages: PDF export of the analysis pages as shown live on MACSQuant Instrument. See **Print to PDF function on page 144**.

1.5.7 Other files



Other files contain sample lists exported to Excel or screenshots made on the MACSQuant Instrument. See **Exporting the sample list to Microsoft® Excel on page 146**.

1.5.8 Log files



Log files contain information about actions executed by the MACSQuantify Software on the MACSQuant Instrument. Log files are created automatically by the software after restart. 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 an MQ Administrator account. They are important for troubleshooting and can be sent to Miltenyi Biotec Technical Support.

1.5.9 System audit trail



System audit trails can be created and exported by an MQ Administrator. Depending on the setup of the MACSQuantify Software, it provides an overview about different user activities. See **Audit trail on page 191** and **Audit trail 21 CFR Part 11 on page 241**.

1.5.10 Crash reports



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

1.6 User roles

The MACSQuantify Software defines access to functions by user roles.

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

- MQ Custom: user of the MACSQuant Instrument with access to the Custom user interface
- MQ Administrator: user of the MACSQuant Instrument with access to the Custom user interface and access to additional administrator specific functions
- MQ Express: user of the MACSQuant Instrument with access to the simplified Express user interface

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

- UM Administrator: role with access to user management related functions
- UM User: role without access to the user management

To access audit trail system (ATS) related functions, following preconfigured role is available:

- ATS User: role with options to view, export, or reset the audit trail

To access the Express Modes, following preconfigured role is available:

- EM Superuser: role with options to access all the installed Express Modes on the MACSQuant Instrument.

The MQ and UM Service preconfigured user roles are intended for service login during a service visit. The MQ System Automation, QC User and QC Analyzer user roles are intended to be used in the context of MB software solutions.

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 10.5** 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 163**.

2 Basic functions

2.1 Switching on the instrument

1 Use the main switch on the right-hand side of the instrument to switch the instrument to standby mode.

A red LED in the lower left corner of the monitor indicates that the instrument is in standby mode.

2 Tap the touchscreen to start the MACSQuantify Software.

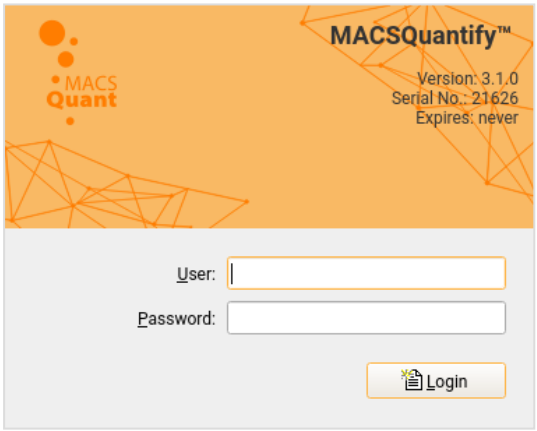
A sign in window appears after a short initialization phase.

2.2 Signing in

1 If the instrument is in standby mode, tap the screen to start the MACSQuantify Software.

2 Enter username and password.

3 Click **Login**.

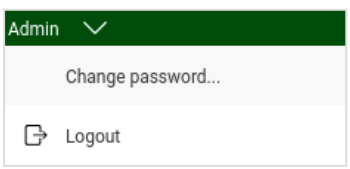


After signing in, the instrument is in data analysis mode. Data acquisition is not yet possible. Prime the instrument before proceeding. See **Priming the instrument on page 41**.

2.3 Signing out

1 To sign out, click the username in the upper left corner of the screen.

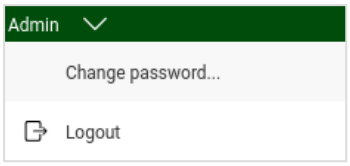
2 Select **Logout** from the dropdown list.



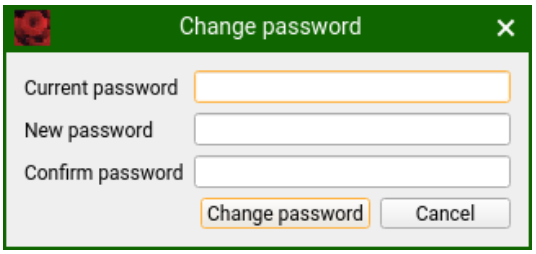
2.4 Changing password

If your instrument is connected to an LDAP system, see **Authentication of LDAP users on page 177**.

1 Click the username in the upper left corner of the screen.



2 Select **Change password...** to open the **Change password** window.



3 Enter the current password. Leave empty if no password is used yet.

4 Enter the new password.

5 Confirm the new password.

6 Select **Change password** to save the new password.

2.5 Switching off the instrument

Before switching off the instrument, start the shutdown procedure. During the shutdown procedure, the fluidic system is cleaned and filled with MACSQuant/MACSima Storage Solution. Automatic shutdown is enabled by default after a defined idle time. An MQ Administrator can change the timer settings in **Edit > Options (default) > Timers > Standby Timer**. See **Timers on page 187**.

1 Click the **Power** button in the upper right corner of the software to shut down manually.

1 To close the MACSQuantify Software on a PC, go to **File > Quit**.

2 Select **Instrument off** to switch the instrument to standby mode, or select **Data analysis mode** to proceed with data analysis after the shutdown procedure. After a seven-minute washing procedure (default setting), the instrument switches to standby or data analysis mode.

3 From data analysis mode, select **Instrument off** to switch to standby mode.

4 From standby, use the main switch on the right-hand side of the instrument to switch off completely.

3
User interface

This chapter describes the structure of the MACSQuantify Software used by the MQ Administrator and Custom users. Elements of the graphical user interface and icons are shown and explained. See **Express user on page 215** for an overview about the user interface for Express users. It is not required that Express users read this chapter.

The MACSQuantify Software user interface allows MQ Custom users and MQ Administrators to program parameters for data acquisition and data analysis. It consists of following elements.

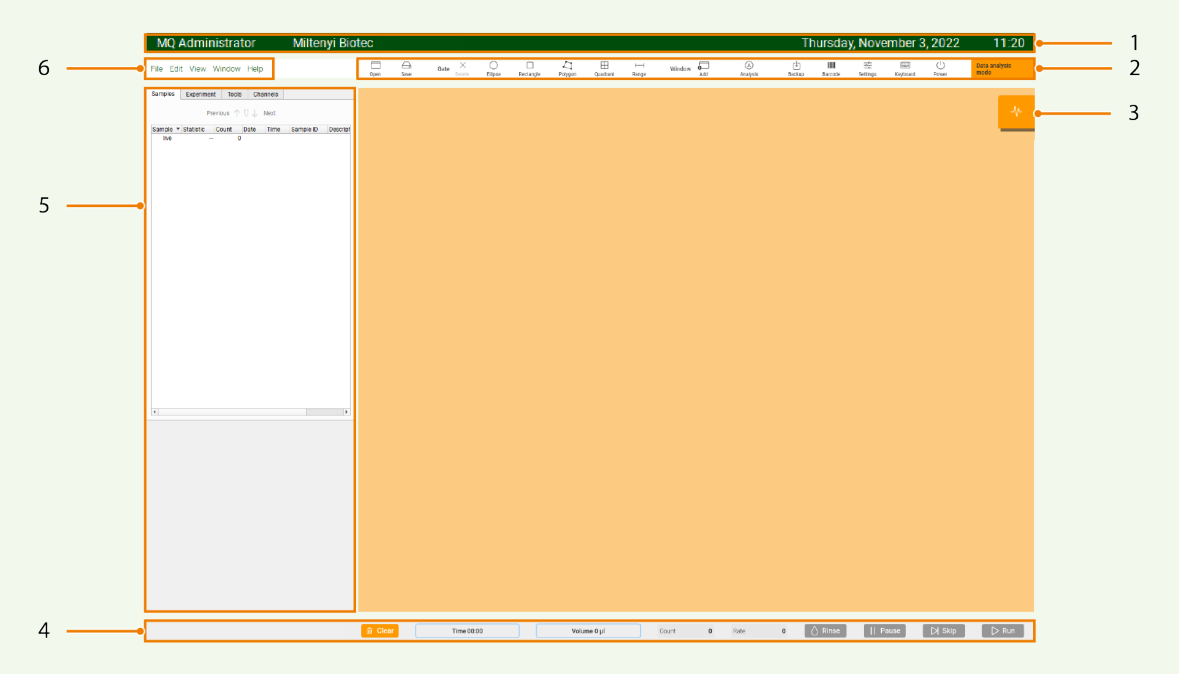


Figure 3.1: The MACSQuantify Software user interface for Custom users and MQ Administrators

1	Title bar	**4**	Instrument status bar
2	Toolbar and status	**5**	Side pane
3	Performance QC & tracking tool	**6**	Menu bar

3.1 Title bar



The title bar shows:

- the signed in user
- the owner hame
- the serial number of the MACSQuant Instrument
- the instrument name (if defined)
- date and time

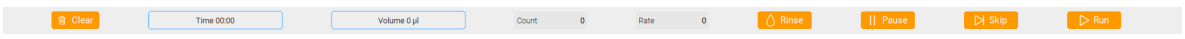
3.2 Instrument status indicator

The current status of the instrument is shown on the right-hand side of the toolbar.

Initializing MACSQuant	The instrument is initializing and not available for any measurement.
Data analysis mode	The instrument is in data analysis mode. The fluidic system is not ready and lasers are switched off. Prime the instrument to switch to acquisition mode for measurement. See **Priming the instrument on page 41**.
Priming MACSQuant	Priming in progress. The instrument is not available for measurement.
Bottle level critical change a bottle	Shown if a bottle has reached a critical level. The affected bottle's LED will be blinking.
Acquisition mode Calibration ok	The instrument is in acquisition mode and ready for measurement. Calibration state can be ok, due, expired, or not calibrated. Calibration status is indicated. See the short instructions **PMT calibration**.
Processing rack Sample 1 of 3	The instrument is processing a sample. The processed sample is indicated.
Processing rack change a bottle	The instrument is processing a sample and a bottle level is critical.
Fatal error occured hardware disabled	Error alert.

Table 3.1: States of the MACSQuant Instrument

3.3 Progress bar



The progress bar shows time, volume, count, and event rate per second of the current sample. It also contains buttons that are required during sample acquisition and to clean the instrument. The buttons are available (orange) when the instrument is in acquisition mode.

Button	Description
Clear	Deletes the current data from the data file during acquisition. Per default, the plot is also reset. To change the default settings, go to **Edit** > **Options** > **Software** > **Acquire**.
Time	Time since starting the measurement.
Volume	Remaining volume of the current sample.
Count	Total events of the current sample.
Rate	Events per second.
Rinse	Starts a needle rinse. Right-click to access the cleaning programs **Clean** and **Flush**. Refer to the MACSQuant Instrument user manual and MACSQuant X user manual.
Pause	Pauses the current sample measurement.
Skip	Stops the current measurement and continues with the next sample in line.
Run	Starts the sample measurement. While a sample is acquired, the **Run** button turns into a **Stop** button. Click the **Stop** button to stop the entire experiment.

Table 3.2: Buttons in the progress bar

3.4 Menu bar

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

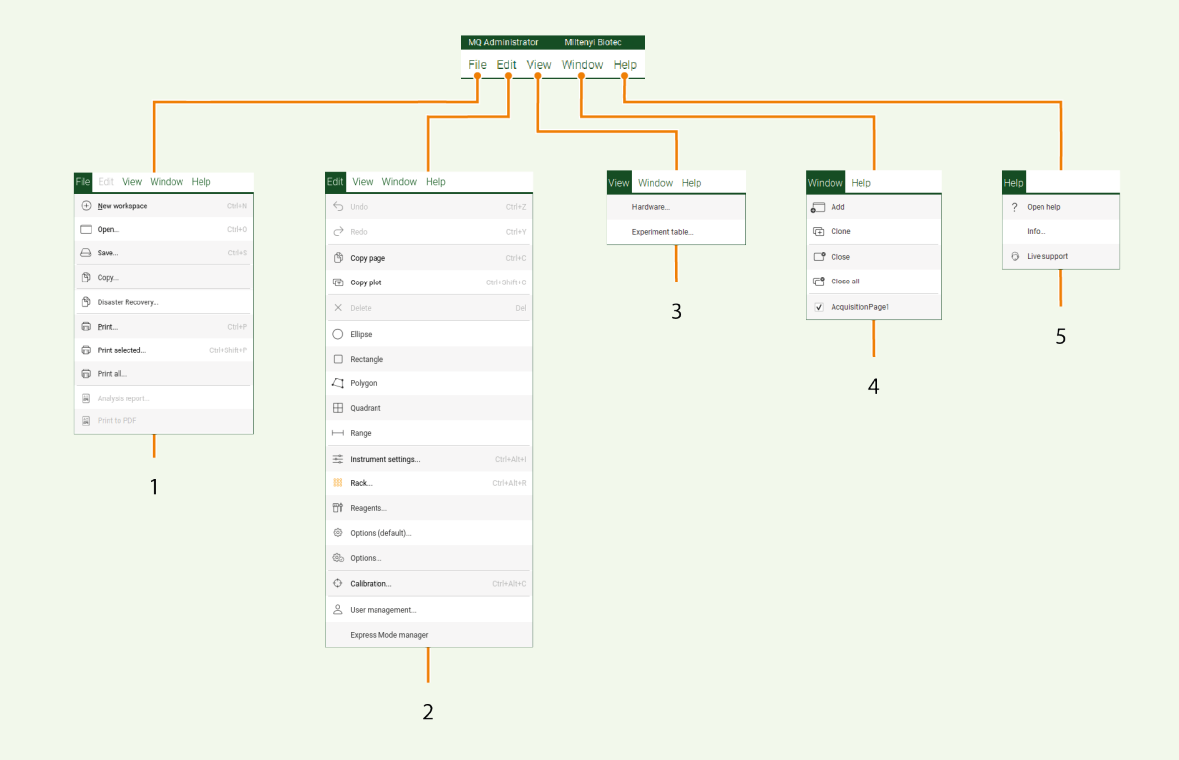


Figure 3.2: The menu bar

1	File menu	**4**	Window menu
2	Edit menu	**5**	Help menu
3	View menu		

3.4.1 File menu

New workspace	Opens a new workspace with the default instrument settings from the last PMT calibration. See **Opening a new empty workspace on page 47**.
Open...	Opens a window to select files including workspaces, instrument settings, experiments, analysis templates, or data files (.mqd). See **Opening files on page 148**.
Save...	Opens a window to save workspaces, instrument settings, experiments, or analysis templates. See **Saving files on page 149**.
Copy…	Opens a window to copy files to and from the instrument. See **Copying files to an external location on page 150**.
Disaster Recovery…	Opens a window to create and restore a complete system backup including all data, properties, configuration files. See **Disaster recovery on page 249**.
Print…	Prints active analysis windows. See **Printing analyzed data files on page 144**.
Print selected...	Prints selected samples in the sample list. See **Printing selected data files on page 145**.
Print all...	Prints all samples in the sample list. See **Printing all data files on page 144**.
Analysis report	Generates a report of analyzed data file(s) in PDF/A format. Only available with 21 CFR part 11. See **Creating analysis reports on page 250**.
Print to PDF	Generates a PDF of the analysis pages as shown live on MACSQuant Instrument. See **Print to PDF function on page 144**.

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

3.4.2 Edit menu

The **Edit** menu is not available if the analysis button icon is orange (activated).

 **Undo**	Cancels the last action.
 **Redo**	Repeats the last action.
 **Copy page**	Copies an analysis window to the clipboard.
 **Copy plot**	Copies a selected plot to the clipboard.
 **Delete**	Deletes a selected region.
 **Ellipse**	Draws a gate of the respective shape. Range is only available for histograms.
 **Rectangle**	
 **Polygon**	
 **Quadrant**	
 **Range**	
 **Instrument settings…**	Opens the **Instrument settings** window. Instrument settings can be adjusted including compensation matrix.
 **Rack...**	Opens the **Rack** window. Sample positions can be defined.
 **Reagents...**	Opens the **Reagents** window. Reagents for autolabeling can be selected.
 **Options (default)...**	Opens the **Options (default)** window to customize global settings that apply to all users. Only accessible for MQ Administrators. See **Editing global software options on page 183**.
 **Options...**	Opens the **Options** window to customize user specific settings. See **Editing software options on page 197**.
 **Configuration…**	Opens the **Configuration** window. Available on PC version to define the optical configuration of the instrument on which data files were acquired or should be acquired.
 **Calibration…**	Opens the **Calibration settings** window. Change axes scales for default PMT calibration. Define settings for **Express Mode Compensation**. See **Express Mode Compensation on page 56**.

	User management…	Opens the **User management** window. Only available for UM Administrators. See **User accounts on page 172**.
	Express Mode manager	Opens the **Express Mode manager** window. See **Express Mode manager on page 158**.

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

3.4.3 View menu

Hardware...	Opens the **Hardware Monitor** window to view the status of **Fluidics**, **Sample uptake unit**, and **Lasers and detectors**. It provides **Supplemental information** and **System information**. See **Hardware monitor on page 233**.
Experiment table...	Provides a tabulated overview of the current experiment settings including **Acquisition**, **Annotations**, **Autolabel**, and **Settings**.

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

3.4.4 Window menu

Add	Opens a new analysis window using pre-defined templates.
Clone	Creates an exact copy of the current analysis window.
Close	Closes an analysis window.
Close all	Closes all currently opened analysis windows.

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

3.4.5 Help menu

Open Help	Opens the MACSQuantify Software user manual.
Info…	Displays information about currently installed MACSQuantify Software version and installed Express Mode version. It also indicates if the software is installed as 21 CFR part 11 setup.
Live support	Opens a window to connect to Miltenyi Biotec Technical Support for MACSQuant Live Support. Allows remote control during a live support session if required. See **MACSQuant Live Support on page 253**. Internet connection is required. The function is only available on the MACSQuant Instrument.

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

3.5 Toolbar

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

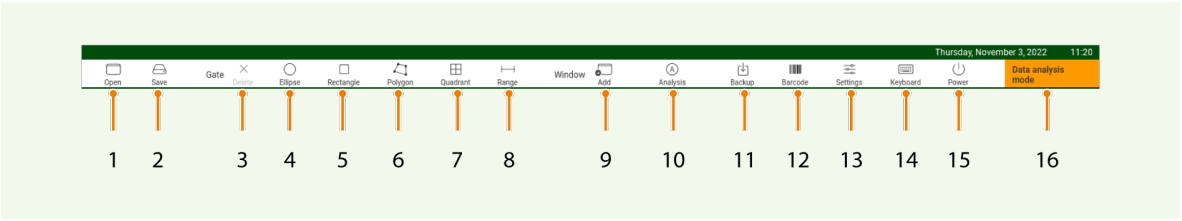


Figure 3.3: Icons in the toolbar

1	Open	**9**	Add
2	Save	**10**	Analysis
3	Delete	**11**	Backup
4	Ellipse	**12**	Barcode
5	Rectangle	**13**	Settings
6	Polygon	**14**	On-screen keyboard
7	Quadrant	**15**	Power
8	Range	**16**	Instrument status

3.5.1 Toolbar functions

 **Open**	Opens workspaces, instrument settings, experiments, analysis templates, or data files (.mqd). See **File types on page 17** and **Opening files on page 148**.
 **Save**	Saves workspaces, instrument settings, experiments, and analysis templates. See **Saving files on page 149**.
 **Delete**	Deletes a selected gate.
 **Ellipse**  **Rectangle**  **Polygon**  **Quadrant**  **Range**	Draws a gate of the respective shape. A range can only be used for histograms. See **Gating tools on page 123**.
 **Add**	Opens a new analysis window.
 **Analysis**	Activates (orange) or deactivates (gray) the **Analysis mode**. When Analysis mode is activated, the analysis settings are locked in the current state to allow the user bulk actions to the performed analysis.
 **Backup**	Opens a backup dialog to save files to a network location or an external USB flash device.
 **Barcode**	Activates the 2D barcode reader to scan a reagent vial.
 **Settings**	Opens the **Instrument settings** window to edit instrument settings.
 **Keyboard**	Opens the on-screen keyboard. Only available on MACSQuant Instrument.
 **Power**	Switches from data analysis mode to acquisition mode or starts shut down procedure and switches off the instrument. Only available on MACSQuant Instrument. See **Priming the instrument on page 41** and **Switching off the instrument on page 24**.
Instrument status	Shows the status of the instrument. See **Table 12.8**.

Table 3.8: Functions in the toolbar

3.6 Side pane

The side pane has four tabs.



Figure 3.4: Tabs of the side pane

3.6.1 Samples tab

Go to the **Samples** tab to view a list of data files available for analysis. If a gating hierarchy was applied (analysis button active), 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 page 133**. To add samples to the sample list, see **Adding data files to the sample list on page 148**.

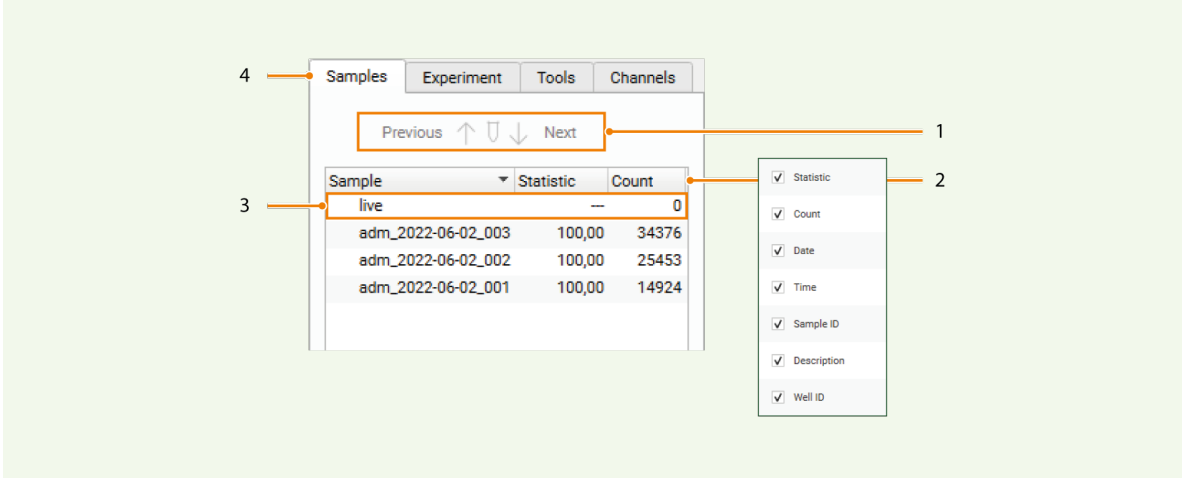


Figure 3.5: The side pane: **Samples** tab options

1	Display previous or next sample	**3**	Live sample and added samples
2	Sample list column parameters	**4**	Samples tab

Sample list columns

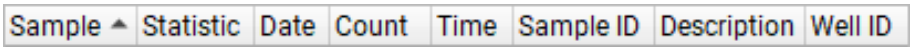


Figure 3.6: Columns of the sample list

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
Well ID	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 the arrow in the **Sample** column to sort the samples in the list 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.
Columns shown	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

3.6.2 Experiment tab

In the **Experiment** tab experimental parameters can be defined for sample acquisition. See **Setting up an experiment on page 47**. It contains also the tabs **Annotations**, **Autolabel**, and **Settings** to define the channel annotations, select reagents for autolabeling, or to select instrument settings or Express Modes. See **Figure 3.7** for an overview of the **Experiment** tab options of a MACSQuant Analyzer 10. The available options can differ slightly between MACSQuant Instrument types.

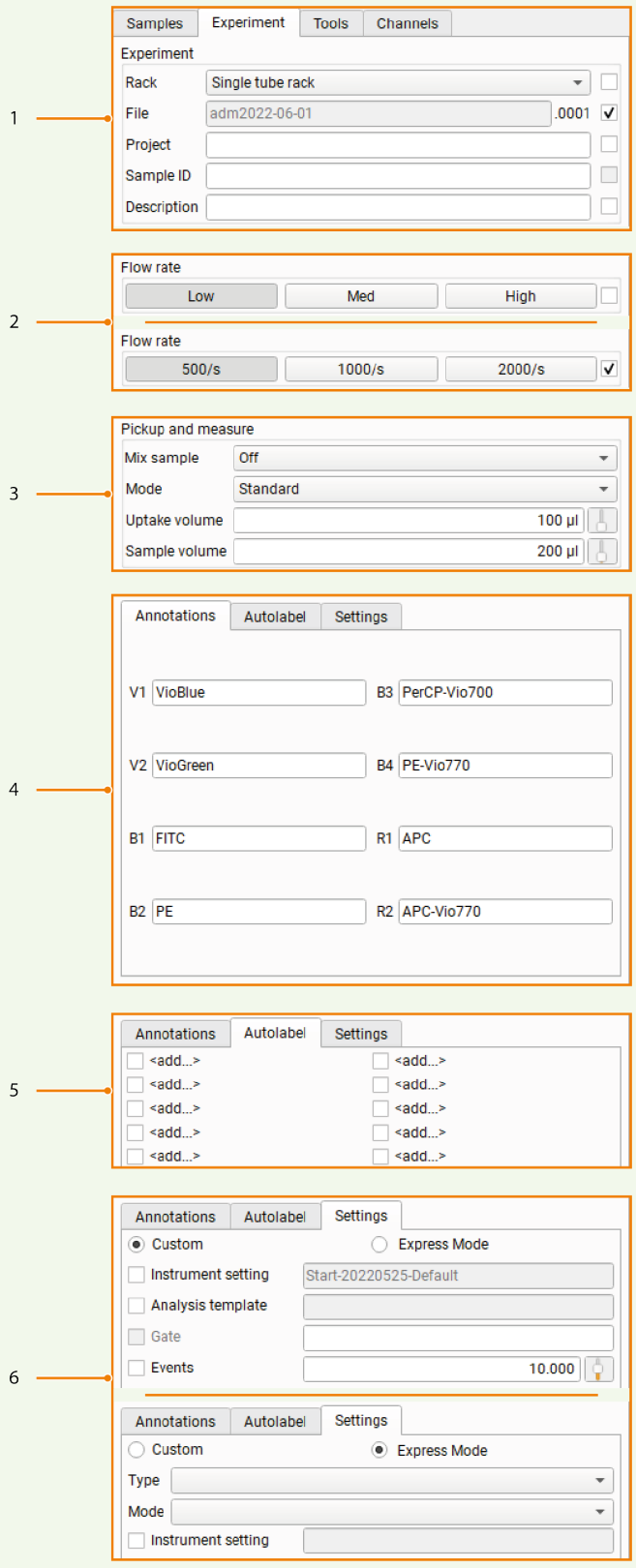


Figure 3.7: The **Experiment** tab options of a MACSQuant Analyzer 10

1	Experiment	**4**	Annotations
2	Flow rate	**5**	Autolabel
3	Pickup and measure	**6**	Settings (Custom and Express Mode)

Experiment

Option	Description
Rack	Select a sample rack. Several racks are available for use with the MACSQuant Instrument. Select the appropriate rack for the experiment from the dropdown list. Only calibrated racks and the related ones are selectable. Select or clear the box on the right to enable or disable automated rack detection. For details refer to the MACSQuant Instrument user manual.
File	Name of the current file. To generate the file name automatically, select the box on the right. See **Identifiers for file naming on page 198** for file naming options. To change the file name, clear the box and enter a new name. See **Using special characters on page 79** for more information about accepted characters.
Project	Enter the name for a project folder in which files should be saved. Select the box 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 79** for more information about accepted characters.
Description	Add additional information about the sample. Select the box to determine a pattern for automated description. See **Using the fragment description dialog on page 94**.

Table 3.11: Options in **Experiment**

Flow rate

It is possible to select the flow rate as low, medium, and high or as events per second. To change the options of the flow rate, select one of the checkboxes on the right-hand side.

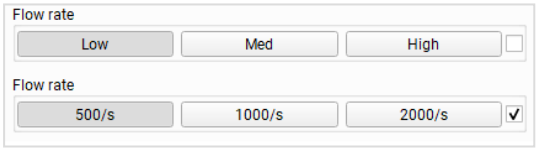


Figure 3.8: Options in **Flow rate**

Select **Low**, **Medium**, or **High** as flow rate. This corresponds to 25, 50, or 100 µL/min. Select **500/s**, **1000/s**, or **2000/s** if the flow rate should be shown as events per second. This corresponds to 25, 50, or 100 µL/min. The flow rate is automatically adjusted during acquisition to approximately reach the selected event rate. The actual event rate depends on the concentration of the sample.

Pickup and measure

Option	Description
Mix sample	Activates sample mixing prior to measurement. The sample volume needs to be specified precisely if mixing is activated to avoid uptake of air bubbles and to ensure effective mixing. MACSQuant Analyzer 10, VYB, and Analyzer 16: Samples are mixed by pipetting a defined volume up and down depending on the sample volume. MACSQuant X: Samples are mixed via needle vibration or orbital shaking of the rack.
Mode	Defines the washing or enrichment mode. Available modes are dependent on the instrument type. Select from **Screen**, **Fast**, **Standard**, or **Extended** for either high throughput or high precision and low carryover. For more information about the modes **Enrich**, **EnrichS**, and **EnrichS2**, see **Pre-enrichment programs on page 229**.
Uptake volume	Defines the volume to be measured. Note that the MACSQuant Instruments pick up additional volume. See **Excess volume on page 210**.
Sample volume	Defines the volume in the vial, tube, or well. The sample volume needs to be specified precisely if mixing is activated or autolabeling is programmed.

Table 3.12: Options in **Pickup and measure**

Annotations tab

Annotations of an experiment can be changed in the **Annotations** tab of the **Experiment** tab. The annotations can be defined individually for each position of a rack. See **Annotations tab on page 83**.

Go to **Edit > Options > Instrument > Annotations** to set the default annotations for the different channels.

Settings tab

The **Settings** tab allows the selection between **Custom** or **Express** settings.
By selecting the **Custom** radio button saved instrument settings and analysis templates can be selected. Stop or live gates and an event limit can be defined. See **Setting a live gate on page 86** and **Setting a stop gate on page 86**.
Select the **Express** radio button to get access to Express Modes. There are Express Modes for the setup of the instrument or for analysis using specific Miltenyi Reagent Kits. See **Table 11.6** for a list of Setup Express Modes.

Autolabel tab

Automated sample labeling can be activated in the **Autolabel** tab. For single tube experiments, only automated dilution of samples is possible. Select the reagent to be added from a dropdown list. See **Autolabeling on page 98** for further instructions.

3.6.3 Tools tab

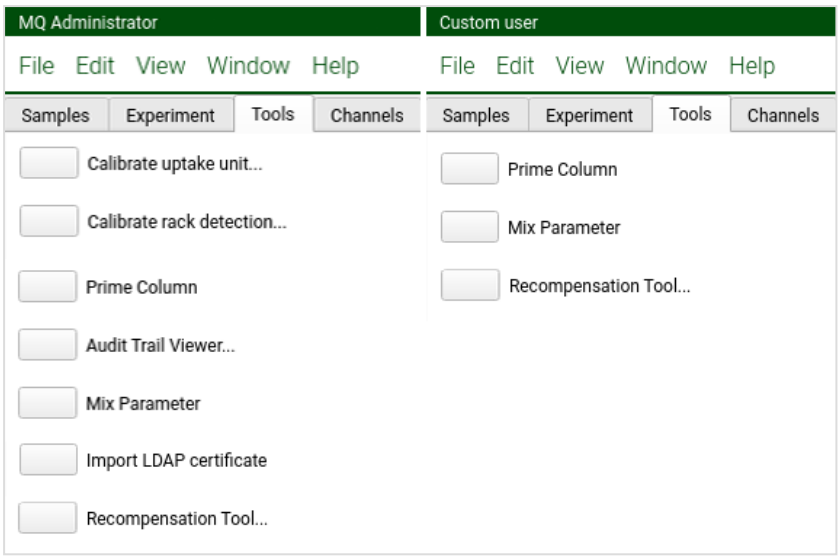


Figure 3.9: Tools tab for MQ Administrator (left) and Custom user (right) on the MACSQuant Instrument

See **Table 3.13** for details about available functions in the **Tools** tab. Some functions require the MQ Administrator user role. The listed options are available on the MACSQuant Instrument. Availability in the PC version differs as described in the table below.

Function	Description	available in PC version	MQ Administrator role required
Calibrate uptake unit	To calibrate the uptake unit. Refer to the MACSQuant Instrument user manual.	No	Yes
Calibrate rack detection	To activate or deactivate the rack detection. Refer to the MACSQuant Instrument user manual.	No	Yes
Prime Column (not available on MACSQuant X)	To prime a MACSQuant Column for rare cell enrichment. Only available on MACSQuant Analyzer 10, VYB, and Analyzer 16. See **Pre-enrichment on page 227** and the MACSQuant Instrument user manual.	No	No
Audit Trail Viewer	To create an audit trail logging user actions. See **Audit trail on page 191**. Only available in 21 CFR part 11 enabled setup.	Yes	Yes
Mix Parameter	To configure sample mixing. Adjust the number of times the sample is pipetted up and down (MACSQuant Analyzer 10, VYB, and Analyzer 16), or the strength of vibrating/shaking (MACSQuant X). See **Changing the mix parameters on page 209**.	No	Yes Custom users can only change the **User variable** parameter.
Import LDAP certificate	To import an LDAP certificate for a secure LDAP connection. Only available with 21 CFR part 11. See **Importing an LDAP certificate on page 248**.	Yes	Yes
Recompensation Tool	To recompensate already measured samples with single stains and have more flexibility for the compensation calculation. See **Recompensation Tool on page 66**.	Yes	No

Table 3.13: The **Tools** tab functions

3.6.4 Channels tab

The **Channels** tab can be used to view and adjust channel settings including setting the trigger threshold. See **Adjusting PMT gains on page 48** and **Creating an instrument setting on page 47**. The **Channels** tab gives also access to the **Advanced** button to define the noise threshold or the doublet processing algorithm. Some features require the MQ Administrator role.

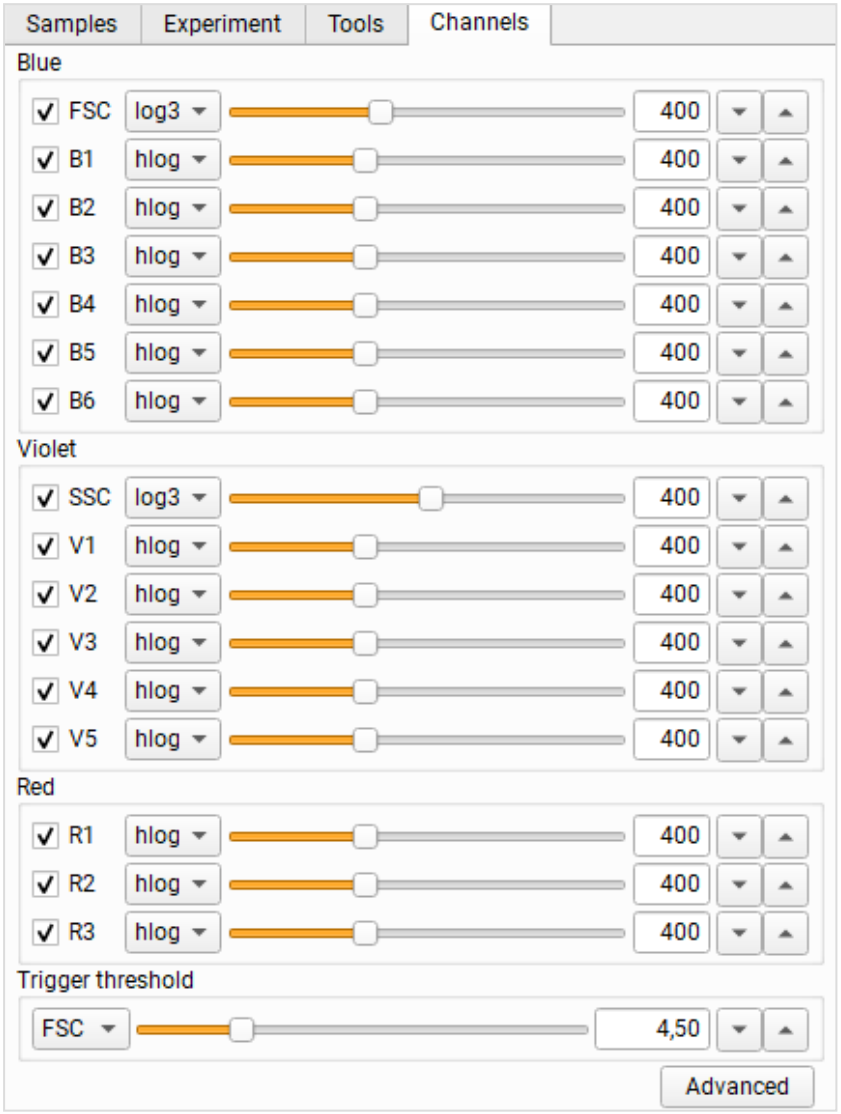


Figure 3.10: Channels tab of a MACSQuant Analyzer 16

4

Setting up the instrument

Before running an experiment, run a PMT calibration and adjust the instrument settings to optimize signal detection for the specific experiment. Make sure the hardware is calibrated properly.

Refer to the MACSQuant Instrument user manual for hardware calibration. For tube length and volume calibration of the MACSQuant X, refer to the MACSQuant X Instrument manual.

4.1 Priming the instrument

1 Click the **Power** button in the upper right corner of the software and select **Acquisition mode** to prime the instrument.

Lasers are switched on and the fluidic system is rinsed.

The MACSQuant instrument can also be primed from the sign in screen.

2 Let the optical bench warm up for at least 30 minutes before starting a measurement.

3 Go to **View** > **Hardware** > **Lasers and detectors** to check the current laser and optical bench temperature.

While the optical bench is warming up, it is recommended to perform a flush cycle followed by a clean program. Refer to the MACSQuant Instrument user manual for details.

4.2 Photomultiplier tube calibration

The MACSQuant Instrument uses photomultiplier tubes (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 emit electrons that are accelerated to hit a downstream array of dynodes. The dynodes serve as a high grade amplifier. Finally, the secondary electrons emitted from the dynodes 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.

Calibrating MACSQuant Instruments using MACSQuant Advanced Calibration Beads (# 130-134-208) is only available for instruments with MACSQuantify Software version 3.1 or later. For earlier software versions, the calibration is done only with MACSQuant Calibration Beads (# 130-093-607).

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. A daily calibration accounts for potential differences in laser performance or PMT sensitivity by adjusting the voltages accordingly. Calibrate the MACSQuant Instruments using MACSQuant Advanced Calibration Beads (# 130-134-208) or MACSQuant Calibration Beads (# 130-093-607). MACSQuantify Software version 3.1 supports MACSQuant Advanced Calibration Beads by default.

MACSQuant Advanced Calibration Beads contain blank beads, dim 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 predefined, lot-specific median fluorescence intensity values for each channel. This is achieved by adjusting the voltages on each PMT during the automated PMT calibration process.

It is recommended to calibrate the instrument on every day the instrument is used.

For information about initial calibration, see **Initial calibration on page 194**.

4.2.1 Smart Gain technology

The MACSQuantify Software version 2.13.0 or higher provides the Smart Gain technology. Smart Gains allow intra- and inter-instrument standardization of multicolor flow cytometry. Based on known median fluorescent intensity of calibration beads, the gain of each detection channel is normalized. The gain is adjusted during calibration to reach the known median fluorescence intensity of the calibration beads. The Smart Gain technology assigns a gain of 400 to each channel during calibration. This standardized gain is saved in the setup-default instrument setting shown in the **Channels** tab. See **Automated PMT calibration on the facing page**.
The default Smart Gains can be adjusted for each application and saved as a new instrument setting. See **Adjusting PMT gains on page 48**. For multicolor applications, a compensation of spectral overlap must be created and saved as part of an application-specific instrument setting. See **Compensating spectral overlap on page 53**.

Smart Gains simplify the following:

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

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

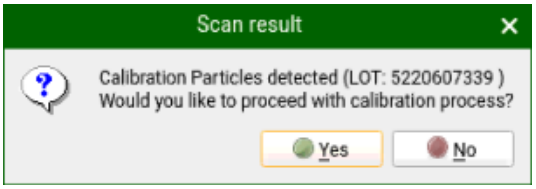
If the application-specific instrument setting is transferred to another MACSQuant Instrument with the same optical bench configuration, then the desired data display is maintained and results can be compared between instruments. For an overview which MACSQuant Instrument types allow sharing of instrument settings, see **Transferring instrument settings on page 79**.
See **Table 4.1** for implementation of Smart Gains.

On the same MACSQuant Instrument	Across MACSQuant Instruments
Perform a PMT calibration.	Perform PMT calibration on MACSQuant Instrument A and B.
Optimize gains of required channels for application.	Optimize gains of required channels for application on MACSQuant Instrument A.
Perform a compensation.	Save as an instrument setting.
Save as an instrument setting.	Transfer and open optimized instrument setting to MACSQuant Instrument B.
Re-use instrument setting.	Perform automated compensation on MACSQuant Instrument A and B.
	Save instrument settings.
	Re-use instrument settings.

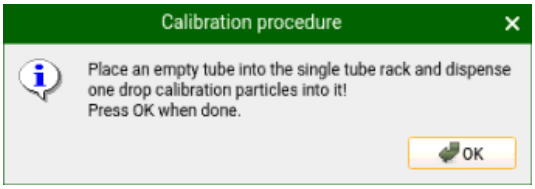
Table 4.1: Implementation of Smart Gains for longitudinal studies

4.2.2 Automated PMT calibration

1. Ensure that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.
2. Attach the Single tube rack to the MACSQuant Instrument.
3. Go to the **Experiment** tab.
4. Select **Single tube rack** from the **Rack** dropdown list.
5. Vortex the MACSQuant Advanced Calibration Beads for 10 seconds to break up aggregates.
6. Click the **Barcode** button in the toolbar to activate the 2D barcode reader.
7. Place the bottle of compatible calibration beads in the right recess of the Single tube rack with the barcode facing the instrument.
8. Scan the 2D barcode printed on the vial label of the MACSQuant Advanced Calibration Beads. A window opens.



9. Select **Yes** to continue with calibration.
10. Follow the instructions in the next window.

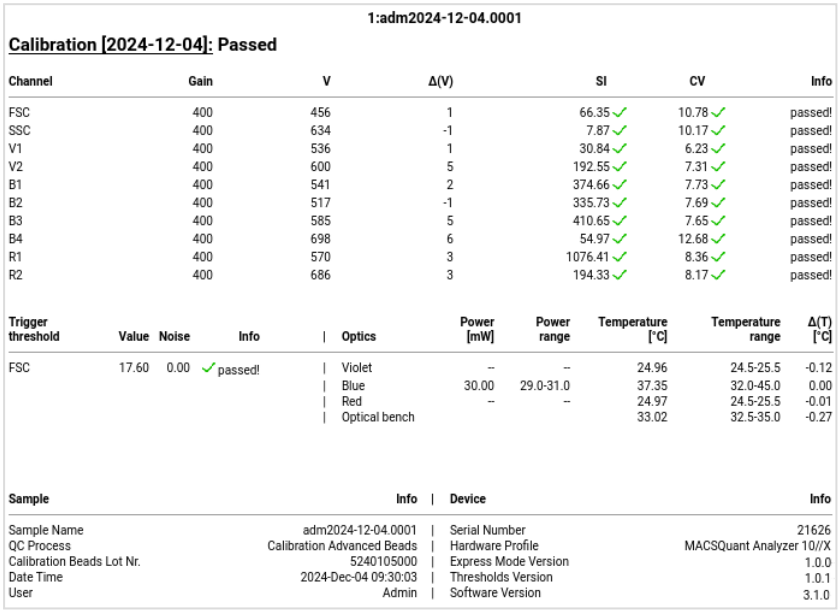


11. Click **OK** to start the calibration. The calibration beads are automatically diluted to a total volume of 300 µL. 250 µL of the diluted calibration beads are injected into the sample injection port. The gain for each channel is automatically adjusted during calibration.

Do not change gates or plot axes during calibration to ensure successful calibration.

12 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary. Click the **Next** or **Previous** button to switch between the analysis windows. See **Figure 4.1** for an example of a successful calibration.

Successful calibration is indicated for each fluorescence channel by a green check mark for the stain index **SI** and the coefficient of variation **CV**. The stain index of side and forward scatter is not taken into account as a factor for the calibration result. Furthermore, the table header of the calibration analysis page shows **Passed** and the instrument status bar reports **Acquisition Mode: Calibration OK**. See **Table 4.2** for details about the shown parameter.



1:adm2024-12-04.0001

Calibration [2024-12-04]: Passed

Channel	Gain	V	Δ(V)	SI	CV	Info
FSC	400	456	1	66.35 ✓	10.78 ✓	passed!
SSC	400	634	-1	7.87 ✓	10.17 ✓	passed!
V1	400	536	1	30.84 ✓	6.23 ✓	passed!
V2	400	600	5	192.55 ✓	7.31 ✓	passed!
B1	400	541	2	374.66 ✓	7.73 ✓	passed!
B2	400	517	-1	335.73 ✓	7.69 ✓	passed!
B3	400	585	5	410.65 ✓	7.65 ✓	passed!
B4	400	698	6	54.97 ✓	12.68 ✓	passed!
R1	400	570	3	1076.41 ✓	8.36 ✓	passed!
R2	400	686	3	194.33 ✓	8.17 ✓	passed!

Trigger threshold	Value	Noise	Info	Optics	Power [mW]	Power range	Temperature [°C]	Temperature range	Δ(T) [°C]
FSC	17.60	0.00	✓ passed!	Violet	--	--	24.96	24.5-25.5	-0.12
				Blue	30.00	29.0-31.0	37.35	32.0-45.0	0.00
				Red	--	--	24.97	24.5-25.5	-0.01
				Optical bench			33.02	32.5-35.0	-0.27

Sample	Info	Device	Info
Sample Name	adm2024-12-04.0001	Serial Number	21626
QC Process	Calibration Advanced Beads	Hardware Profile	MACSQuant Analyzer 10//X
Calibration Beads Lot Nr.	5240105000	Express Mode Version	1.0.0
Date Time	2024-Dec-04 09:30:03	Thresholds Version	1.0.1
User	Admin	Software Version	3.1.0

The PMT calibration is automatically saved as default instrument setting in **Public**.

Voltages and trigger of the PMT calibration are loaded as default instrument setting when a new workspace is generated via **File > New workspace** and after every sign in.

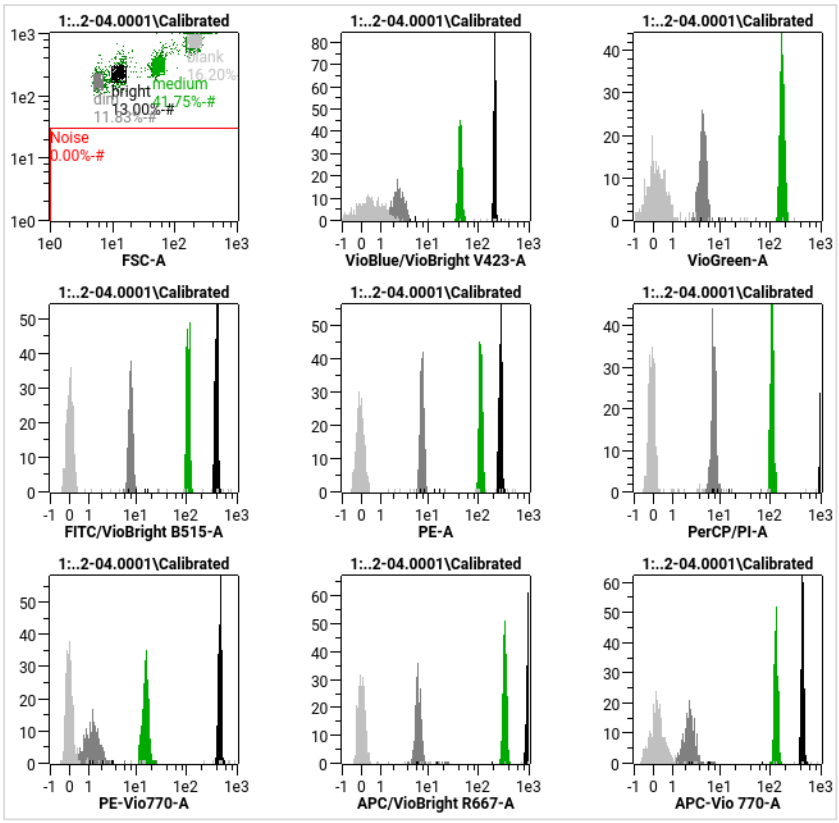


Figure 4.1: Calibration results of a MACSQuant Analyzer 10. Results look slightly different for other MACSQuant Instrument types.

Parameter	Explanation
V	Voltage on PMT
Δ(V)	Difference from last successful calibration
SI	Stain index
CV	Coefficient of variation based on MFI
Δ(T)	Difference in temperature from the start of calibration to the end of calibration
Temperature range	Acceptable range of temperature change during calibration
Temperature	Measured temperature
Power range	Acceptable range of laser power
Power	Power of the laser

Table 4.2: Parameter of PMT calibration shown in analysis template

4.2.3 Manual PMT calibration

If the instrument is unable to read the barcode on the vial label of the MACSQuant Advanced Calibration Beads, a manual PMT calibration can be performed.

If the PMT calibration is started manually, i.e. without scanning calibration beads, one of the previously scanned calibration lot can be selected as reference for the manual calibration.

1 Ensure that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.

2 Attach the Single tube rack to the MACSQuant Instrument.

3 Go to the **Experiment** tab.

4 Select **Single tube rack** from the **Rack** dropdown list.

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

6 Dispense one drop into an empty tube.

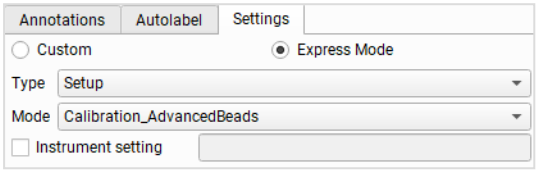
7 Add 270 µL MACSQuant Running Buffer.

8 Place the tube in the Single tube rack.

9 Select the **Settings** tab and select **Express**.

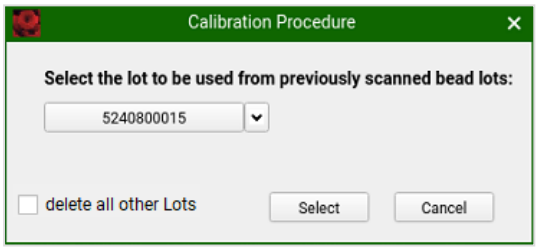
10 Go to the **Type** dropdown list and select **Setup.**

11 Go to the **Mode** dropdown list and select **Calibration_AdvancedBeads**.



12 Select the calibration lot to be used as reference for the manual calibration.

To delete all the other calibration lots, except for the selected one, select the checkbox **delete all other Lots**.



13 Click the **Run** button in the instrument status bar. 250 µL of the diluted calibration beads are injected into the sample injection port. The gain for each channel is automatically adjusted during calibration.

Do not change gates or plot axes during calibration to ensure successful calibration.

14 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary. Click the **Next window** or **Previous window** button to switch between analysis windows. See **Figure 4.1** for an example of a successful calibration.

15 Successful calibration is indicated for each channel by a green check mark for the stain index **SI** and the coefficient of variation **CV**. Furthermore, the table header of the calibration analysis page shows **Passed** and the instrument status bar reports **Acquisition Mode: Calibration OK**. See **Table 4.2** for details about the shown parameter.

16 Gains and threshold are automatically saved as a default instrument setting in **Public**.

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

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

5 Setting up an experiment

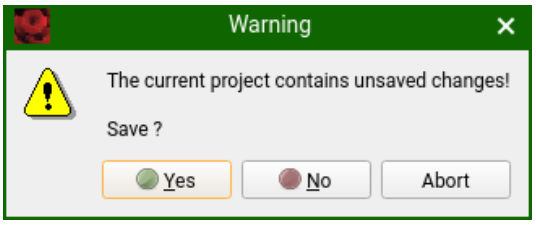
This chapter describes the experiment setup in the **Experiment** tab for advanced flow cytometry users. MQ Custom and MQ Administrator users can define customized experiments including measurement parameters and autolabeling. It is also possible to assign instrument settings and analysis templates to samples.
Less advanced users can run experiments as an Express user. See **Express user on page 215** for details.

5.1 Opening a new empty workspace

Opening a new workspace is recommended before a new acquisition is started. This closes scripts that might run in the background and closes all open analysis windows. This is particularly recommended after running a PMT calibration or automated compensation to increase the performance of the software. A new empty workspace loads the default setting from the last successful PMT calibration.

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

2 If the current workspace contains unsaved changes, a warning message appears. Click **Yes** to save current settings. A new empty workspace opens. The instrument setting of the last successful PMT calibration is loaded.



3 Optional: Open an appropriate instrument setting for the next measurement, for example, including compensation.

5.2 Creating an instrument setting

Before running an experiment, it is necessary to create an instrument setting for optimal results. An instrument setting contains all information defined in the **Channels** tab plus compensation settings. Adjust the default instrument setting from the PMT calibration for optimal signal detection. Changes can be saved as a new instrument setting. To create an instrument setting following steps are required:

- Calibration of PMTs
- Selecting data display scales for each channel using the **Channels** tab
- Adjusting of forward and side scatter PMT gains using the **Channels** tab
- Adjusting of fluorescence channel PMT gains using the **Channels** tab

- Setting trigger and noise threshold to exclude unwanted events using the **Experiment** tab and **Advanced** button
- Automated or manual compensation of spectral overlap for multicolor experiments
- Optional: Addition of height and/or width (pulse processing) using the **Advanced** button, for example, to detect doublets

All settings and adjustments can be saved as an **Instrument setting**. See **Saving files on page 149** for details. To review the instrument setting, click the instrument setting icon in the toolbar. To check an existing instrument setting based on sample material, see **Checking instrument settings on page 78**.

5.3 Adjusting PMT gains

After PMT calibration, it might be necessary to adjust the PMT gains for a specific application. This ensure that the settings are optimized for the used cells or particles and expected fluorescence signal intensities. Depending on the specific application, sample material, and antibody panel, only FSC and SSC but not the fluorescence channels need adjustment. For very dim or bright signals or high fluorescence spillover signals, adjustment might be necessary. When decreasing PMT gains from the calibrated values, sensitivity for weakly stained antigens might be lost. It is not recommended to adjust the PMT solely due to autofluorescence. Adjust the PMT to bring the brightly positive events onto the display scale and ensure that each fluorescence signal is the brightest in its designated channel.

5.3.1 Adjusting FSC and SSC gains

1. Ensure that the MACSQuant Instrument is in acquisition mode and calibrated.
2. Optional: Go to **File > New workspace** to open a new workspace.
3. Select the **Add** icon in the toolbar to open a new analysis window.
4. Select a plot.

By default, plots display FSC-A and SSC-A.

5. In the **Experiment** tab, select **Single tube rack** from the **Rack** dropdown list.
6. Place a sample in the Single tube rack.
7. Optional: Select a project, add a sample ID, and select a mixing level.
8. Define an uptake volume.
9. Open the **Channels** tab.
10. Click the **Run** button in the instrument status bar to start acquisition.
11. Adjust the gains for FSC and SSC in the **Channels** tab to bring the population of events into your desired area of the dot plot. To adjust gains, do one of the following:
    - Slide the scroll bar left and right to change the gains.
    - Click the scroll bar to adjust the gains in increments of 10 units.
    - Use the up and down arrows to change the value by increments of 1 unit.
    - Double-click the value box, type in a value, and press **Enter**.
12. Optional: After adjustment during a running measurement, it is recommended to clear the previous events to only visualize events with the new settings. Click the **Clear** button on the instrument status bar to refresh the events.

13 Adjust the trigger threshold to minimize debris. If the sample contains lots of debris, it can be difficult to see the cells of interest.

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

15 Save as instrument setting with appropriate name for recalled use.

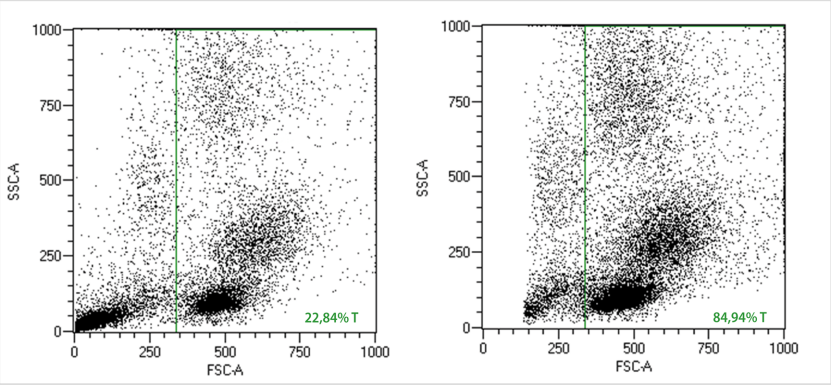


Figure 5.1: Adjustment of FSC and SSC PMT gains. Left: PBMCs prior to adjustment. Right: PBMCs after setting a trigger threshold to exclude debris.

If the desired population of events is unknown, it can be difficult to adjust FSC and SSC gains. Use a cell sample labeled with a fluorescent marker to identify the cells of interest. Use this marker for back gating to determine the proper FSC and SSC adjustment. See **Back gating on page 132**.

5.3.2 Adjusting gains of fluorescence channels

It is recommended to use a sample with both a negative and a positive population when adjusting the gains of fluorescence channels. Keep both populations on scale and only make adjustment if one population is not on scale or if the signal is not the brightest in its designated channel. In general, PMT adjustments of fluorescence channels might be necessary in following cases:

- Using CFSE or other bright amine-binding dyes.
- Looking at highly expressed fluorescent proteins such as GFP.
- Highly expressed antigens are stained for detection in the V1, V2, or B1 channels in which autofluorescence is highest (i.e., for dendritic cells).

1 After adjustment of FSC and SSC, place a sample in the Single tube rack. An optimal sample should contain a negative and a positive population for each of the used fluorescence channels in the experiment. It is recommended to use the same sample material as used during the experiment. The gains of the fluorescent channels can be adjusted using single stains or using a fully stained sample to consider potential spillover effects.

2 Format one of the dot plot displays to view the first fluorescent parameter.

3 Go to the **Experiment** tab. From the **Rack** dropdown list, select **Single tube rack**.

4 Enter an uptake volume.

5 Optional: Select a project, add a sample ID, activate sample mixing.

6 Place a stained sample in the Single tube rack.

7 Open the **Channels** tab.

8 Click the **Run** button.

9 Determine the position of positive and negative events. Optionally, draw a region around the appropriate population in the FSC versus SSC dot plot. Select the region from the dropdown list of the plot title in a fluorescence plot.

10 Increase or decrease the gains for the fluorescence channel in the **Channels** tab to have both negative and positive populations on scale, achieve an appropriate signal to noise, and ensure that the positive fluorescence signal is brighter in this channel than any spillover channel. To adjust gains, do one of the following:

- Slide the scroll bar left and right to change the gains.
- Click the scroll bar to adjust the gains in increments of 10 units.
- Use the up and down arrows to change the value by increments of 1 unit.
- Double-click the value box, type in a value, and press **Enter**.

11 Continue with the next fluorescence channel.

12 Save as instrument setting with appropriate name for recalled use.

It is not recommended to decrease the gains in a way that the negative population appears on the far left of the plot. This may decrease sensitivity for weakly expressed antigens.

Always adjust fluorescence channel PMT gains before running a compensation.

5.4 Threshold

Threshold is a minimum parameter value of a scatter or fluorescence channel that can be defined by a user when running a measurement. A threshold enables the user to exclude unwanted cells or debris. During data acquisition the set threshold value limits the electronic data record to events of interest originating from cells or particles. Events with properties falling below a set threshold value in a scatter or fluorescence channel are not recorded. Selecting an appropriate threshold value is a prerequisite for high quality data sets. Unwanted cells or debris can be too small in size (FSC), exhibit low granularity (SSC), or show poor fluorescent signals, for example, due to insufficient antibody labeling or background signals. Therefore, inappropriate threshold values can lead to incorrect count results, high coincidence, or other measurable effects. This can artificially reduce or increase the number of detectable events. Commonly, a value for FSC or SSC is used as threshold channel to exclude debris from the sample.

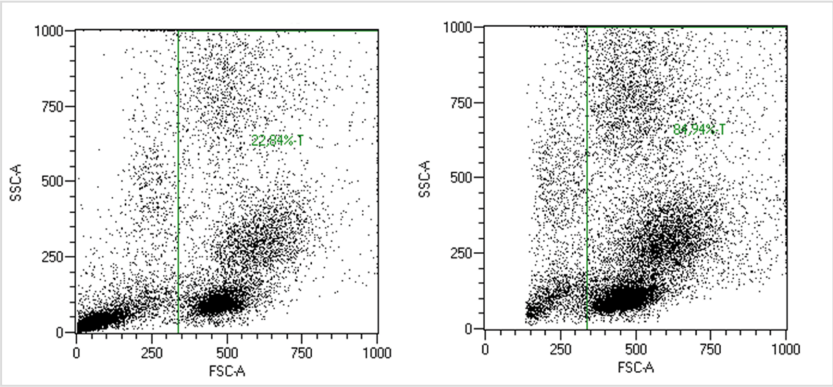


Figure 5.2: Threshold to exclude debris. Left: Debris represents 77% of sample. Right: After setting a threshold, debris represents 15% of sample.

The MACSQuantify Software allows to define two thresholds:

- **Trigger threshold**: All scatter and fluorescence channels can be used to set a trigger threshold. This value can be set prior to measurement or can be changed during acquisition.

- **Noise threshold**: The noise threshold can be activated if a second channel should be used additionally to the trigger threshold. The noise threshold is by default set to auto.

For details how to adjust the thresholds, see **Setting the trigger threshold below** and **Setting the noise threshold on the next page**.

In the MACSQuantify Software versions before 3.0.1, the trigger threshold was called primary trigger, the noise threshold was called secondary trigger.

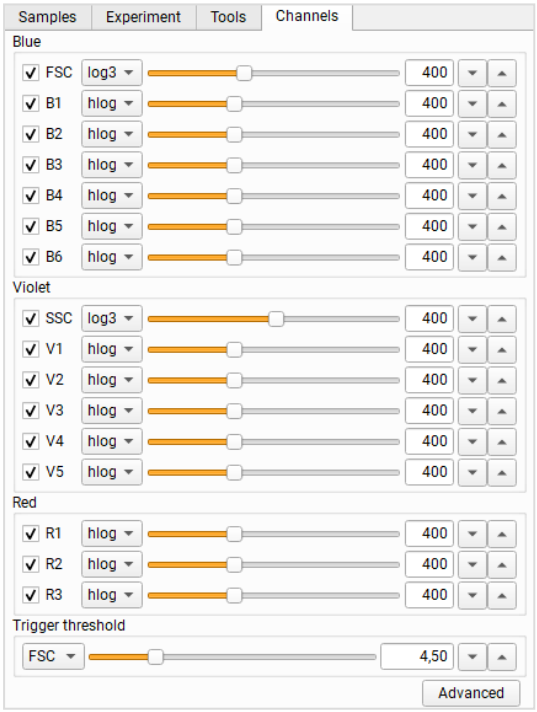
5.4.1 Setting the trigger threshold

Any optical parameter (FSC, SSC, or fluorescence channel) can be used as trigger threshold. Use a fluorescence channel as threshold if a large number of undesirable events can be distinguished by the lack of a fluorescence marker, i.e., triggering on CD45 expression to exclude red blood cells and platelets. It is highly recommended to use the trigger threshold to increase data quality.

The trigger threshold can be set and adjusted at any time during data acquisition via the **Channels** tab.

1 Go to the **Channels** tab.

2 Select the desired channel from the **Trigger threshold** dropdown list. By default, the trigger threshold is set to FSC.



3 Click the **Add** button to open an analysis window and select an analysis window template.

4 Select the parameter for the trigger threshold in one of the plots.

5 Click the **Run** button to start data acquisition. As data become visible on the plot, adjust the trigger threshold in the **Channels** tab.

Default FSC trigger threshold value is adjusted upon calibration.

6 To adjust the trigger threshold do one of the following:

- Slide the trigger threshold toggle bar right and left to change the value.
- Click the toggle bar to adjust the value in increments of 5 units.
- Use the up and down arrows to change the value by increments of 0.1 unit.
- Double-click the value box, type in a value, and press **Enter**.

7 Click the **Clear** button in the instrument status bar to remove all events acquired with the old trigger threshold. The plot refreshes and shows only events above the new set trigger threshold.

5.4.2 Setting the noise threshold

The noise threshold can be used as an additional threshold to ignore events that are not relevant. It can be set on a second optical parameter (FSC, SSC, or fluorescence channel). The channel can be identical to or different from the trigger threshold.

> On a MACSQuant Analyzer 16, only FSC, SSC, V1 (450/50), and R1 (667/30) are selectable as noise threshold channels.

The noise threshold is accessible via the **Advanced** button in the **Channels** tab. To set the noise threshold select one of the following options:

- **Auto**: This option is the default trigger setting. Using **Auto** synchronizes the trigger threshold and the noise threshold if both are set to the same channel. If the trigger threshold is adjusted during acquisition, the noise threshold is also set to this value.

  > It is recommended to use this option only if both thresholds are set to the same channel.

- **On**: This option should be used if the noise threshold is set to a channel different from the trigger channel. The noise threshold must be defined before starting a measurement because the **Advanced** button in the **Channels** tab is not available during acquisition. Using **On** allows to change the trigger threshold independently from the noise threshold during acquisition. Events which do not exceed the minimum threshold values in one and/or the other channel are not recorded.
- **Off**: Noise threshold is not active. Only the trigger threshold is used.

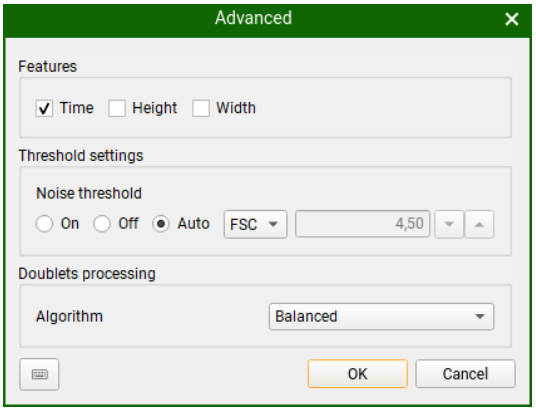
To set the noise threshold:

1 Set a trigger threshold as described in **Setting the trigger threshold on the previous page**.
2 Go to the **Channels** tab and click the **Advanced** button.
3 Go to the **Threshold settings** section and check the box **On**.
4 From the dropdown list select the desired channel for the noise threshold.
5 Enter a value for the noise threshold.
6 Click **OK**. The noise threshold appears in bold in the **Channels** tab.

If the value for the noise threshold is not yet known:

1 Go to the **Channels** tab and click the **Advanced** button.

2 Go to the **Threshold settings** section and select the box **Auto**.



3 Close the **Advanced** window.

4 Go to the **Trigger threshold** in the **Channels** tab.

5 Set the trigger threshold channel to the desired noise threshold channel, for example, V1.

6 Start a measurement.

7 Identify the optimal noise threshold value by adjusting the trigger threshold in the **Channels** tab.

8 If the value is defined, stop the measurement.

9 Click the **Advanced** button.

10 Select **On** and set the noise threshold to the desired noise threshold channel, for example, V1.

11 Enter the value for the noise threshold that was defined using the trigger threshold.

12 Click **OK**. The noise threshold appears in bold in the **Channels** tab.

13 Set the trigger threshold to a second channel, for example, FSC.

14 Go to **File > Save**.

15 Save as an instrument setting.

In addition to trigger and noise threshold, a live gate can be set to filter out events outside the live gate.
See **Setting a live gate on page 86**.

5.5 Compensating spectral overlap

Multiparameter cell analysis requires simultaneous detection of different fluorochromes. 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 into a different detection channel. For example, FITC fluorescence is mainly detected in the B1 channel but is also detected in the B2 (PE) channel due to spectral overlap. Compensation of spillover is necessary to produce accurate and consistent results for multicolor analysis. Compensation can be done automatically on the MACSQuant Instrument. Compensation adjustments can be set in different ways:

- automated compensation using the Compensation Express mode (**Express Mode Compensation on page 56**)
- automated compensation using the CompensationMultiColor Express Mode (**Express Mode CompensationMultiColor on page 59**)

- automated compensation using the CompensationMultiColor_flex Express Mode (**Express Mode CompensationMultiColor_flex on page 64**)
- semi-automated compensation using the Recompensation Tool (**Recompensation Tool on page 66**)
- manual compensation using the compensation matrix (**Compensation matrix on page 70**)
- manual offline compensation and recompensation after data acquisition (**Manual compensation on page 70**)

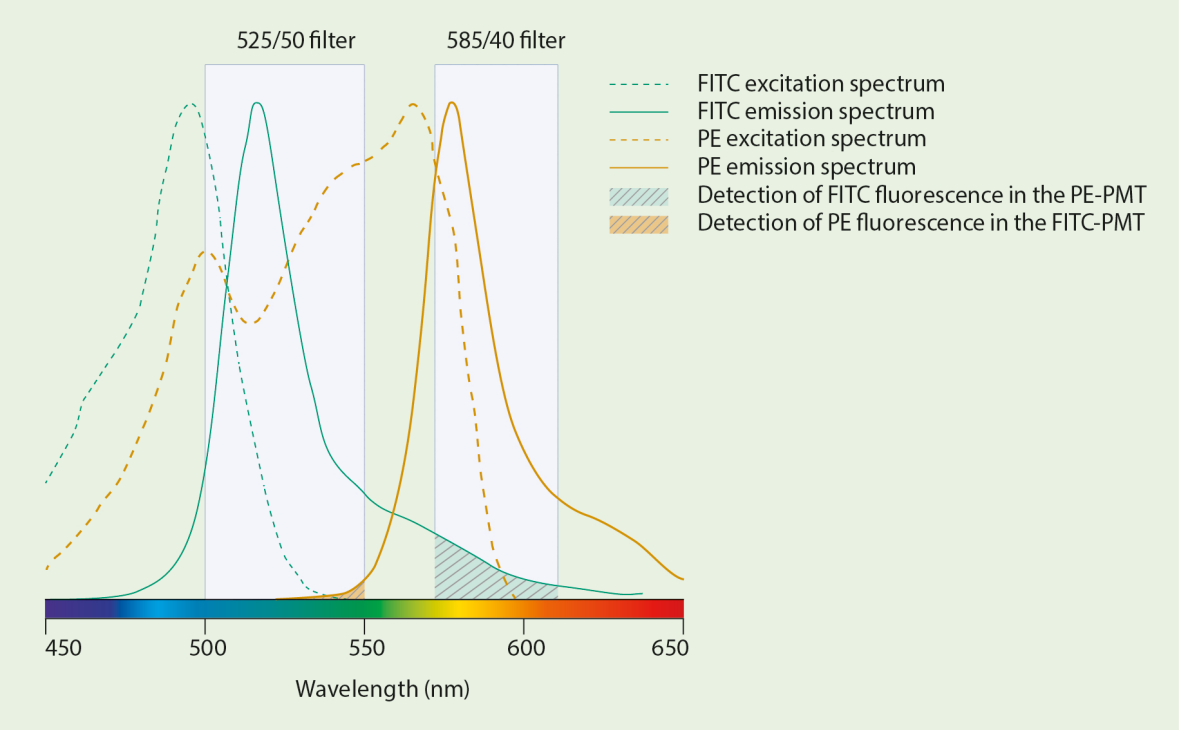


Figure 5.3: Emission spectra of FITC (green solid line) and PE (red solid line). The green hashed area represents the amount of FITC signal detected in B2 (585/40 filter). The red-hashed area represents the amount of PE signal detected in B1 (525/50 filter).

5.5.1 Compensation guidelines

It is mandatory to use single stained samples for compensation.

Compensation controls can be cells, pre-stained compensation beads, or antibody-capture compensation beads, for example, MACS MultiSpecies Comp Beads (# 130-137-398) or MACS All-in-One Comp Bead Kit (# 130-138-867). 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 display fluorescence intensity levels equal or brighter than the samples used in the experiment.
- 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 re-evaluation of compensation values.
- The negative and positive population must 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, the exact same vial from the same manufacturer must be used to set compensation adjustments. 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 recommended when visualizing compensated data.

5.5.2 Example for compensation

To compensate for spectral overlap, cells or antibody-capture compensation beads that are stained with one fluorochrome only are used, for example, MACS MultiSpecies Comp Beads (# 130-137-398) or MACS All-in-One Comp Bead Kit (# 130-138-867). 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 detection channels that are used during the experiment.

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 the median 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 the median of the negative population.

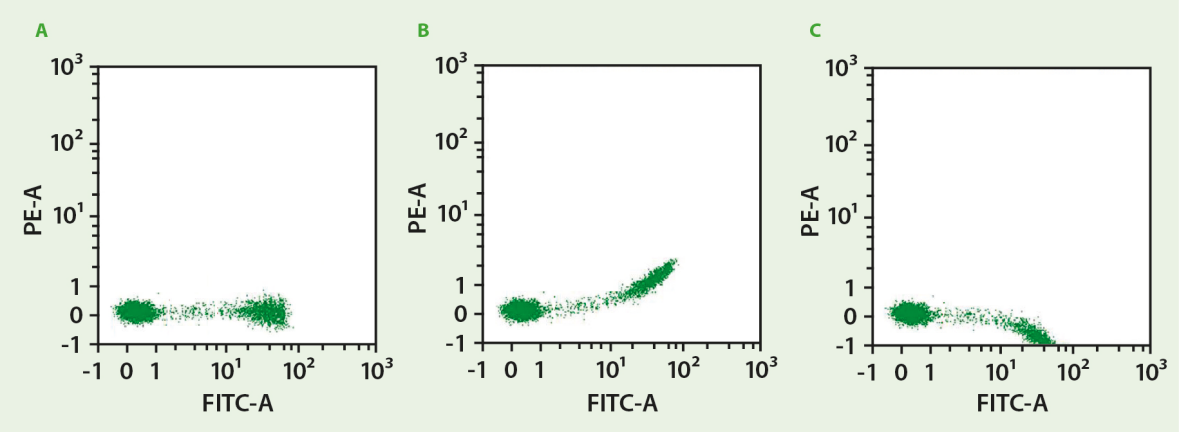


Figure 5.4: A: Compensated, B: Undercompensated, and C: Overcompensated sample.

5.5.3 Automated compensation

Express Mode Compensation

Materials required

It is recommended to use the Express Mode MultiColorCompensation. The Express Mode Compensation can be used if no minisampler is available.

Do not use more than 8 colors for the Express Mode Compensation to ensure 10% positive cells for each population. If more colors are required, use the Express Mode CompensationMultiColor instead.

- Single-stained controls representing all fluorochromes used in the experimental staining panel combined into one tube. Ensure that there is a comparable positive and negative population for setting compensation.
- 12×75 mm round bottom or 1.5 mL tubes.
- Single tube rack.

Mix the single stained controls into the tube shortly before starting the compensation. Do not store the controls before starting the compensation.

Protocol

1 Ensure that the MACSQuant Instrument is calibrated.

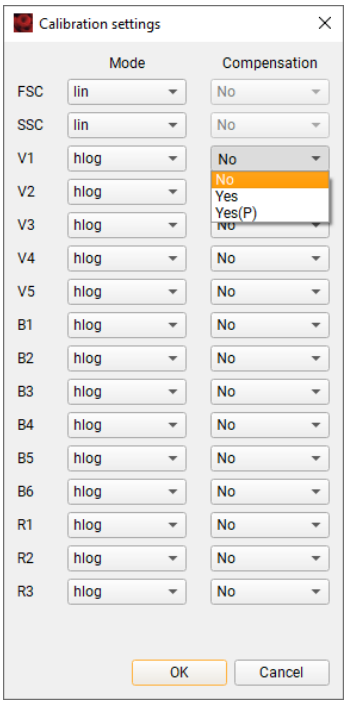
2 Go to **File > New workspace** to open an empty workspace.

3 Adjust all PMT voltage channels or open an instrument setting via **File > Open**. Each fluorescent signal must be the brightest signal in its designated channel.

4 Go to **Edit > Calibration**.

5 Select one of the following options from the dropdown list:

- **No** = No compensation required.
- **Yes** = Compensate spillover out of this channel, but no fluorochrome is present in the compensation sample. If viability dyes are used, then select this option.
- **Yes(P)** = Compensate spillover out of this channel and substract the fluorochrome signal from other channels.



Make sure to select at least one **Yes(P)** in a channel.

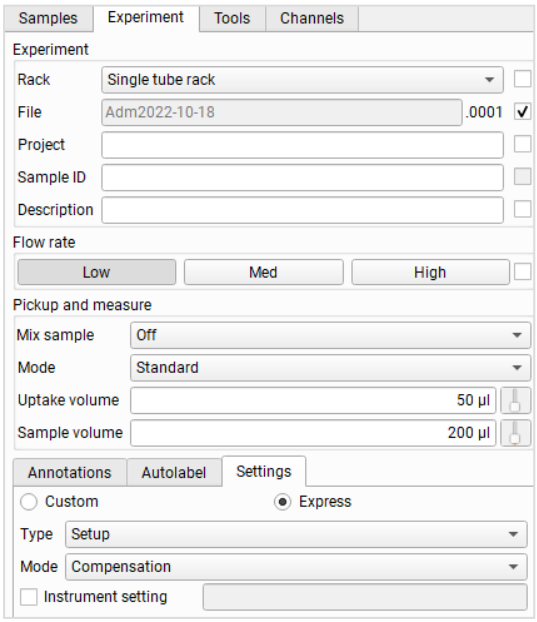
6 Go to the **Experiment** tab.

7 Select **Single tube rack** from the **Rack** dropdown list.

8 Go to the **Settings** tab and select the **Express** radio button.

9 Select **Setup** from the **Type** dropdown list.

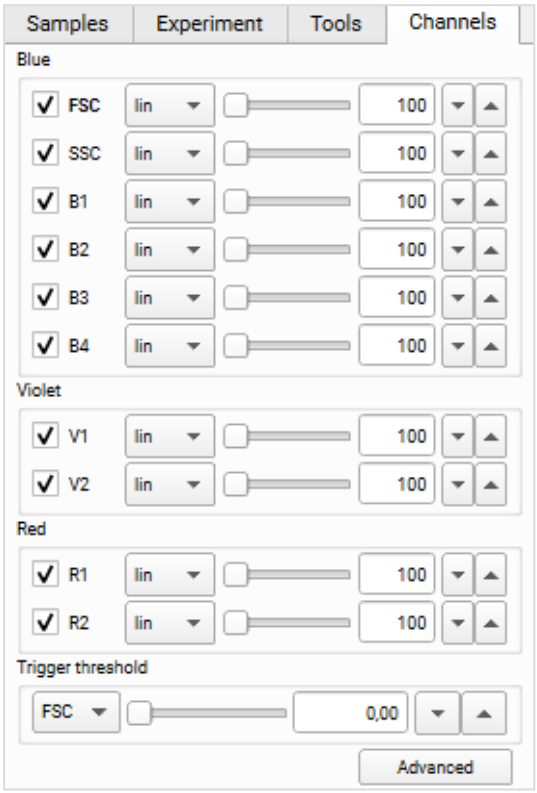
10 Select **Compensation** from the **Mode** dropdown list.



11 Optional: Enter sample ID, project folder, uptake volume etc.

12 Place the sample in the Single tube rack.

Make sure that all channels are selected before starting automated compensation. Do not clear unused channels in the **Channels** tab.



13 Click the **Run** button to start the acquisition.

14 A window opens. Draw a region in the scatter plot around the cells or beads of interest. Include only populations that are relevant for compensation and data analysis.

Do not close the window without drawing a region of interest to allow optimal compensation results.

15 Click the **Continue** button.

16 At the end of the process enter a name for the instrument setting file or use the default naming.

17 Optional: Select **private** or **public** as storage location.

18 Click **Save**.

Express Mode CompensationMultiColor

Read the **Compensation guidelines on page 54** to ensure proper setup of compensation. Incorrect compensation can result in low quality data analysis.

The automated multicolor compensation corrects spillover for every channel.

Sample material

- Single-stained controls representing all fluorochromes used in the experiment. Ensure comparable positive and negative populations for setting compensation. Use cells or compensation beads as sample material.
- One unstained sample as blank for all samples or a negative (unstained) population in each tube.
- Optional: one unstained sample for compensation of viability dye channel, for example, propidium iodide (PI).

Protocol

1 Ensure that the MACSQuant Instrument is calibrated.

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

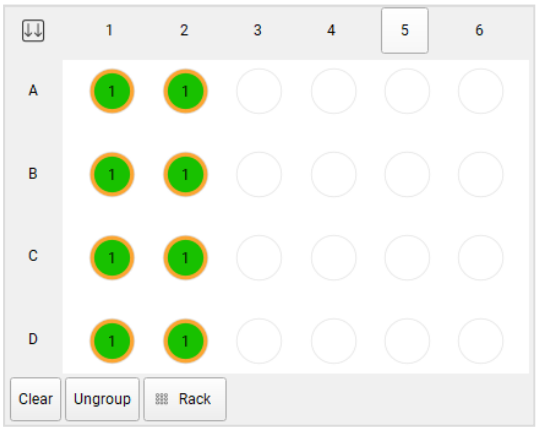
3 Adjust all PMT values based on a fully stained sample or using single-stained controls. Alternatively, open an instrument setting via **File > Open**. Each flurorescent signal must be the brightest signal in its designated channel.

4 Select an appropriate sample rack from the dropdown list in the **Experiment** tab. Typically, the Chill 5 Rack or the Chill 96 Rack is used.

5 Go to the rack scheme in the **Experiment** tab.

6 Select the appropriate number of sample positions to match the number of samples used for compensation. See **Programming sample positions on page 93**. Note the rack processing order indicated by the symbol in the upper left corner.

7 Click the **Group** button. Each of the selected rack positions should now be labeled with the number 1.



Only rack positions that are adjacent can be grouped. See **Grouping of samples on page 96**.

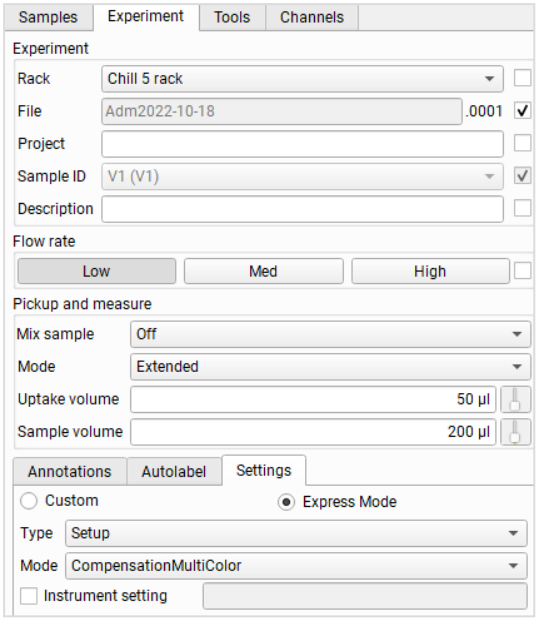
8 Place the samples in the rack accordingly.

9 From the **Experiment** tab, go to the **Settings** tab and select the **Express Mode** radio button.

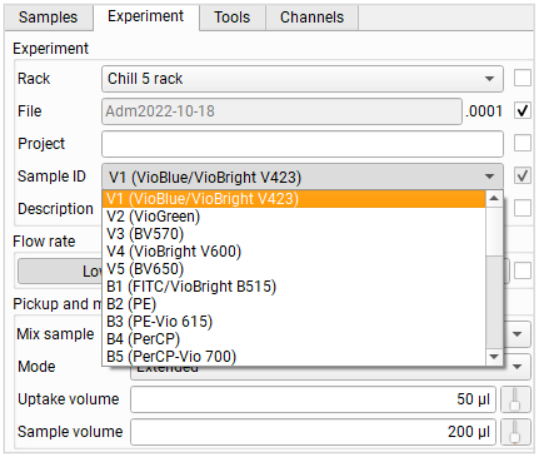
10 Select **Setup** from the **Type** dropdown list.

11 Select **CompensationMultiColor** from the **Mode** dropdown list.

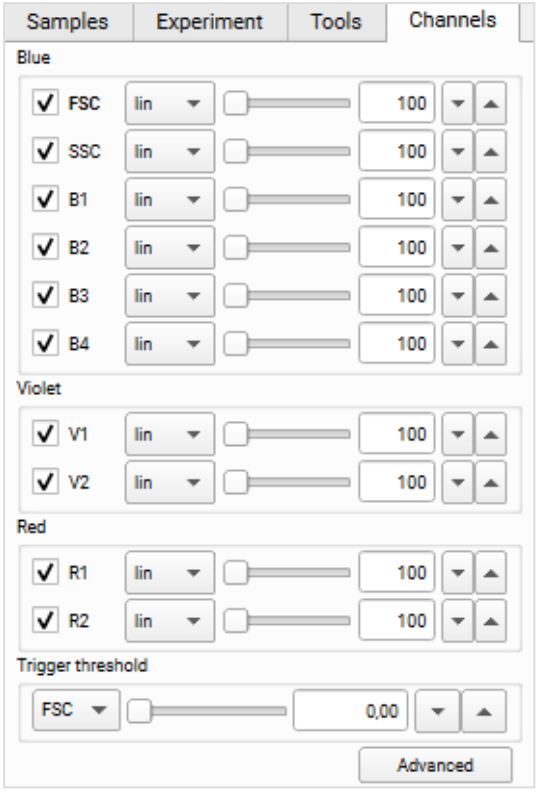
12 Optional: Select mixing, change the flow rate, change the mode, or change the uptake and sample volume if required.



13 Select a single sample position in the rack window. Select the channel that represents the fluorescent stain in the sample from the **Sample ID** dropdown list matching the order of the samples in the rack. The fluorochromes in brackets are based on the entries in the **Annotations** tab. Each fluorochrome in the dropdown list represents a specific detection channel, for example, FITC represents channel B1. If using another B1–compatible fluorochrome, for example, VioBright B515, select FITC. See **Annotations on page 199**.



Ensure that all channels are selected in the **Channels** tab before starting automated compensation. Do not deselect unused channels.



14 Optional: If a viability dye is used that only labels dead cell, select the respective viability channel from the Sample ID dropdown list. The spillover of other selected fluorescence channels is compensated out of the viability dye channel. Do not add the viability dye.

15 Optional: If one blank sample is used for all stained controls, select **Blank** from the dropdown list.

If different blanks are needed due to different sample material/autofluorescence, ensure a blank and positive control is present in each sample.

16 Check the levels of MACSQuant Running Buffer and waste.

17 Click the **Run** button to start the acquisition.

Once 5,000 events are measured, a pop-up window asking to draw your region of interest appears.

18 Draw a region in the scatter plot around the population of interest. Include only populations that are relevant for compensation and data analysis. Click **OK**.

Do not close the pop-up window without drawing a region of interest to allow optimal compensation results.

Ensure that the first sample contains a minimum of 10,000 events in the uptake volume and 5,000 events in the subsequent samples.

19 Click the **Continue** button.

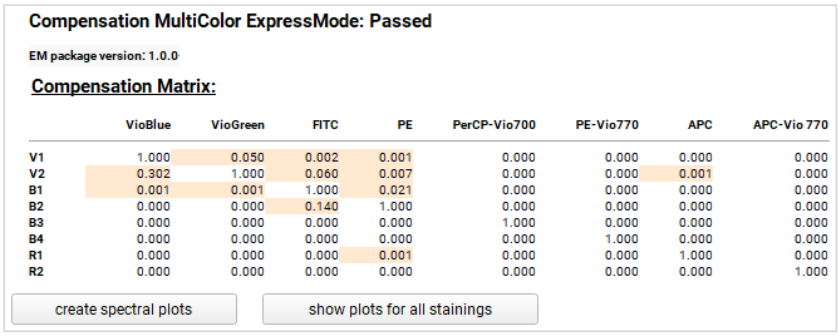
20 At the end of the process enter a name for the instrument setting file or use the default naming.

21 Optional: Select **private** or **public** as storage location.

22 Click **Save**.

Spectral plots

After completion of the CompensationMultiColor, the compensation matrix is shown in the analysis window. Applied compensation values are highlighted orange in the matrix (**Figure 5.5**).



Compensation MultiColor ExpressMode: Passed

EM package version: 1.0.0

Compensation Matrix:

	VioBlue	VioGreen	FITC	PE	PerCP-Vio700	PE-Vio770	APC	APC-Vio 770
V1	1.000	0.050	0.002	0.001	0.000	0.000	0.000	0.000
V2	0.302	1.000	0.060	0.007	0.000	0.000	0.001	0.000
B1	0.001	0.001	1.000	0.021	0.000	0.000	0.000	0.000
B2	0.000	0.000	0.140	1.000	0.000	0.000	0.000	0.000
B3	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
B4	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
R1	0.000	0.000	0.000	0.001	0.000	0.000	1.000	0.000
R2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

create spectral plots | show plots for all stainings

Figure 5.5: Compensation matrix

To receive more information about spectral overlap:

- Select **create spectral plots** to show spectral overlap for each channel (**Figure 5.6** and **Figure 5.7**).
- Select **show plots for all stainings** to show spillover of one fluorescence channel against all other used channels for compensation (**Figure 5.8**).

Create spectral plots

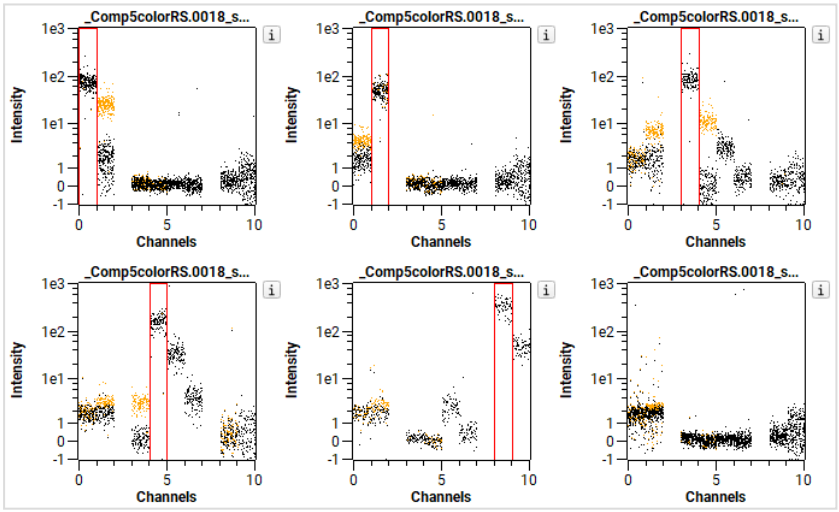


Figure 5.6: Spectral plots of a MultiColorCompensation sample. The red frame highlights the color that had spillover correction out of other channels. Fluorescence channels used for MultiColorCompensation Express Mode are designated in orange. Orange is signal prior to compensation; black is signal after applied compensation.

Legend for Spectral plots

Value	Channel	Annotation
0	V1	(VioBlue)
1	V2	(VioGreen)
3	B1	(FITC)
4	B2	(PE)
5	B3	(PerCP-Vio700)
6	B4	(PE-Vio770)
8	R1	(APC)
9	R2	(APC-Vio 770)

Colored events represent intensities BEFORE compensation
Black events represent intensities AFTER compensation

Figure 5.7: Legend for spectral plots. Annotations are shown as defined for the sample in the **Annotations** tab.

Show plots for all stainings

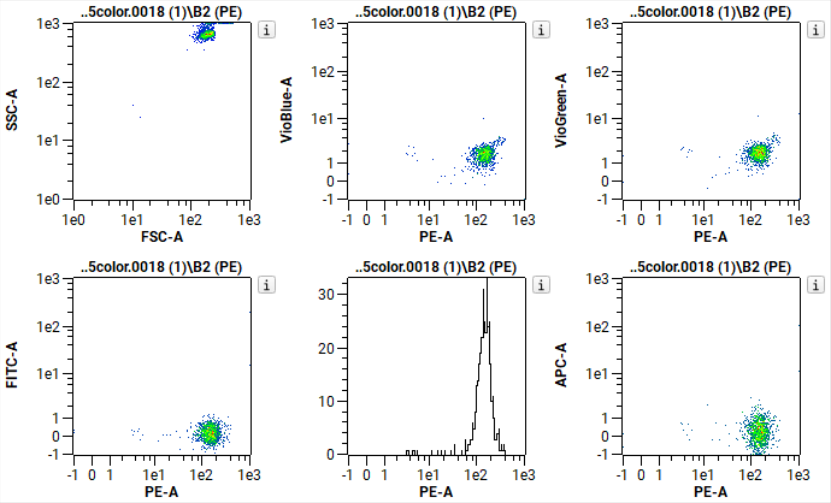


Figure 5.8: Plots of compensated PE channel against all other fluorescence channel used for MultiColorCompensation on a MACSQuant Analyzer 10.

Express Mode CompensationMultiColor_flex

Read the **Compensation guidelines on page 54** to ensure proper setup of compensation. Incorrect compensation can result in low quality data analysis.

The automated multicolor compensation corrects spillover for every channel. The CompensationMultiColor_flex Express Mode expands the CompensationMultiColor Express Mode process and measures the whole uptake volume. It allows the user to adjust the automatically generated gating for each channel individually. This enables compensation of both cells and beads simultaneously, in the same experiment.
Uptake volume, Sample volume and in general all the Experiment settings can be individually modified for each sample after selecting the Express Mode.

Sample material

- Single-stained controls representing all fluorochromes used in the experiment. Ensure comparable positive and negative populations for setting compensation. Use cells or compensation beads as sample material.
- One unstained sample as blank for all samples or a negative (unstained) population in each tube.
- Optional: one unstained sample for compensation of viability dye channel, for example, propidium iodide (PI).

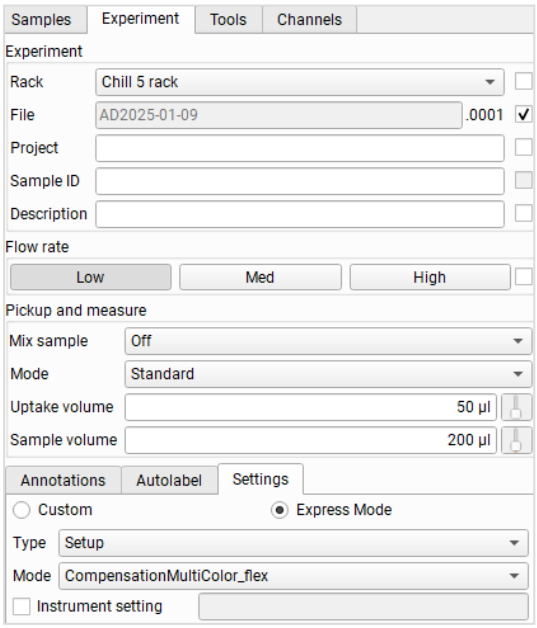
Protocol

1 Perform the steps **1** through **10** of the Express Mode CompensationMultiColor **Protocol on page 59**.

2 Select **CompensationMultiColor_flex** from the **Mode** dropdown list.

3 Optional: Select mixing, change the flow rate, change the mode, and change the uptake and sample volume as needed.

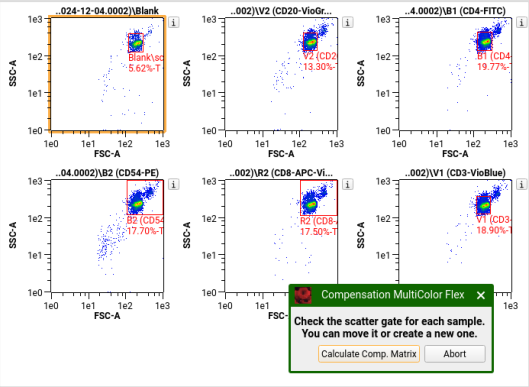
When running the CompensationMultiColor_flex Express Mode, the entire uptake volume defined in the experiment settings is measured.



4 Perform the steps **13** through **16** of the Express Mode CompensationMultiColor **Protocol on page 59**.

5 Click the **Run** button to start the acquisition.

Results are automatically shown on the analyis page. An analysis page is created for each compensation sample.



6 Review, modify, create, or delete the automatically created gates as needed. Ensure that there is one gate per compensation sample to allow clear identification of the relevant population.

Delete the existing gate and draw a new one as needed. The newly drawn gate can be of each shape, not only a polygon.

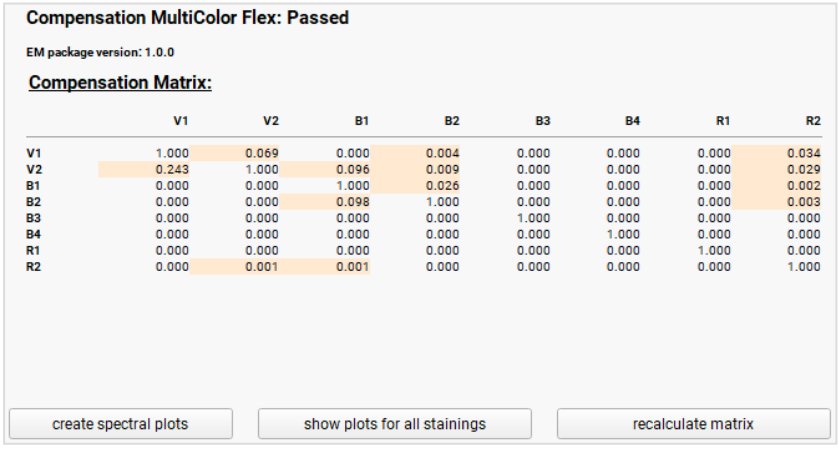
7 If needed to set the same gate for all regions of interest, first delete the gates in the individual plots, then set the gate on the selected plot. The gate appears in all plots.

8 Click on **Calculate Comp. Matrix**.

9 When compensation is completed, a dialog box opens to save the instrument setting. Enter a name for the instrument settings file and click **Save**.

Spectral plots

After completion of the MultiColorCompensation_flex the compensation matrix is shown in the analysis window. Applied compensation is highlighted orange in the matrix (**Figure 5.9**).



	V1	V2	B1	B2	B3	B4	R1	R2
V1	1.000	0.069	0.000	0.004	0.000	0.000	0.000	0.034
V2	0.243	1.000	0.096	0.009	0.000	0.000	0.000	0.029
B1	0.000	0.000	1.000	0.026	0.000	0.000	0.000	0.002
B2	0.000	0.000	0.098	1.000	0.000	0.000	0.000	0.003
B3	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
B4	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
R1	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
R2	0.000	0.001	0.001	0.000	0.000	0.000	0.000	1.000

Figure 5.9: Compensation matrix

To receive more information about spectral overlap::

- Select **create spectral plots** to show spectral overlap for each channel (**Create spectral plots on page 62**).
- Select **show plots for all stainings** to show spillover of one fluorescence channel against all other used channels for compensation (**Show plots for all stainings on page 63**).
- Select **recalculate matrix** to change the gate for all groups or selectively for a specific group and repeat the compensation.

5.5.4 Recompensation Tool

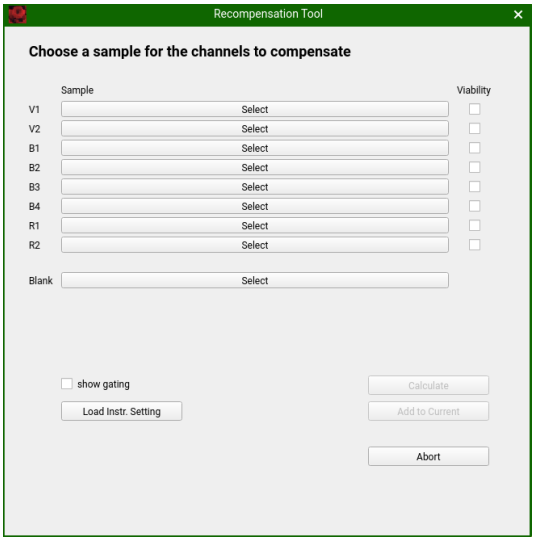
The Recompensation Tool adds flexibility to the compensation calculation by allowing users to adjust an existing matrix or create a new one from already measured single-stained samples.

The tool offers two types of compensation:

- Calculates the compensation based on already measured single-stained samples via the **Calculate** button. See **Calculating a new compensation on the facing page**.
- Adds additional single-stained samples in the calculation of a compensation, and into the currently loaded compensation matrix via **Add to Current** button. See **Adding single-stain samples to current compensation on page 68**.

Protocol

1. Load the single-stained samples into the **Sample** list. See **Opening files on page 148**.
2. Ensure that all the single-stained samples were measured with the same instrument settings.
3. Apply the instrument setting of one of the loaded samples. See **Applying instrument settings from a data file on page 136**.
4. Go to the **Tools** tab.
5. Select **Recompensation Tool**.

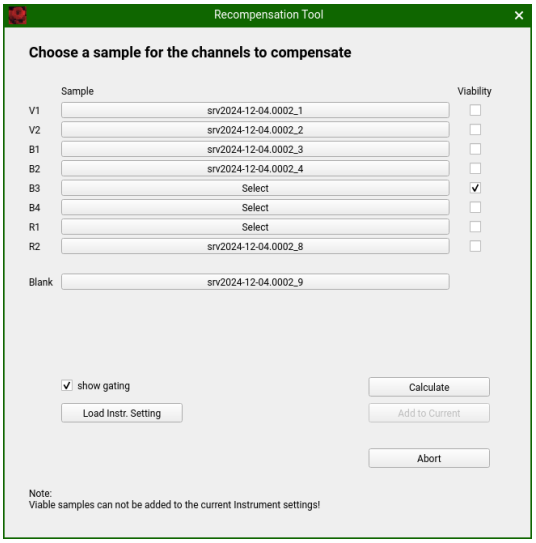


Calculating a new compensation

1 Select the single-stained samples and assign them to the target channels in which they were stained.

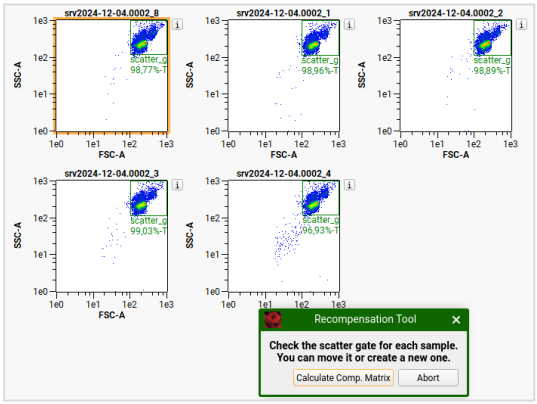
2 Optional: If a viability dye is used, select the checkbox **Viability** for compensating the spillover of other selected fluorescence channel out of your viability channel.

3 Optional: To show and adapt the gates in the histograms for the positive and negative population, select the checkbox **show gating**.



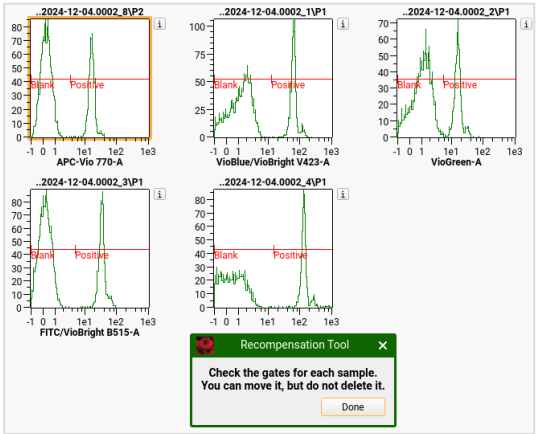
4 To start the calculation of the compensation, click **Calculate**.

A pop-up window to check the gate for each sample appears. The tool shows scatter plots of all samples and creates a gate for the biggest population.



5 Check and adapt the gate for all samples, or selectively for a specific sample as needed.

6 Click on **Calculate Comp. Matrix**. If the checkbox **show gating** was selected, histograms with positive and negative populations are shown.

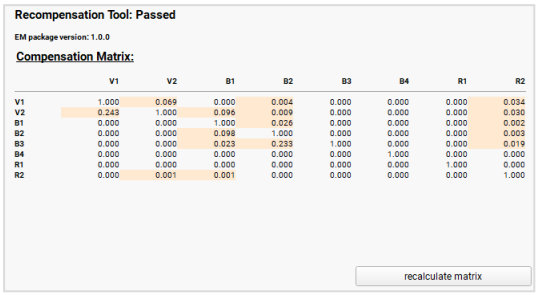


7 Check and adapt the gates as necessary.

8 Click **Done** to finish the compensation.

9 When the compensation is completed, a dialog box opens to save the instrument setting. Enter a name for the instrument settings file and click **Save**.

The compensation matrix is shown in the analysis window. Applied compensation is highlighted orange in the matrix.



10 To repeat the compensation, change the gates and click **recalculate matrix**.

Adding single-stain samples to current compensation

This feature adds single-stained samples, measured with the same instrument settings, to the current current compensation matrix. For example, in case of exchanging only one staining or if one sample was not compensated properly. This feature is especially beneficial for updating a compensation when working with tandem dyes.

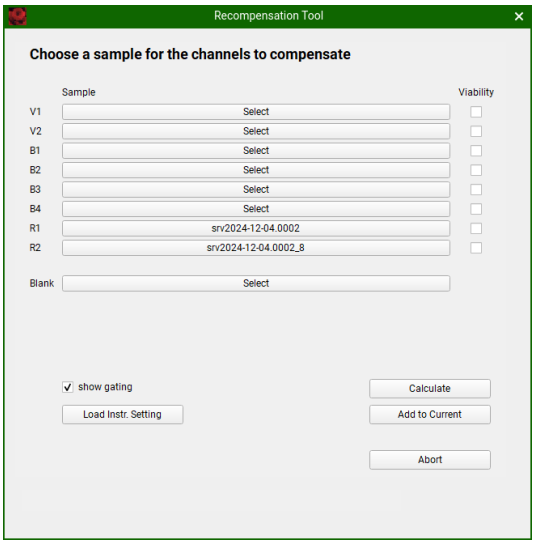
1 Ensure that all the single-stained samples were measured with the same instrument settings.

2 To load the instrument settings which should be updated with the additional single-stained sample, click **Load Instr. Setting**.

3 Select the single-stained samples and assign them to the target channels in which they were stained.

> Viability dye compensation via the **Viability** checkbox is not availble in this mode.

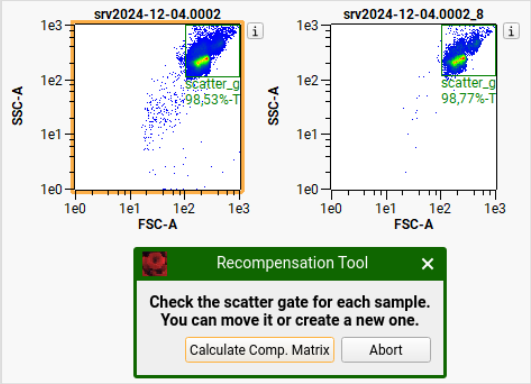
4 Optional: To show and adapt the gates in the histograms for the positive and negative population, select the checkbox **show gating**.



5 Click on **Add to Current**.

A pop-up window to check the gate for each sample appears.

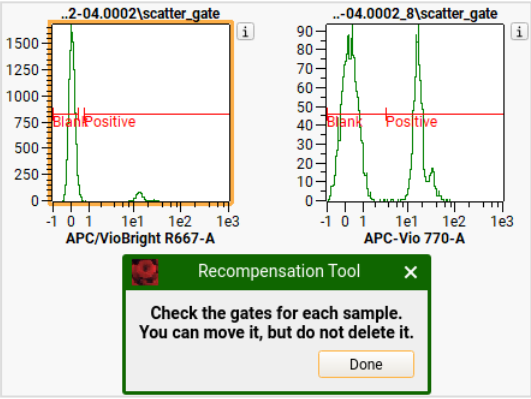
The tool shows scatter plots of the added samples and creates gate for the biggest population.



6 Check and adapt the gates as necessary.

7 Click on **Calculate Comp.Matrix**.

If the checkbox **show gating** was selected, histograms with positive and negative populations are shown.

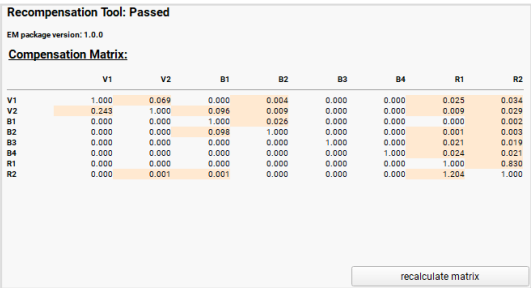


8 Check and adapt the gates as necessary.

9 To finish the compensation, click **Done**.

10 When compensation is completed, a dialog box opens to save the instrument setting. Enter a name for the instrument settings file and click **Save**.

The newly added single-stained samples are included in the current compensation matrix.



11 To repeat the compensation, change the gates and click **recalculate matrix**.

5.5.5 Compensation matrix

MACSQuantify Software uses a compensation matrix, which organizes the detection of individual fluorescence signals in columns. Column headers identify representative fluorochromes detected on the MACSQuant 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 fluorescence.

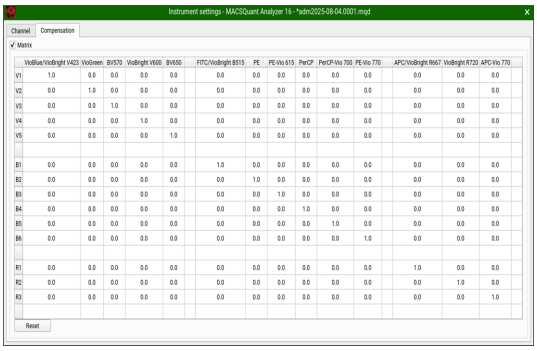


Figure 5.10: The compensation matrix of the MACSQuant Analyzer 16

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 compensation 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 compensation matrix select the field to be changed. For details about adjustment, see **Manual compensation below** or **Recompensation Tool on page 66**.

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

5.5.6 Manual compensation

Materials required

- Single-stained controls representing all fluorochromes used in the experimental staining panel. Ensure that there is a comparable positive and negative population for setting compensation. Each sample must contain a negative and a positive population.
- 12×75 mm round bottom or 1.5 mL tubes.
- Single tube rack.

Protocol manual compensation

1. Ensure that the MACSQuant Instrument is in acquisition mode and calibrated.
2. Adjust all PMT values based on a fully stained sample or using single-stained controls. Alternatively, open an instrument setting via **File > Open**. Each fluorescent signal must be the brightest signal in its designated channel.
3. Open a new analysis window by clicking the **Add** button in the toolbar. To change one of the dot plots into a statistics table, click the **'i'** button next to a plot and select **Statistic**.
4. Change one plot into a histogram.
5. Display the respective channel of the stained sample. For example, use B1 for FITC.
6. Add a new analysis window showing a statistics table.

It is highly recommended to use the hlog scale for performing compensation.

7 In the **Experiment** tab, define parameters, for example, mixing, sample ID, and uptake volume.

8 Open the compensation matrix by clicking the **Instrument settings** icon in the toolbar. Select the **Matrix** checkbox. The columns represent the measured fluorochrome, the rows represent the detection channels, from which spillover fluorescence is substracted.

9 Place the single-stained sample into the Single tube rack.

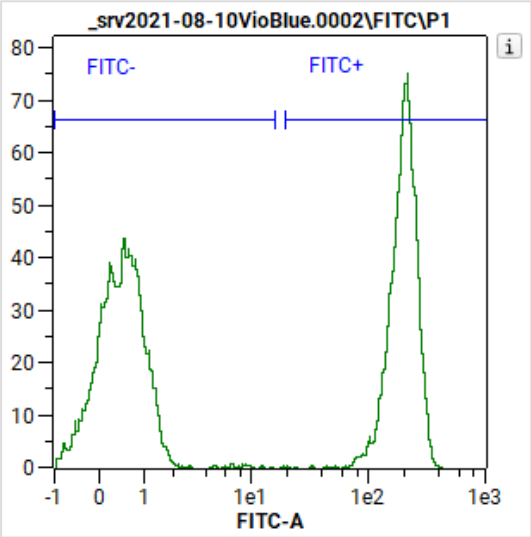
10 Click the **Run** button.

11 When events start to appear on the plots, click the **Pause** button.

12 Draw a region around the population of interest within the FSC versus SSC dot plot.

13 Select the region from the plot title of the histogram plot.

14 Draw range regions around the negative and positive population.



15 Optional: Rename the regions. Right-click the region and select **Region properties**.

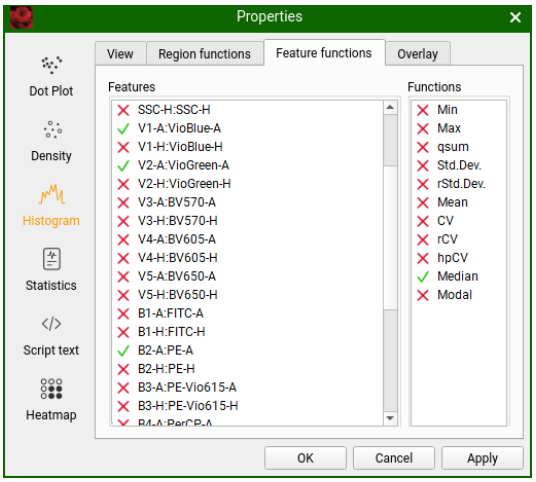
16 Click the **'i'** button of the statistics table.

17 Go to the **Region Functions** tab.

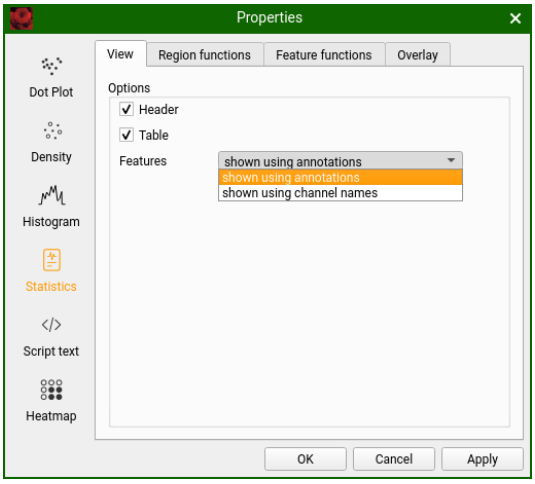
18 Select the regions for the negative and positive population.

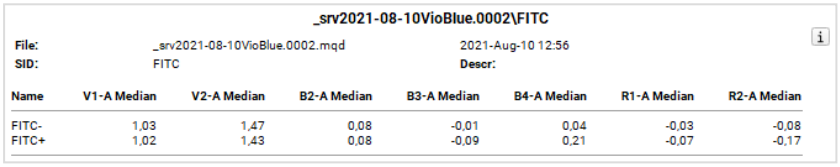
19 Go to the **Feature Functions** tab.

20 Select channels and **Median** to simultaneously show the medians for all channels.



21 Optional: Go to the **View** section and select **shown using channel names**. B1 is shown in the table instead of the annotation.





_srv2021-08-10VioBlue.0002\FITC

File: _srv2021-08-10VioBlue.0002.mqd 2021-Aug-10 12:56
SID: FITC Descr:

Name	V1-A Median	V2-A Median	B2-A Median	B3-A Median	B4-A Median	R1-A Median	R2-A Median
FITC-	1,03	1,47	0,08	-0,01	0,04	-0,03	-0,08
FITC+	1,02	1,43	0,08	-0,09	0,21	-0,07	-0,17

22 Click the **Pause** button to resume measurement.

23 To adjust compensation, select the appropriate cell in the matrix. See **Figure 5.11**. For example, to correct the spillover of a FITC-stained sample into the PE channel, go to the cell FITC/B2.

24 Adjust the values until the median fluorescence for the positive and negative populations are equal.

25 Adjust the matrix values doing one of the following:

- Click the toggle bar and move the slider up and down to change the value.
- 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.
- Double-click the value box, type in a value, and press **Enter**.

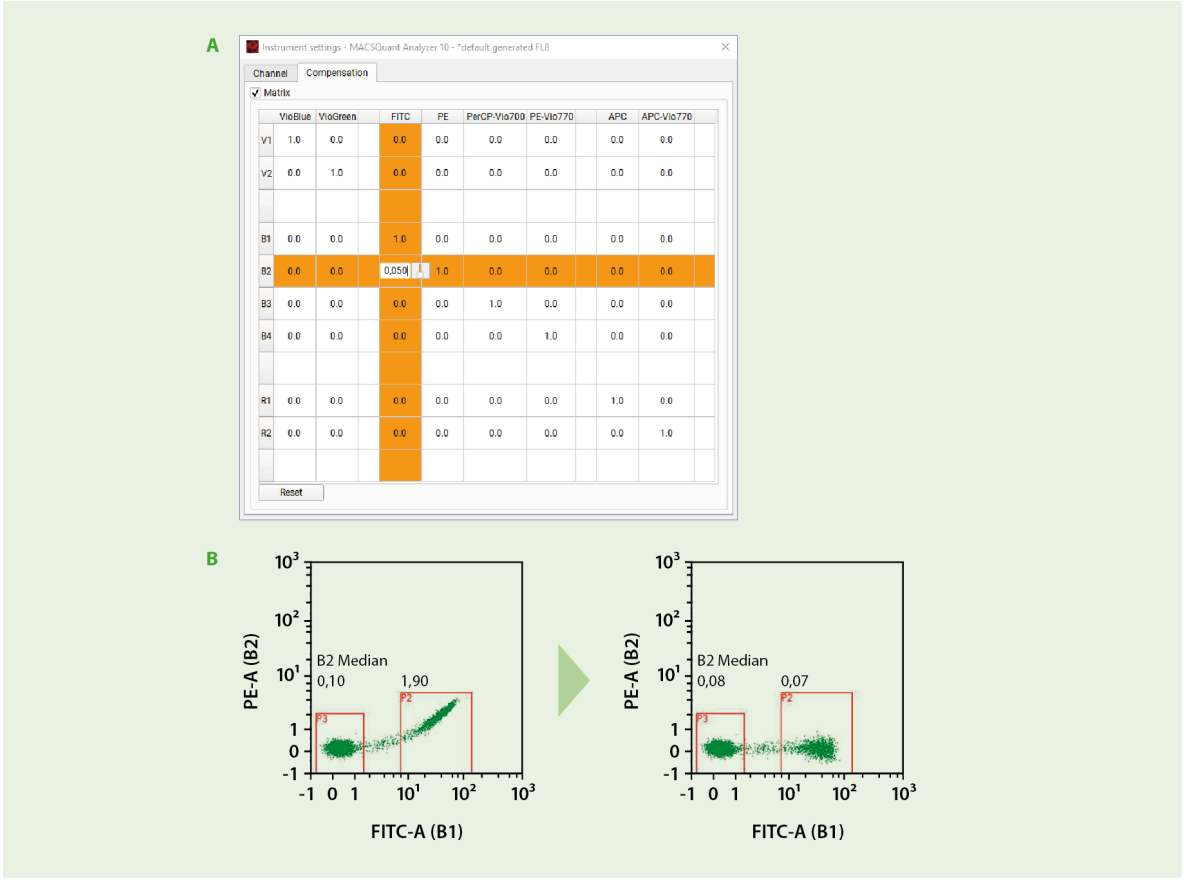


Figure 5.11: Manual compensation of the FITC spillover signal in the B2 channel. (A) Adjustment of compensation values in the compensation matrix. The value of the cell FITC/B2 is increased to reduce spillover of the FITC staining into the B2 channel. (B) Left panel: Dot plot of a FITC-stained sample prior to compensation. The higher B2 median for region P2 indicates a spillover of the FITC signal into the B2 channel. Right panel: Dot plot of the FITC-stained sample after adjusting the compensation matrix. Equal PE median values indicate that the spillover is properly compensated.

26 Increase the value if the median of positive population is higher than the median of negative population. Decrease the value if the median of positive population is lower than the median of negative population.

27 Once the compensation is adjusted for one fluorochrome, continue with compensation for all other fluorochromes used in the experiment.

28 Save as instrument setting.

5.6 Pulse processing

As particles move through the laser path, the pulse increases until the cell is fully illuminated, and then decreases until the cell passes fully through the laser path. The measurement of the light scatter is output as voltage pulses. On MACSQuant Instruments, three voltage measurements are available for all optical channels.

- The **Area** under the curve represents the total light emitted by the particle.
- **Height** represents the maximum signal intensity.
- **Width** represents the time of a particle traveling through the laser path. It is also called "time of flight" and represents the relative size of the particle.

By default, the pulse area is the default parameter for fluorescence or scatter. On the MACSQuant Instruments, the pulse area of the channel parameter is represented by the annotation followed by **-A**, i.e., **FITC-A**. In some cases, voltage pulse height and width are advantageous, for example, for discrimination of doublets during cell cycle analysis. For the MACSQuant Instruments, height and width must be selected prior to measurements to be saved in the data file.

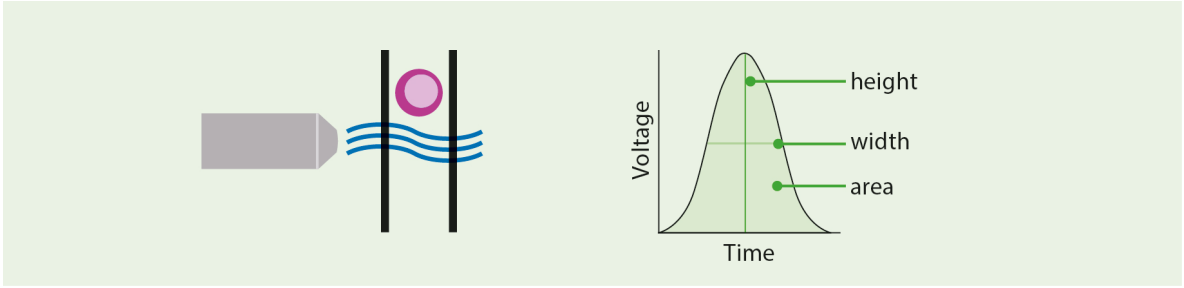
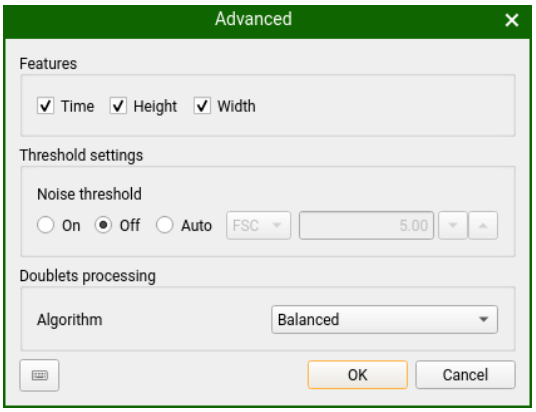


Figure 5.12: Three different parameters can be used to measure a voltage pulse: height, width, or area.

5.6.1 Selecting height and width as part of an instrument setting

1 Go to the **Channels** tab in the side pane.

2 Select the **Advanced** button.



3 Select **Height** or **Width** byselecting the box next to the desired parameter.

4 Click **OK**. The additional parameters are now active and are saved in the data file.

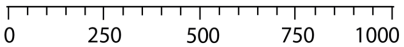
5 To display these parameters in plots, select the annotation ending with "**-H**" or "**-W**".

If a saved instrument setting is used, it is recommended to first open this setting as the active setting on the instrument and then activate height or width as additional parameters. See **Using one active instrument setting on page 77**. To save it permanently in the instrument setting go to **File > Save** and select **Instrument setting**.

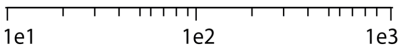
5.7 Adjusting data display scales

To properly visualize data the MACSQuantify Software offers various options for scaling. MACSQuant Instruments can display data in a variety of formats. FSC and SSC can be shown in lin, log2, log3, log4, log 5, and hlog scale. Fluorescent channels can be shown in lin, log3, log4, log5, and hlog scale.

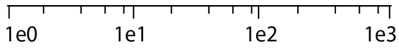
lin – Linear scale



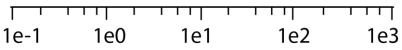
log2 – Logarithmic scale of two decades



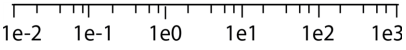
log3 – Logarithmic scale of three decades



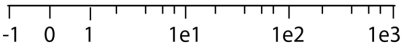
log4 – Logarithmic scale of four decades



log5 – Logarithmic scale of five decades



hlog – Biexponential scale



Standard dot plots showing side scatter and forward scatter are typically shown using linear scales since different cell populations often do not vary a lot in their light scatter signal intensity. Use a logarithmic scale when analyzing cell lines or samples that include very large particles as well as small particles such as bacteria or microvesicles.

Linear scaling is often not appropriate when displaying fluorescence intensity of different cell populations. Fluorescence signal intensities that extend over several orders of magnitude must be shown in a logarithmic scale.

Some fluorescence intensities may be below zero. As digital processing allows the assignment of true zeros and negative fluorescence values, data values below zero are not shown properly using a conventional logarithmic scale, even though all calculated statistics are correct. To improve visualization of data close to or below zero, the MACSQuantify Software offers a hyperlog (hlog) scale. In an hlog scale, the upper values of the scale are logarithmic while the lower values are linear around the zero point.

Occasionally, the difference between fluorescence values in a dataset can be relatively small, for example, when using fluorescent probes to measure quantitative changes in cellular DNA during cell cycle. It is recommended to use a linear scale to visualize these subtle changes in fluorescence intensity.

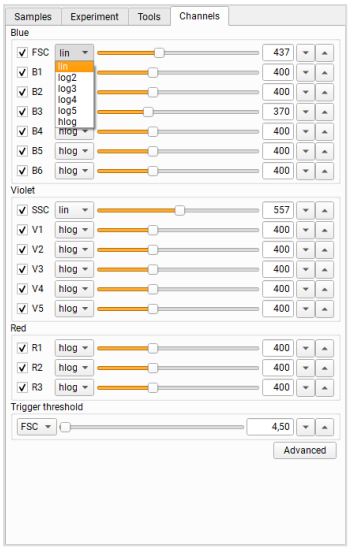
5.7.1 Adjusting scales prior to data acquisition

The data display scales can be set prior to acquisition using the **Channels** tab of the side pane. The selected scale is used during data acquisition.

Changing the scale in the dot plot properties changes the display of the scale but not the scale for the data acquisition.

1 Go to the **Channels** tab.

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



Scales can be also adjusted after acquisition to change the axis scaling that was used during acquisition. For details, see **Resampling: Change scales on page 142**.

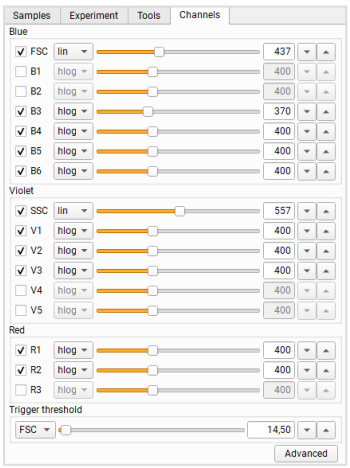
5.8 Reducing the number of channels

If only a few detection channels are being used in an experiment, the unused channels can be turned off to reduce the size of data files.

If a saved instrument setting is used for data acquisition, this setting must be opened as an active setting on the instrument prior to deselecting the unused channels.

1 Go to the **Channels** tab.

2 Clear the boxes next to the unused detection channels.



Upon data acquisition, only the selected detection channels are recorded. In this example, B1, B2, V4, V5, and R3 are cleared and are no longer available for data analysis.

5.9 Using an instrument setting

The MACSQuantify Software offers two ways of using instrument settings. An instrument setting can be used as an active instrument setting applied to all samples in an experiment. Alternatively, an existing instrument setting can be assigned to samples individually based on the rack position.

5.9.1 Using one active instrument setting

The active instrument setting can be changed in the MACSQuantify Software before starting the acquisition. The changes are applied to the samples unless there is another instrument setting assigned. If required, the changes can be permanently saved as an instrument setting for later use.

To use an active instrument setting, select one of the following options:

- Adjust instrument settings in the **Channels** tab.
- Load a saved instrument setting file via **File > Open...**. See **Opening an instrument setting file below**.
- Load an instrument setting from a data file in the sample list. See **Opening an instrument setting from a data file below**.

Opening an instrument setting file

1 Click the **Open** button in the toolbar.

2 Select **Instrument settings**.

3 Select the **Public** or **Private** directory.

> Depending on the user rights, only the **Private** directory may be accesible.

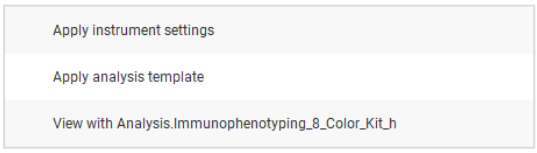
4 Select the instrument setting from the options on the right and click **Open**.This setting is now an active setting on the MACSQuant Instrument. All data are acquired using this setting. Adjustments to the settings can be made if necessary for the specific experiment and are applied during measurement.

5 Optional: Click the **Save** button in the toolbar and select **Instrument setting** to update the instrument setting or to save it as new instrument setting for later use.

Opening an instrument setting from a data file

1 Click the **Open** button in the toolbar and open the data file with the desired instrument setting.

2 Go to the **Samples** tab, right-click the data file, and select **Apply instrument settings**.



The instrument setting of the data file is loaded as the active setting on the MACSQuant Instrument. All subsequently acquired samples are run with this instrument setting. Changes to the active instrument setting are applied to samples during acquisition.

5.9.2 Using different instrument settings

It is possible to load an instrument setting file via the **Experiment** tab. This is necessary if more than one instrument setting should be used in an experiment.

1 Go to the **Experiment** tab.

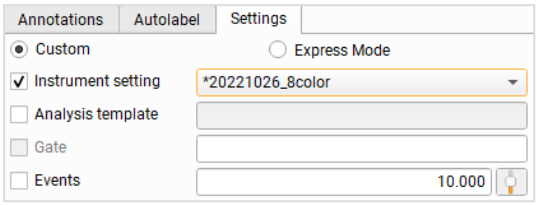
2 Go to the **Settings** tab.

3 Select the **Custom** radio button.

4 Select the box **Instrument setting**.

5 Highlight the sample positions in the **Rack** window which should be run with the same instrument setting.

6 Select the desired instrument setting from the dropdown list. Instrument settings saved in private are marked with an asterisk.



If the instrument setting needs to be adjusted, make sure to save the change. When acquisition is started, the software applies the instrument setting selected from the dropdown list. Changes are ignored unless they are saved.

5.9.3 Checking instrument settings

It is recommended to check the instrument settings including compensation regularly to ensure that the settings still meet the application specific requirements. The following situations may require an additional check and adjustment of instrument settings:

- After a service visit.
- After exchange of hardware components of the optical bench, for example, exchange of a PMT.
- When using a new or a different lot of fluorochromes.
- If altered spillover properties are expected due to aging of reagents, for example, when using tandem-dyes such as PE-Vio770.
- If changes in the scatter properties of the material is expected, for example, due to different treatment of cells.

Follow the instructions below to check if the instrument setting is still appropriate for the application.

1 Run a PMT calibration.

2 Optional: Load a saved instrument setting.

3 Check and adjust the FSC and SSC based on an unstained or stained sample. See **Adjusting FSC and SSC gains on page 48**.

4 Adjust the threshold settings if required.

5 Check and adjust gains for every fluorescence channel used in the application based on a fully stained or single stained sample(s). See **Adjusting gains of fluorescence channels on page 49**.

Changes of fluorescence channel gains require adjustment of the compensation.

6 Review compensation settings based on single-stained samples. See **Manual PMT calibration on page 45**.

Adjustment of compensation can be done post-acquisition if single stained controls are acquired in the experiment.

7 Go to **File > Save**.

8 Select **Instrument Setting**.

9 Click **Save**.

5.9.4 Transferring instrument settings

Instrument settings can be transferred between instruments according to **Table 5.1**.

MACSQuant type		Analyzer 16	Analyzer 10		VYB		X	
	Serial number	All	<21200	≥21200	<3100	≥3100	<5020	≥5020
Analyzer 16	All	Yes	No	No	No	No	No	No
Analyzer 10	<21200	No	Yes	No	No	No	Yes	No
	≥21200	No	No	Yes	No	No	No	Yes
VYB	<3100	No	No	No	Yes	No	No	No
	≥3100	No	No	No	No	Yes	No	No
X	<5020	No	Yes	No	No	No	Yes	No
	≥5020	No	No	Yes	No	No	No	Yes

Table 5.1: Transfer of instrument settings between MACSQuant Instruments

5.10 Defining experiment parameters

Experiment parameters can be defined in the different sections of the **Experiment** tab.

5.10.1 Using special characters

Use the ASCII characters A-Z, a-z, 0-9 for any entries. Use maximum 254 characters for file naming, sample ID, description fields in experiment tab, or fragment fields. Only special characters listed in **Table 5.2** are allowed to be used. Other special characters are restricted, and cannot be typed in. Same restriction applies when saving a file via **File > Save**.

German special characters **ä, ü,ö, Ä, Ü,Ö, ß** are replaced automatically by **ae, ue, oe, Ae, Ue, Oe, ss**.

Character	Name
.	Full stop
#	Number sign
()	Left and right parenthesis
+	Plus sign
-	En dash
_	Underscore

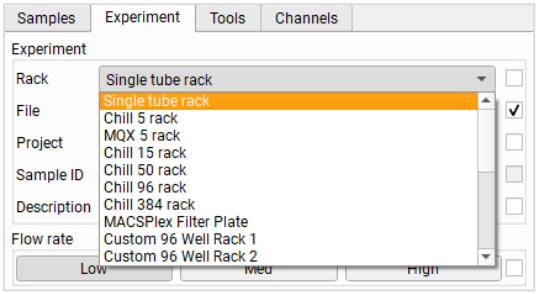
Table 5.2: Allowed special characters in the MACSQuantify software

5.10.2 Experiment tab

This chapter describes general settings for an experiment in a single tube format. See **Processing multiple samples on page 87** for programming the sample rack position for multiple samples.

1 Go to the **Experiment** section.

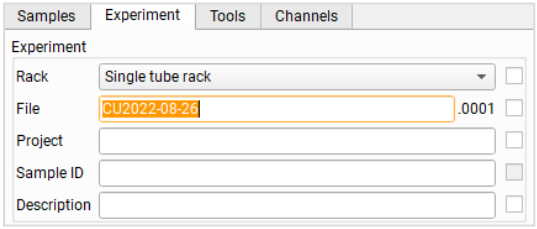
2 Go to the **Rack** dropdown list and select a rack type. If the box to the right of the field is selected, then the instrument automatically detects the used rack via the rack detection. To activate rack detection, see the MACSQuant Instrument or MACSQuant X Instrument user manual.



Only calibrated racks are shown in the dropdown list.

Available rack types differ between instrument types. For a detailed list, see **Selecting a rack on page 87**.

3 Optional: Adjust the name of the file. Clear the box to manually change the file naming. Use alphanumeric text only. By default, a checkbox is selected indicating an automated file naming.

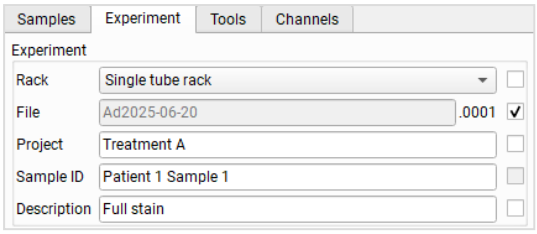


By default, the MACSQuantify Software uses the user's initials and date to construct the file name. To learn more about file naming options and how to change the default file name see **Changing path or file naming on page 197**.

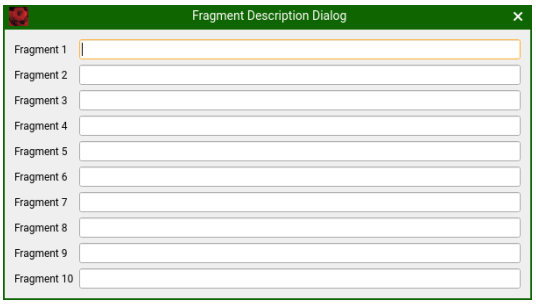
4 Optional: Enter a name for a project folder in the field **Project**. All data are saved in this folder. Select the box next to **Project** to see a dropdown list of all project folders of the signed in user.

Use only the ASCII chararcters A-Z, a-z, 0-9, left and right parenthesis, plus sign, en dash and underscore (# ()+-_) for file, project, sample ID, and description.

5 Optional: Enter alphanumeric text as sample ID.



6 Optional: Enter alphanumeric text in the field **Description**. Select the box on the right-hand side to determine a pattern for automated description. See **Using the fragment description dialog on page 94**.

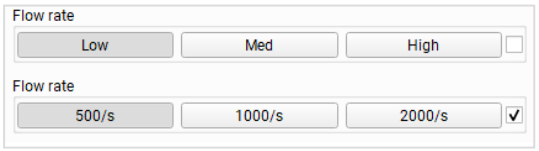


5.10.3 Flow rate

Keep the event rate below 15,000 events per second for accurate measurements. 1000–5000 events/s is ideal.

The flow rate can be adjusted by doing one of the following:

- Select **Low**, **Medium**, or **High** depending on the cell concentration and research question. Select **Low** for high cell concentrations, for example, 10^7 cells/mL.
- Select the box on the right-hand side to switch to events per second. The flow rate is then automatically adjusted to low, medium, or high during acquisition to approximately reach the selected event rate.



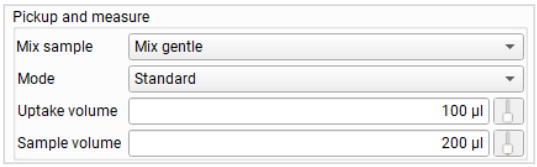
Flow rate (µL/min)	MACSQuant Running Buffer (sheath) consumption (mL/measurement)
Low: 25	18
Med: 50	13
High: 100	8.6

Table 5.3: Flow rate and MACSQuant Running Buffer (sheath) consumption for a 100 µL sample, run in Standard mode.

5.10.4 Pickup and measure

1 Go to the **Experiment** tab.

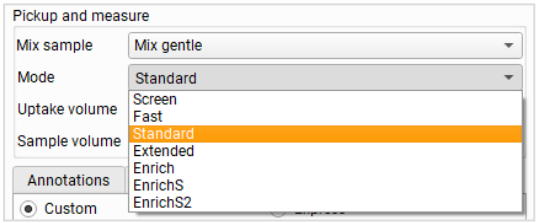
2 Go to the dropdown list **Mix sample** to select a mixing option. Available mixing options differ between the instrument types. See **Mix parameters on page 209**.



3 Select a mode from the **Mode** dropdown list: **Screen**, **Fast**, **Standard**, **Extended**, **Enrich**, **EnrichS**, or **EnrichS2**. Available modes differ between the instrument types. The selected mode defines the washing steps between samples or during enrichment using the MACSQuant Column.

The **Screen** mode and the enrich programs are only available on MACSQuant Analyzer 10, VYB, and Analyzer 16.

If an uptake volume larger than 50 µL is selected in **Screen** mode, a message appears on the screen.



4 Enter an uptake volume and a sample volume. The uptake volume defines the amount of sample that is taken up for acquisition. The sample volume refers to the amount of sample in the tube. The maximum uptake volume is 450 µL on the MACSQuant Analyzer 10, VYB, and Analyzer 16, and 5 mL on the MACSQuant X.

If mixing is selected on a MACSQuant Analyzer 10, VYB, or Analyzer 16, it is necessary to accurately define the sample volume to avoid the uptake of air bubbles.

Mode	Total buffer volume (µL)	Total time (s)
Screen	2508.4 + 750 per sample	4 (between samples) 22 (after final sample)
Fast	4,916.60	12
Standard	6,575	25
Extended	11,050	72

Table 5.4: Buffer volume and time needed to washing the needle between samples. Applicable for MACSQuant Analyzer 10, VYB, and Analyzer 16

Mode	Total buffer volume (µL)	Total time (s)
Fast*	1,200	<1
Standard*	3,200	<10
Extended	10,800	25

Table 5.5: Buffer volume and time needed to washing the needle between samples. Applicable for MACSQuant X
*If using single tube holder for sample acquisition, Fast and Standard modes perform similarly to Extended mode

5.10.5 Annotations tab

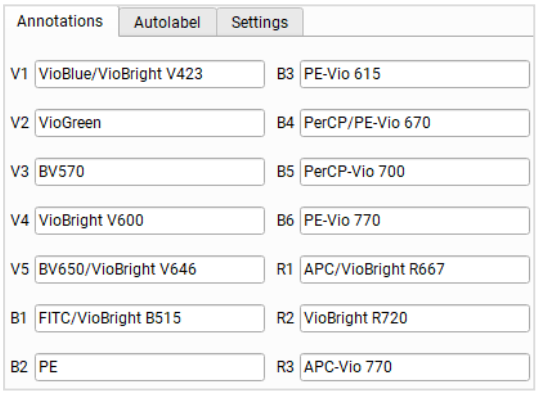
The annotations of the fluorochrome channels can be changed in the **Annotations** tab. The entries are shown on the plot axes.

1 Go to the **Experiment** tab.

2 Select the **Annotations** tab.

3 Optional: Select sample positions first to change annotations for samples individually.

4 Click into the field of a channel to change its annotation.



Annotations may not appear on the plot display window until acquisition starts.

If using a saved instrument setting the annotations are reset to the annotations of the saved instrument setting.

Annotations are automatically set when using selected Miltenyi reagents in the **Settings** tab.

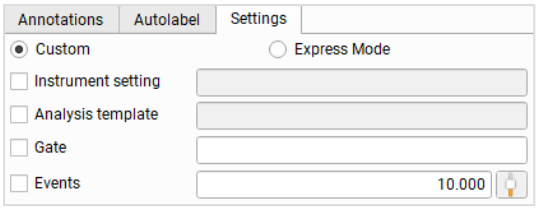
5.10.6 Settings

Custom analysis

1 Go to the **Experiment** tab.

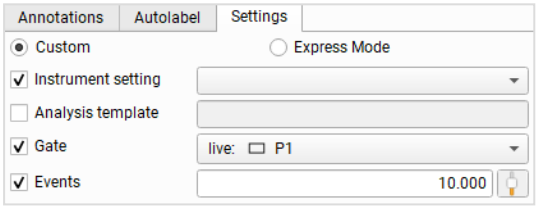
2 Select the **Settings** tab.

3 Select the **Custom** radio button.



4 Select the checkboxes next to **Instrument setting**, **Analysis template**, **Gate**, and **Events** to activate these features. When setting up an acquisition of multiple samples, make sure to select the sample positions which should be run with the selected options. When cleared, the instrument runs all samples with the current active instrument setting and analysis template. To create an analysis template, see **Creating an analysis template on page 105**

> The annotations in the instrument setting file override any annotations set in the experiment tab, including the **Fragment Description Dialog**.



- **Instrument setting:** Select the checkbox to use saved instrument settings. This function allows usage of different instrument settings within one experiment.

> If the instrument setting is changed after loading the setting, the changes must be saved to be applied.

- **Analysis template:** Select the checkbox to use saved analysis templates.
- **Gate:** Select the checkbox to activate gate settings. A gate must be drawn in a plot to enable selection of the checkbox. **Stop gate**: Used in combination with a defined event limit. When the event limit is reached in the selected gate, the measurement stops. Data are saved automatically including events outside the stop gate. **Live gate**: Only events within the selected gate are saved in the data file. Can optionally be used in combination with a defined event limit. If an event limit was defined, the measurement stops when the event limit within the live gate is reached. See **Setting a stop gate on page 86** and **Setting a live gate on page 86**.
- **Events:** Select the checkbox to stop data acquisition and save the data file after a defined number of total events were acquired; in this example, 10,000 events. Can also be used in combination with a **Stop gate** or **Live gate**. Values in events are represented by European numbering.

> It is recommended to limit measurement by volume because volumetric measurements allow absolute cell counting. If using an event rate to limit measurement, ensure that sufficient uptake volume is defined to reach the desired number of events. This depends on the concentration of the cell suspension.

Selecting an Express Mode

1 Load an appropriate instrument setting.

2 Go to the **Experiment** tab.

3 Select a rack and highlight the positions for the samples. This is not required if the Single tube rack is used.

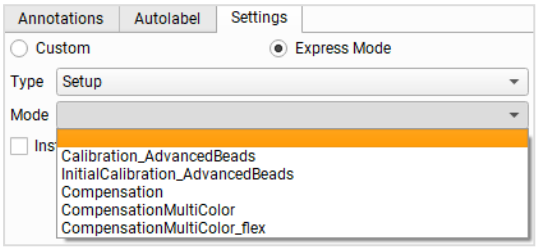
4 Optional: Group the samples. See **Grouping of data files on page 138**.

Do not group more positions than sample IDs available.

5 Select the **Settings** tab.

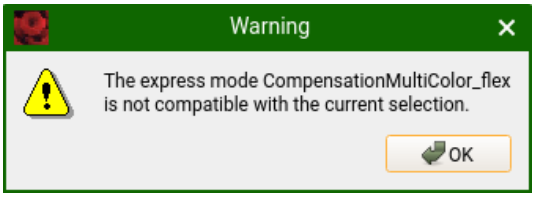
6 Select the **Express** radio button.

7 Select **Setup** from the **Type** dropdown list to use an Express Mode for calibration or compensation processes. See **Table 11.6** for a list of **Setup Express Modes**.



8 Select **Setup** from the **Type** dropdown list to use Express Modes for analysis of cells with Miltenyi reagents such as MACS Antibody Cocktails. Experiment settings such as mixing, mode, uptake volume, and sample volume are loaded automatically. Custom users or MQ Administrators can change these experiment settings manually.

9 Optional: If the message below is shown when selecting the Express Mode, do one of the following:



- Select an appropriate Chill Rack.
- Group the sample.
- Check if the number of grouped samples is in accordance with the number of sample IDs.

10 Optional: Enter a **Sample ID** and **Description**.

Use only the ASCII chararcters A-Z, a-z, 0-9, left and right parenthesis, plus sign, en dash and underscore (# ()+-_) for file, project, sample ID, and description.

11 For grouped Express Modes, select the sample ID from the **Sample ID** dropdown list.

Do not assign the same sample ID twice to avoid difficulties during automated sample analysis.

12 Start the measurement. The acquisition page of the Express Mode is shown automatically.

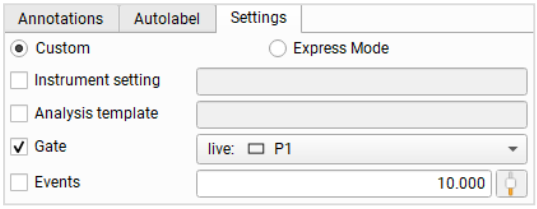
13 The Express Mode analysis is performed and automatically shown after measurement of the current sample. To change the default behaviour, go to **Edit > Options... > Software > Acquire > Apply Express Mode analysis automatically**. Click the arrow in front of **Software** to access all options.

For more details about Express Modes see **Express Modes on page 157**.

5.10.7 Setting a live gate

Only events within a live gate are acquired and saved in the data file. Live gates can be used when large data sets are being acquired to reduce the size of the data file. For example, experienced users can exclude debris by live gating.
However, it is recommended to acquire and save all data to avoid unintended data loss. Live gating strategies can be saved for future use as analysis templates.

1 Click the **Add** button in the toolbar and select an analysis window template.
2 Define the **Experiment settings**. A region must be drawn in the analysis template to be able to define a live gate.
3 Go to the **Settings** tab and select the **Gate** checkbox.
4 Select **live:** of the desired region from the dropdown list.
5 Optional: Select the Events checkbox and enter a value to define an event limit.



6 Click the **Run** button.
7 Move the gate if required to show the events of interest.
8 Click **Clear**to refresh the plot after moving the gate. Events are also deleted from the data file when clicking **Clear**. To change this behaviour, see **Reset sample on clear view on page 200**.
9 Optional: Save the **Analysis template** for future use.

5.10.8 Setting a stop gate

If a stop gate is used in combination with the **Events** option, data are acquired from the entire analysis window until a pre-defined number of events is reached within the stop gate. Stop gating strategies can be saved as analysis templates.

1 Click the **Add** button in the toolbar and select an analysis window template.
2 Define the **Experiment settings**. A region must be drawn in the analysis template to be able to define a stop gate.
3 Go to the **Settings** tab and select the **Gate** checkbox.
4 Select **stop:** of the desired region from the dropdown list.
5 Enter the required number of events for the associated stop gate.



For absolute cell counting it is recommended to limit measurement by volume and not by an event limit.

If using an event rate to limit measurement, ensure that sufficient uptake volume has been selected to acquire the desired number of events.

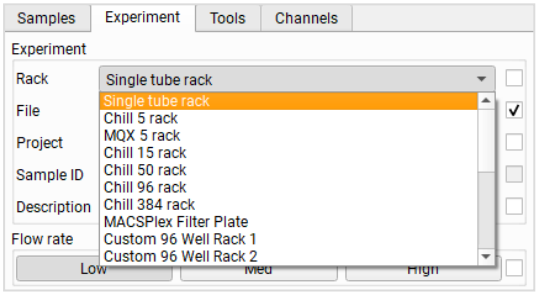
5.11 Processing multiple samples

Use the MACSQuant Analyzer 10, VYB, or Analyzer 16 in combination with the MACS MiniSampler Plus and one of the Chill Racks to measure multiple samples. Use the MACSQuant X in combination with the MACSQuant X Orbital Shaker and the MACSQuant X 5 Rack or appropriate plates to measure multiple samples.
Different sample racks are available, allowing for processing up to 96 (MACSQuant Analyzer 10, VYB, or Analyzer 16) or 384 samples (MACSQuant X) in a single experiment. Different types of cell samples or analysis panels can be configured on the same rack. Samples can be automatically labeled with fluorochrome-conjugated antibodies, cocktails, or fluorescent dyes prior to measurement. Use the Universal Reagent Rack for labeling samples with up to four reagents.

5.11.1 Selecting a rack

1 Go to the **Experiment** tab.

2 Select the required rack type from the **Rack** dropdown list.



Only calibrated racks and related ones are shown in the dropdown list.

A scheme of the selected rack is shown at the bottom of the **Experiment** tab.

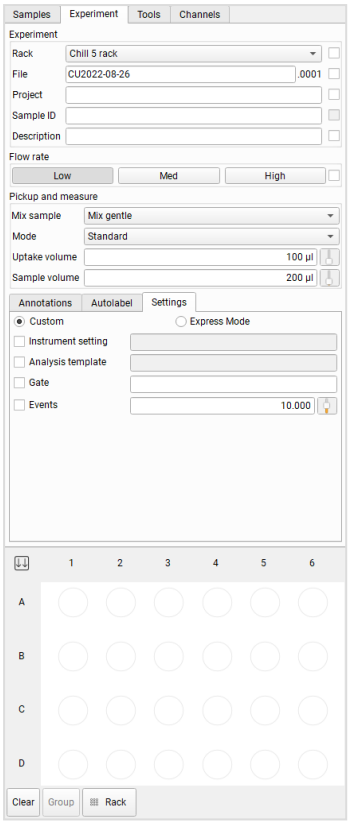


Figure 5.13: Experiment tab with rack scheme

Rack	Select from drop-down	Slots	Compatible tubes and plates
Single tube rack	Single Tube Rack	1×5 mL	5 mL tubes 1.5 mL or 2 mL reagent tubes
Chill 5 Rack	Chill 5 Rack	24×5 mL	5 mL tubes
Chill 15 Rack	Chill 15 Rack	15×15 mL 5×5 mL	15 mL tubes 5 mL tubes
Chill 50 Rack	Chill 50 Rack	6×50 mL 3×15 mL 3×5 mL	50 mL tubes 15 mL tubes 5 mL tubes
Chill 96 Rack	Select an entry matching the plate design, for example, Chill 96 Rack.	Up to 96 wells	6-, 12-, 24-, 48-, and 96-well plates
MACSPlex Filter Plate	MACSPlex Filter Plate	96 wells	MACSPlex Filter Plate, 96-well plates

Table 5.6: Racks available for the MACSQuant Analyzer 10, VYB, and Analyzer 16

Rack	Select from drop-down	Slots	Compatible tubes and plates
Single tube rack	Single Tube Rack	1×5 mL	5 mL tubes 1.5 mL or 2 mL reagent tubes
MACSQuant X 5 Rack	MQ 5 rack	24×5 mL	5 mL tubes
Microtiter plate no rack	Select an entry matching the plate design, for example, Chill 384 Rack.	Up to 384 wells	6-, 12-, 24-, 48-, 96-, and 384-well plates
MACSPlex Filter Plate no rack	MACSPlex Filter Plate	Up to 96 wells	MACSPlex Filter Plate, 96-well plates

Table 5.7: Racks available for the MACSQuant X

Refer to the MACSQuant Instrument user manual and MACSQuant X Instrument user manual for an overview of racks included in the delivery. Further racks can be ordered separately.

5.11.2 Using the Chill 15 and Chill 50 Rack

The Chill 15 and Chill 50 Rack are available from the **Rack** dropdown list if the Chill 5 Rack is calibrated. Refer to the MACSQuant Instrument user manual for instructions.
To avoid sample carry over, do not exceed following sample volumes. The volume must not be exceeded at any time during measurement. If dilution of sample or autolabeling is active, take the final volume into account.

Rack type	Max. allowed volume
Chill 15 Rack	7 mL
Chill 50 Rack	27 ml

Table 5.8: Maximum allowed sample volume for using Chill 15 and Chill 50 Rack

5.11.3 Sample rack positions

All rack positions are defined by coordinates: columns are identified by numbers, rows by letters. To select a single rack position, click the desired position. Alternatively, the MACSQuant Instrument touchscreen can be used. Click the same position to activate the position for editing. Positions are marked with colors depending on their status.

Sample position color code

Clear		**Default open circle indicates no operation.**
Closed green circle		**Sample used for measurement. Click circle to select for editing.**
Closed green circle with orange rim		**The orange rim indicates that the sample is selected and activated for editing.**
Closed yellow circle		**Processing of sample has started, for example, sample is diluted and incubated.**
Closed blue circle with orange rim		**Measurement is in progress.**
Closed gray circle		**Measurement has been finished.**

Table 5.9: Sample position color code

Selecting a sample position

Several rack positions or an entire rack, row, or column can be selected, deselected, or cleared. Highlighted sample position can be edited.

The rack is shown in the **Experiment** tab. Click the **Rack** button to open an enlarged rack scheme in a separate window.

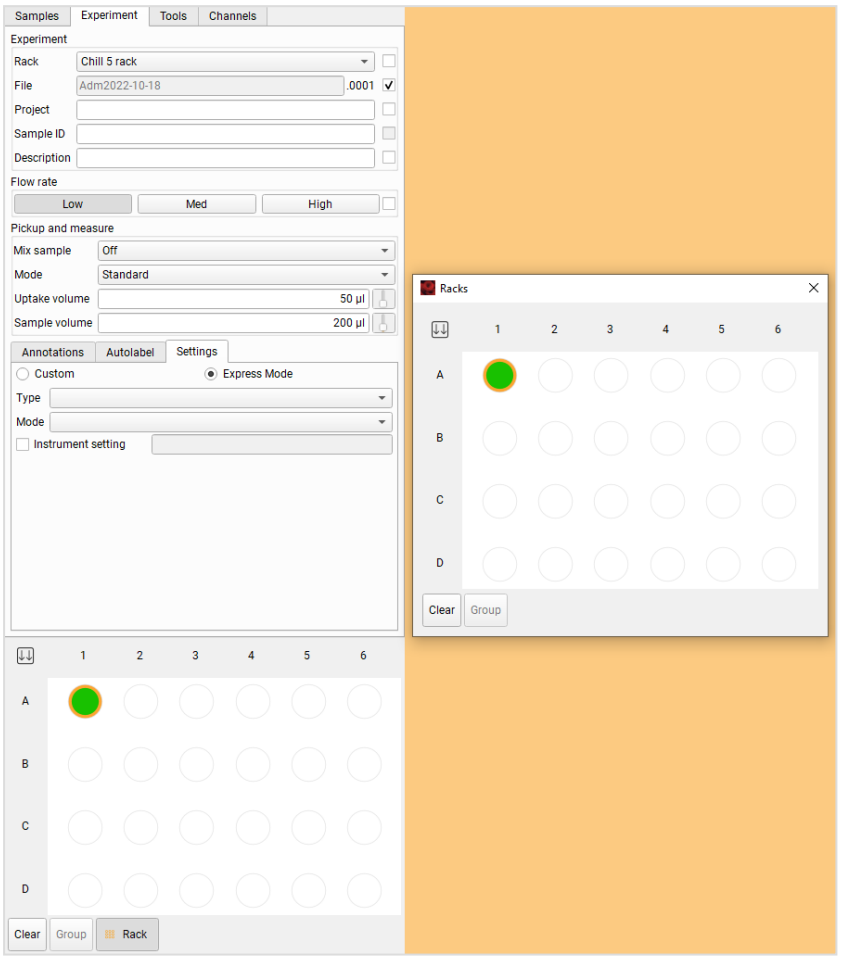
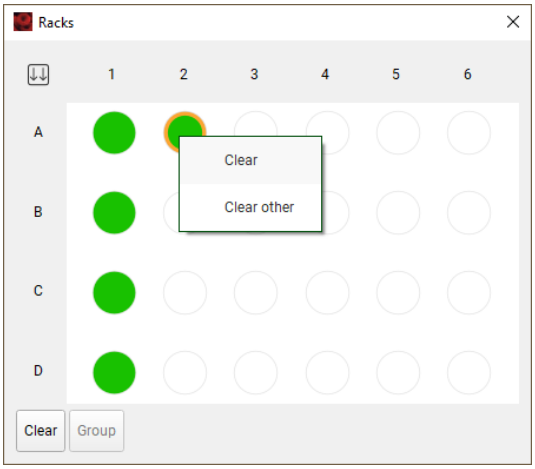


Figure 5.14: Rack scheme shown in the **Experiment** tab and in a separate window

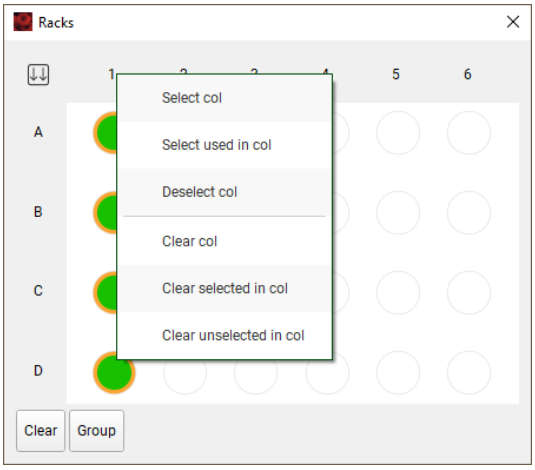
To select or clear single sample positions:

- Click a sample position. Highlighted positions are indicated by an orange rim.
- Right-click a sample position and select **Clear** to clear the highlighted position.
- Right-click a sample position and select **Clear other** to clear all wells except the selected well.
- Use the **Clear** button in the lower left corner of the **Racks** window to clear selected sample positions.



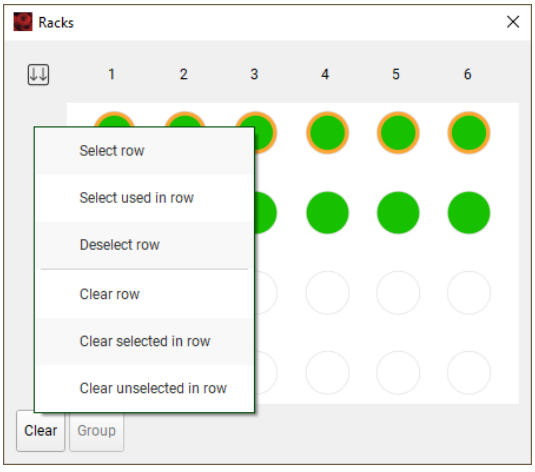
To select or clear a column or positions in a column:

- Right-click a column number and select from the available options **Select col**, **Select used in col**, **Deselect col**, **Clear col**, **Clear selected in col**, or **Clear unselected in col**.



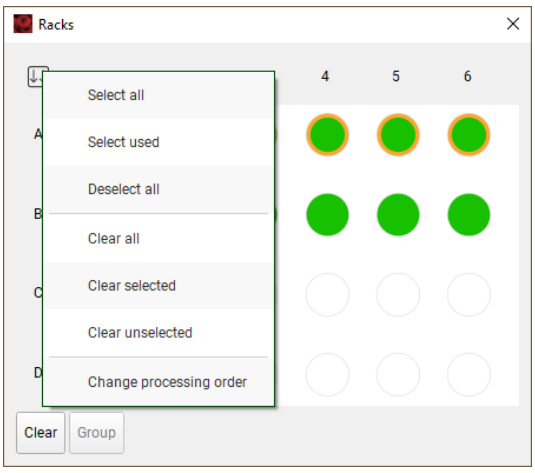
To select or clear a row or positions in a row:

- Right-click a row letter and select from the available options **Select row**, **Select used in row**, **Deselect row**, **Clear row**, **Clear selected in row**, or **Clear unselected in row**.



To select or clear all position:

- Right-click the **Sample menu** button in the upper left corner of the **Racks** window to select from the options **Select all**, **Select used**, **Deselect all**, **Clear all**, **Clear selected**, **Clear unselected**, or to **Change processing order**. The sample menu button indicates the current processing order.



The processing order cannot be changed for the Chill 50 Rack.

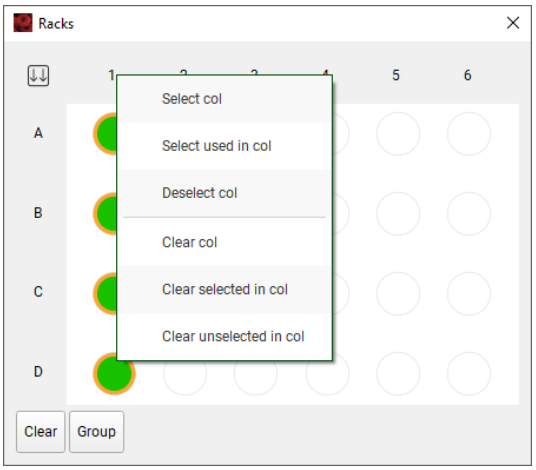
The default processing order is vertical. To change the default processing order to horizontal, go to **Edit > Options > Experiment**.

Programming sample positions

Program sample positions in the **Experiment** tab. Selected samples indicated by an orange rim can be edited. Selected samples can be programmed at the same time, or each position or group of positions can be programmed individually.

1 Select the sample position(s).

2 Optional: right-click a number or letter to select an entire column or row.



3 Adjust the **Experiment**, **Flow rate**, and **Pickup and measure** options.

4 Go to **View > Experiment table** to check the experiment settings for all sample positions.

5 Before starting the run check the following:

- The instrument is correctly calibrated and compensated. See **Automated PMT calibration on page 43** and **Automated compensation on page 56**.
- Each sample is correctly positioned on the sample rack.
- Sufficient quantities of reagents and buffers are provided.
- The waste bottle is empty.
- Lids are removed from microtiter plates.
- If autolabeling is selected: Reagents are assigned to a position on the Universal Reagent Rack. See **Automated reagent entry using the 2D barcode reader on page 98**.
- Reagents for autolabeling are placed on the Universal Reagent Rack.
- Caps are removed from the reagents.

5.11.4 Using the fragment description dialog

The **Fragment Description Dialog** allows to enter several descriptions for samples that share the same specifications. Examples for specifications are treatment type, staining conditions, or sample material. Entered fragment descriptions are automatically shown in the **Description** field for each sample. The fragments are defined based on the sample position.

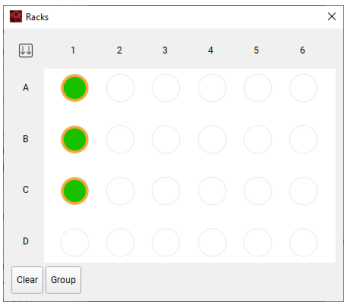
1 Go to the **Experiment** tab.

2 Select a rack for multiple samples from the **Rack** dropdown list.

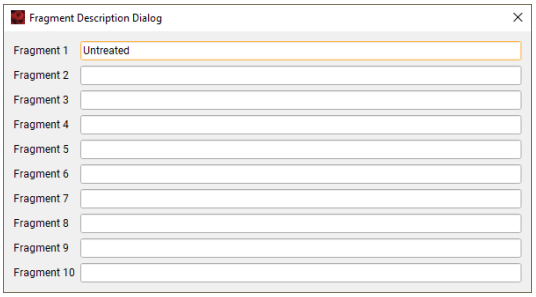
3 Select the checkbox on the right-hand side of the **Description** field.

4 Select the positions in the **Rack** window for all samples.

5 Highlight the wells with the same description fragment. Highlighted wells appear with an orange rim.



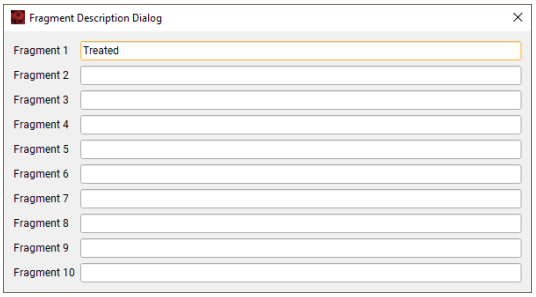
6 Enter a description into **Fragment 1**, for example, Untreated.



7 Highlight the next wells with the same description fragment.

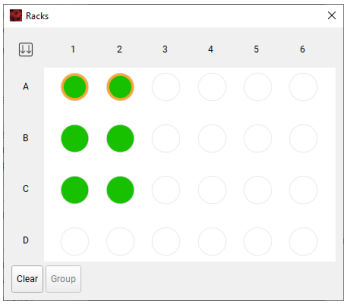


8 Enter the description into **Fragment 1**, for example, Treated.

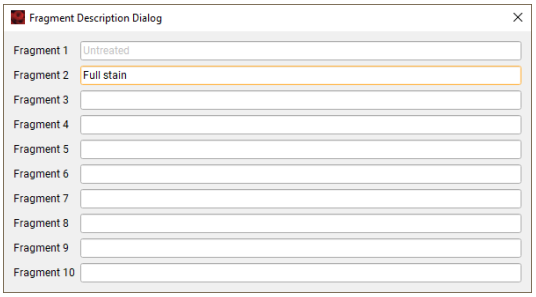


The same fragment can be used to enter the description if the Well IDs do not overlap.

9 Continue to highlight wells to add another description, for example, Full Stain.



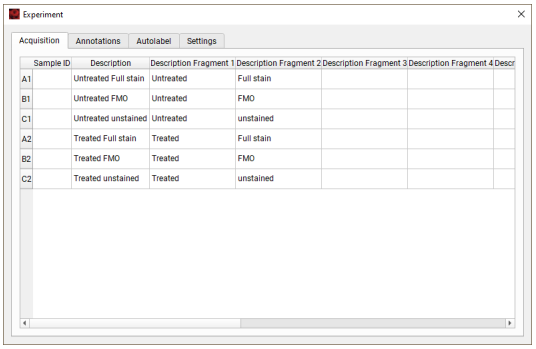
10 Enter the description into **Fragment 2**.



11 Continue with other samples and fragments as required.

12 Go to **View > Experiment table...** in the menu bar.

13 Select the **Acquisition** tab to check the correct assignment of the description fragments.



If the description is part of the automated file naming, the fragments are included in the file name. See **Identifiers for file naming on page 198**.

5.11.5 Grouping of samples

By grouping samples, the acquired samples of the group are combined into a single file. Use this function if samples of one experiment shall be analyzed together, for example, original, positive, and negative fraction of a sample separated using MACS MicroBeads. Additionally, if the sample size exceeds the maximum sample volume of the MACSQuant Instrument, aliquots of the sample can be spanned over two or more tubes. Some Express Modes also require grouping before acquisition, for example, CompensationMultiColor.

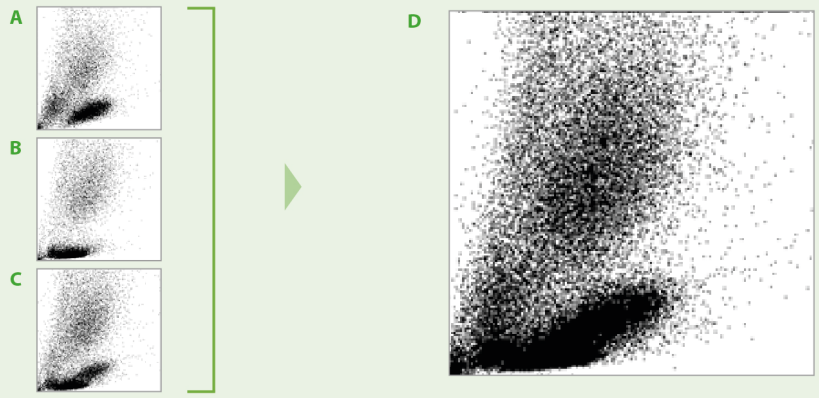


Figure 5.15: Sample grouping prior to acquisition

When a grouped data set is opened, the gates are automatically applied to all samples within the group.

To group or ungroup files after acquisition, see **Grouping and ungrouping of data files on page 138**.

1 Go to the **Experiment** tab.

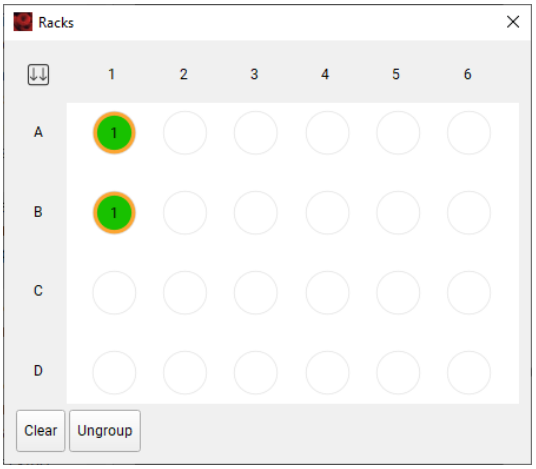
2 Select the desired rack from the **Rack** dropdown list.

3 Select sample positions for grouping. Selected samples must be adjacent either in a column or in a row, depending on the processing order. To change the processing order, see **Selecting a sample position on page 91**.



4 Click **Group** in the lower left corner.

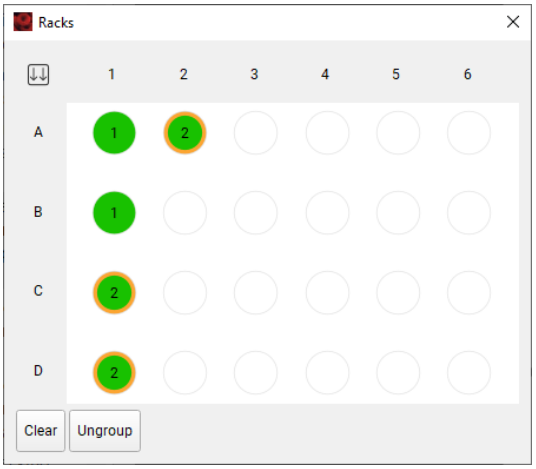
5 The wells of one group are marked with the same number.



6 Enter the sample information in the **Experiment** tab.

7 Optional: Select additional rack positions to perform further grouping:

- Add selected positions to an existing group: highlight the group and the new wells and click **Group**.
- Add a new group: select positions and click **Group**.



The entire group can be selected by double-clicking one sample position of the respective group.

8 Optional: To ungroup a selected group, click **Ungroup**.

9 Close the **Racks** window.

5.12 Autolabeling

The MACSQuant Instrument can add reagents such as antibodies, viability dyes, or MACSQuant Running Buffer to samples using the robotic needle arm. The Universal Reagent Rack is used in combination with the MACS MiniSampler Plus or MACSQuant X Orbital Shaker for autolabeling of cells. Up to four reagents can be placed in the Universal Reagent Rack.
Autolabeling can be used for following activities:

- Add fluorochrome-conjugated antibodies or antibody cocktails.
- Add viability dyes such as propidium iodide.
- Magnetically label rare cell populations for subsequent pre-enrichment and analysis.
- Dilute a sample with MACSQuant Running Buffer.

Reagents can be entered using the 2D barcode reader or manually via the **Reagents** window. See **Automated reagent entry using the 2D barcode reader below** and **Selecting and assigning reagents manually below**.

5.12.1 Automated reagent entry using the 2D barcode reader

Use the 2D barcode reader for scanning reagents. Reagent vials are automatically recognized and logged by the MACSQuantify Software.

1 Click the **Barcode** button in the toolbar.

2 Tilt the reagent vial slightly and present it in front of the 2D barcode reader, with the 2D barcode facing the blinking code reader light. The optimal reading distance is 0.5–2.5 cm. Scanned reagents are reported to the **Reagents** window.

3 Place the reagent onto the corresponding position (1-4) on the Universal Reagent Rack.

4 Optional: If the 2D barcode reader fails to recognize the 2D barcode, enter the information directly into the MACSQuantify Software **Reagents** window.

Scanning MACSQuant Calibration Beads initiates the calibration procedure of the MACSQuant Instrument. For more information on calibration, see **Automated PMT calibration on page 43**.

5.12.2 Selecting and assigning reagents manually

It is recommended to use the 2D barcode reader to scan reagents as this prevents incorrect reagent entries. If the reagent label or data sheet insert is damaged or an individual staining strategy should be applied, it may be necessary to select reagents manually using the **Reagents** window. Incubation time, titer, and order can be adjusted.

1 Place a reagent onto the Universal Reagent Rack, noting its position.

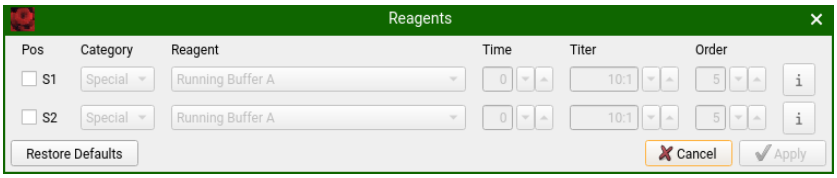
2 Go to the **Experiment** tab.

3 Select a multisample rack type.

4 Go to the **Autolabel** tab.

5 Select any **<add…>** box to open the **Reagents** window.





6 Select the reagent and Universal Reagent Rack position from the dropdown list of the **Reagents** window.

7 Check the desired reagent position **Pos R1, R2, R3, R4, S1**, or **S2**. R1-R4 positions are located on the Universal Reagent Rack. S1 and S2 can be used if MACSQuant Running Buffer should be added to the sample. MACSQuant Running Buffer is taken directly from the buffer bottle to dilute the sample as defined.

Annotations are automatically set when using selected Miltenyi reagents in the **Settings** tab.

8 Optional: To dilute samples automatically by the MACSQuant Instrument prior to measurement, select a buffer dilution from **S1** or **S2**.

9 Go to the **Category** dropdown list to select a reagent type.

- **Calibration**: MACSQuant Advanced Calibration Beads for the calibration of the instrument settings.
- Species and conjugated fluorochrome, for example, Human – APC, Mouse – PE.
- **Isotype Control**: Isotype control antibodies are raised against non-mammalian epitopes and can be therefore used as a negative control for non-specific binding.
- MACSPlex Reagents: These reagents are components of MACSplex Kits and are used in the respective assays.
- **Universal**: for generic labeling strategies using tags such as biotin, histidin, or propidium iodide.

Propidium iodide (PI) is listed under **Universal**.

10 Go to the **Reagent** dropdown list and select a reagent. A dropdown list of available reagents is shown in accordance with the selected category.

11 Go to **Time** to change the incubation time if required. A default incubation time is shown in a black font type. Change the incubation time by using the adjacent arrows.

12 Go to **Titer** to change the reagent titer if required. A default sample titer is shown in a black font type. Change the titer by using the adjacent arrows.

13 Go to **Order** to change the order if required. The shown number indicates in which order the reagents are used during the label procedure. Reagents with lower numbers are added first.

Changed titers or times appear in red font.

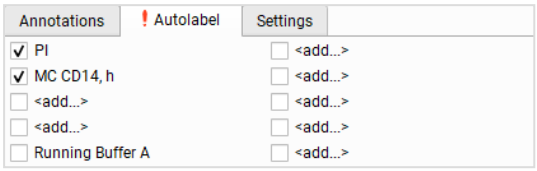
14 Click **Apply** to confirm.

15 Go to the **Rack** window to select one or more wells for autolabeling.

16 Go to the **Experiment** tab and select the **Autolabel** tab.

17 Select the reagent(s) to be added to the selected wells. If the box of the required reagent is not selected, the reagent is not added to the sample.

A red exclamation mark indicates that autolabeling is activated.



18 Repeat for other samples and reagents if required.

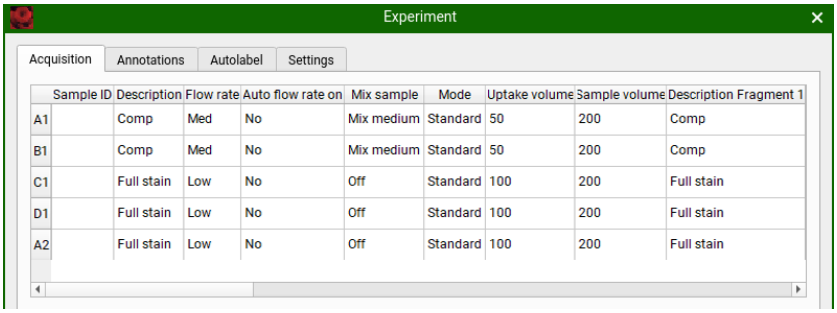
It is possible to assign a predefined analysis template to a sample position when using analysis cocktails such as CD14 Monocyte Analysis Cocktail, anti-human, for autolabeling. Select the corresponding Analysis Express Mode in the **Settings** tab, for example, MC_CD14_h.

5.13 Reviewing experiment settings

All experiment settings can be reviewed in the experiment table.

1 Go to **View > Experiment table**.

2 Switch between **Acquisition**, **Annotations**, **Autolabel**, or **Settings** to review the respective settings.



Sample ID	Description	Flow rate	Auto flow rate on	Mix sample	Mode	Uptake volume	Sample volume	Description Fragment 1
A1	Comp	Med	No	Mix medium	Standard	50	200	Comp
B1	Comp	Med	No	Mix medium	Standard	50	200	Comp
C1	Full stain	Low	No	Off	Standard	100	200	Full stain
D1	Full stain	Low	No	Off	Standard	100	200	Full stain
A2	Full stain	Low	No	Off	Standard	100	200	Full stain

5.14 Cell counting

Each MACSQuant Instrument is equipped with a precise fluidics system that enables automated and accurate uptake of sample volumes. Cell counting is included in every measurement without the need of counting beads. During data analysis, the MACSQuantify Software provides the cell count for each sample and population. Results can be shown as absolute numbers or as concentration. To achieve optimal results, consider the following recommendations:

- Program an uptake volume of at least 25 µL.
- Use **Low** as flow rate.
- Keep the events below 5000 events per second. If required, dilute the sample.
- Do not stop the measurement during acquisition.
- Do not use a stop gate.
- See **Compatibility of coincidence processing algorithms on page 193** to select the proper algorithm on the MACSQuant Instrument used.

6 Data analysis

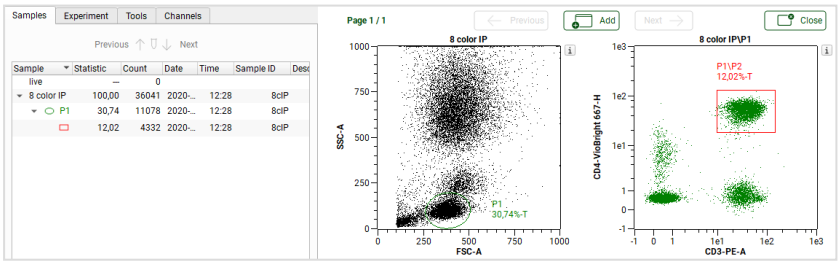
For optimal data analysis results, compare only data analyzed with the same MACSQuantify Software version.

Acquired data are shown and analyzed in an analysis window. Analysis templates consist of an analysis window template and, if required, a gating strategy. Depending on which template is applied, the analysis windows contains dot plots, density plots, histograms, statistics, and text tables. Several analysis windows can be opened at one time. These can contain several experiments or represent a single experiment with a complex gating strategy. Gating strategies can be created before sample acquisition and saved for future use, or they can be created after data acquisition. To change plots or analysis templates, deselect the **Analysis mode** button in the toolbar.

6.1 Hierarchical gating

Create a hierarchical gating strategy.

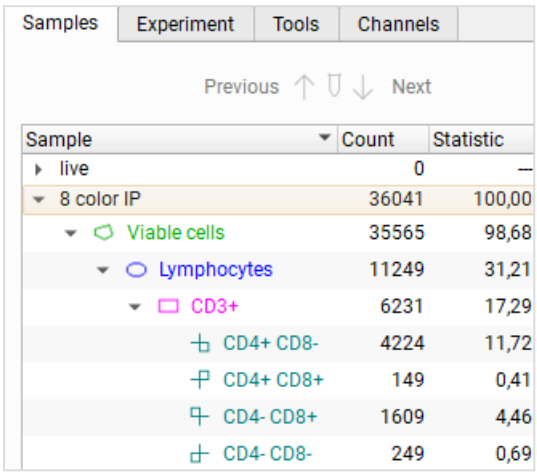
1. Draw a gate (P1) in the first plot.
2. Click the plot title of the next plot and select P1. Only events of P1 are shown.
3. Draw a gate (P2) within the plot displaying the events from P1.
4. Optional: Open the gating hierarchy by clicking the arrow icon next to the sample in the sample list in the **Samples** tab. The hierarchy level, gate type, name, and color are shown in the hierarchy tree.



The gating hierarchy does not change if the plot title is changed after drawing the gate.

6.1.1 Example for hierarchical gating

In this example, the 8-Color Immunophenotyping Kit, human REAfinity (# 130-120-640) was used for manual analysis of T cells from human lysed blood.



1 Optional: Open a data file.

2 Click the **Add** button in the toolbar to open a new analysis window.

3 Select the analysis window(s) required for analysis. See **Creating an analysis template on page 105**.

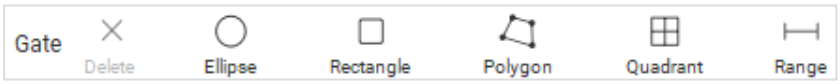
4 Select the plot of interest. An orange border indicates the selected plot.

5 Click the **Analysis** button in the toolbar.

6 Double-click the data file in the sample listor select the data file from the plot title. See **Displaying data in a plot on page 106**.

7 Click the **Analysis** button.

8 Select a gating tool from the toolbar.



9 Use the appropriate button to select a geometrical shape for gating. See **Gating tools on page 123**.

Ellipse, **Rectangle**, **Polygon**, and **Quadrant** are for 2D plots only. **Range** can only be used in histograms.

10 Optional: Change the axis parameters of plots See **Figure 6.14**.

11 Draw a region around a population.

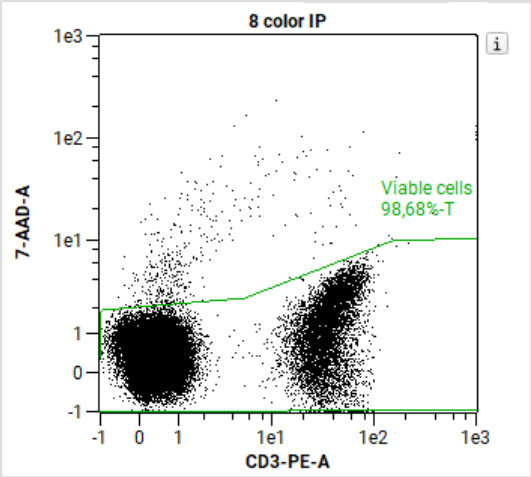
12 Select the parent region in the next plot from the plot title dropdown list to show only events of this region. See **Figure 6.14**.

13 Optional: Click the **'i'**-button of a plot to change the plot type See **Figure 6.14**.

14 Continue with hierarchical gating as required.

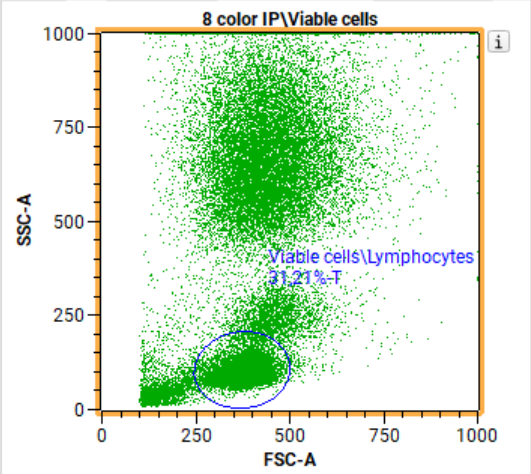
15 Draw a polygonal region to select viable cells.

16 Optional: Right-click the region and rename the region.



17 Select the region of viable cells in the plot title of the next plot.

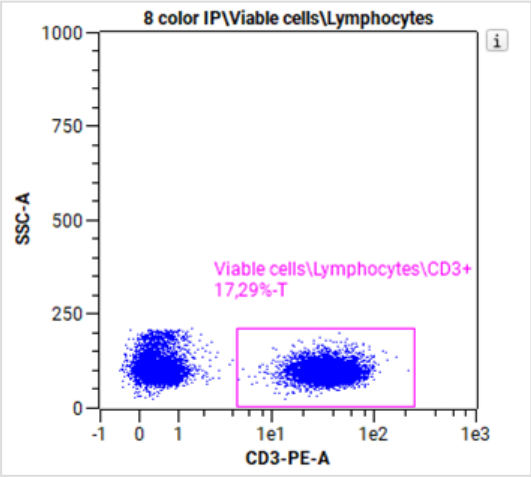
18 Draw an elliptical region within the viable cells to select viable lymphocytes.



19 Select the lymphocyte region in the plot title of the next plot.

20 Change the x-axis to CD3-PE-A. See **Plot properties on page 115**.

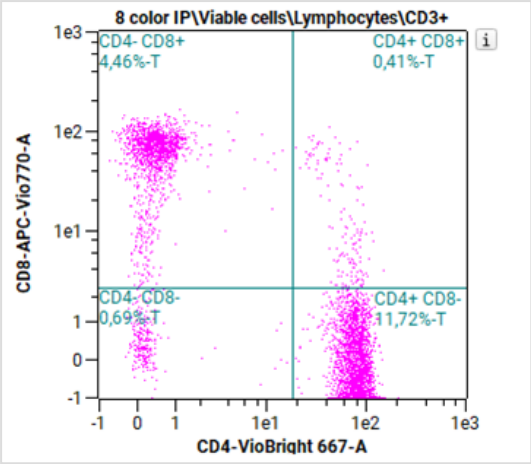
21 Draw a rectangle region to select CD3+ viable lymphocytes.



22 Select the CD3+ viable lymphocytes from the plot title in the next plot.

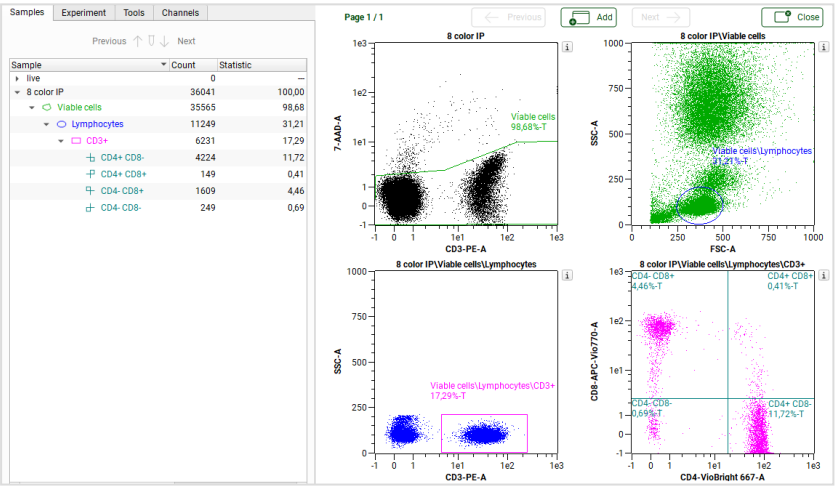
23 Change the axes to CD4-VioBright 667 versus CD8-APC-Vio770.

24 Draw a quadrant gate to separate different T cell populations.



25 Optional: Click the arrow next to the sample in the sample list to expand the gating tree.

The gating tree is shown. Regions in this gating strategy are not on the same level in hierarchy. Regions are created within regions, creating children, grandchildren, and great-grandchildren of the primary gated region.



26 Optional: Save the analysis template for later use.

27 Optional: Click the **Save** button in the toolbar. See **Saving files on page 149**.

28 Select **Analysis templates** to save the gating strategy as an analysis template.

29 Select the file location **Public**, **Private**, or **External**.

30 Name the file.

31 Click **Save**.

6.2 Displaying data

6.2.1 Creating an analysis template

An analysis template is a predefined layout for acquired data and can consist of plots, statistics, and tables. It usually includes gating strategies, region names, and selected statistics. See **Applying analysis templates on page 135**.

Pre-defined analysis window templates are available to select from. The MQ Administrator can define new analysis window templates or change the pre-defined analysis window templates. See **Templates on page 188**. Depending on the selected analysis window template, the analysis window contains dot plots, density plots, histograms, or statistics. Text tables can also be added by changing the plot type. For details, see **Plot types on page 108**.

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

2 Select an analysis window template from the **Create new plot** window.

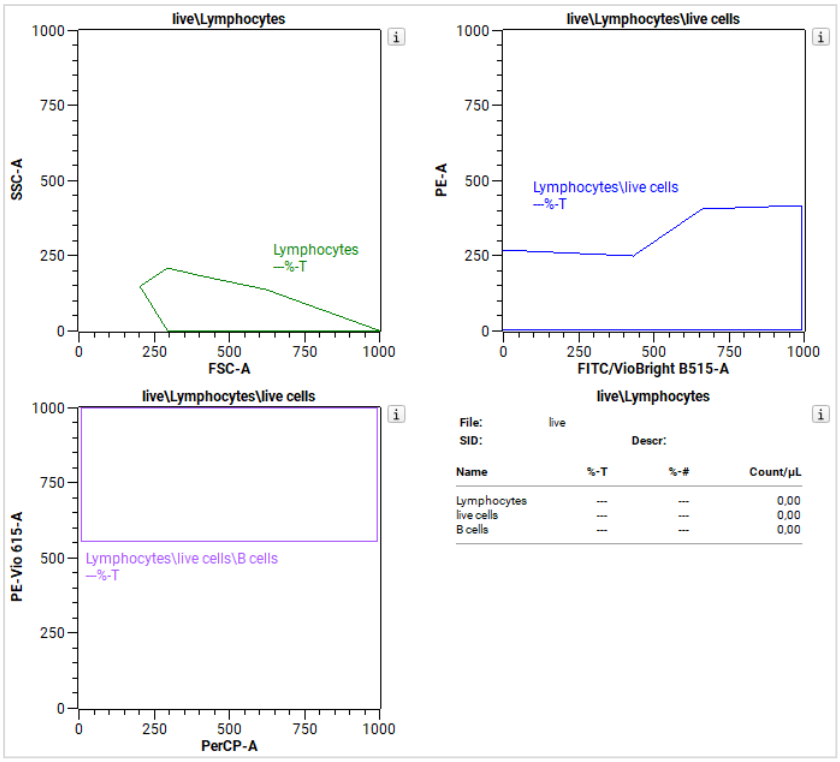


3 If more than two analysis windows are open, use the **Previous** and **Next** buttons to switch between analysis windows.

4 Optional: To close the selected analysis window, select **Close**.

5 Optional: To add a new analysis window subsequent to the analysis window currently selected, click the **Add** button in the toolbar.

6 Add regions as required. See **Gating tools on page 123**.



7 Use the created analysis template during acquisition.

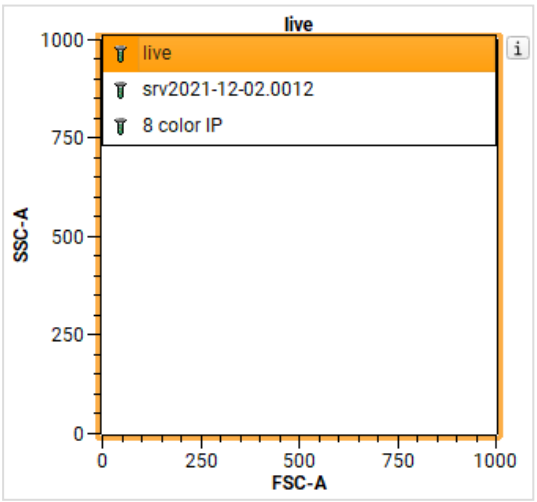
8 Go to **File > Save** to save the analysis template for later use.

6.2.2 Displaying data in a plot

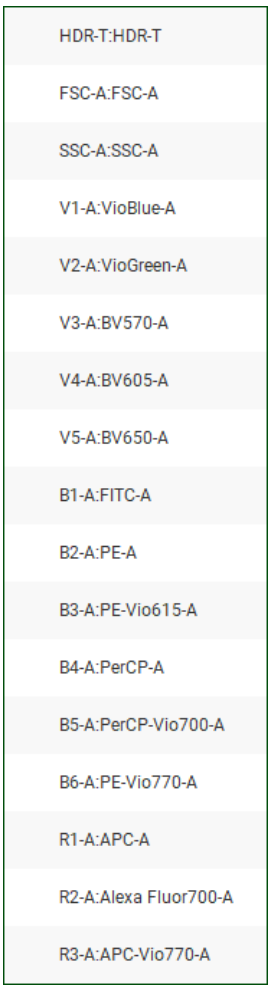
1 Open or add a data file to the sample list. See **Opening files on page 148** and **Adding data files to the sample list on page 148**.

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

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



3 Click the axis label and select one of the saved parameters in the dropdown list to change the shown parameters. The dot plot displays FSC-A versus SSC-A per default.



If fluorescence channels were deselected or parameters such as height or width were not activated, then these parameters are not selectable from the dropdown list.

6.3 Plot types

Flow cytometry data can be shown in different formats: As a **Dot plot**, **Density plot**, **Histogram**, **Statistics table**, or as a **Heat map**. Additionally, a **Text box** can be used to show additional information.

1 Click the **'i'** button next to a plot to open the **Properties** window.

2 Select a plot type from the left-hand side pane to change the plot type. See **Plot properties on page 115**.

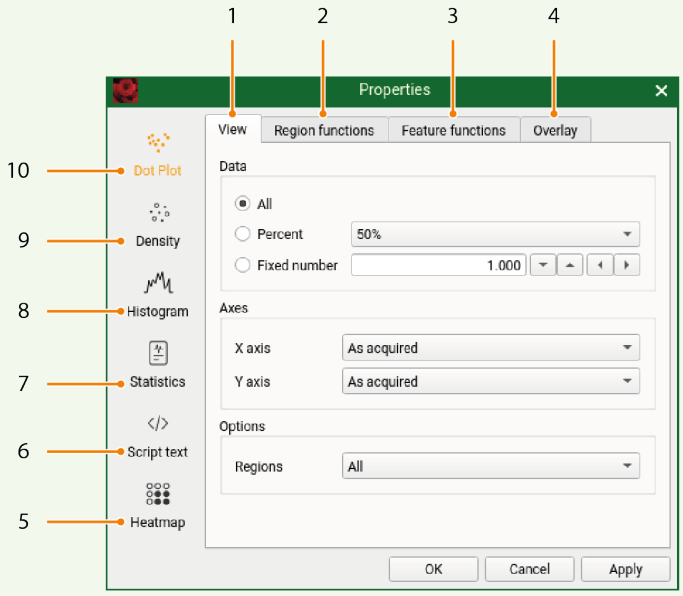


Figure 6.1: Properties window to switch between plot types or change their appearance

1	Change general plot options	**6**	Select script text and add information about a sample
2	Select region functions	**7**	Select statistics and show statistical parameters
3	Select feature functions	**8**	Select and adjust histogram as plot type
4	Adjust overlay options	**9**	Select and adjust density plot as plot type
5	Select and adjust heat map as plot type	**10**	Select and adjust dot plot as plot type

6.3.1 Dot plots and density plots

Dot plots and density plots are bivariate plots as they both show two parameters. Density plots additionally show the cell distribution as a color-coded gradient. Use density plots if the number of cells is large.

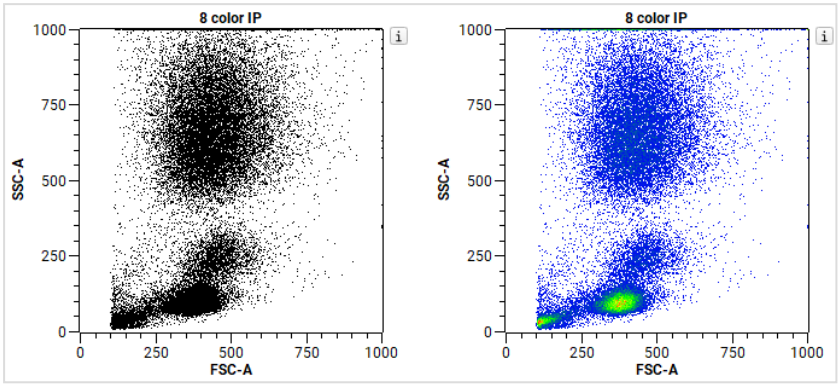


Figure 6.2: Example of a dot plot (left) and a density plot (right)

Use the key combination **Ctrl**+ the arrows to move events within a selected plot. Use **Ctrl**+**Fn**+**Pos1** or **Home** to reset the plot.

6.3.2 Histograms

Histograms are univariate plots which display one parameter.

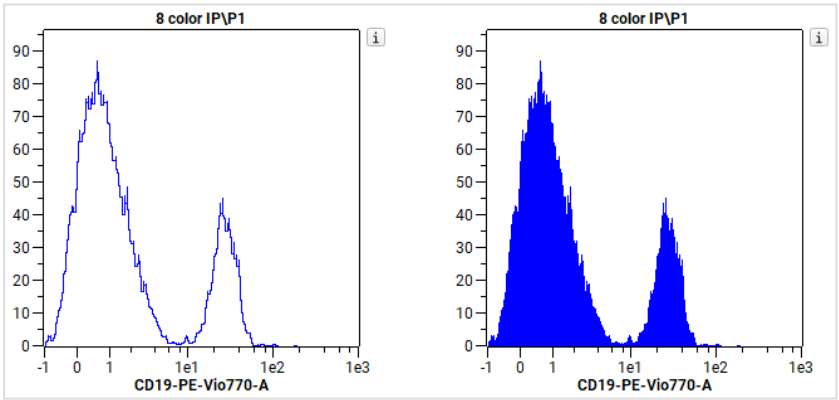


Figure 6.3: Examples of a histogram

6.3.3 Statistics tables

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



8 color IP

File:	8 color IP.mqd	2020-Jan-28 12:28	
SID:	8cIP	Descr:	

Path	%-T	%-#	Count	Count/mL
8 color IP	100,00	---	36041	1,07e6
Lymphocytes	26,40	26,40	9514	2,83e5

Figure 6.4: Example of a statistics table

6.3.4 Script text

Script text can be used to enter free text. It is also possible to select one of the pre-defined scripts to show additional information. To use script text select one of the options from the **Script text** dropdown list.

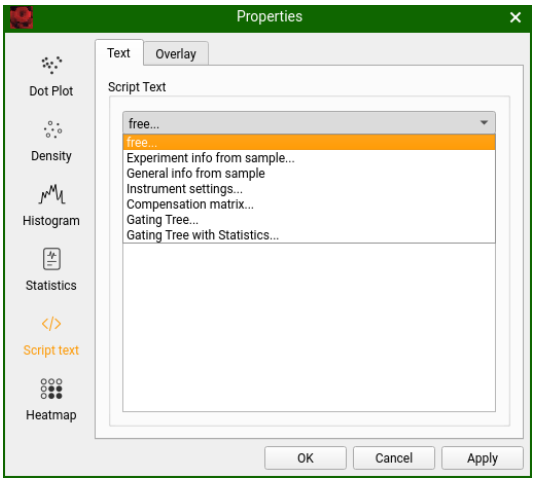


Figure 6.5: Options for Script text

- **Free…** – Enter free text into the empty text field. Use HTML (hypertext mark-up language) to write own scripts. Statistics about a region or gate can be automatically shown using a script text. Specific regions can be removed from or added to a text table by using the **Overlay** tab.
  The option **Gating tree with statistics** can be modified via **Free...** to add more info and to change the layout. See **Gating tree with statistics on the facing page**.
- **Compensation matrix**: Values from the compensation matrix is shown as a table. Fields with active compensation are highlighted in orange.

8 color IP

Compensation Matrix:

	V1	V2	B1	B2	B3	B4	R1	R2
V1	1.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000
V2	0.328	1.000	0.018	0.004	0.000	0.000	0.000	0.000
B1	0.000	0.001	1.000	0.020	0.000	0.001	0.000	0.000
B2	0.000	0.000	0.050	1.000	0.000	0.007	0.000	0.000
B3	0.000	0.000	0.007	0.207	1.000	0.022	0.006	0.001
B4	0.000	0.000	0.001	0.021	0.000	1.000	0.001	0.012
R1	0.000	0.000	0.000	0.000	0.000	0.002	1.000	0.040
R2	0.000	0.000	0.000	0.000	0.000	0.083	0.174	1.000

Figure 6.6: Example for script text showing a compensation matrix

- **Experiment info from sample**: Experimental settings, such as uptake and sample volume, flow rate, mixing, sample ID, and description are shown in a table.

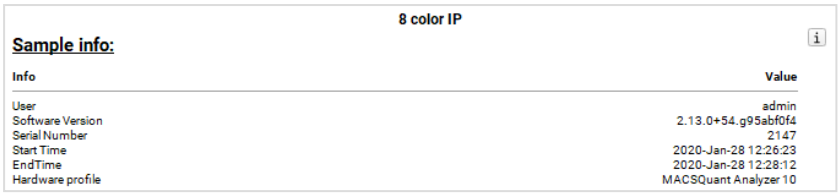
8 color IP

Experiment info:

Info	Value
Hardware profile	MACSQuant Analyzer 10
Project	Flow1
Racktype	Chill 5 rack
Rack position	A1
Sample ID	8cIP
Description	
Measure mode	Standard
Flow rate	Low
Mixing	Mix gentle
Uptake volume	50
Sample volume	1000
Live/Stop gate	---
Event Limit	---

Figure 6.7: Example for script text showing experiment info

- **General info from sample**: General information from sample such as user or software version is shown in a table.



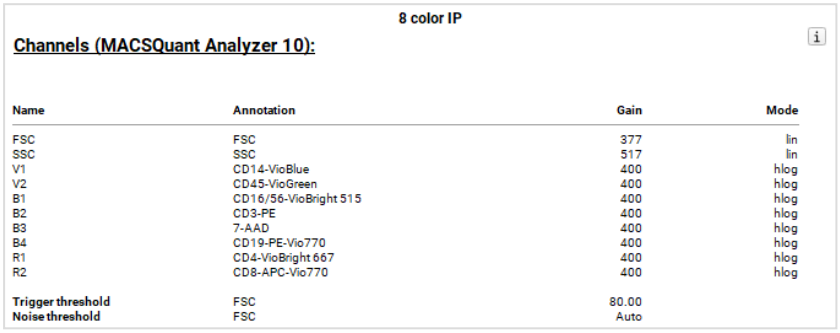
8 color IP

Sample info:

Info	Value
User	admin
Software Version	2.13.0+54.g95abf0f4
Serial Number	2147
Start Time	2020-Jan-28 12:26:23
EndTime	2020-Jan-28 12:28:12
Hardware profile	MACSQuant Analyzer 10

Figure 6.8: Example for script text showing sample info

- **Instrument settings**: The current instrument setting is shown in a table.



8 color IP

Channels (MACSQuant Analyzer 10):

Name	Annotation	Gain	Mode
FSC	FSC	377	lin
SSC	SSC	517	lin
V1	CD14-VioBlue	400	hlog
V2	CD45-VioGreen	400	hlog
B1	CD16/56-VioBright 515	400	hlog
B2	CD3-PE	400	hlog
B3	7-AAD	400	hlog
B4	CD19-PE-Vio770	400	hlog
R1	CD4-VioBright 667	400	hlog
R2	CD8-APC-Vio770	400	hlog
Trigger threshold	FSC	80.00	
Noise threshold	FSC	Auto	

Figure 6.9: Example for script text showing the instrument setting

- **Gating Tree**: The gating tree using the colors of the gates is shown in a table.

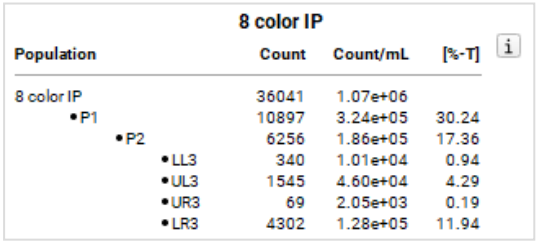


8 color IP

8 color IP
- Lymphocytes
  - CD3+
    - CD4-CD8-
    - CD4-CD8+
    - CD4+CD8+
    - CD4+CD8-

Figure 6.10: Example for script text showing a gating tree

- **Gating Tree with Statistics**: The gating tree in black and the statistical parameters Count, Count/mL, and percent gated of all events (Total) are shown in a table.



8 color IP

Population	Count	Count/mL	[%-T]
8 color IP	36041	1.07e+06	
• P1	10897	3.24e+05	30.24
• P2	6256	1.86e+05	17.36
• LL3	340	1.01e+04	0.94
• UL3	1545	4.60e+04	4.29
• UR3	69	2.05e+03	0.19
• LR3	4302	1.28e+05	11.94

Figure 6.11: Example for script text showing a gating tree with statistics

Gating tree with statistics

The option **Gating Tree with Statistics** allows to show the parameter listed in **Table 6.1**.

1. Click the **'i'** button of a plot type to open the plot properties.
2. Select **Script text**.
3. Select **free...** from the dropdown list.
4. Enter the text **$$textblocks.GatingWithStat.createGatingTree(sample)$$** as minimal requirement. The table shows the same parameter like the option **Gating Tree with Statistics...**. This means, the gating tree in color is shown together with statistics Count, Count/mL, and Percent gated (parental).
5. Add statistical parameters. Change which parameters should be shown by setting them True or False. See **Table 6.1**.

Parameter	Default value	Definition
colored	True	True: text inherits the color of the gate as in the gating hierarchy False: text is black
distances	2	Number of spaces for left alignment of levels
Count	True	All events
Count_ml	True	Count per mL
Count_µl	False	Count per µL
perc_T	False	Percent total
perc_1	True	Percent gated parental
perc_2	False	Percent gated grand parent
perc_3	False	Percent gated great grand parent
perc_4	False	Percent gated great-great grand parent

Table 6.1: Parameters for a customized gating tree with statistics

Example: Gating tree with statistics

$$textblocks.GatingWithStat.createGatingTree(sample, colored=False, perc_T=True, perc_1=False)$$ was entered as free text.

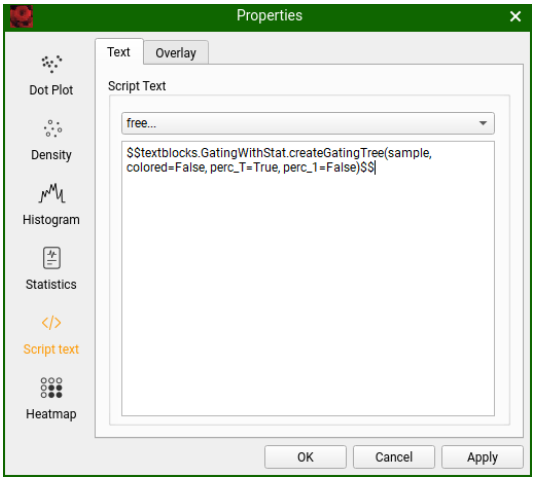


Figure 6.12: Manually entered text in **free...** for a gating tree with statistics

This results in a black gating tree with statistics Count, Count/mL, Percent gated from all events (%-T, Total). Percent gated from parental gate are not shown.

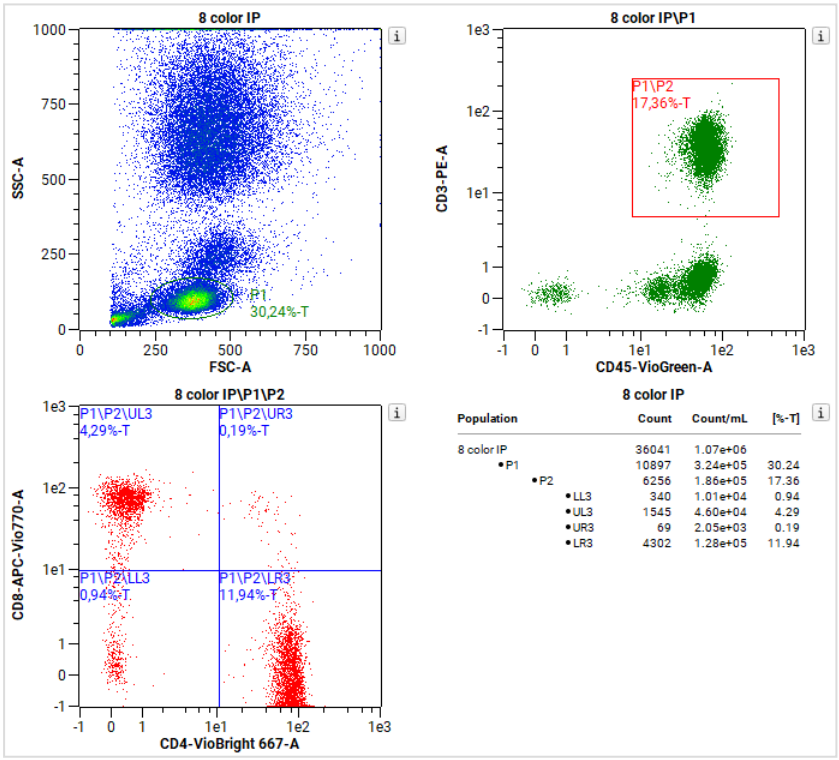


Figure 6.13: Example of a gating tree with statistics

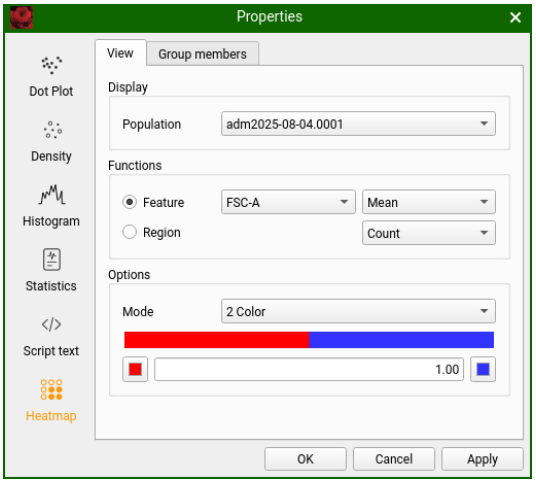
6.3.5 Heat maps

Only grouped data can be shown as a heat map. If data sets are grouped manually and sample positions overlap or two plates are grouped, a 96-well template is used and filled according to the sample list.

1 Click the **'i'** button next to the plot.

2 Go to the **View** tab.

3 Select a subpopulation from the **Display** dropdown list.



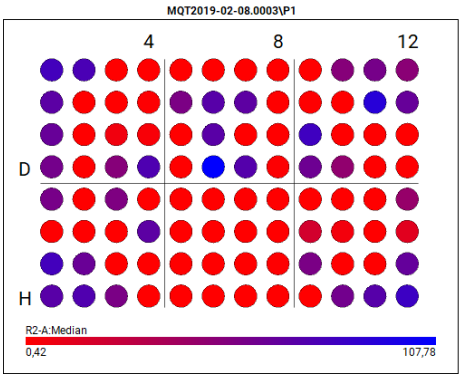
4 Activate the radio button next to **Feature** or **Region** in the **Function** section to show the respective data in the heat map:

- **Feature**: Select the feature and a numeric feature function.
- **Region**: Select a numeric region function.

5 Select one of the available display modes from the **Options** section:

- color gradient
- 2 color heat map
- 3 color heat map

6 Pick colors and set thresholds if required.



6.4 Plot properties

To change the properties of a plot, click the parameters shown in **Figure 6.14**. Double-click a plot to enlarge the plot. Double-click again to return to the original view.

- Plot title: Use the dropdown list to select the parent region to be shown.
- **'i'** button: Select the **'i'** button to open the plot properties window to change the plot type and other options of the plot.
- x-axis: Click the axis label to change the shown parameter.
- y-axis: Click the axis label to change the shown parameter.

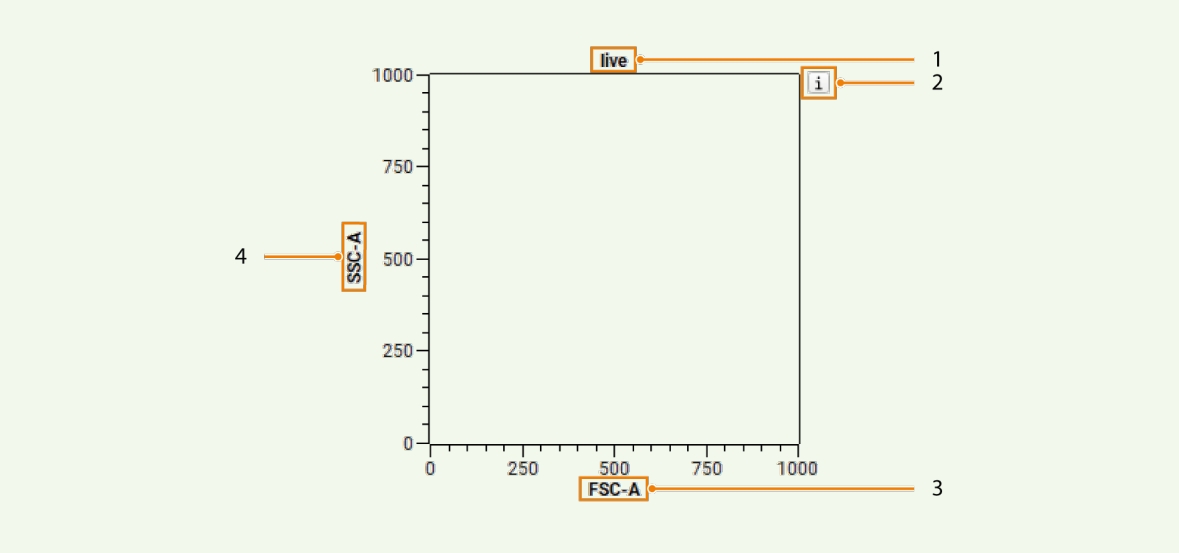


Figure 6.14: Plot properties

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

6.4.1 View

In the **View** section of the **Properties** window the number of shown events can be defined. Furthermore, the display of the axes scales and regions are selectable.

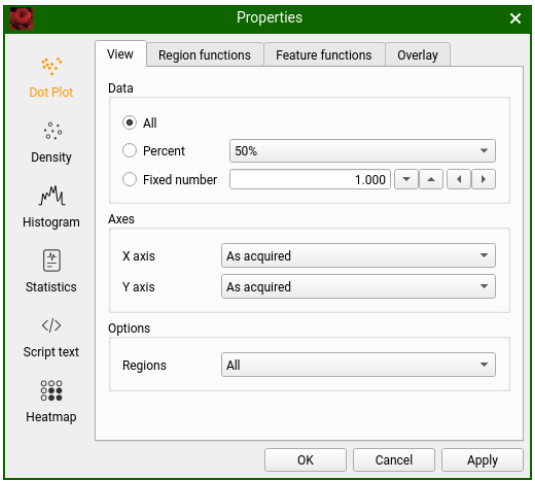


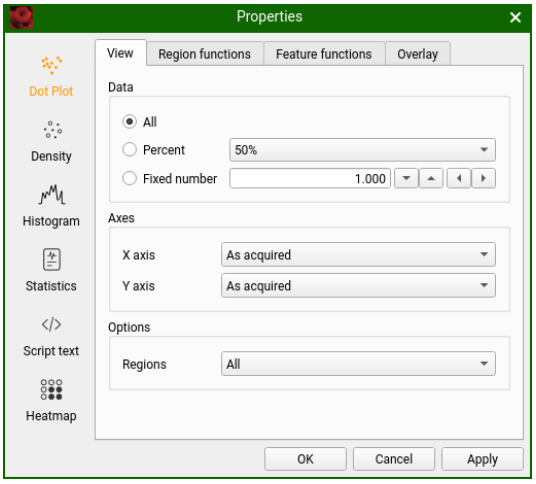
Figure 6.15: Properties window for plots

Data

If the number of events is very large, display only a fraction of all acquired events. The number of events can be adjusted for dot plots, density plots, and histograms.

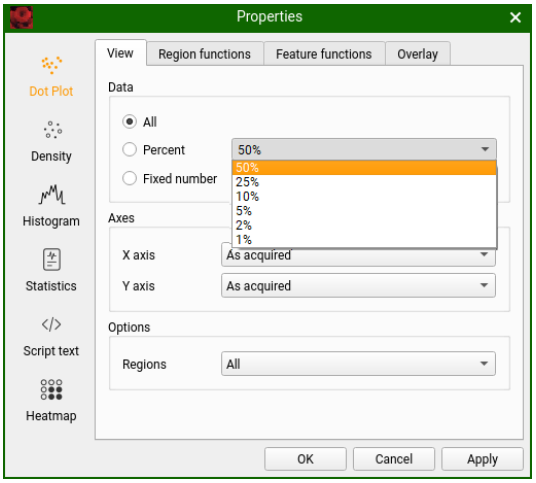
1 Click the **'i'** button next to the plot.

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% of the total events are shown.
- **Fixed number**: A fixed number of events is shown. Enter the desired number or change the default value using the arrows.



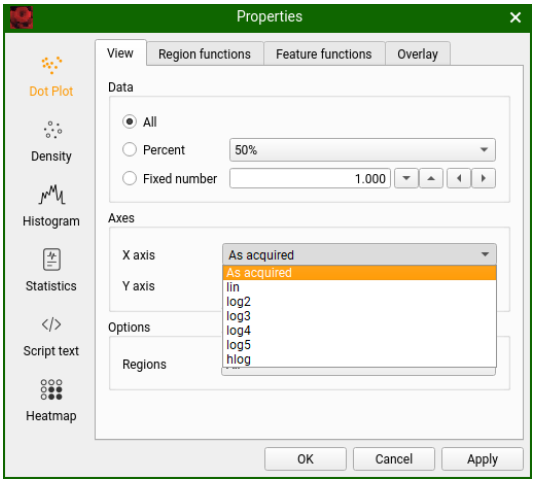
Axes

1 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 instrument settings
- **lin**: linear scale
- **log2-5**: logarithmic scales from 2-5 decades
- **hlog**: bi-exponential scale



Changing the axes scale in the **Properties** window changes only the display of data. The data file remains unchanged. To change the scale in the data file, see **Resampling: Change scales on page 142**.

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 the **'i'** button next to the plot.

2 Go to the **View** tab.

3 Go to the **Options** section.

4 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. Use this function if two plots have the same axes label but the parental gate is on different hierarchy levels.

For dot and density plots **All**, **This**, and **None** can be selected.

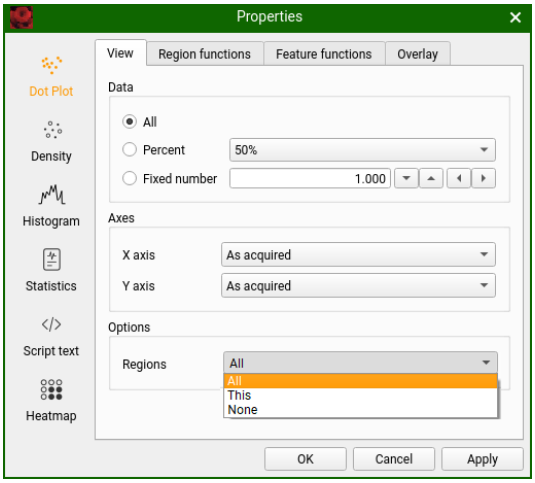


Figure 6.16: Options for dot and density plots

- **All**: All regions ("gates") drawn in either of the plots is shown.
- **This**: Only the region ("gate") drawn in the selected plot is shown. This is the default option
- **None**: No regions ("gates") are shown in the plot.

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

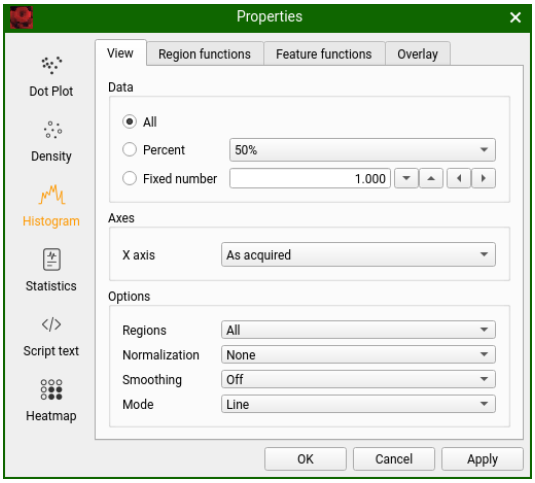


Figure 6.17: Options for histograms

- **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 adjust smoothing of the curve.

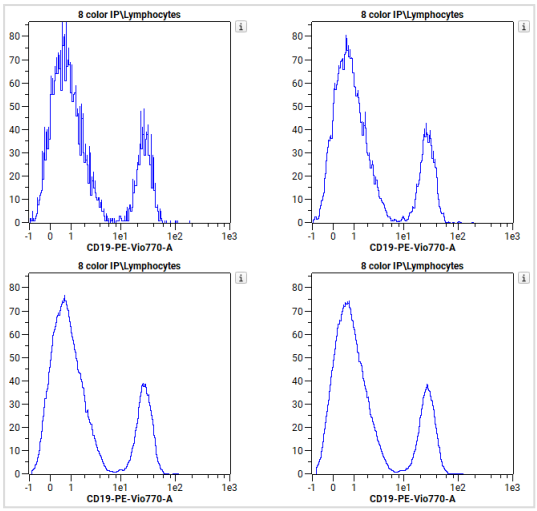


Figure 6.18: Smoothing options for histograms: **Off** (upper left), **Light** (upper right), **Medium** (lower left), and **Strong** (lower right)

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

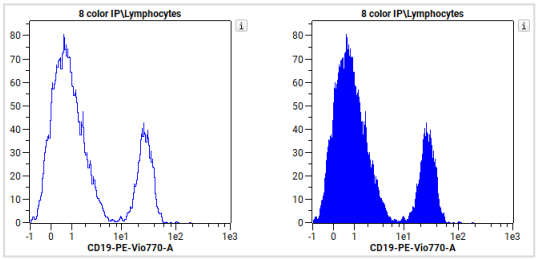


Figure 6.19: Histogram as line chart (left) or bar chart (right)

Options for statistics tables

The appearance of a statistics table can be adjusted.

- **Header**: Select the box to show a header.
- **Table**: Select the box to show a table with statistics.
- **Feature**: Select **shown using annotations** or **shown using channel names** from the **Feature** dropdown list. Annotations are defined by the user in the **Experiment** tab.

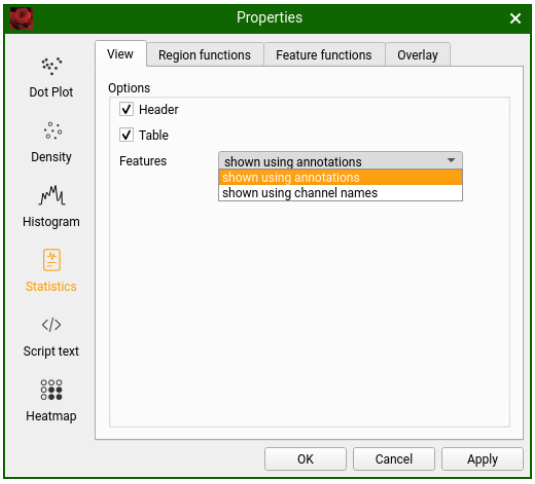


Figure 6.20: Options for statistics

6.4.2 Region functions

Parameters of a region such as **Count** or **Sample ID** can be shown in a plot. Region functions are available for dot plots, density plots, histograms, and statistics tables. See **Table 6.2** for available region functions.

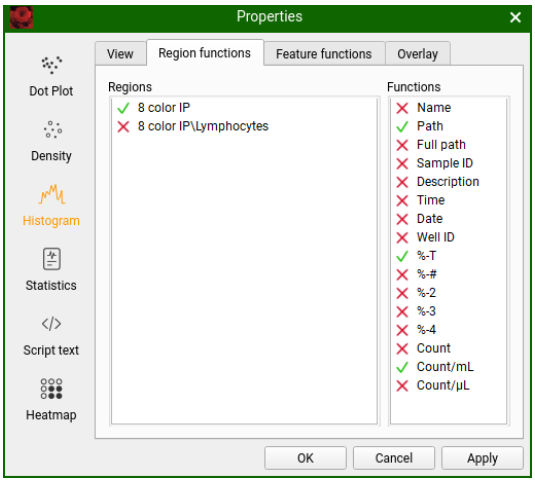


Figure 6.21: Region functions window

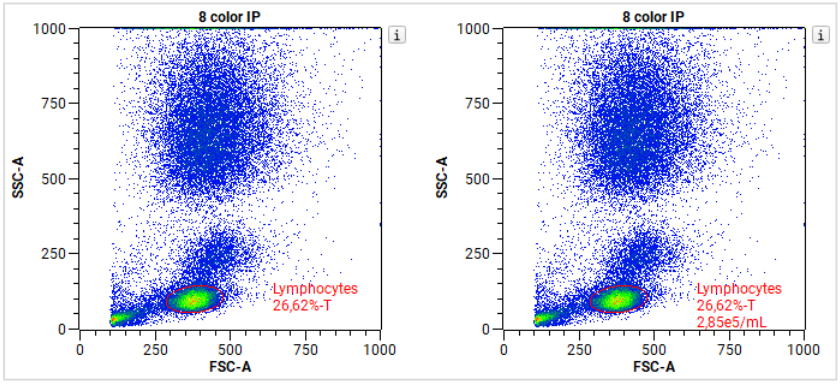
1 Click the **'i'** button next to the plot.

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

3 Select or deselect a region.

4 Select or deselect the function for the selected region.

5 Click **Apply** to confirm and **OK** to close. The selected functions are shown in the plot.



By default, the region functions **Path** and **%-T** are shown.

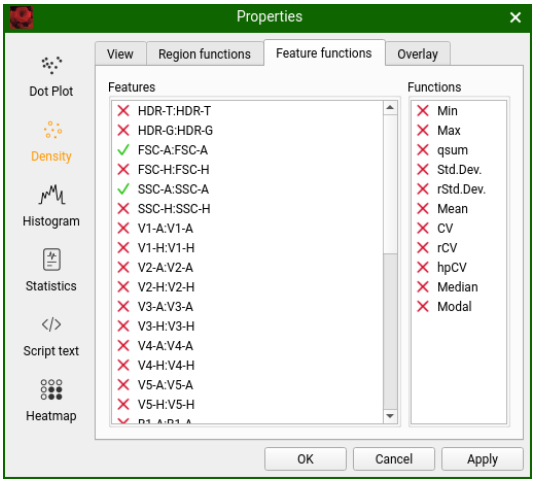
Name	Description
Name	Sample\\P1\P2: P2
Path	Sample\\P1\P2: P1\P2
Full path	Sample\\P1\P2: Sample\\P1\P2
Sample ID	Sample ID as defined in the experiment tab
Description	Description as defined in the experiment tab
Time	Time in the format HH:MM
Date	Date in the format YYYY-MMM-DD
Well ID	Well ID in the format RC; R represents row, C represents column
%-T	Percent total
%-#	Percent gated parent
%-2	Percent grand parent
%-3	Percent great grand parent
%-4	Percent great-great grand parent
Count	Number of events
Count/mL	Number of events per mL
Count/µL	Number of events per µL

Table 6.2: Available region functions

6.4.3 Feature functions

Statistical values of a channel such as standard deviation or median can be shown in a plot or table. The feature functions are available for dot plots, density plots, histograms, and statistics tables. See **Back gating on page 132** for an exemplary application.

1. Click the **'i'** button next to the plot or table.
2. Go to the **Feature functions** tab.
3. Go to **Features** and select or deselect channel parameters.
4. Go to **Functions** and select or deselect the statistical values to be shown in the plot.
5. Click **Apply** to implement changes and **OK** to close the window. The selected statistical value is shown in the plot or table.



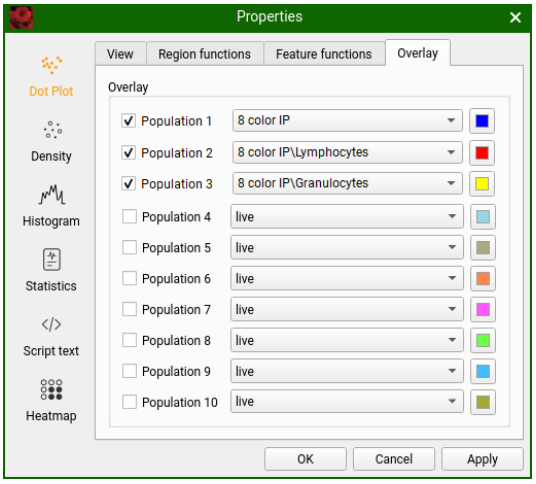
Name	Description
Min	Minimum value
Max	Maximum value
qsum	Sum of the squares of the values
Std.Dev.	Standard deviation of the values
rStd.Dev.	Robust standard deviation calculated as 50 (Intensity at 84.13%ile - Intensity at 15.87%ile) / Median
Mean	Mean value (sum of all intensity values divided by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

Table 6.3: Available feature functions

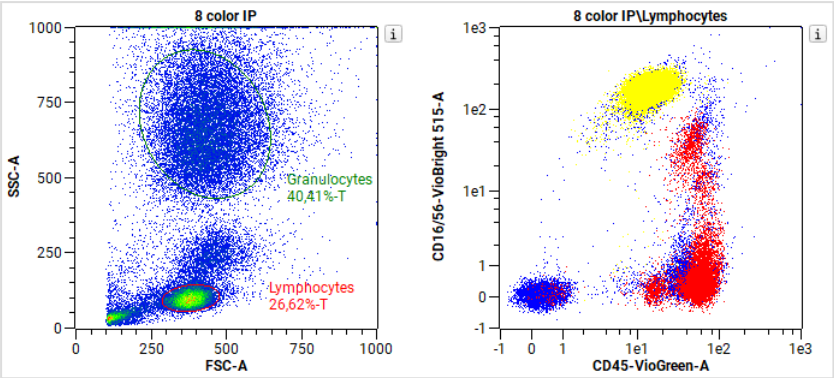
6.4.4 Overlay

The **Overlay** function in the **Properties** window allows the display of several regions or samples in one plot or a table. The **Overlay** function is available for all plot types except heat maps. If using script text or statistics tables, the selected samples are shown in rows within a table.

1. 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: Select the box to the right to change the color.



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

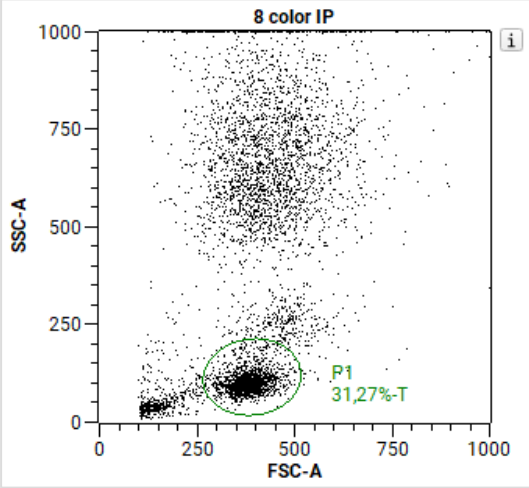


6.5 Gating tools

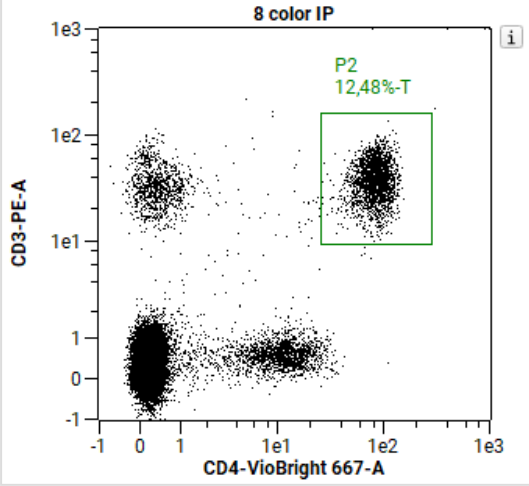
Gating tools can be selected in the toolbar to create a region around a population. Created regions in a hierarchical gating can be selected to show only events of interest.

1 Select a gating tool from the toolbar.

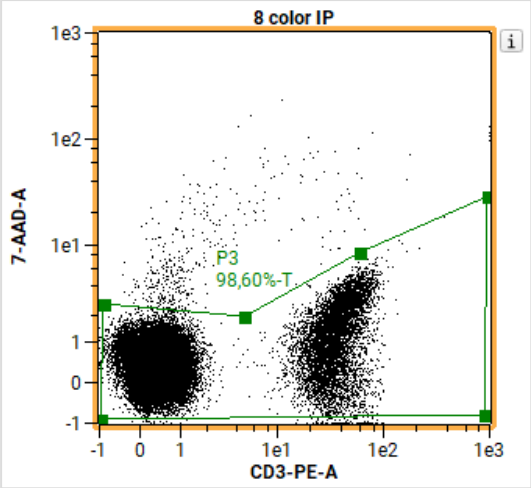
- **Ellipse**



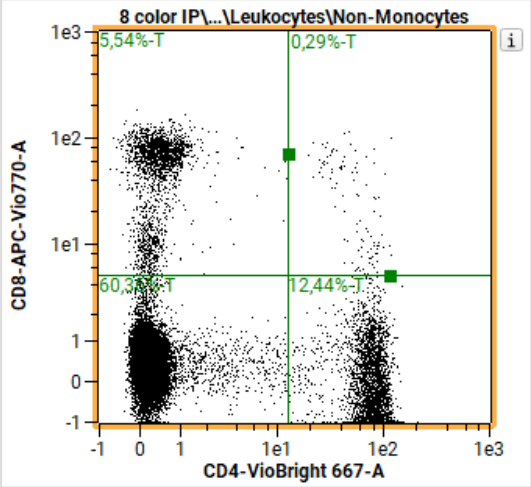
- **Rectangle**



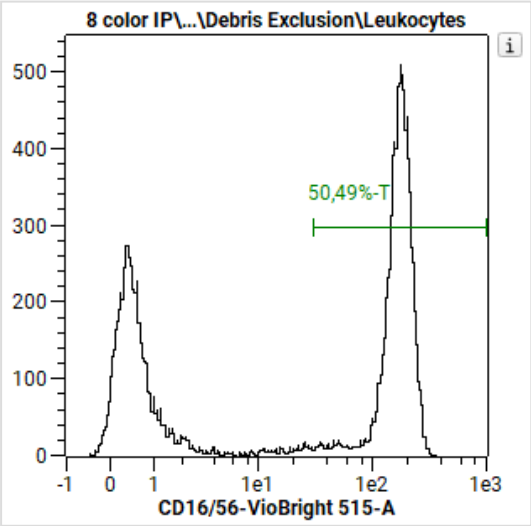
- **Polygon**



- **Quadrant**



- **Range**: Ranges are only available for histograms.



2 Draw a region of the respective shape around the cell population of interest.

- Ellipse, rectangle, quadrant, or range: 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 region. It is not possible to add additional points to a polygon once it is created.

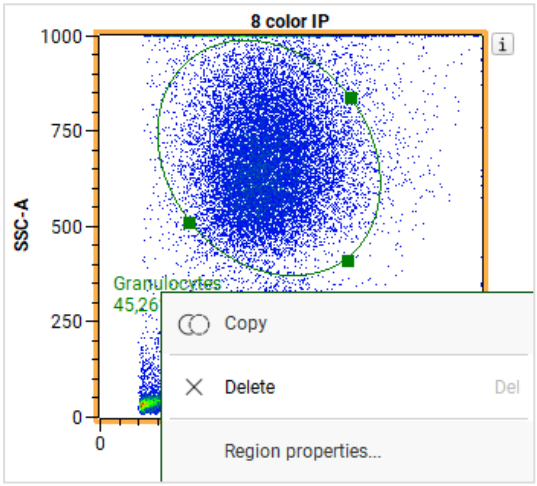
3 To change the appearance of a region, select the region in the plot and do one of the following:

- Click and drag any edit points to adjust the size of the region.
- Click and hold to drag the region and move it to a new position.

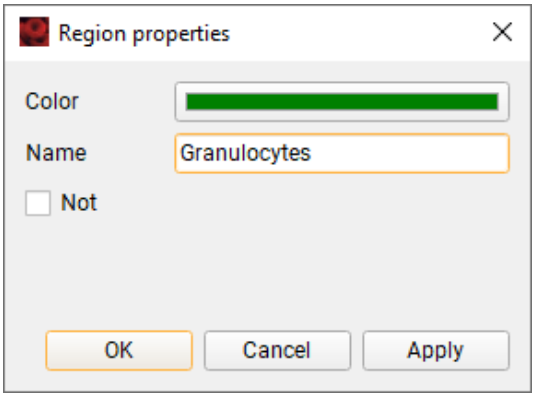
6.5.1 Changing region properties

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 on the next page**.

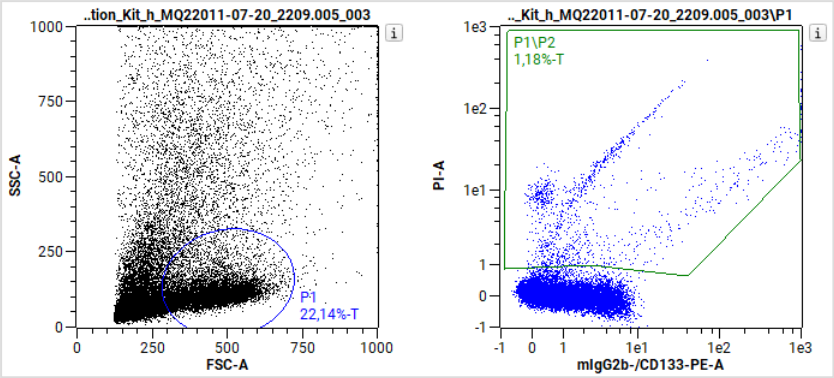


4 Click **Apply** to apply the changes and **OK** to close the window.

6.5.2 Setting a Not gate

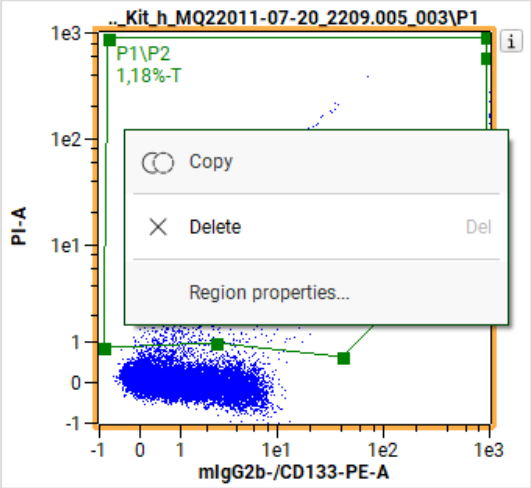
Not gates are used to exclude a cell population from analysis and are indicated by a dotted line.

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

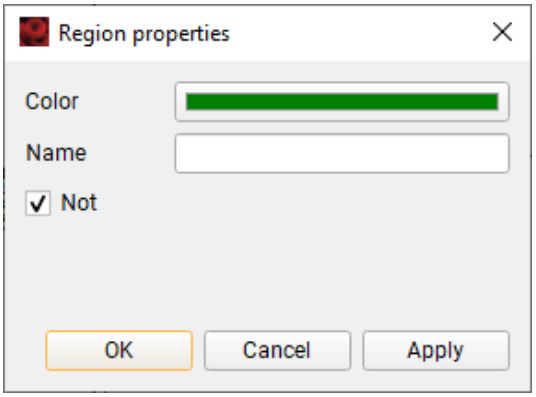


2 Right-click the region.

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



4 Select the box **Not**.

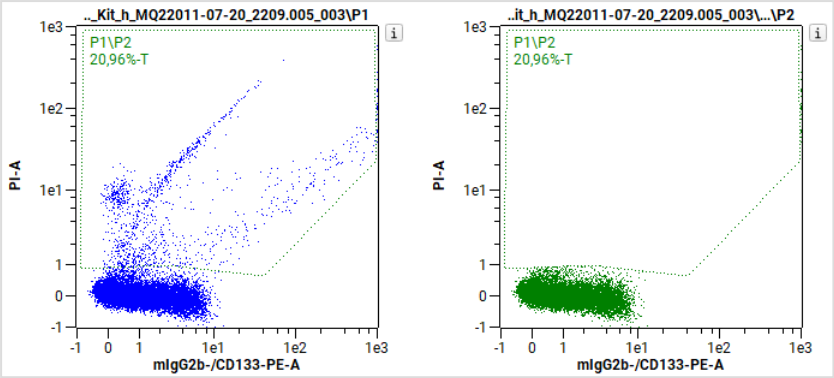


5 Optional: Select a color or name the Not gate.

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

7 Select the Not gate from the plot title dropdown list to exclude the events within the Not gate.

8 In subsequent plots, the events within the Not gate are excluded. In the example below P2 is a Not gate.



6.5.3 Copying regions and gating strategies

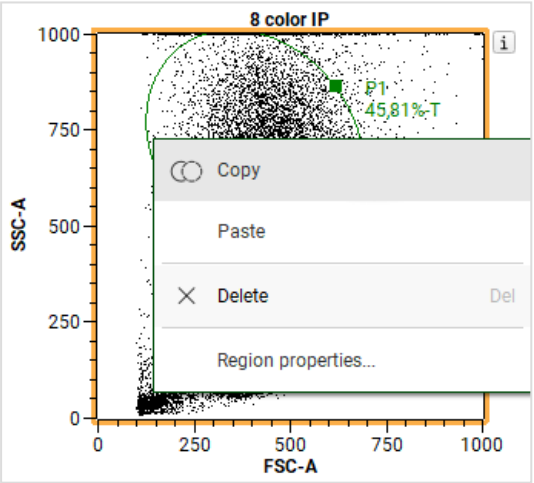
Regions can be copied and pasted from one plot to another plot. Regions or entire gating strategies can be also copied to other files via drag-and-drop within the sample list.

Copying and pasting regions

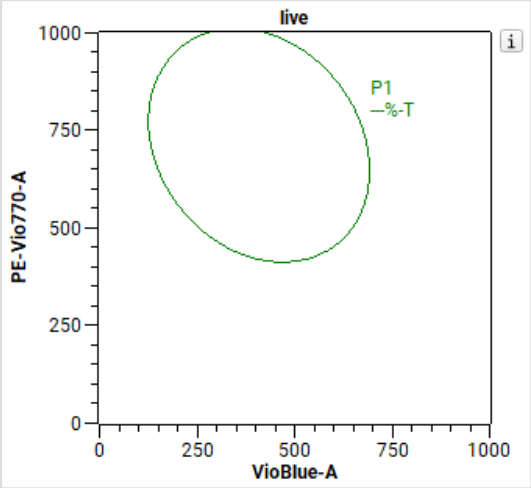
Copy a region from one plot and paste it to the same or a compatible plot. A region from a dot plot can be pasted to a dot plot or density plot, but not to a histogram. The same physical coordinates are used for the region, even if the plot has different scales.

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.



Copy 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.

3 Regions that are copied via drag-and-drop are linked per default. Moving a linked gate leads to a message on the screen. To change the settings and behaviour of linked regions see **Regions on page 203**.

Moving a region within a gating hierarchy

After establishing a gating strategy within a data file, click a region 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.

Sample	Statistic	Count
live	—	0
8 color IP	100,00	36041
P1	28,90	10415
P2	28,72	10350
P3	3,90	1406

2 Select P3 and drag-and-drop under P1.

3 The region under P1 is renamed to P4. It has the same coordinates and plot association as P3 but different statistics.

4 If the name of Region P3 or P4 is changed under region properties, names of both regions are changed.

Sample	Statistic	Count
live	—	0
8 color IP	100,00	36041
P1	28,90	10415
P4	3,99	1439
P2	28,72	10350
P3	3,90	1406

Sample	Statistic	Count
live	—	0
8 color IP	100,00	36041
P1	28,90	10415
T cells	3,99	1439
P2	28,72	10350
T cells	3,90	1406

Dividing a parental gate in two regions with the same hierarchy

It is possible to create two different regions from one parental gate. Regions drawn in a single plot have the same hierarchy. In the example below, regions P3 and P4 (bottom left) were both defined within region P2 (top right) and therefore have the same hierarchy.

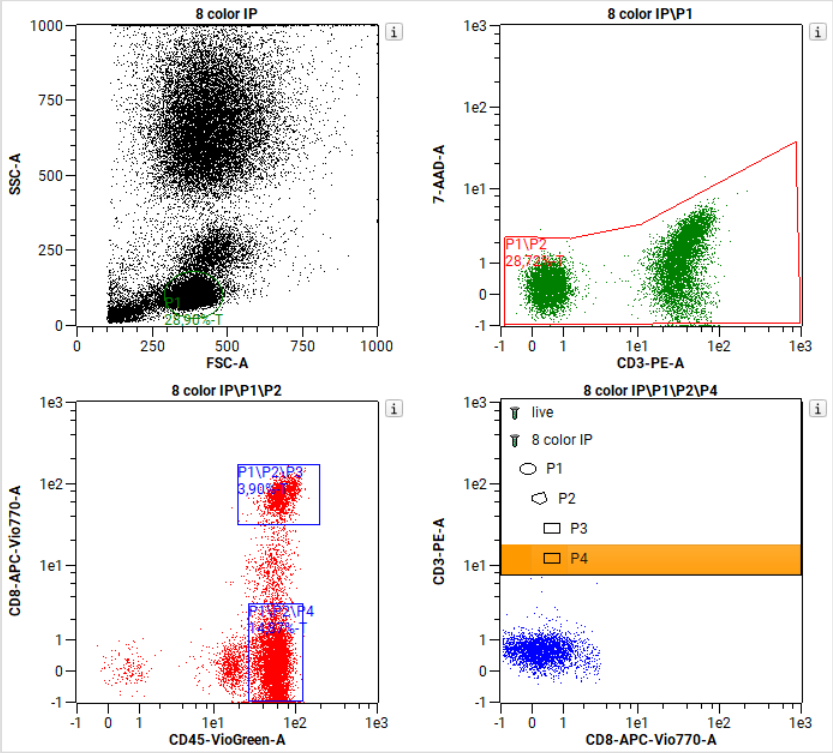
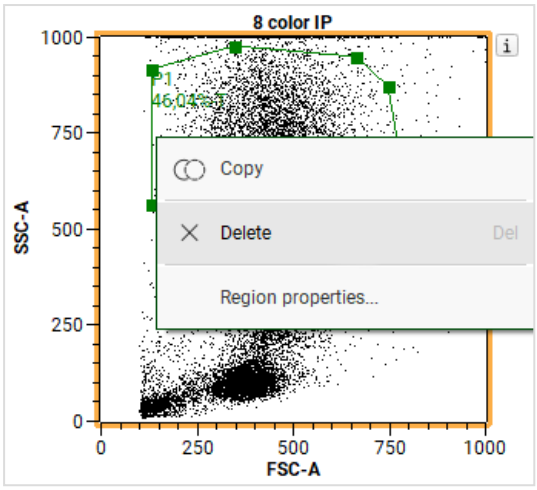


Figure 6.22: Regions P3 and P4 have the same hierarchy level. P2 is the parental gate of both.

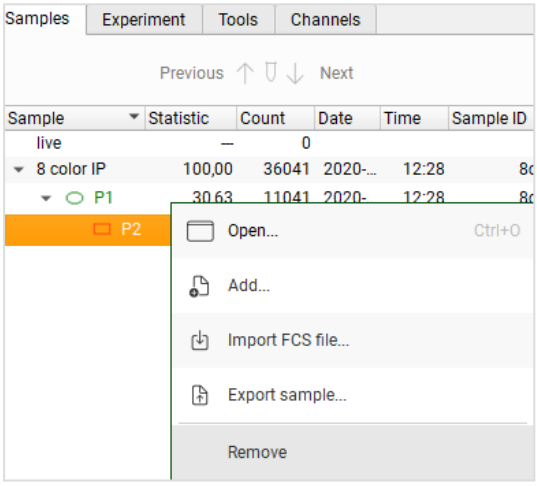
6.5.4 Deleting gates

To delete a gate, select the gate in the plot and do one of the following:

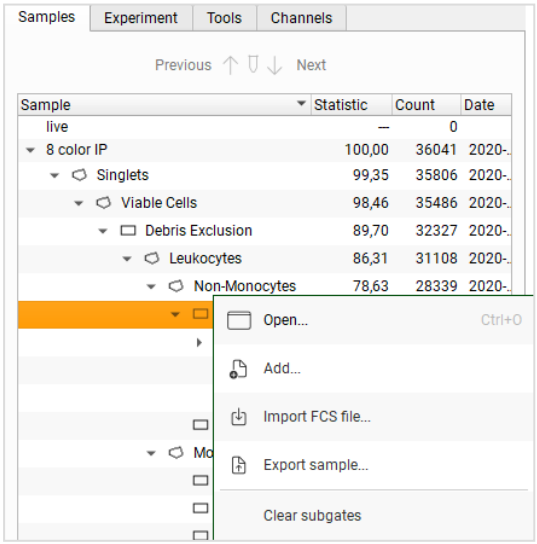
- Press **Delete** on the keyboard.
- Select the **Delete region** button in the toolbar.
- Right-click the gate and select **Delete**.



- Select the gate in the sample list, right-click, and select **Remove**.



- All regions under a file or under other regions can be deleted by right-clicking the file or the top region in the hierarchy and selecting **Clear subgates**.



6.5.5 Multilayer mode display

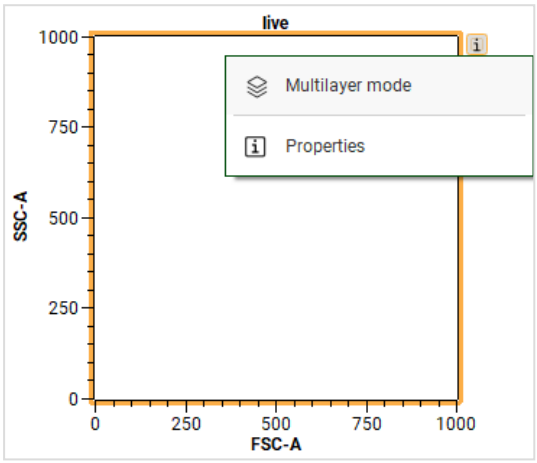
Gated events within dot plots are shown in their respective gate color. While establishing a gating strategy, each subsequent plot is formatted to show only events of the parental gate. By using the multilayer mode, all events with parent populations can be shown in one plot and are identified by their region color.

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

2 Define a hierarchical gating strategy.

3 Right-click the **'i'** button of a plot.

4 Select **Multilayer mode**.



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

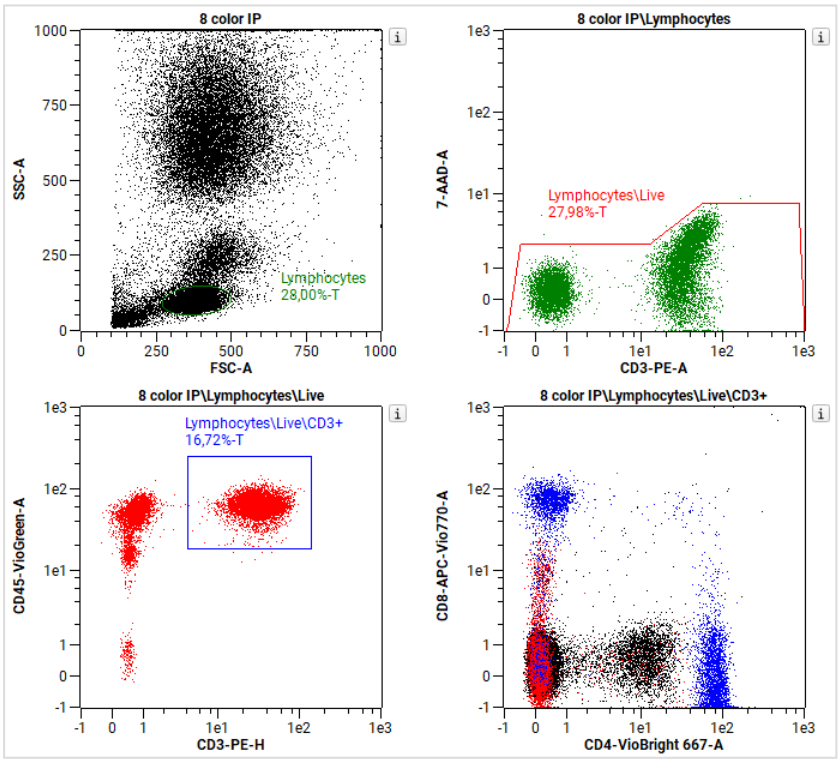


Figure 6.23: Multilayer mode gating strategy and display. Cells were stained with the 8-Color Immunophenotyping Kit, anti-human REAfinity. Cells were gated on lymphocytes in the scatter plot (upper left) and for all live cells (upper right). Live lymphocytes were gated on CD3+ cells (lower left). CD8+ and CD4+ T cells are shown in a dot plot with multilayer mode (lower right) in their specific region colors (Black: All cells: Green: Lymphocytes; Red: Lymphocytes/Live cells; Blue: Lymphocytes/Live cells/CD3 pos). Depending on the frequency, some colors might not be visible in multilayer mode.

6.5.6 Back gating

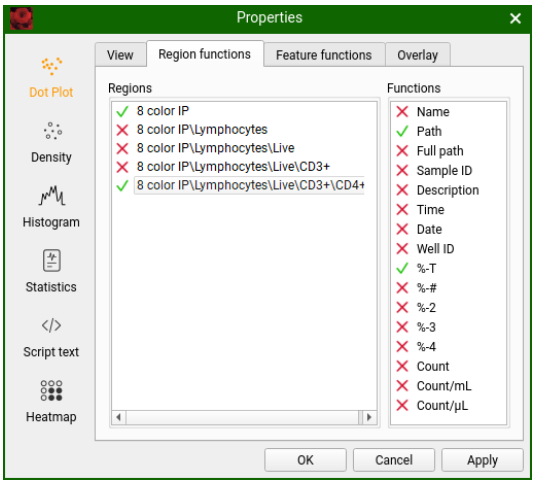
Back gating means that a specific subpopulation of cells defined by a particular gating strategy can be viewed in a previous plot. Use this function to identify where within the previous plot the cells of interest are shown.

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

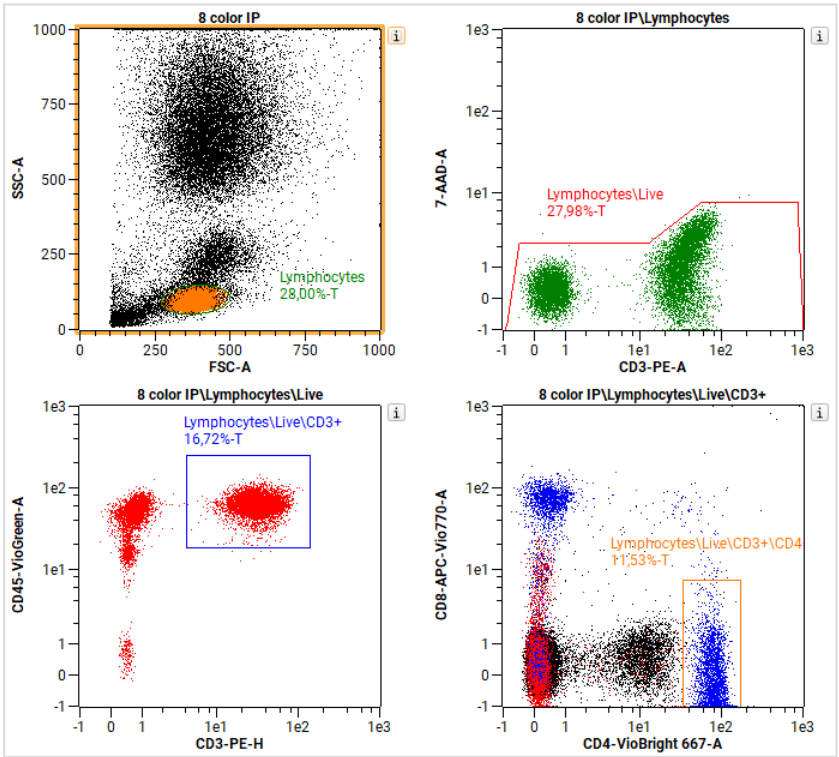
2 Define a gating strategy.

3 Click the **'i'** button of the plot in which a region lower in the hierarchy should be shown.

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



Gated events with their associated region color are shown on top of all other events within the plot. The region of CD4+ T cells in the lower right plot was selected for display in the upper left plot.



6.6 Post-acquisition data analysis

6.6.1 Sample context menu

Right-click a sample in the sample list to access the sample context menu. The number and type of functions available in the sample context menu may vary depending on the sample type. Some functions are only available in the PC version. See the table below for all sample context menu functions.
For details about the options if you right-click in the sample list, see **Adding data files to the sample list on page 148**.

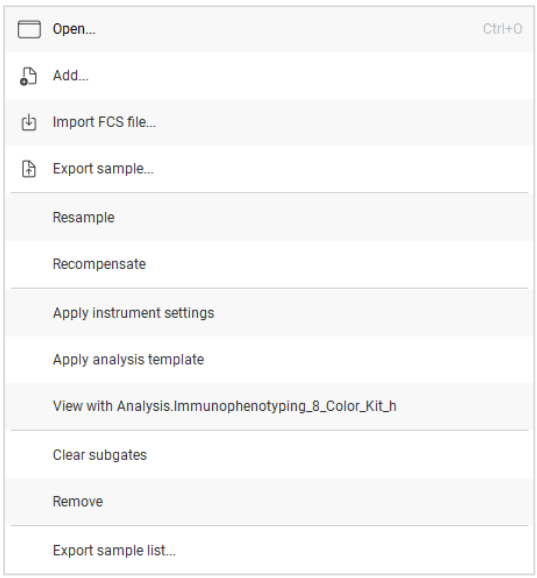


Figure 6.24: Sample context menu after right-clicking a sample

Option	Description
Open…	Opens files such as workspaces, instrument settings, experiments, analysis templates, or data files (.mqd). See **Opening files on page 148**.
Add...	Adds .mqd data files to the sample list. Only available if using the MACSQuantify Software on a PC. Browse to any location on your PC to select data files. See **Adding data files to the sample list on page 148**.
Export sample …	Exports samples in .csv (comma-separated values) or .fcs (flow cytometry standard) format. See **Export as .FCS on page 201**.
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 144**. 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 145**. A printer must be configured to enable printing.
Group	Groups samples. Available if two or more samples are selected. Generates a new sample file that contains selected samples. See **Grouping and ungrouping of data files on page 138**.
Ungroup	Ungroups a grouped sample and generates a separate file for each sample of the group. See **Grouping and ungrouping of data files on page 138**.
Resample	Displays data on a scale that differs from the acquisition scale. This feature also allows to change annotations if there were mistakes during sample acquisition. See **Resampling: Change annotations on page 141**.
Recompensate	Applies changes of the compensation matrix to selected samples. Data files (.mqd) can be re-analyzed using a different compensation matrix if data were acquired using incorrect instrument settings. See **Offline compensation and recompensation on page 139**.
Apply instrument settings	Applies the instrument settings that were run with a selected data file. See **Applying instrument settings from a data file on page 136**.
Apply analysis template	Applies the analysis template to the selected sample if an analysis template has been selected prior to acquisition. The feature is only available for files acquired with a MACSQuant Instrument. See **Post-acquisition data analysis on the previous page**.
View with Analysis <Name of the Express Mode>	Applies the Express Mode to the selected sample that was used during acquisition. The option is available if a sample was acquired with an Express Mode. For analysis on a PC, the Express Mode must be installed on the PC. Not available in **Analysis Mode**. See **Data analysis using Express Modes on page 136**.
Analyze with Analyze with <Name of Compensation Express Mode>	Analyzes a sample acquired with a Compensation Express Mode. For analysis on a PC, the Express Mode must be installed on the PC. Not available in **Analysis Mode**. See **Analyzing with Compensation Express Mode on page 138**.
Restore group gates	Restores original group gates from files. Existing gates are maintained, added gates are linked to all group members. Not available in **Analysis mode**.
Clear subgates	Clears all associated gates and regions. Available if a sample with associated gates is selected. Not available in **Analysis Mode**.
Remove	Removes selected samples from the sample list. Not available in **Analysis Mode**.
Export sample list ...	Exports the sample list and associated statistics to Microsoft Excel. See **Exporting the sample list to Microsoft® Excel on page 146**.

Table 6.4: Options of the sample context menu

6.6.2 Applying analysis templates

Saved analysis templates can be applied to samples after acquisition. See **Creating an analysis template on page 105**. Plots and the gating strategy are opened. For samples acquired with an Express Mode, see **Data analysis using Express Modes on the next page**.

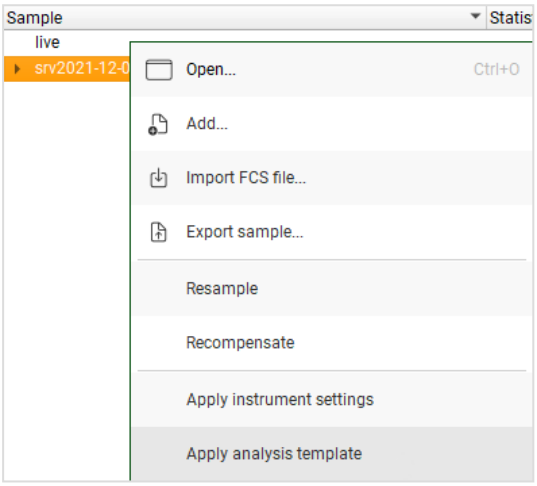
To apply an analysis template, do one of the following:

- View and analyze data using the analysis template used at acquisition:

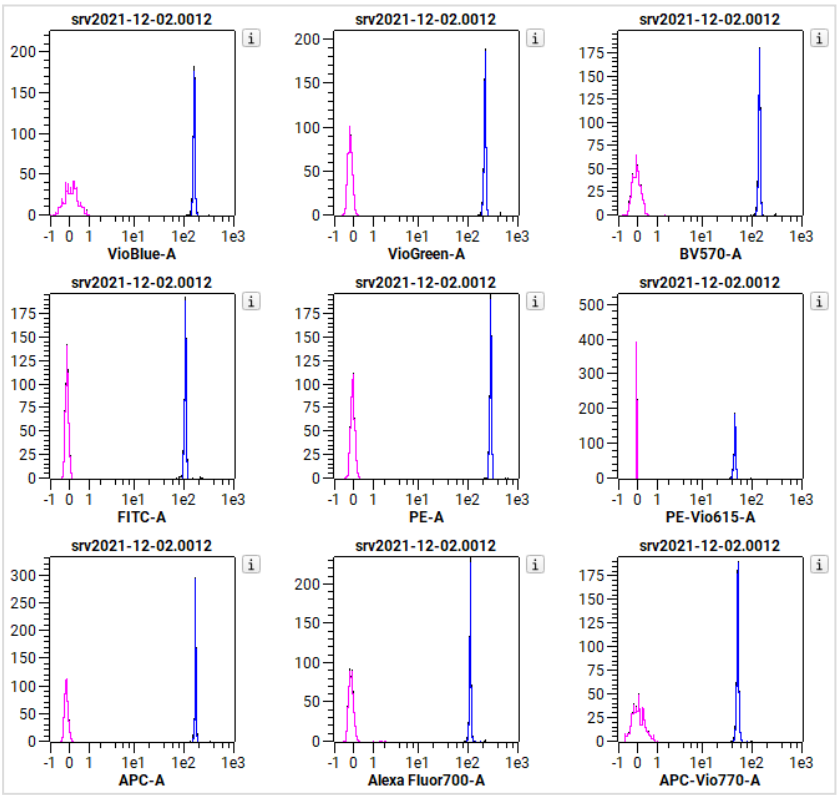
  1 Right-click the data file in the sample list.

  2 Select **Apply analysis template**.

  The data file and corresponding analysis template is loaded. The **Analysis** button is activated.



  3 Optional: Save the analysis template as an analysis template file.



- View or analyze data using a saved analysis template:

  1 Right-click a sample or highlight multiple samples in the sample list and right-click one of them.

  2 Select **Open** and select the desired analysis template.

3 Click **Open**.

The analysis template is applied to the selected data file. The analysis mode is automatically selected.

4 Optional: To adapt an analysis template, for example move a region, the **Analysis button** must be inactivated (gray).

- If no analysis template exists:

1 Create a new analysis template. See **Creating an analysis template on page 105**.

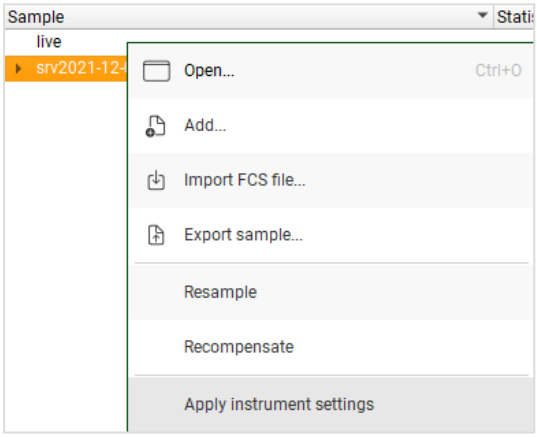
6.6.3 Applying instrument settings from a data file

Apply instrument settings associated with a data file.

1 Open a data file. The file appears in the sample list.

2 Right-click the data file in the sample list.

3 Select **Apply instrument settings**.



4 The instrument setting associated with the data file is loaded.

The instrument setting can be used as an active instrument setting for measurement. It can also be saved as an instrument setting file.

6.6.4 Data analysis using Express Modes

For optimal analysis results it is recommended to perform the analysis on a PC. Some functions, like export to Excel, are only available in the PC version.

It is necessary that the Express Modes are installed in the MACSQuantify Software. Make sure that both the installed software version and the Express Mode package on the instrument and the PC are the same. Go to **Help > Info...** to check the current versions. See **Express Mode manager on page 158** for details about Express Mode updates.

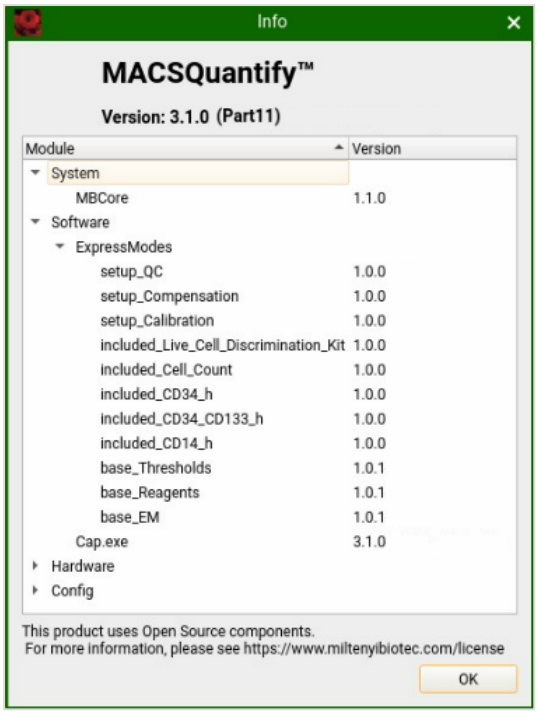


Figure 6.25: Installed Express Modes shown in **Help** > **Info**

The data file resulting from a measurement with an Express Mode contains all relevant information for analysis. Express Mode settings, acquisition page(s), and a reference to the used Express Mode are saved within the data file and do not need to be saved separately.

The gating is performed for each file individually. If an analysis is repeated, it results in the same statistics. During re-analysis with the Express Mode, the same algorithm is used resulting in identical gating. This applies also if the analysis is repeated or done on a PC if the same software version and Express Mode version is used for acquisition and analysis. If required, regions can be moved manually after Express Mode analysis. To document manual changes, it is necessary to save the new gating as an analysis template.

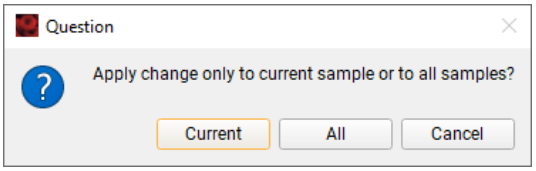
Applying an Express Mode

1 Right-click a sample in the sample list acquired with an Express Mode.

2 Select **View with Analysis <Name of Express Mode>**.

3 The gating is applied to the sample. For some Express Modes popup windows may appear, for example, to enter a dilution factor. Follow the instructions on the screen.

4 Optional: Adjust the gate by drag-and-drop. The **Analysis**button must be inactive (gray). If a gate is moved for a grouped data file, a popup message appears.



5 Select **Current** to apply the changes to the current sample. Select **All** to apply the changes to all samples of the group.

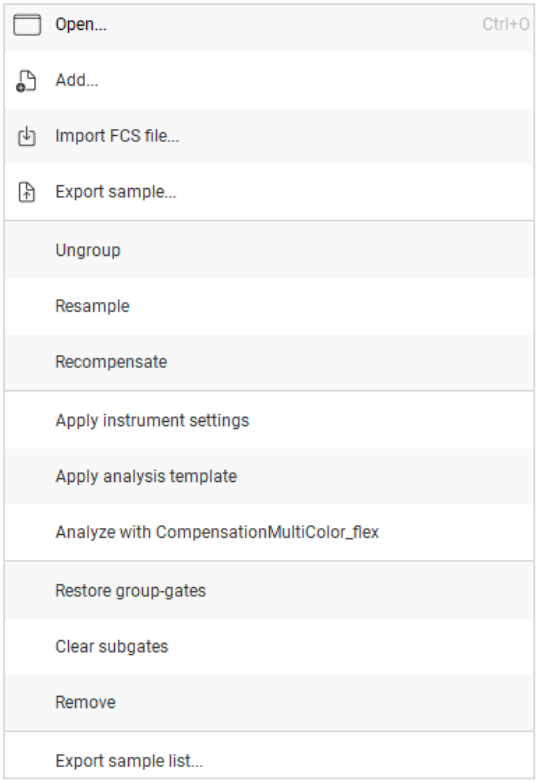
6 Optional: If the Express Mode allows export of results, then adjust gates as required before starting the export.

Analyzing with Compensation Express Mode

1 Right-click a data file in the sample list acquired with a Compensation Express Mode.

2 To open the compensated data file, acquired with a Compensation Express Mode, select **Apply analysis template**. See **Applying analysis templates on page 135** for details.

3 To analyze the uncompensated data file and adjust the compensation matrix, acquired with a Compensation Express Mode, select **Analyze with <Name of Compensation Express Mode>**. The compensated data file is indicated by an underscore in the file name.



4 For instructions on how to compensate and analyze your data, see **Express Mode CompensationMultiColor on page 59** or **Express Mode CompensationMultiColor_flex on page 64**, depending on the Compensation Express Mode used.

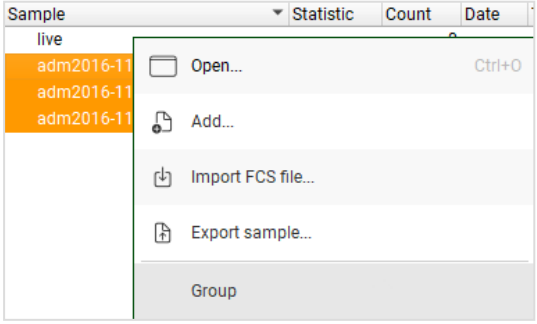
6.6.5 Grouping and ungrouping of data files

Data files can be grouped or ungrouped after acquisition for analysis as group or single files, respectively.

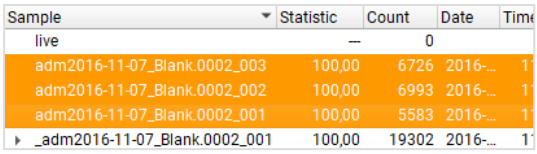
Grouping of data files

1 In the **Samples** tab, highlight each file in the sample list that should be grouped.

2 Right-click the files and select **Group**.



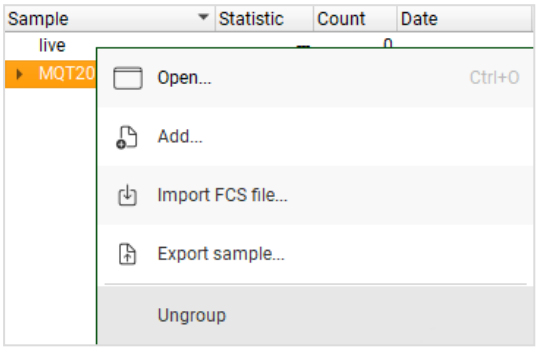
The resulting grouped file is highlighted and starts with an underscore (_).



Ungrouping of data files

1 In the **Samples** tab, highlight the file(s) in the sample list that should be ungrouped.

2 Right-click the data file(s) and select **Ungroup**.



Ungrouped files are shown in the sample list. The files start with an underscore (_). All individual files are saved in the same folder like the original grouped file. The original file remains unchanged.

6.6.6 Offline compensation and recompensation

Compensation can be performed on any file acquired on the MACSQuant Instrument. Files can be compensated after acquisition based on single-stained samples. Furthermore, already compensated files can be recompensated if the applied compensation is not correct.

1 Go to the **Samples** tab.

1 Select a file in the sample list for compensation or recompensation.

2 Right-click the file and select **Apply instrument settings**. This loads the compensation matrix linked to this file.

3 Click the **Add** button in the toolbar.

4 Select a new analysis window template, for example, **'Plot3'**.

5 In the left dot plot, select a file from the plot title dropdown list.

6 Click the **'i'** button next to the right plot window and change the plot type to histogram.

7 Select the first fluorochrome requiring compensation in the histogram, for example, FITC.

8 Click the **Instrument settings** button in the toolbar to open the compensation matrix.

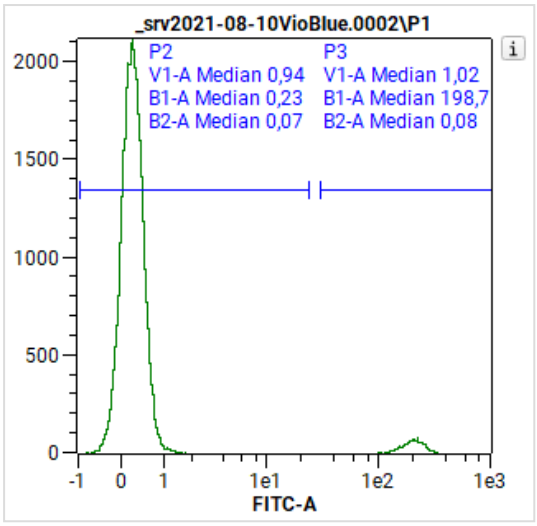
9 Select the **Compensation** tab.

10 Select the box **Matrix** to show the compensation matrix.

11 Draw a scatter gate around the population of interest within the FSC versus SSC dot plot. This is P1.

12 Select P1 from the the plot title to show only events in P1 in the histogram plot.

13 Draw a range around the negative (P2) and positive (P3) populations.

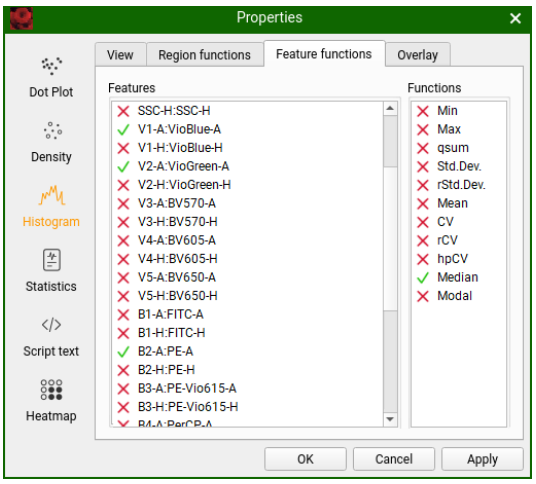


14 Optional: Rename P2 and P3 regions to negative and positive, respectively. Right-click the region and select **Region properties**.

15 Click the **'i'** button to format statistics table properties.

16 Go to the **Region Functions** tab and select P2 and P3.

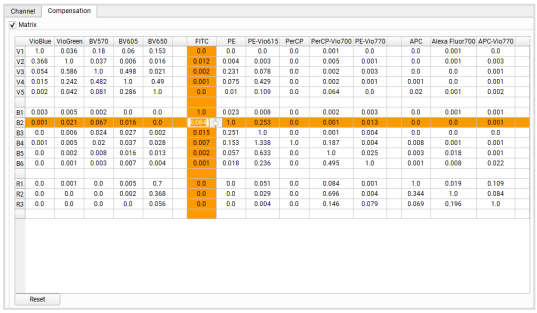
17 Go to the **Feature Functions** tab. Select the spillover channels used in the experiment and select **Median**.



18 Click **OK**.

19 Select the appropriate cell in the compensation matrix to adjust the value. See **Manual compensation on page 70**.

20 Use the arrows to change the value or type in a value. Press enter.



21 Select the file for compensation or recompensation from the sample list.

22 Right-click the file and select **Recompensate**. A new file is created with an underscore (_) in front of the file name. The original data file is not changed. The currently open compensation matrix is applied to the new file.

23 Continue to adjust values until the median fluorescence values for the positive and negative populations are equal for the spillover channel.

24 Adjust values for other spillover channels if necessary.

25 Right-click the new file and select **Apply analysis template** to show the compensated data file.

26 Optional: Copy the gating strategy from the original file to the file with underscore.

To avoid creation of multiple files, continue to apply the recompensation to the original file (file without underscore).

6.6.7 Resampling: Change annotations

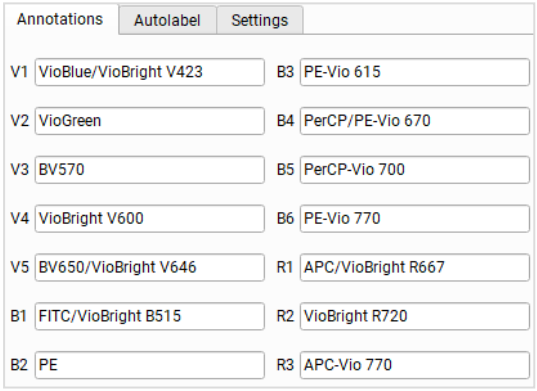
Annotations can be changed after acquisition. Use this function to change the axis label if it was not correctly aligned prior to acquisition.

1 Open a data file.

2 Right-click the data file that requires the annotation change.

3 Select **Apply instrument settings**.

4 Go to the **Annotations** tab of the **Experiment** tab and type in the correct annotations.



5 Highlight all files requiring the changed annotations.

6 Right-click the data files and select **Resample**.

If several files should be resampled to change their annotations, make sure that the selected files were acquired with the same instrument setting.

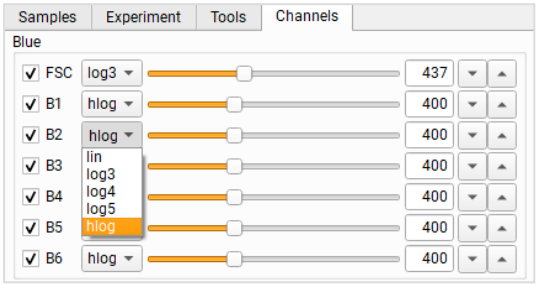
Recompensated data files are created with an underscore (_) in front of the file name. The underscore file contains the new annotations. The original data file remains unchanged.

Sample	Statistic	Count	Date	Time	Sample
live	—	0			
srv2021-13-02.0012	100,00	7334	2021-...	14:16	
srv2021-12-02.0012	100,00	3667	2021-...	14:16	
_srv2021-13-02.0012	100,00	7334	2021-...	14:16	
_srv2021-12-02.0012	100,00	3667	2021-...	14:16	

Figure 6.26: Resampled files in the sample list with an underscore

6.6.8 Resampling: Change scales

1. Open data files.
2. Right-click the data file that requires the scale change.
3. Select **Apply instrument settings**. The scales are shown as used during acquisition.
4. Go to the **Channels** tab.
5. Select the scale from the dropdown list of the respective channel.



6. Highlight all files requiring the changed scales.
7. Right-click the data files and select **Resample**.

> If several files should be resampled to change their annotations, make sure that the selected files were acquired with the same instrument setting.

Resampled data files are created with an underscore (_) in front of the file name. The underscore file contains the new scales. The original data file remains unchanged.

Sample	Statistic	Count	Date	Time	Sample
live	—	0			
srv2021-13-02.0012	100,00	7334	2021-...	14:16	
srv2021-12-02.0012	100,00	3667	2021-...	14:16	
_srv2021-13-02.0012	100,00	7334	2021-...	14:16	
_srv2021-12-02.0012	100,00	3667	2021-...	14:16	

Figure 6.27: Resampled files in the sample list with an underscore

7 Reporting data

The MACSQuantify Software offers several ways to create reports. Single plots or entire pages can be copied and pasted into external software, such as Microsoft® Word. If the instrument is connected to a printer, reports can be printed directly, or, alternatively, saved as .pdf. The sample list can be exported to Microsoft® Excel.

7.1 Copying pages or plots

7.1.1 Copying a page

1 Analyze data.

2 Click **Edit > Copy page**. The selected analysis window is copied to the clipboard.



3 Open a program for pasting.

4 Right-click and select **Paste**.

5 The page including all plots and statistic tables are pasted.

It is recommended to use scaling of 100% on your PC system to avoid problems with data display and resolution.

7.1.2 Copying a plot or table

1 Analyze data.

2 Click the specific plot or statistic table for copying. The plot is highlighted indicated by an orange rim.

3 Click **Edit > Copy plot**. The plot is copied to the clipboard.

4 Open a program for pasting.

5 Right-click and select **Paste**.

6 The plot or table including gating and annotations are pasted.

It is recommended to use scaling of 100% on your PC system to avoid problems with data display and resolution.

7.2 Printing analyzed data files

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 Instrument, to the PC running the MACSQuantify Software, or via a network connection.

See **Print on page 203** to change the numbers of analysis pages printed per sheet.

7.2.1 Printing a single data file

To print the analysis of a data file, open or add a data file.

If the file was acquired with an analysis template:

1 Right-click the file and select **Apply analysis template**.

2 Go to **File > Print**.

If no analysis template is linked to the data file:

1 Open a new analysis window and create a new data analysis. Alternatively, open a saved analysis template and load a sample from the sample list by using the dropdown list of the plot title.

2 Go to **File > Print**.

7.2.2 Printing all data files

All samples can be printed if the same analysis template is applied to all samples in the sample list. The **Print all...** option is only accessible in **Analysis mode**.

1 Open data files.

2 Right-click one sample and select **Apply analysis template**.

3 Optional: create a new analysis or open a saved analysis template. For further details see **Applying analysis templates on page 135**.

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

5 Use the previous and next sample arrows to apply the analysis template to all files in the sample list.

6 Go to **File > Print all...**.

7.2.3 Print to PDF function

This function allows to generate a .pdf file of analysis pages as they are shown live on MACSQuant Instrument. The generated .pdf files have an automatic naming convention **Analysis_printout_<accountID><YYYY-MM-DD_hhmmss>.pdf**. The files can be accessed via **File > Copy > Reports**.

1 Go to **File > Print to PDF**.

2 The below message appears. Click **OK**.



7.2.4 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 Open data files.

2 Right-click one sample and select **Apply analysis template**.

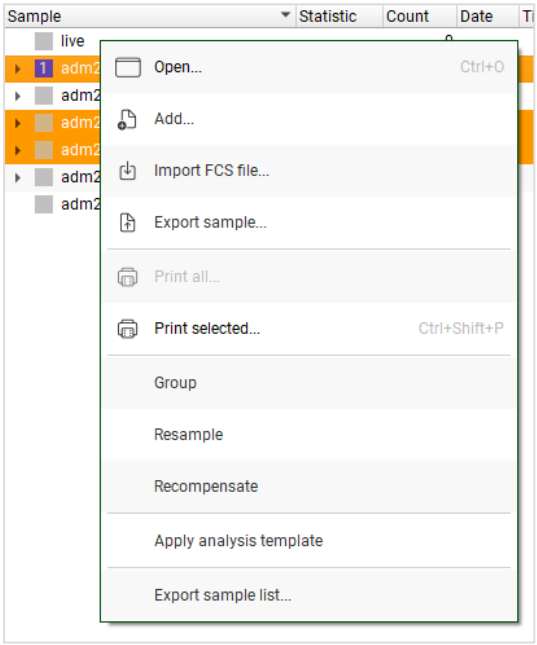
3 Optional: create a new analysis or open a saved analysis template. For further details see **Applying analysis templates on page 135**.

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

5 Use the previous and next sample arrows to apply the analysis template to other files in the sample list.

6 Highlight one or more samples.

7 Right click the selected samples and select **Print selected...**. Alternatively, go to **File > Print selected...**.

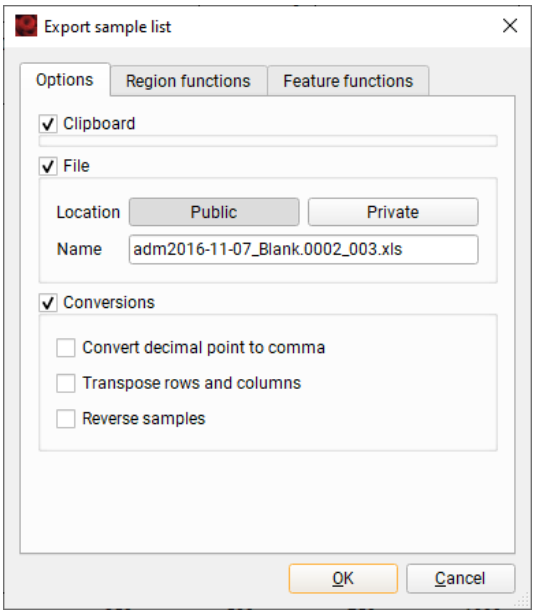


7.3 Exporting the sample list to Microsoft® Excel

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**. A dialog box opens to select the parameters to export.



3 Select one or both of the following:

- Select the box **Clipboard** to save to the clipboard.
- Select the box **File** to save data as an Microsoft Excel file. Depending on the selected location, the file is saved within the data folder of the MACSQuantify Software either in **Public** or **Private** location.

4 Optional: Select the box next to **Conversions** and all desired parameters.

5 Go to the **Region functions** tab to select the region for export and the % and count statistics to be exported.

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

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

7 Click **OK**. If **Clipboard** was selected, open Excel and select paste.

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

8 File management

This chapter describes file management for Custom users and MQ Administrators. For information about file management for Express users, see **Express user on page 215**.

8.1 Data storage

Apart from flow cytometry data files, MACSQuantify Software uses other files to save and re-open user settings. Files can be saved and opened from different locations. From within MACSQuantify Software, these locations are referenced as Public, Private, and External.

If files saved in a network location or on an external drive should be opened, the files must be saved in the same folder structure as in the MACSQuantify folder structure.

- Public files are located on the local hard drive of the MACSQuant Instrument and can be accessed by any user.
- Private files are located on the local hard drive of the MACSQuant Instrument and are only accessible by the signed in user. A folder named with the **Display Name** of each user is created by default.
- External files are located on an independent file storage device, for example, a USB flash drive. They can be accessed when the external device is connected to the MACSQuant Instrument.

Access rights defined for a user determine the right to read and save files or to only read files from and to **Public** or **Private** locations. If an external location is available, then, depending on the user rigts, an user can save, copy, and open files to and from this location.
Workspaces, instrument setting, experiments, analysis templates can be saved and opened with the **Open** and **Save** dialogs. Upon completion of a measurement, .mqd files are automatically saved in the user's private or public directory depending on the file access rights. .mqd files can be exported as .fcs and .csv files. Files can be copied and backed up to an external location with the **Copy** or **Backup** dialog.

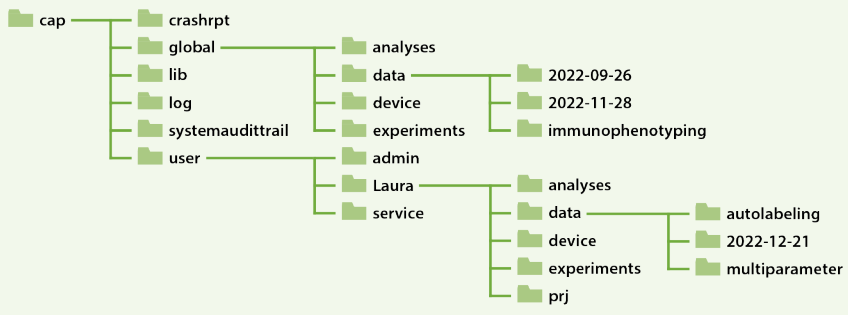


Figure 8.1: Folder structure of MACSQuantify software installation on the Windows analyis PC

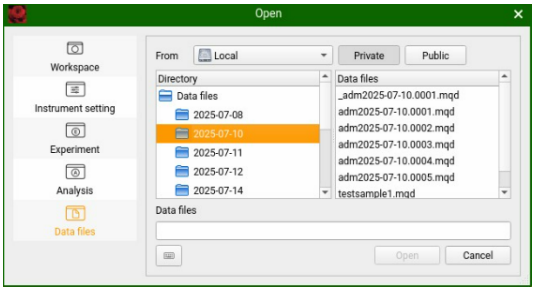
8.2 Managing files

8.2.1 Opening files

Workspaces, instrument settings, experiments, analysis templates, and data files can be opened in the MACSQuantify Software.

1 To open files, do one of the following:

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



2 Select one of the file types workspaces, instrument settings, experiments, analysis templates, or data files on the left-hand side of the dialog box.

3 Select the appropriate file location **Local** with the options **Private** and **Public**, or **External** to open files from an external medium.

4 Select the desired file(s). It is possible to open several data files at once.

5 Select **Open**.

6 If files saved in a network location or on a USB flash drive should be opened, the files must be saved in the same folder structure as in the MACSQuantify folder structure to be visible. See **Data storage on the previous page**.

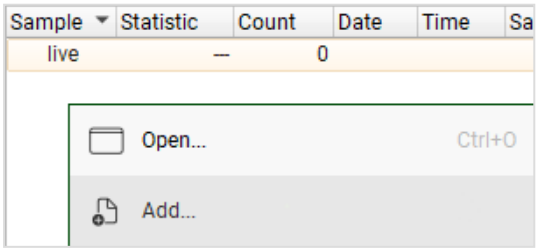
8.2.2 Adding data files to the sample list

If the MACSQuantify Software is installed on a PC, it can be used to view and analyze previously generated .mqd files. If files are located outside the MACSQuantify folder structure, for example, on an external storage device, they can be opened by adding them to the sample list. The **Add** function is only available in the PC version of the MACSQuantify Software.

1 Go to the **Samples** tab.

2 Right-click within the sample list.

3 Select **Add...** and browse to the storage location of the data file(s).



4 Select file(s).

5 Select **Open**. Data file(s) are added to the sample list.

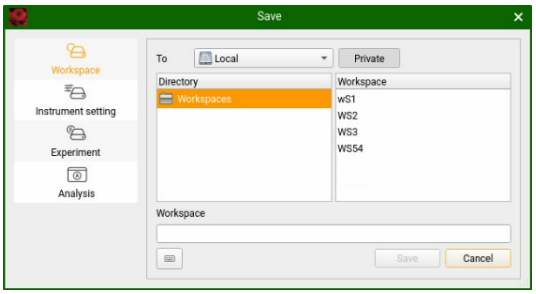
8.2.3 Saving files

Depending on the access permissions of the user, files can be saved in the global folder (public files) or in the user folder (private files). Global files are visible for all users and can be shared. When **public** is selected as storage location, the file is saved in the **global** folder. When **private** is selected as storage location, the file is saved in the **user** folder. The folders are only visible in the PC version.
Instrument settings, **Experiment**, and **Analysis files** can be saved as both public and private files as defined under **User management**. Files can also be saved to external locations.

Workspaces can only be saved as private files in the respective user folder.

Data files are either saved automatically as public or private files depending on the access permissions of the user. A user with both rights will have the choice where to save the data files at first sign in. The location is saved and used afterwards for automatic data saving. It can be changed from **Edit > Options (default) > Files**. For an overview about file access rights for the preconfigured MQ user roles, see **Table 10.2**.

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



2 Select **Workspace**, **Instrument setting**, **Experiment**, or **Analysis** as file type.
3 Select the appropriate file location **Local** with the options **Private** and **Public**, or **External** to save files to an external medium.
4 Name the file.
5 Click **Save**.

8.2.4 Management of overwriting rights

Depending on the access permissions, the user can overwrite an existing file from the global folder (public files) or from the user folder (private files). If the user has the delete right, then the user can choose to overwrite a file. If the user has only the read/write right, then overwriting a file is not possible.
Only the MQ Administrator user role has the overwriting rights by default. For details on which files can be overwritten by the MQ Administrator, see **Table 10.2**. If needed, additional user roles with overwriting rights can be set up and assigned to users according to **Table 10.5**.

For 21 CFR Part 11 compliant setup, an entry in the audit trail is generated when a file is overwritten. See **Audit trail 21 CFR Part 11 on page 241**.

8.2.5 Exporting FCS or CSV files

MQD files can be exported as FCS or CSV files. An MQD file can be automatically exported to FCS file after acquisition, see **Export as .FCS on page 201** To adjust the settings for FCS export, see **Export on page 201**.

1 Right-click a data file in the sample list and select **Export sample...**.
2 Select the desired file type from the dropdown list.

3 Optional: Clear the box **Skip subpopulations** if you prefer a separate FCS file for each subgated population of events. The FCS file is saved in the same folder where the original MQD file is located. It can be accessed via **File > Copy** in **Data files**. The CSV file can be accessed via **File > Copy** in **Other files**.

8.2.6 Copying files to an external location

Use the copy function to transfer files between MACSQuant Instruments, the PC version, or external sources such as a network or external drive. See **Copying files from an external location on the facing page** for details on how to copy files from an external drive or a network location. Access to file types is dependent on the access rights of the user. For details see **File access rights on page 166**.

Make sure that the external drive is formatted as exFAT for compatibility with the operating system. If required, change the format on a PC. If the external drive is formatted on a MAC system, remove the .trash folder.

An MQ Administrator can copy following file types:

- all files including (private) files of all users
- all files in public locations
- administrator files in a private location
- log files
- crash reports
- system audit trails

For more information of copying and deleting files of all users see **Deleting files on page 153**.

1 If you need to copy from an external drive, insert it into a USB port of the instrument.
2 Wait for the drive to be recognized.
3 Go to **File > Copy...**.
4 Select **To** from the left-hand side dropdown list to copy files to the selected destination. The folder structure is preserved at the destination location.

Depending on the user rights, the folder structure can look different.

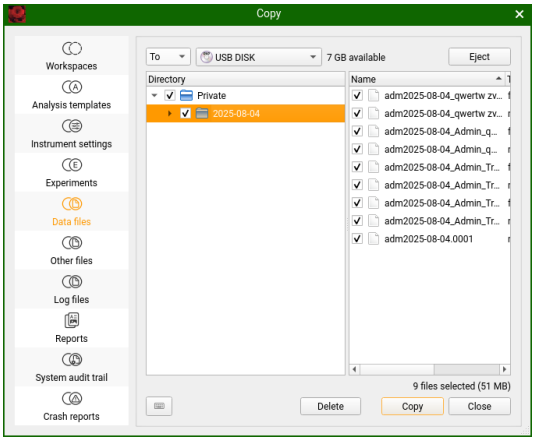
5 Select the destination from the right-hand side dropdown list in the dialog box.

An external drive (if mounted) or a private/public network drive location can be selected.

6 Select the file type.

7 Go to **Directory** and do one of the following:

- Click the arrow in front of a folder to expand the folder structure.
- Click a folder to show the files and select the files to be copied in the right panel of the dialog box.
- Select the checkbox in front of a folder to select all files in a folder.



The files are selected only when the checkbox in front of them is selected. Make sure that the checkbox in front of the files is selected.

8 Click **Copy**. A dialog window appears when all files are copied.

9 Select **Eject and close** or **Close**. If selecting **Close** ensure to eject the external drive via **File > Copy** later for safe removal.

The files must be organized in the same folder structure as in the destination folder to be properly imported.

Recreate users with the same username and user role to see the files in the MACSQuantify Software.

8.2.7 Copying files from an external location

The copy function can be used to copy files from an external location such as: a backup location, a public/private network drive or an external drive. Access to file types is dependent on the access rights of the user. For details see **File access rights on page 166**.

Files that have been backed up and archived can be transferred to the MACSQuant Instrument via the copy function. Specific folders are shown according to the type of backup that has been carried out and the access rights of the user.
Different MACSQuant Instrument can share the same backup location. Folders with the respective serial number of the different instruments are created automatically. The backed up files can be accessed via the copy function. See the **Figure 8.2** and **Table 8.1** for a detailed description of the copy function directory.

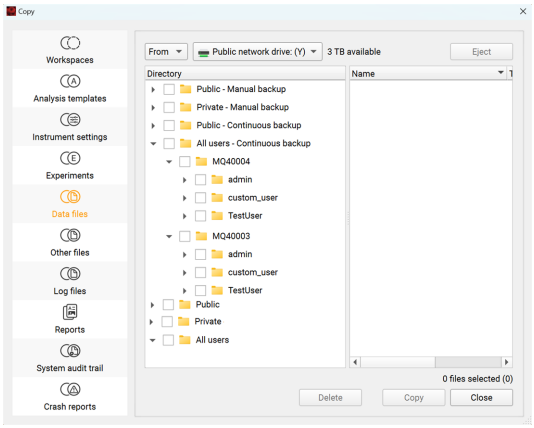


Figure 8.2: Overview of the copy function directory

Directory	Description
Public - Manual backup	Location where the files backed up in public folder are stored after a manual backup. See **Manual backup on page 155**.
Private - Manual backup	Location where the files backed up in private folder of the signed in user are stored after a manual backup. See **Manual backup on page 155**.
Public - Continuous backup	Location where the files backed up in public folder are automatically stored during the continuous backup. See **Continuous backup on page 240**.
All users - Continuous backup	Location where all the files backed up in user private folders are automatically stored during the continuous backup. User folders are visible according to the assigned user rights of the currently sign in user. See **Continuous backup on page 240**.
Public	Location where public files are stored and can be accessed by any user. See **Data storage on page 147**.
Private	Location where private files are stored and are only accessible by the signed in user. See **Data storage on page 147**.
All users	Location where all the files available on the external location are stored as in the private directory of all users account. User folders are visible according to the assigned user rights of the currently sign in user. See **Data storage on page 147**.

Table 8.1: Overview of the copy function directory

1 If you need to copy from an external drive, insert it into a USB port of the instrument.

2 Wait for the drive to be recognized.

3 Go to **File > Copy...**.

4 Select **From** from the left-hand side dropdown list.

5 Select the files to be imported.

Depending on the user rights, the folder structure can look different.

6 Click **Copy**. A dialog window appears when all files are copied.

7 If copying from an external drive, click **Eject** to ensure safe removal of the drive.

8.2.8 Deleting files

Files can be permanently deleted from the hard drive of the instrument by an MQ Administrator. See **Table 10.2** for details which files can be deleted by the MQ Administrator.

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

1 Go to **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 box in front of a folder to select all files in a folder or select single files within a folder.
6 Click **Delete**. Selected files are permanently deleted.

8.3 Data backup

It is recommended to back up data to a remote storage location and to remove data from the MACSQuant Instrument regularly to avoid a full hard drive. To back up data from the MACSQuant Instrument to a network location, a network path must be defined.
Data can be transferred from the MACSQuant Instrument to an external storage location, such as network folders, or external physical drives. Use the **Backup** or **Copy** function. The folder structure on the MACSQuant Instrument is automatically copied to the remote storage location.

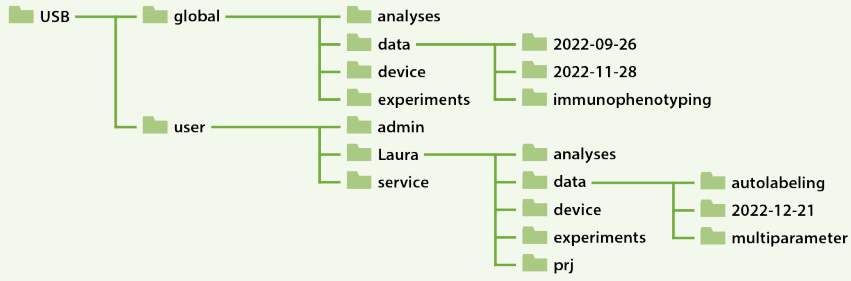


Figure 8.3: Folder structure on the remote storage location after backup

An MQ Administrator can define a global backup location. This location can be selected by every user as target location to backup or copy files.
A custom user can define a private backup location which is only selectable for the user who entered the location. The global and private backup location must always be different.
Which files are available for manual backup depends on the user role and file access rights.
When continuous backup is activated, files generated on the system are continuously backed up.

Different MACSQuant Instrument can share the same backup location. Folders with the respective serial number of the different instruments are created automatically. For full traceability, the data that has been backed up on a mounted network location, can be accessed via the Copy dialog. See **Copying files from an external location on page 151**.

User type	Write rights		Files available for backup
	Private data	Public data	
Custom user	Yes	No	Own private data folder Own private subfolders (analysis, device, experiment, prj)
Custom user	No	Yes	All public data folder
MQ Administrator	Yes	No	Own private data folder Own private subfolders (for example, analysis, device, experiment, prj)
MQ Administrator	No	Yes	All public data folder All public subfolders (for example, analysis, device, experiment)
Express user	Yes	No	Own private data folder
Express user	No	Yes	All public data folder

Table 8.2: Manual backup of files depending on the preconfigured MQ user roles and file access permissions

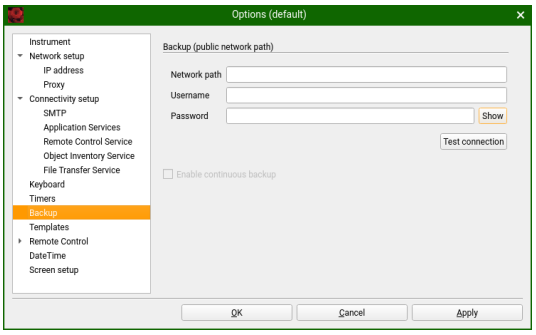
8.3.1 Setting up a public network location

A public network location can be used for continuous and manual backup and applies to all users of the MACSQuant Instrument. The backup location is selectable as available drive when the manual backup is started. Private files of the signed in user, as well as the public data files, can be backed up during the process. An MQ Administrator can additionally back up files from all users. This public network location can also be used for copying the files of MACSQuant Instruments on an external location. See **Copying files from an external location on page 151** for details.

For the 21 CFR Part 11 compliant setup, a continuous backup can be configured to automatically save all MACSQuant Instrument files on a dedicated network location. An MQ Administrator can specify the backup network location and activate/deactivate the feature. See **Continuous backup on page 240** for details about the backup procedure.

1 Sign in as MQ Administrator.

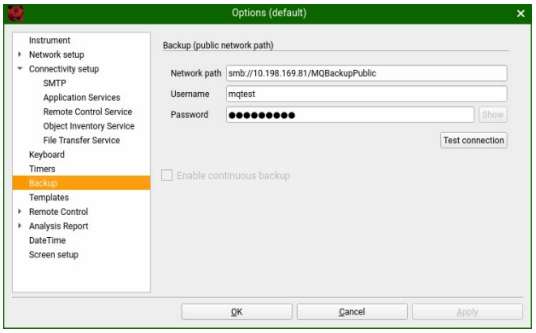
2 Go to **Edit > Options (default) > Backup**.



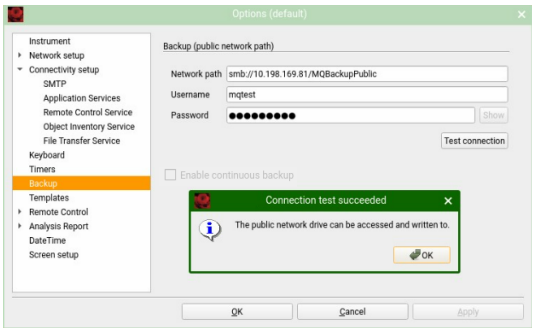
The checkbox **Enable continuous backup** is only available for the 21 CFR Part 11 compliant setup.

3 Click in the field **Network path** and enter the full path of the network location. Enter a location on the network that can be accessed by all users of the MACSQuant Instrument. Use the following path format \\RemoteServerIP\RemoteFolder.

4 Enter the username and password of the network location.



5 Click **Test connection** to ensure that the network location is accessible.



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

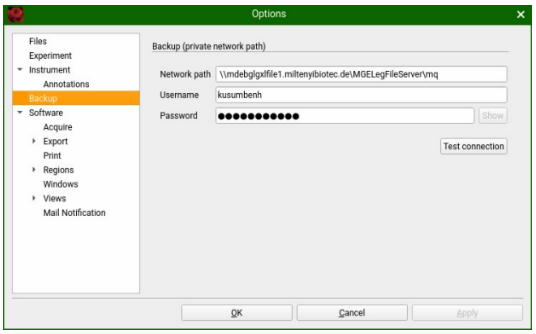
8.3.2 Setting up a private network location

A private network location can be used for manual backup. It applies to the user account in which the private network location is defined. Each user needs to set up its own private network location.

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

2 Click the field **Network path** and enter the full path of the network location. Use the following path format \\RemoteServerIP\RemoteFolder.

3 Enter the username and password of the network location.



4 Click **Test connection** to ensure that the network location is accessible.

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

8.3.3 Manual backup

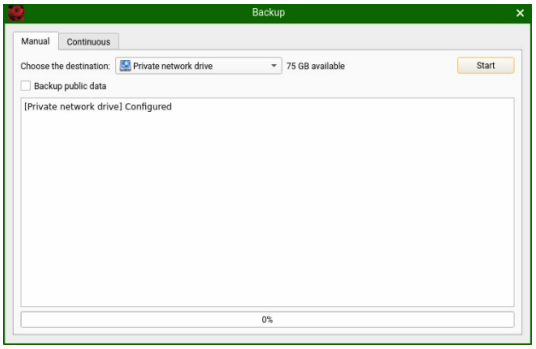
Manual backup of files is possible to an external drive or to a defined public or private network location. The manual backup is available for the MQ Administrator, MQ Custom user and MQ Express user.

For the 21 CFR Part 11 compliant setup, an entry in the audit trail is generated. See **Audit trail 21 CFR Part 11 on page 241**.

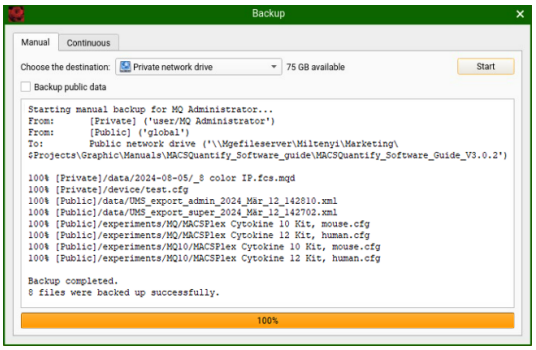
1 Click on the **Backup** button on the toolbar. See **Toolbar on page 31** for details.

2 Select the desired backup location.

3 Optional: click **Backup public data** to also back up the files saved in the public location. By default, only the files of the signed-in user are backed up.



4 To start the manual backup, click **Start**. The files are backed up in the defined network path, in the folder _**mb_\MQ_SerialNumber**.



8.3.4 Continuous backup

For the 21 CFR Part 11 compliant setup, a continuous backup is available to automatically save all MACSQuant Instrument data files from private and public folder on a dedicated network location. For details, see **Continuous backup on page 240**.

9 Express Mode

9.1 Express Modes

Express Modes are data acquisition and analysis tools included in the MACSQuantify Software available for all user types. Express Modes use predefined experiment settings as well as analysis templates. The analysis with Express Modes applies an automated gating strategy to a sample. The gating is adjusted to each sample individually to achieve optimal results. The data file contains all relevant information such as acquisition pages, the instrument setting, and a reference to the used Express Mode.

The MACSQuantify Software allows the use of different Express Mode types. Each software version contains a set of installed Express Modes that consists of Setup Express Modes and Included Express Modes.
Setup Express Modes can be used for calibration of the instrument and to perform multicolor compensation.
For a list of Setup Express Modes, see **Setup Express Modes on page 212**.
Included Express Modes are compatible with Miltenyi Biotec reagent kits such as MACS Control Antibody Cocktails, or other reagent kits like the 8-Color Immunophenotyping Kit, anti-human REAfinity.
Additionally to the included Express Modes, there are Add-on Express Modes available for purchase, for example, for CAR T cell analysis.
It is also possible to use the Miltenyi Biotec Custom Express Mode development to develop automated acquisition and analysis for specific applications. See **www.miltenyibiotec.com** for further details.
Some Express Modes require grouping of samples, for example, the included Express Modes EPC_Enrichment_Enumeration_Kit_h and MC_CD34_CD133_h. Using Express Modes with a control staining, grouping is mandatory to allow gate calculation for the experimental sample based on the control. More information about the Express Mode can be found in the corresponding data sheet of the reagent.

Before running an Express Mode, make sure to run a PMT calibration and to set up an appropriate instrument setting including compensation. The Express Mode only adjusts some instrument setting but does not contain channel gains or compensation.

For details about selecting an Express Mode, see **Selecting an Express Mode on page 84**.

For details about data analysis using Express Modes, see **Data analysis using Express Modes on page 136**.

9.2 Express Mode manager

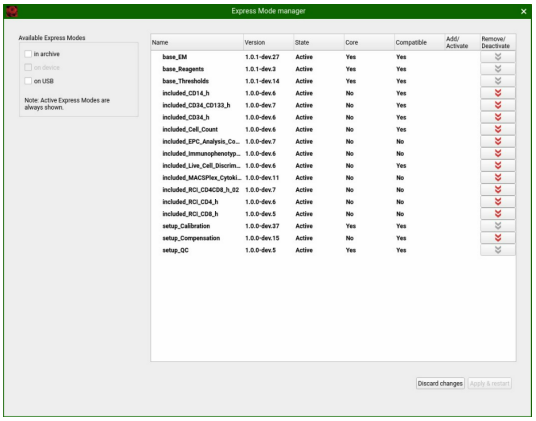
Express Modes installation, activation and deactivation is managed via the **Express Mode manager**. The Express Mode manager is only available in data analysis mode. New Express Modes can be added or updated from an USB flash drive or another external drive. To show the Express Modes available on an external drive, click the checkbox **on USB**.
Deactivated Express Modes are still present on the MACSQuant Instrument, but they are not available for user usage. To show the deactivated Express Modes, click the checkbox **in archive**.

Assign the added Express Modes to the users according to **Adding a new user role on page 168**. Otherwise the users will not be able to run the new Express Mode.

The MQ Administrator user role has the Express Mode management rights by default. If needed, additional user roles with Express Mode packages management right can be set up and assigned to users according to **Table 10.5**. In an 21 CFR Part 11 compliant setup, Express Mode manager actions are tracked in the Audit Trail. See **Audit trail 21 CFR Part 11 on page 241** for details.

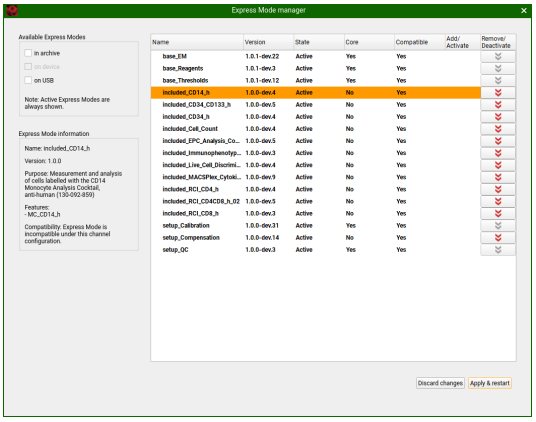
9.2.1 Deactivating and deleting an Express Mode

1 Go to **Edit > Express Mode manager**.



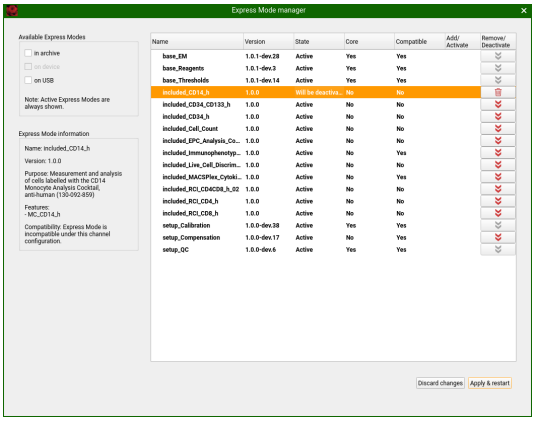
Express Modes serving core functionalities of the MACSQuant Instrument are marked **Yes** in the **Core** column and cannot be deactivated and deleted. A deleted Express Mode cannot be reactivated and needs to be installed again from an external drive.

2 Select the Express Mode to be deactivated.



3 To deactivate the Express Mode, click the red arrow in the column **Remove/Deactivate**.

The **State** of the Express Mode changes to **Will be deactivated**. In column **Remove/Deactivate**, the red arrow changes to a red recycle bin button.



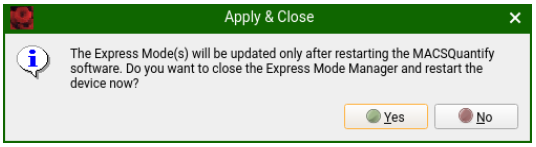
4 To remove the Express Mode from the MACSQuant Instrument, click on the red recycle bin button in the column **Remove/Deactivate**.

The **State** of the Express Mode package changes to **Will be removed**.

5 To discard the changes without saving, click **Discard changes**.

6 To apply the changes, click **Apply & restart**.

7 Click **Yes** to restart. Make sure to save your work, before clicking **Yes**.

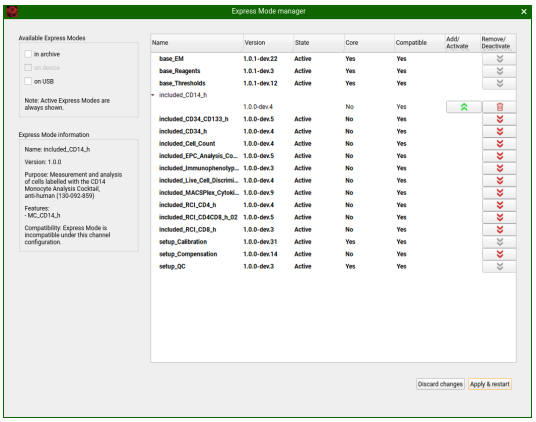


8 On a PC, close the software and manually restart the PC.

9.2.2 Reactivating an Express Mode

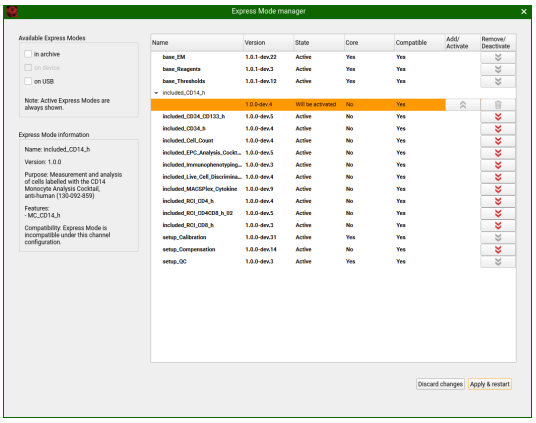
1 Go to **Edit > Express Mode manager**.

2 Select the checkbox **in archive** to show the deactivated Express Mode still present on MACSQuant Instrument.



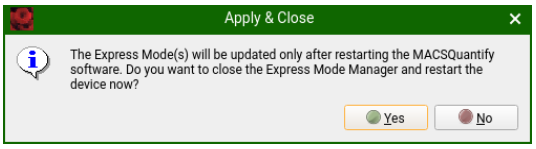
3 To reactivate the Express Mode, click the green arrow in the column **Add/Activate**.

The **State** of the Express Mode changes to **Will be activated**.



4 To apply the changes, click **Apply & restart**.

5 Click **Yes** to restart. Make sure to save your work, before clicking **Yes**.



6 On a PC, close the software and manually restart the PC.

9.2.3 Adding an Express Mode from a USB flash drive or another external drive

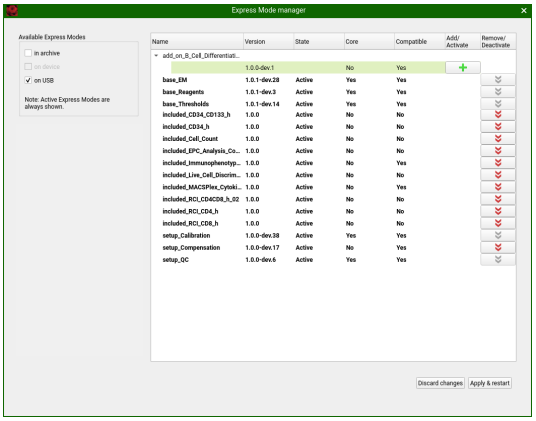
On the external drive, the Express Modes need to be in the folder **em_install** in order to be detected.

Assign the added Express Modes to the users according to **Adding a new user role on page 168**. Otherwise the users will not be able to run the new Express Mode.

1 Insert an external drive into a USB port of the MACSQuant Instrument.

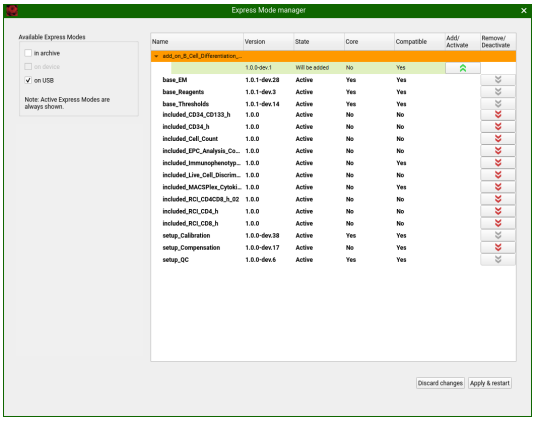
2 Go to **Edit > Express Mode manager**.

3 Select the checkbox **on USB**. The Express Mode ready to be added on the MACSQuant Instrument is highlighted on green.



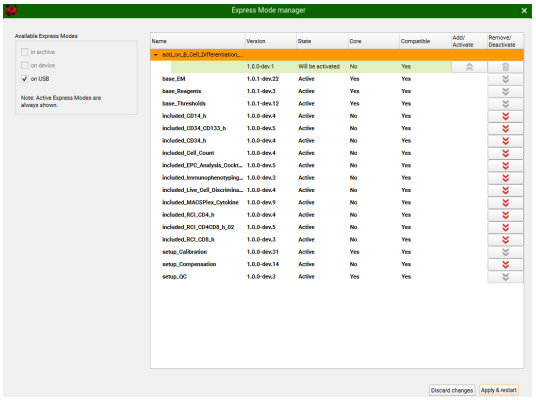
4 To add the Express Mode to the MACSQuant Instrument, click the green plus sign in the column **Add/Activate**.

The **State** of the Express Mode changes to **Will be added**.



5 To activate the Express Mode, click the green arrow in the column **Add/Activate**.

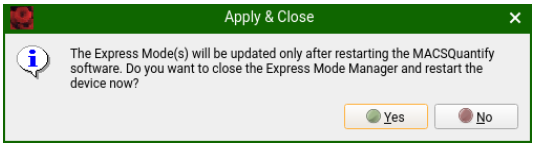
The **State** of the Express Mode changes to **Will be activated**.



6 To discard the changes without saving, click **Discard changes**.

7 To apply the changes, click **Apply & restart**.

8 Click **Yes** to restart. Make sure to save your work, before clicking **Yes**.



9 On a PC, close the software and manually restart the PC.

10 Administrator

This chapter describes functions that require an administrator role. The MACSQuantify Software uses two administrator roles:

- MACSQuant (MQ) related functions are bundled in the **MQ Administrator** role.
- User management (UM) related functions are bundled in the **UM Administrator** role.

Both administrator roles can be assigned together to one user account or separately from each other to different user accounts.
See **Table 10.1** for available functions based on the MQ user roles. See **Table 10.3** for available functions based on the UM user roles.

An account with the MQ Administrator user role has the same software interface for acquisition and data analysis like a MQ Custom user. Additionally, the MQ Administrator can change user and software settings that apply to all users. For individual settings within the MQ Administrator account, see **Custom user on page 197**.

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

User rights define access to functions within the MACSQuantify Software, access permissions for file types, access to the user management system, and access to the audit trail system. The user rights are defined within a user role. See **Table 10.3** and **Table 10.2** 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 171**.

MQ Custom user role

MQ Custom 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 PMT calibration and run a multicolor compensation. They can create instrument settings, analysis templates, experiments, data files, and workspaces.

MQ Administrator role

The MQ Administrator role includes the same functions of a Custom user. The user interface is the same. The MQ Administrator can define personal software settings and additionally global software settings that apply to all users. The MQ Administrator has acces to additional files and can delete/overwrite certain file types. Furthermore, he has the right to create Performace QC and Tracking reports and manage the Express Modes installation.

MQ Express user role

The MQ Express user role offers a way to operate the instrument for less experienced users. The Express user interface allows access to selected functions of the software. Express users can run experiments using analysis templates, experiments, or instrument setting created for example by an MQ Administrator. Managing different file types is restricted for Express users. For details see **Express user on page 215**.

MQ System Automation user role

The MQ System Automation user role is dedicated to the MACSQuant Instrument integrated into an automation platform. For integration into an automation platform, contact Miltenyi Biotec Technical Support.

QC User and QC Analyzer user roles

QC User and QC Analyzer user roles are intended to be used in the context of MB software solutions.

Function	Description	Pre-configured role		
		MQ Administrator	MQ Custom	MQ Express
Access to **Edit > Options**	To define user specific software settings	Yes	Yes	No
Access to **Edit > Options (default)**	To define global software settings for all users	Yes	No	No
Export audit trail events	To export a report about user activities that is saved as public file	Yes	No	No
Delete audit trail events	See **Table 10.4**	No	No	No
Trigger disaster recovery	To create and restore a complete system backup including all data, properties, and configuration files.	Yes	No	No
Access to all Express Modes	To access all the included Express Modes installed on the MACSQuant Instrument	Yes	No	No
Manage Express Mode	To add, activate, deactivate, and remove an Express Mode	Yes	No	No
Create Performance QC and Tracking reports	To create, edit and suspend values in Performance QC and Tracking charts.	Yes	No	No
Copy files of other users	To copy files of other users to an external drive	Yes	No	No
Run a PMT calibration	To calibrate the instrument	Yes	Yes	No
Perform automated compensation	To perform a multicolor compensation	Yes	Yes	Yes
Run initial calibration	To run an initial calibration. Required after first installation of the instrument or if the instrument is not serviced every 6 months	Yes	No	No
Run remote experiments	To validate and execute experiments and analyze the date in quality control.	Yes	No	No
Create analysis report (21 CFR part 11 only)	To create a PDF/A report of analyzed sample(s)	Yes	No	No
Access to log files	To copy log files to a remote storage location	Yes	No	No
Access to crash reports	To copy crash reports to a remote storage location	Yes	No	No
Access to hardware calibration	To calibrate the uptake unit, rack types, and plates	Yes	No	No

Function	Description	Pre-configured role		
		MQ Administrator	MQ Custom	MQ Express
Access to rack detection	To enable rack detection	Yes	No	No
Delete files	To delete files on the instrument	Yes	No	No

Table 10.1: Access to functions of the preconfigured MQ roles

File access rights

File type	Pre-configured role					
	MQ Administrator		MQ Custom		MQ Express	
	Private location	Public location	Private location	Public location	Private location	Public location
Analysis template	Write, delete/overwrite	Write, delete/overwrite	Write	Read	Write	Read
Data	Write, delete/overwrite	Read	Write	Read	Write	Read
Experiment	Write, delete/overwrite	Write, delete/overwrite	Write	Read	Write	Read
Instrument setting	Write, delete/overwrite	Write, delete/overwrite	Write	Read	Write	Read
Other files	Write, delete/overwrite	Write, delete/overwrite	Write	Read	Write	Read

Table 10.2: File access rights of the preconfigured MQ roles

UM Administrator role

The UM Administrator role is required to get access to the user management of the MACSQuantify 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.

User management rights

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

Function	Description	Pre-defined 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
Overwrite user/role details	To overwrite detailed information of existing users and roles	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
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 own user account	Yes	No
Update role	To change a user role	Yes	No
Update user details	To update detailed information of existing users	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

ATS user role

The ATS role allows to read and export the audit trail entries.

Function	Description	Pre-configured role	
		MQ Administrator	ATS User
Reset audit trail events	To delete audit trail events permanently	No	No
Export audit trail events	To export audit trail events as report	Yes	Yes
Read audit trail events	To read, search, and filter the audit trail events	Yes	Yes

Table 10.4: Access to audit trail functions of preconfigured roles

EM Superuser role

The EM Superuser role allows access to all of the installed Express Modes on the MACSQuant Instrument. These rights are assigned by default to the MQ Administrator and MQ Custom user roles.

10.1.2 Adding a new user role

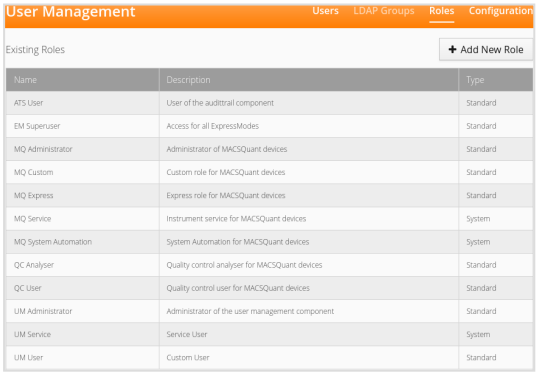
An UM Administrator can add new user roles besides the preconfigured user roles. See **Table 10.3** for a description of user management rights.

1 Sign in as UM Administrator.

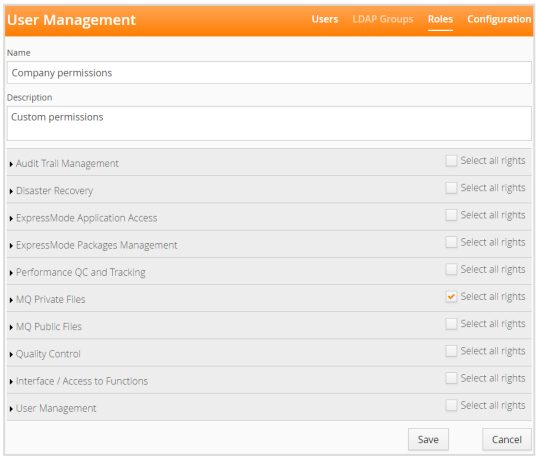
2 Go to **Edit > User management**.

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 Select the box **Select all rights** on the right-hand side to select all rights at once.

8 Assign the rights as needed. See **Table 10.5**.

9 Click **Save**.

Section of user role	Minimum requirement
Audit Trail Management	See **Table 10.4**.
Disaster Recovery	To enable the user to create and restore a complete system backup.
ExpressMode Application Access	Select which Express Modes the user has access to.
ExpressMode Packages Management	To enable the user to add, activate, deactivate, and remove an Express Mode.
Performance QC & Tracking	To enable the user to create charts and suspend values in the Performance QC & Tracking charts.
MQ Private Files	Select **Write** to enable the user role to save and open the selected file type. Select **Read** to enable the user role to open the selected file type. Select **Delete** to enable the user role to delete and overwrite the selected file type (only recommended for MQ Administrator).
MQ Public Files	Select **Write** to enable the user role to save and open the selected file type. Select **Read** to enable the user role to open the selected file type. Select **Delete** to enable the user role to delete and overwrite files (only recommended for MQ Administrator).
Quality Control	To enable the user to validate and run remote experiment in Miltenyi Biotec environment
Interface / Access to Functions	Select at least one of the three options **Administrator access**, **Custom access**, or **Express access**.
User Management	Select the options **Read details of own account** and **Read rights of own account** to allow the user with this role to sign in.

Table 10.5: Right selection for new user roles

10.1.3 Changing a user role

1 Sign in as UM Administrator.

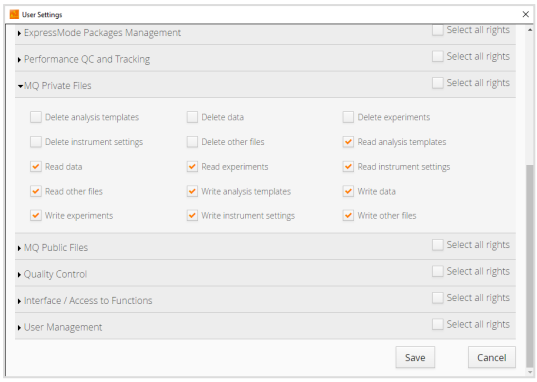
2 Go to **Edit > User management**.

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.



6 Select the checkboxes to assign rights as needed. See **Table 10.3** and **Table 10.2**.

7 Click **Save**.

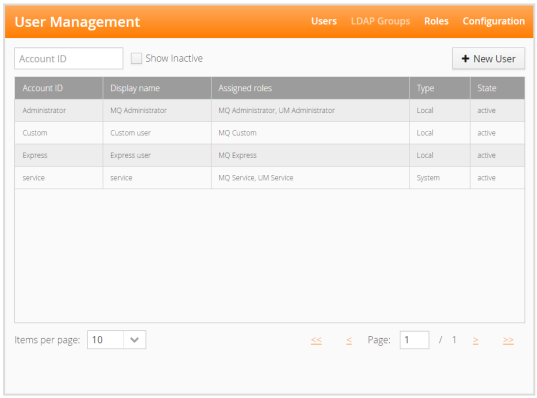
10.1.4 Assigning or changing user roles of a user account

1 Sign in as UM Administrator.

2 Go to **Edit > User management > Users**. A list of all users is shown.

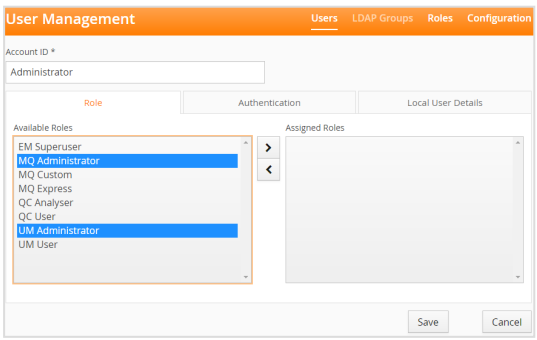
Users that have roles assigned only via a group assignment are not shown here. See **Assigning user roles via an LDAP group on the facing page**.

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 or click on the right facing arrow to move it from **Available Roles** to **Assigned Roles**.



A selection of both UM and MQ user role is required to be able to sign in.

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

7 Click **Save**.

10.1.5 Assigning user roles via an LDAP group

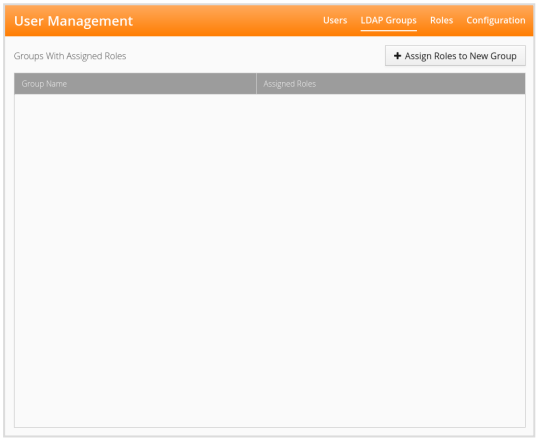
For the 21 CFR Part 11 compliant setup, user accounts can be connected to an existing user management system, for example, a Microsoft Active Directory or Lightweight Directory Access Protocol (LDAP).

1 Sign in as UM Administrator.

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

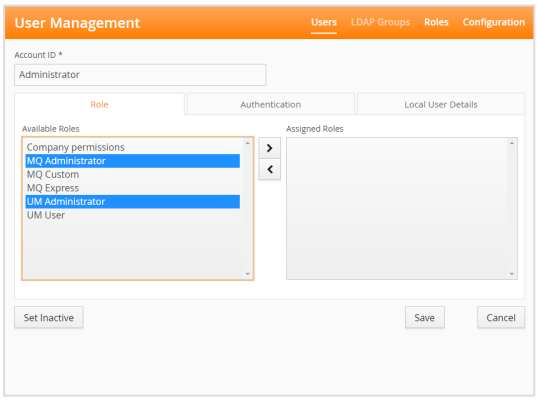
3 Select **LDAP Groups**.

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 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 Software. Creating local user accounts is also possible in the research use only version of the software.

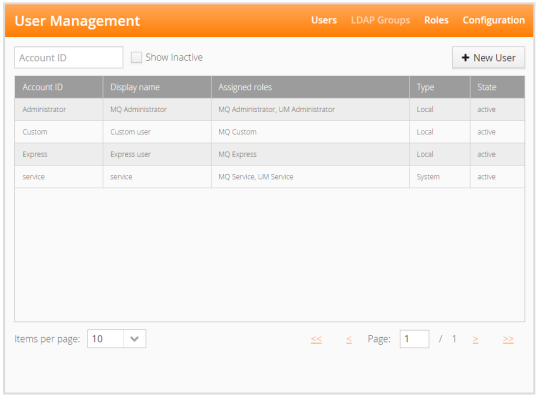
See **Adding a new LDAP user account on page 176** for setup, authentication, and modification of LDAP user accounts.
See **Adding a new local user account on the facing page** for setup, authentication, and modification of local user accounts.
See **LDAP configuration on page 244** for information on 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

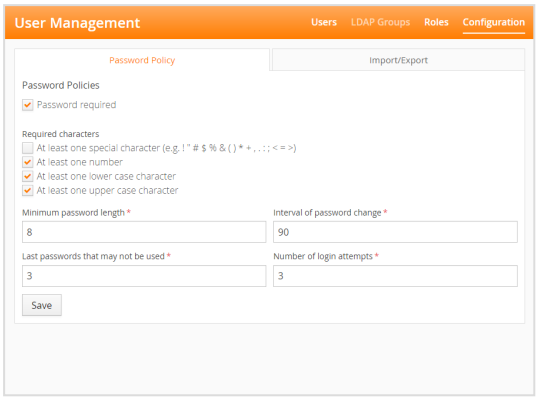
The MACSQuantify Software offers the possibility to create local user accounts without connection to an LDAP system. If 21 CFR part 11 compliance is required with local user accounts, password settings and user information must be set up in a specific way.

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.

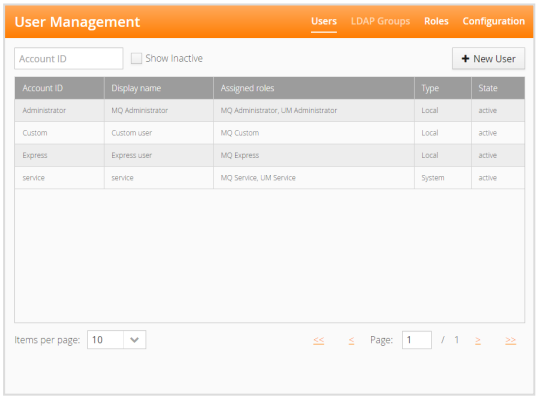
6 Click **Save**.

Adding a new local user account

1 Sign in as UM Administrator.

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

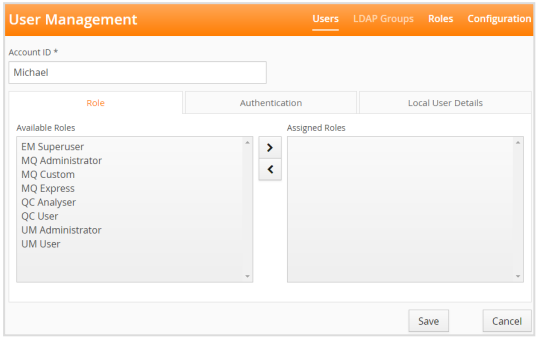
3 Select the **Users** tab.



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

5 Enter an username. Click outside the **Account ID** field. The account ID is not case sensitive. A confirmation message appears.

The username must be unique for each user account and not contain any not restricted special characters or spaces. For a list of allowed special characters, see **Using special characters on page 79**



6 Assign user roles as described in **Assigning or changing user roles of a user account on page 170**.

7 Proceed with step **5** of **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**.

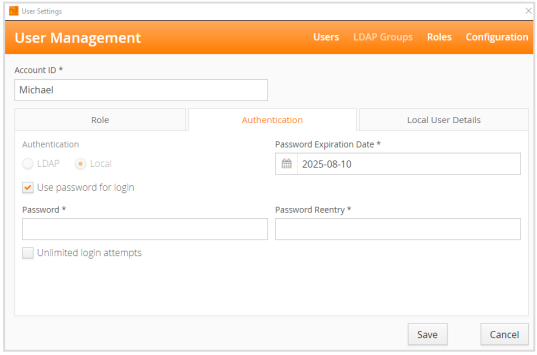
1 Sign in as UM Administrator.

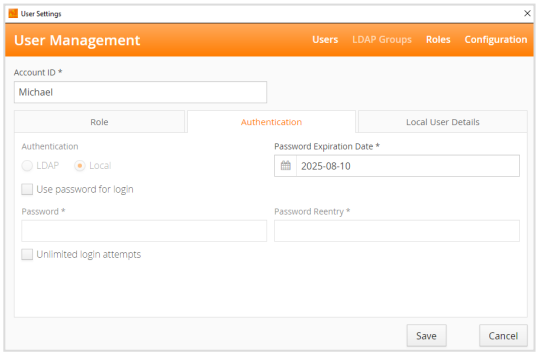
2 Go to **Edit > User management**.

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.

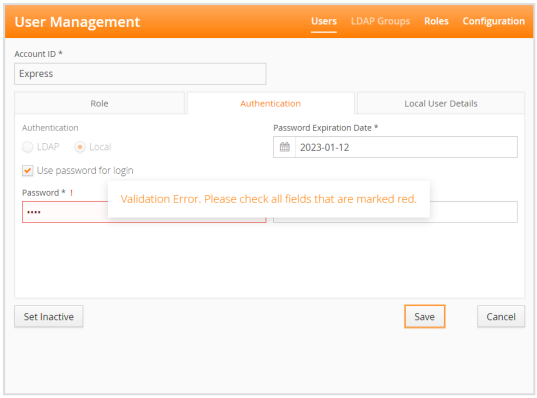
5 Go to the **Authentication** tab. If a password is required, the left screenshot is shown. If no password is required, the right screenshot is shown. The user has the possibility to select the checkbox **Use password for login** to set a password.





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 the previous page**.

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

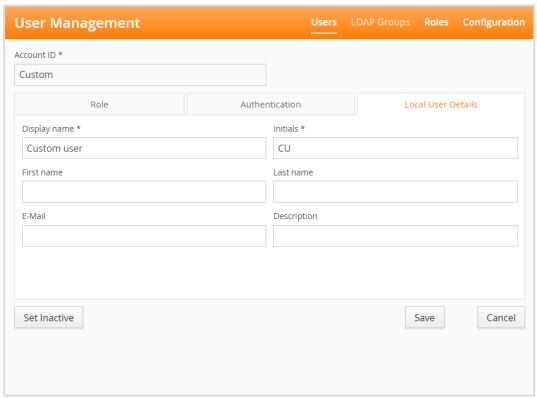
1 Sign in as UM Administrator.

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.

The display name and initials for each user account must be unique and not contain special characters or spaces.

7 Click **Save**.

Inactivating a local user account

User accounts that were set up locally on the instrument cannot be deleted, only inactivated. User details of a new user account need to be different from inactivated accounts. This prevents that a newly set up user account has the same user details as a previously deleted account.

1 Sign in as UM Administrator.

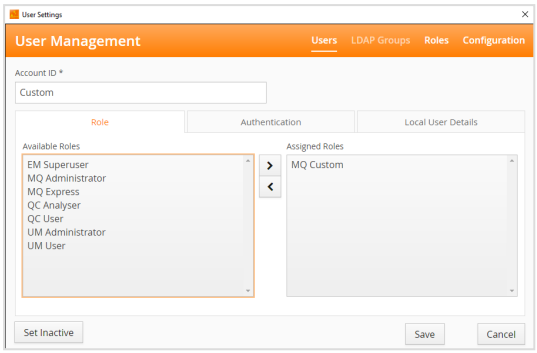
2 Go to **Edit > User management**.

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 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 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 Software passes requests to the user management system to validate the username and password. When creating user accounts on the MACSQuant Instrument, account settings such as password policy already existing in the company's LDAP system are used. Users sign into their MACSQuantify accounts with the username 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 244**.

Once the MACSQuant 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 171**.
If user accounts are added individually in the MACSQuantify 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.

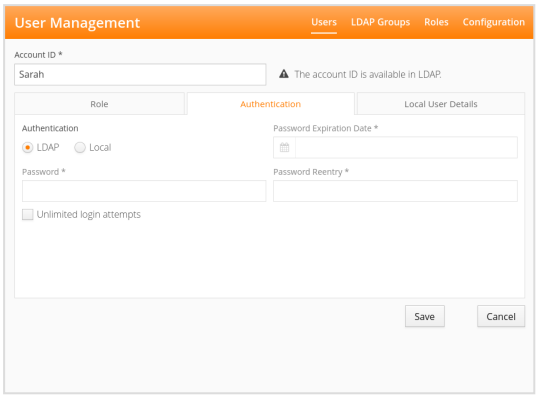
2 Go to **Edit > User management**.

3 Select the **Users** tab.

4 Click the **+ New User** button to add a new user.

5 Enter the username 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, a confirmation message appears.

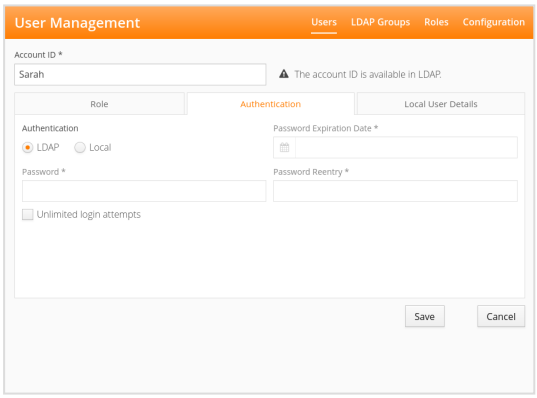


7 Optional: Add local user details. See **Adding local user details to an LDAP account below**.

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.

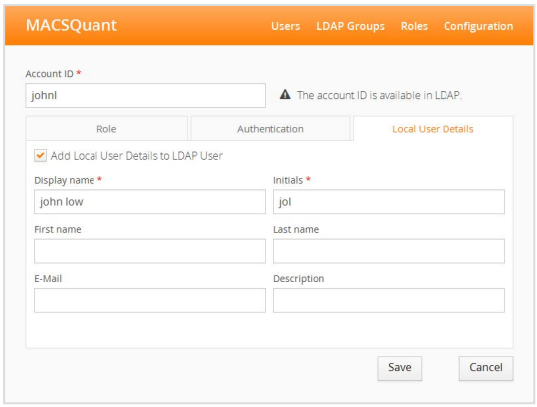
4 Select a user.

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

6 Select the box **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.

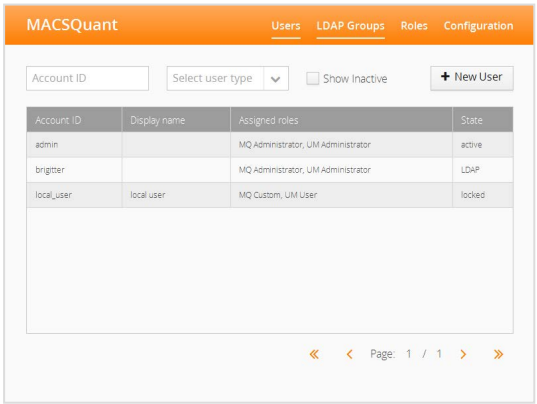
1 Sign in as UM Administrator.

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 box **Show inactive** to show deactivated users only.



5 Select the user to be deleted.

6 Click **Delete**.

7 Click **Save**.

Unlocking an user account

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

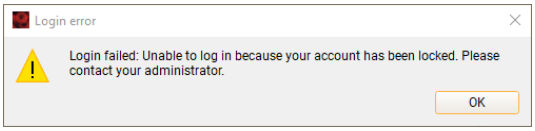
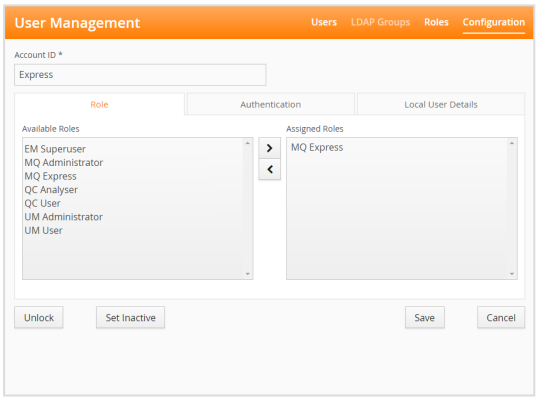


Figure 10.1: Message about locked user account

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 173**.

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 an 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 box **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 password on page 24**.

10.1.7 Importing and exporting user settings

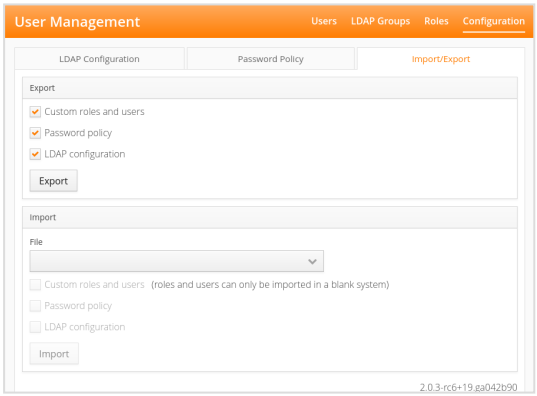
User accounts can be transferred between MACSQuant Instruments running MACSQuantify Software version 3.1. The accounts and their settings can be exported from a MACSQuant Instrument and imported into another MACSQuant Instrument.

Exporting user accounts

Export of user accounts is only available between instruments with MACSQuantify 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 boxes **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 Click **Export** to start the 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 Go to **File > Copy** and click **Eject**.

Importing user accounts

Export of user accounts is only available between instruments with MACSQuantify 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 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.

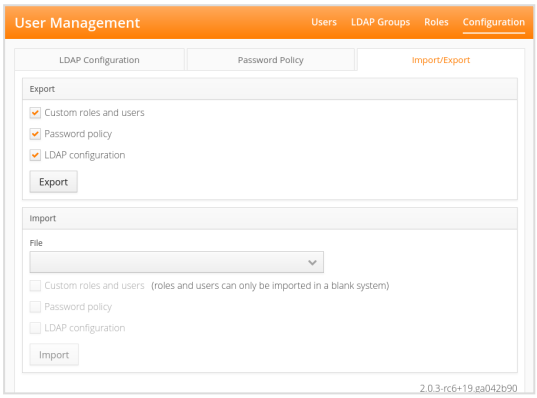
9 Go to **Edit > User management**.

10 Go to the **Configuration** tab.

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

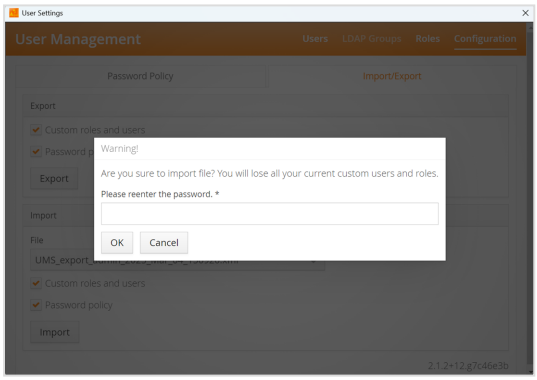
12 Go to the section **Import**.

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



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

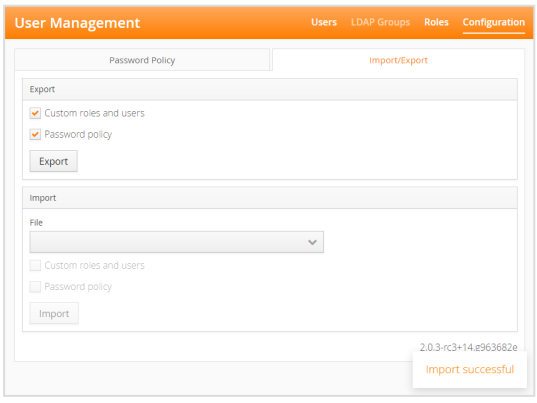
15 Click **Import**. A warning that the custom users and roles will be lost appears.



When a new file is imported, any any user accounts present on the system will be overwritten

16 To confirm the import, enter your password.

17 If import is successful, a message is shown.



18 Go to **File > Copy** to eject the USB flash drive.

19 Select the USB flash drive from the dropdown list.

20 Go to **File > Copy** and click **Eject**.

Next time the sign in 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 MQ Administrator

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

10.2.1 Editing global software options

This chapter describes the **Edit > Options (default)** menu that is accessible by an MQ Administrator. Software settings in this menu apply to all users of the instrument.
The available options can differ between the MACSQuant Instrument and the PC version.

The options can be accessed via **Edit > Options (default)** or by double-clicking on the time and date in the right corner of the MACSQuantify Software.

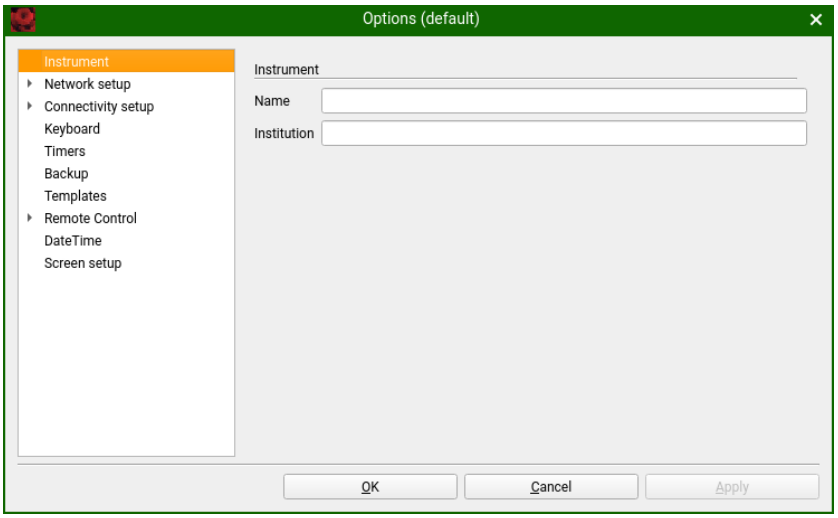


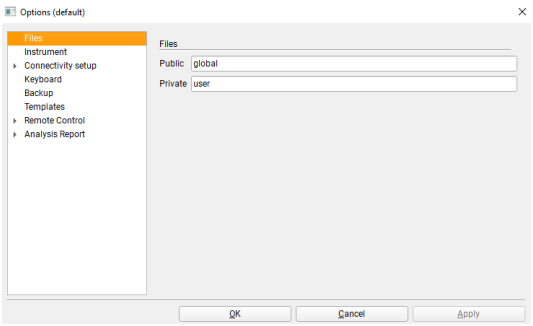
Figure 10.2: The **Edit > Options (default)** window

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

Files

The MQ Administrator can assign default file management values for data storage.

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



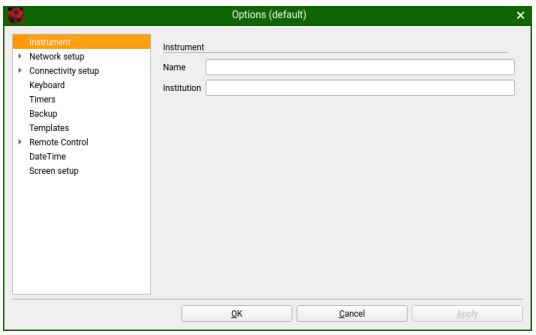
Change the default values for Public and Private locations only if using MACSQuantify Software on a PC.

2 Assign a folder for **Public** and **Private** locations on a network. Public data is stored in **Public** locations. **Private** locations are used for user specific data.

Instrument

By default, the MACSQuant Instrument is unnamed. Assign an instrument name and institution if several MACSQuant Instruments are run in an institution, or if the data is analyzed on a PC. The instrument's name is shown in the sign in dialog and in the green title bar. The institution name can be shown by clicking **View > Hardware**.

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



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

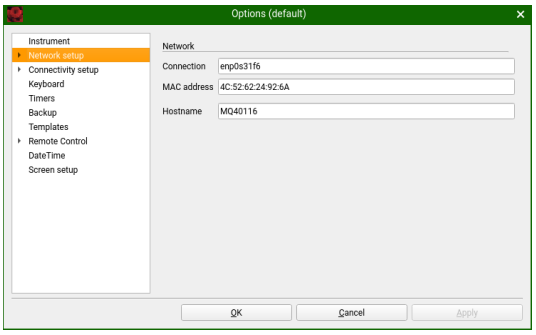
Network setup

Connect the MACSQuant Instrument to a network if the MACSQuant Live Support or backup to a shared location are used. The network setup is only available on the MACSQuant Instrument.

The MACSQuant 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 Instrument. The MACSQuant Instrument manages user rights and permissions locally as part of MACSQuantify Software in order to allow different user rights.

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

Entries in this section should not be changed.



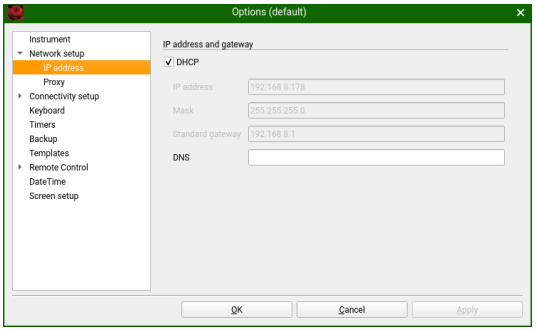
2 Click the arrow to access options for **IP address** and **Proxy**.

IP Address

Connect the MACSQuant via the port LAN1 (local area network 1) on the rear side of the instrument. Using the network setup configures LAN1 but not LAN2.

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 box **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 box **DHCP** if static IP addresses are used.

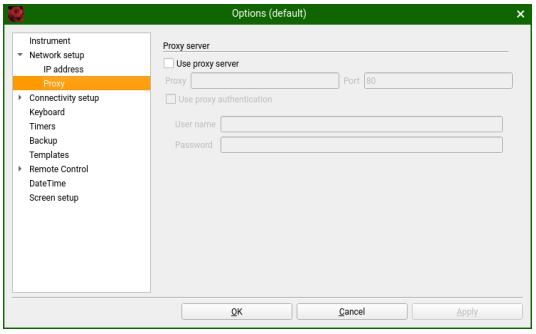
- Enter **IP address**, **Mask**, **Standard gateway**, and **DNS** server address. Contact your local IT for details.

5 Click **Apply** to implement 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 box **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 box **Use proxy authentication**. If user credentials are needed to access the proxy server, the system asks for it.

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

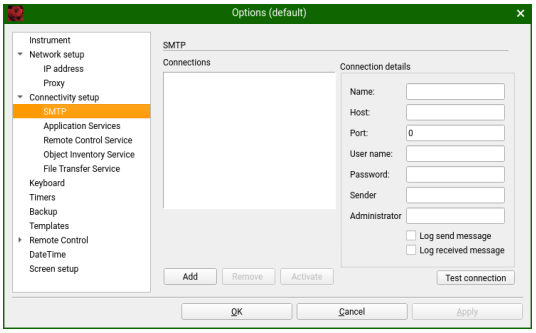
SMTP Server

The SMTP server is required for email notifications. Required information are specific to the local network. Ask your local IT for details.

Email notifications are only available if the used SMTP server does not use an encrypted protocol.

1 Go to **Edit > Options (default) > Connectivity setup**.

2 Click the arrow to access options for **SMTP Server**.



3 Enter a name for the server.

4 Enter an SMTP server host

5 Enter a port.

6 Optional: Enter sign in credentials for the SMTP server if required.

7 Enter a sender email address. It is required to assign an email address by the local IT to the MACSQuant Instrument.

8 Enter the email address of one or more MQ Administrator to send error notifications by email to them. Separate email addresses by a comma without space. For details see **Mail notification on page 208**.

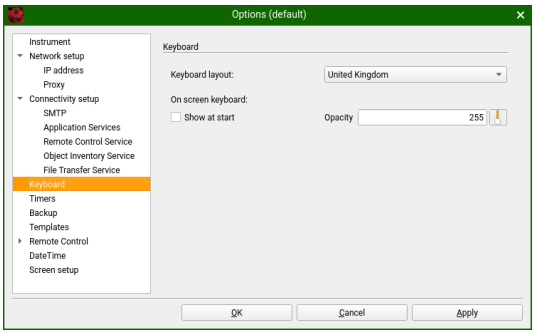
9 To add the SMTP server to **Connection** column, click **Add**.

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

Keyboard

This function is only available on the MACSQuant Instrument.

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



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

3 Select the box **Show at start** to automatically activate the on-screen keyboard during the startup of the MACSQuant Instrument.

4 Adjust opacity.

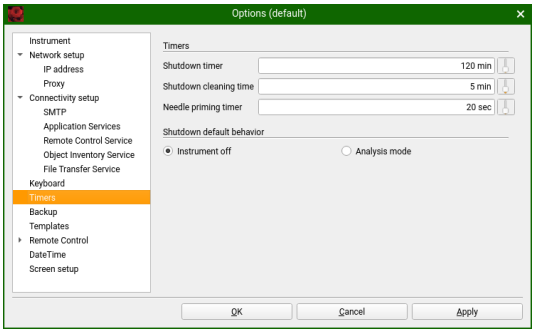
5 Click **Apply** to implement changes and **OK** to close the window.

Timers

This function is only available on the MACSQuant Instrument.

By default, MACSQuant Instruments perform an automatic shutdown procedure after a defined idle time. This ensures proper cleaning and shutdown of the instrument. A defined shutdown timer reduces the time in which lasers are switched on although they are not in use. Refer to the MACSQuant Instrument user manual for instructions on manual shutdown of the instrument.

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



2 Change timers as needed.

- **Shutdown timer**: Defines the idle time (no fluidic operation) before automated shutdown begins.
- **Shutdown cleaning time**: Defines the time of incubation of the fluidics system with MACSQuant Washing Solution before automated shutdown begins. The recommended incubation time is at least 5 minutes.
- **Needle priming time**: Defined to ensure that no air is introduced during measurement due to evaporation while the uptake needle is idle. The recommended priming time is 20 seconds.
- **Lock screen time**: Defines the idle time (no user input) before the screen is locked automatically. The lock screen is only available in the 21 CFR part 11compliant setup of the MACSQuantify Software.
- **Shutdown default behavior**: Select from two options: **Analysis mode** or **Instrument off**. If **Analysis mode** is selected, the instrument is cleaned but remains in data analysis mode. The fluidic system is not ready. Data can be analyzed.

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

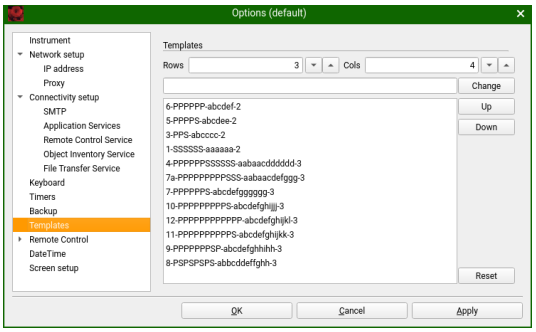
Backup

Define a public network path for continuous and manual backup. For details on how to set up a public network path, see **Setting up a public network location on page 154**.

Templates

Define new analysis window templates or change the default appearance of a pre-defined analysis window template.

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



2 Double-click a pre-defined analysis window template. The format appears in the field on the left-hand side to the **Change** button.

3 Optional: Enter a format for a new template in the field on the left-hand side to the **Change** button.

4 Use the following entries to create a new analysis page template:

- The first number defines the number of total elements per analysis window.
- The last number defines the number of elements per row.
- The capital letter defines the type of element. See **Table 10.6**.
- The small letter defines the relative position of the element. Letter a corresponds to the upper left corner. If using the same letter more than once, the element is enlarged over several positions.

5 Click **Change** to save the changes of the template.

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

Click **Reset** to restore the original settings.

Example analysis window template

The pre-defined analysis window template Plot7 is defined as 7-PPPPPPS-abcdefggg-3.

- Plot 7 consists of seven elements, defined by the first number.
- It contains six dot plots (P) and one statistics table (S).
- Each row contains three elements as defined by the last number.
- Each small letter corresponds to a separate position in the template.
- If a small letter is used more than once, the corresponding element is enlarged within a row. In the given example, the statistics table covers three plot positions as defined by ggg.

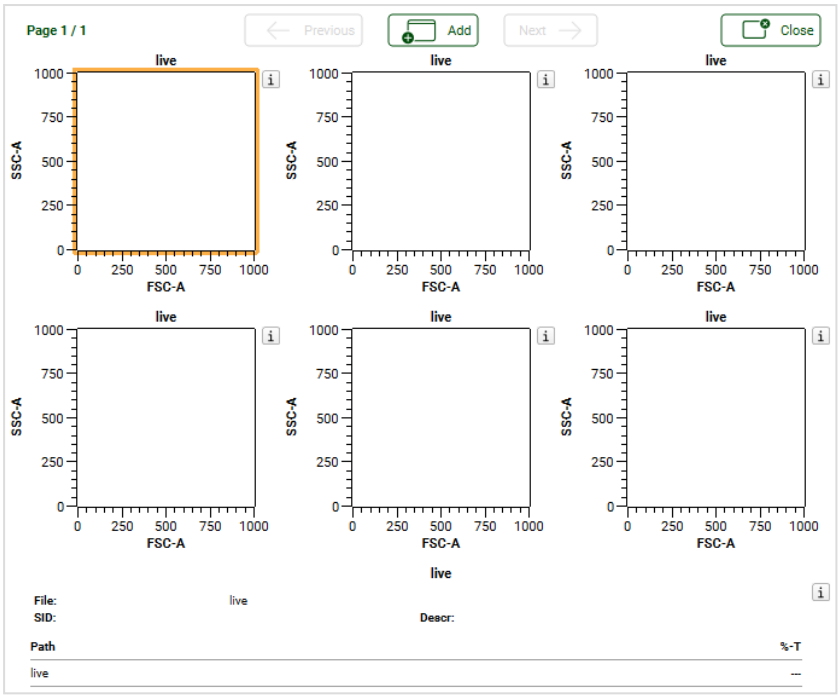


Figure 10.3: Plot 7 from the pre-defined analysis window templates

Capital letter	Type of element
P	Dot plot
H	Histogram
D	Density plot
S	Statistics table
T	Text
N	None (blank)

Table 10.6: Letter code for plots, statistics table, and text for analysis window templates

Connectivity setup and Remote Control

The sections **Remote Control** and **Connectivity setup** with subsections **Application Services**, **Remote Control Service**, **Object Inventory Service**, and **File Transfer Service** are required if the MACSQuant Instrument is integrated into a liquid handling system or automation platform. For integration into one of the platforms, contact Miltenyi Biotec Technical Support.

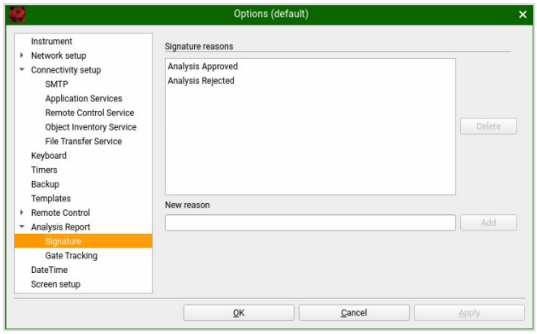
Analysis Report

Edit signature and gate tracking settings for the analysis reports.

Signature

Delete existing or create new signature for signing analysis reports.

1 Go to **Options (default) > Analysis Report> Signature**.



2 Select the respective signature and click **Delete**.

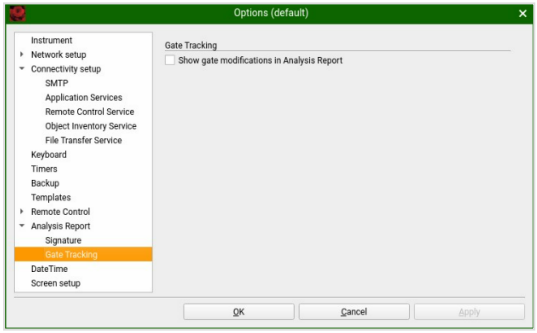
3 To create a new signature reason, enter a new reason.

4 Click **Add**.

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

Gate Tracking

1 Go to **Options (default) > Analysis Report> Gate Tracking**.



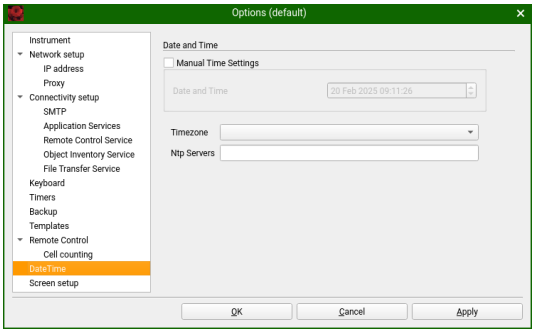
2 Select or deselect the checkbox **Show gate modification in Analysis Report** as needed.

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

In a 21 CFR Part 11 compliant setup, gate changes in the audit trail are always tracked. Optionally they can also be visualized in the exported analysis report

Setting date and time

1 Go to **Edit > Options (default) > DateTime** to change the date and time on the MACSQuant Instrument.



2 Select the box **Manual Time Settings** to enter date and time manually.

3 Select a time zone from the **Timezone** dropdown list. The time is set automatically.

4 Optional: Enter an NTP (network time protocol) server address. The NTP server provides the time settings including daylight settings such as winter and summer time of the selected time zone.

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

6 Restart the MACSQuant Instrument.

7 Wait until the sign in window appears.

8 Sign in.

Screen setup

For details on how to set up an external monitor connected to the MACSQuant Instrument, see **Configuring an external monitor on page 194**.

10.2.2 Audit trail

The audit trail can be created by an user with access to audit trail functions. For the details about the audit trail viewer of the 21 CFR part 11 compliant setup, see **Audit trail 21 CFR Part 11 on page 241**.
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	Set user inactive/reactivate user
Export system audit trail	Roles added, changed or deleted
Performance QC & tracking exports and point suspension	Change password
Activation, deactivation, and installation of Express Mode packages	

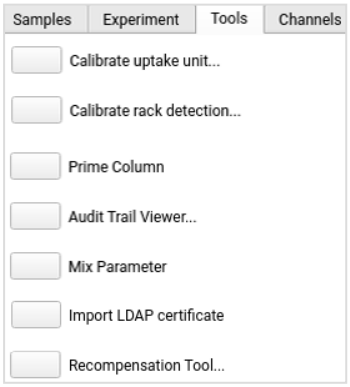
Table 10.7: Tracked activities in the audit trail

Exporting an audit trail

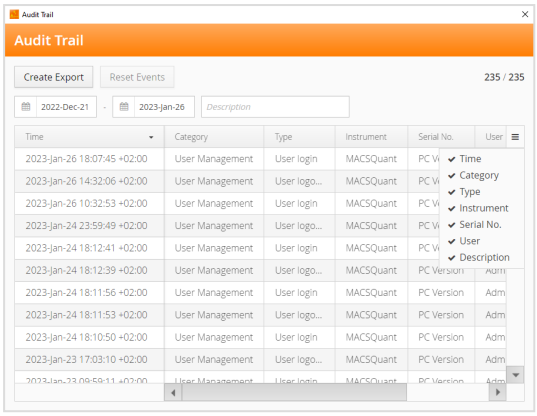
1 Sign in as user with access to the audit trail system.

2 Go to the **Tools** tab.

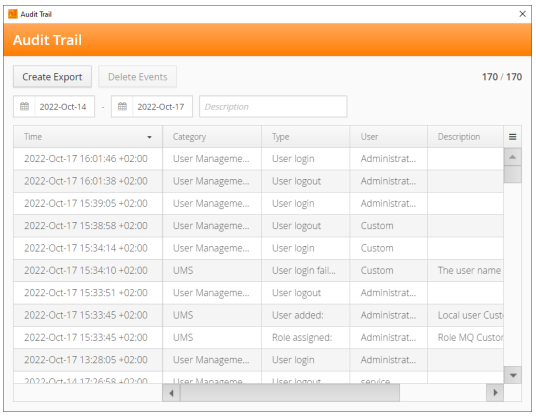
3 Select the checkbox **Audit Trail Viewer**.



4 To customize the shown columns, click on the symbol in the right-hand corner. Select the boxes to show the content of a column.

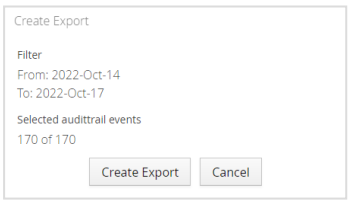


5 Define the time span in the **Audit Trail Viewer** window.



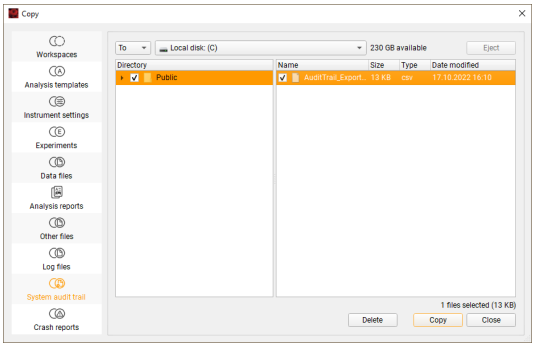
6 Optional: Type in search criteria in the **Description** field to limit the number of events. Filter will apply to the **Description** field.

7 Click **Create Export.**



PDF export of Audit Trail report is limited to 999 entries. In case more events are listed in the Audit Trail viewer, a compressed ZIP file is generated. Separate PDF exports of different time spans can otherwise be generated, each containing less than 999 events.

8 Go to **File > Copy** and select **System audit trail**.



9 Select the file.

10 Click **Copy**.

See **Copying files to an external location on page 150** for details how to copy files between instruments or a PC.

10.2.3 Hardware calibration

The MQ Administrator role is required to calibrate the uptake unit, rack types, or plates. Refer to the MACSQuant Instrument user manual for instructions.

10.2.4 Coincidence processing algorithms

The MACSQuantify Software offers three different algorithms for handling coincidence events. If two particles cross the laser at the same time, the event can be recognized as one coincident event. All algorithms detect coincident events but differ in data display. Detected events including coincident events are saved in the data file and are always considered for the volumetric cell count. Events that appear in a dot plot are considered for the absolute cell count. Depending on the algorithm, the absolute cell count includes coincident events or does not. Changes in the coincidence handling algorithm do not usually affect the volumetric cell count or population frequencies. However, there might be differences in the absolute cell count.

Coincidence processing algorithm	Description
Optimal yield	All coincident events are detected and shown in dot plots.
Doublet rejection	All coincident events are detected but not shown in dot plots.
Balanced	Detection of coincident events starts in **Doublet rejection** mode. If coincidence occurs with a frequency of >10,000 events per second, the MACSQuantify Software switches to **Optimal yield**.

Table 10.8: Coincidence processing algorithms

Changing the coincidence processing algorithms

1. Sign in as MQ Administrator.
2. Go to the **Channels** tab.
3. Click **Advanced**.
4. Select the doublets processing algorithm from the **Algorithm** dropdown list.
5. Click **OK** to apply the changes. The selected algorithm is applied to all users.

Compatibility of coincidence processing algorithms

The MACSQuantify Software offers different algorithms for coincidence processing. The algorithm **Balanced** is not compatible with all instrument types.

The **Optimal yield** coincidence processing algorithm is not recommended to be used on a MACSQuant Analyzer 16.

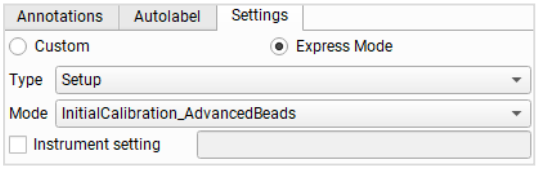
MACSQuant type	Serial number
MACSQuant Analyzer 16	All
MACSQuant Analyzer 10	≥21200
MACSQuant VYB	≥3100
MACSQuant X	≥5020

Table 10.9: Instruments compatible with coincidence processing algorithm **Balanced**

10.2.5 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 every six months by an MQ Administrator if the instrument is not serviced.

1 Sign in as MQ Administrator.
2 Attach the Single tube rack.
3 Prime the instrument by switching into acquisition mode. Wait for at least 30 minutes before starting the calibration.
4 Go to the **Experiment** tab.
5 Go to the **Settings** tab.
6 Select the **Express** radio button.
7 Select **Setup** from the **Type** dropdown list.
8 Select **InitialCalibration_AdvancedBeads** from the **Mode** dropdown list.



9 Thoroughly vortex the MACSQuant Advanced Calibration Beads to break up any aggregates
10 Dispense two drops into an empty tube, and place it in the Single tube rack.
11 Click the **Run** button to start the calibration. The calibration beads are automatically diluted to a total volume of 500 µL. 350 µL of the diluted calibration beads are injected into the sample injection port. During calibration, the gain for each respective channel is automatically adjusted.

Do not change gates or plot axes during calibration to ensure successful calibration.

12 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on several pages. Click the **Next window** button or **Previous window** button to switch between analysis pages.

Successful calibration for each channel is indicated by a green check mark. When the process is successfully completed, the MACSQuant Instrument Status bar reports **Acquisition Mode: Calibration OK**. All settings are automatically saved as default settings.

10.2.6 Configuring an external monitor

Only one external monitor can be connected to the MACSQuant Instrument.

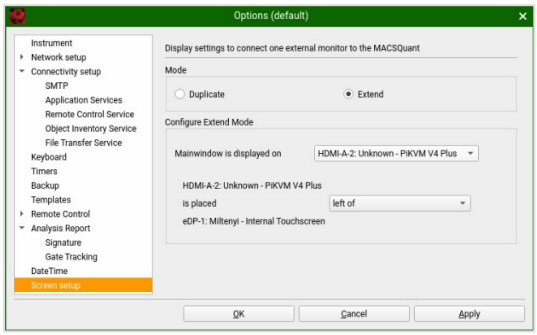
1 Make sure that the MACSQuant Instrument is switched off and not in standby mode.

2 Connect an external monitor to the Display Port of the MACSQuant Instrument.

3 Switch the MACSQuant Instrument on.

It is recommended to change the external monitor settings or switch off the connected monitor only when the MACSQuant Instrument is in idle state.

4 Go to **Options (default) > Screen setup**



5 Duplicate or extend the external monitor as needed.

6 Click **Apply** .

7 To activate the new screen setup and close the window, click **OK** .

To reset the screen setup configuration, use the keyboard combination **Ctrl**+**Alt**+**Shift**+**D**.

10.2.7 Installing a printer

Printers are installed by Miltenyi Biotec service personnel during a service or maintenance visit. Printers that support the printer driver hplip can be used. The printer can be networked or directly connected via USB to the rear side of the MACSQuant Instrument or to the PC running MACSQuantify Software.

11 Custom user

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

11.1 Editing software options

This chapter describes the **Edit > Options** menu. Software settings in this menu apply to the signed in user.

Changes in **Edit > Options** are effective after sign out and sign in of the user of the MACSQuantify Software. This applies to the PC version and the software installed on the MACSQuant Instrument.

11.1.1 Files

In the **Edit > Options > Files** menu, the Custom user can define the settings of the folder structure for data storage and the file naming, using specific identifiers. See **Table 11.1** for a list of file naming identifiers.

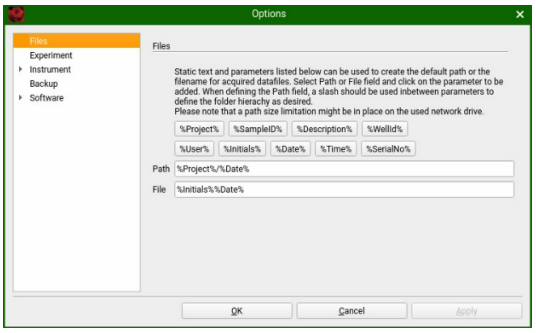
Using the default setting, data files are stored in a date folder (YYYY-MM-DD) or, in a project folder, if a project is selected in the **Experiment** tab.

Ensure to keep a maximum of two folders in the **Path** structure.

The default file name structure **%Initials%%Date%** it is composed of the initials of the signed in user, the acquisition date, and an index that counts up beginning with 0001.

Changing path or file naming

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



2 Change the default path or file name in the **File** section using the identifiers. See **Identifiers for file naming on the next page**.

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

Ensure to keep the forward slash properly placed, i.e. **%Project%/%Date%** when changing the **Path**. Avoid spaces between or behind the identifiers.

Identifiers for file naming

To define a path or automatic file name enter free text or the following case-sensitive commands listed in **Table 11.1**. The default file naming is **%Initials%%Date%**.

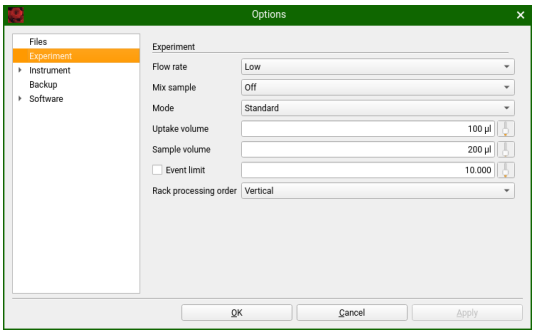
Use the commands exactly as they are listed below to ensure a correct file naming. The commands are case-sensitive.

Command	Description
%Initials%	Initials of currently signed in user
%User%	Name of the currently signed in user
%Date%	Acquisition date in the format YYYY-MM-DD
%Description%	Text entered as the **Description**
%Project%	Project name
%Time%	Time in the format hh-mm-ss
%SerialNo%	Serial number of the MACSQuant Instrument
%SampleID%	Text entered as the **Sample ID**
%WellId%	Position of the well, for example, A1

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

11.1.2 Experiment

1 Go to **Edit > Options > Experiment** to access and assign default values for experiment settings. The settings are shown in the **Experiment** tab. For more information, see **Experiment tab on page 34**.



2 Change the default values for **Flow rate**, **Mix sample**, **Mode**, **Uptake volume**, **Sample volume**, **Event limit**, and **Rack processing order** as required.

Select the box **Event limit** to limit data acquisition to the specified number of events. For accurate volumetric cell counting this function should be disabled.

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

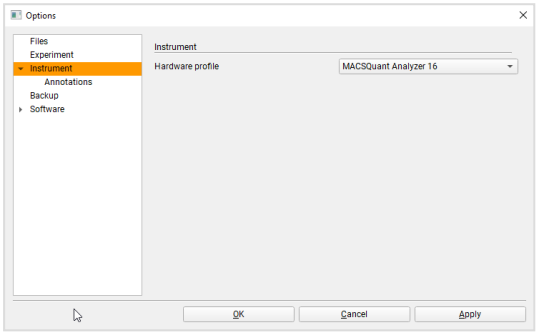
11.1.3 Instrument

The hardware profile can be defined in the MACSQuantify Software version on a PC.

It is required to select the appropriate instrument to handle files generated with or prepare experiments for different MACSQuant Instruments.

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

2 Select the appropriate instrument type from the **Hardware profile** dropdown list.

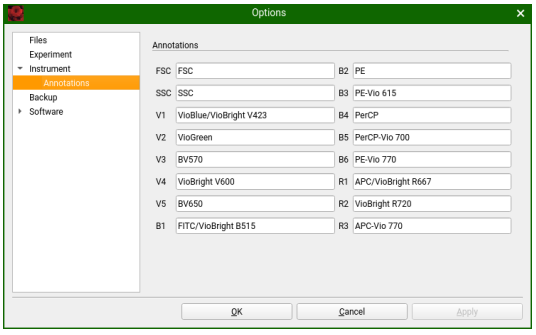


3 Click **Apply** to implement changes and **OK** to close the window.

Annotations

Annotations provide descriptions of the optical analysis channels that are available on the MACSQuant Instruments. The default values for the annotations can be changed if other than the shown fluorochromes are used. If working with the PC version, take care to select the required configuration of the instrument in **Edit** > **Configuration**.

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** tab.

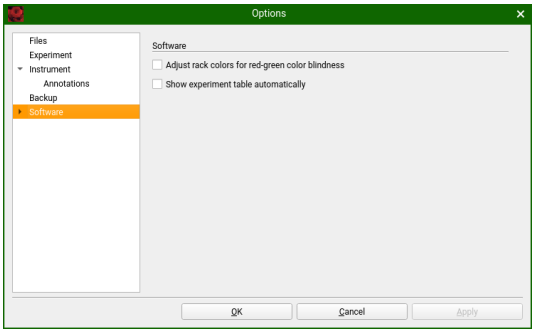
11.1.4 Backup

Define a private network path for manual backup. For details on how to set a private network path, see **Setting up a private network location on page 155**.

11.1.5 Software

Options for the color scheme of the MACSQuantify Software and automatic display of the experiment table can be defined.

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



2 Select the checkbox **Adjust rack colors for red-green color blindness**.

Green color is replaced with gray, for example during selection of rack positions.

3 Select the checkbox **Show experiment table automatically**.

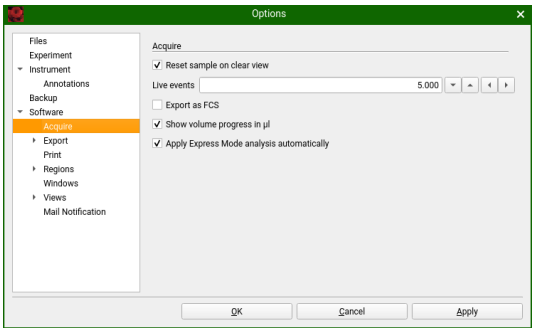
The experiment table is displyed automatically when a saved **Experiment** file is opened.

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

Acquire

Settings for data display during acquisition can be changed. Furthermore, automatic display of an Express Mode analysis or **Export as FCS** file can be activated.

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



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

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

Reset sample on clear view

Define the default behaviour when the **Clear** button in the status bar is used during data acquisition.

- Cleared: only the events shown in the plot are cleared. The events in the file are not discarded.
- Selected: events shown in the plot are cleared. Events in the file are also discarded (default setting).

Live events

Define the number of live events shown in a plot during acquisition.

1 Enter the desired value or change the existing value by using the arrows.

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

Export as .FCS

Define if the .mqd file should be exported as .fcs per default.

- Cleared: The .mqd file is not exported as .fcs. Later export as .fcs is possible.
- Selected: The .mqd file is automatically exported as an .fcs file after acquisition.

Show volume progress in µL

Define if the volume progress is shown in percent or in µL.

- Cleared: Volume progress is shown in percent.
- Selected: Volume progress is show in µL.

During sample uptake and processing the Instrument status bar continually displays the remaining sample uptake volume.

Apply Express Mode analysis automatically

Define if the Express Mode analysis template is automatically opened after acquisition of a sample.

- Cleared: The file is not analyzed automatically with the Express Mode.
- Selected: The file is analyzed automatically with the Express Mode and the analysis page(s) of the Express Mode are shown.

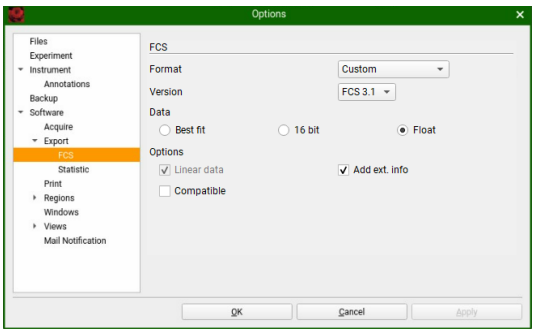
Export

FCS

The MACSQuant Instrument saves all acquisition data in the .mqd file format. In addition, data files can be saved automatically as .fcs file. See **Export as .FCS above**.
If manual export is required, follow the instructions below.

1 Go to **Edit** > **Options** > **Software** > **Export** > **FCS**.
2 Select **FCS3**, **FCS 3.1**, **FCS 3.1 Compatible**, or **Custom** from the **Format** dropdown list.
3 If **Custom** is selected, individual parameters can be selected from the dropdown lists.

- **Version**: **FCS 3.0** or **FCS 3.1**
- **Data**: **Best fit**, **16 bit**, or **Float**. 16 bit is compatible with most data handling software.



4 Depending on the selection in the **Data** section, different **Options** can be selected or cleared.

- **Linear data**: Save data without logarithmic conversions.
- **Add ext. info**: Save extended data information about the file format, time and date, and the file type to the text header of the data file. As this information varies according to the size of the data file, the text header may also vary in size, which some flow cytometry data handling software are unable to work with. It is therefore recommended to disable this function by default.
- **Compatible**: Save data for use with other flow cytometry analysis software.

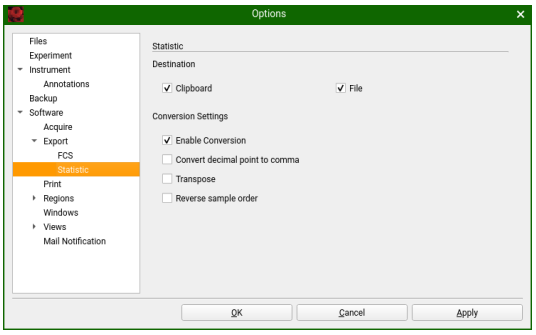
For optimal compatibility with any other third party such as FlowJo® or FCS Express™, software export to FCS3.1 is recommended.

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

Statistic

Default export settings for sample statistics can be changed.

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



2 Go to the section **Destination**.

3 Select from the options for data export :

- **Clipboard**: Copy data to the clipboard. Only available in PC version.
- **File**: Export data to an Excel file. Only available in PC version.

4 Go to the section **Conversion Settings**.

5 Select from the options for conversion:

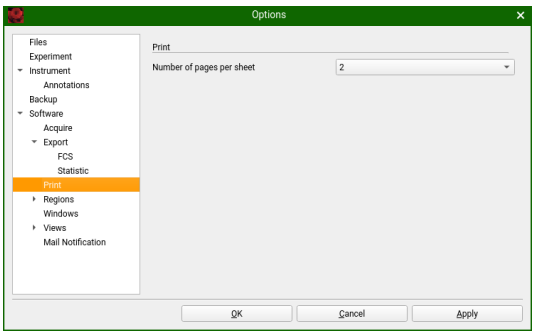
- **Convert decimal point to comma**: Improves compliance with regional language settings.
- **Transpose**: Invert rows and columns.
- **Reverse sample order**: Sort samples in ascending instead of descending order.

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

Print

Go to **Edit** > **Options** > **Software** > **Print** to change the number of analysis pages printed per sheet.

1 Select the desired number of pages per sheet from the dropdown list.



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

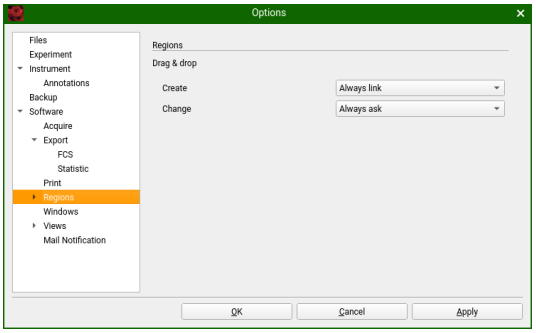
Regions

Define the default settings for regions moved by drag-and-drop and the default colors of a region.

Creating a region

Define the default behavior if a new region is copied by drag-and-drop in the sample list.

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



2 Go to the **Create** dropdown list.

3 Select from the following options:

- **Always link** (default setting): A link to the region is created.
- **Always copy**: A copy of the region is created.
- **Always ask**: A dialog box appears to select one of the options: **Link**, **Copy**, or **Cancel**.

If regions are linked, they are related to more than one population. Both populations P1 and P2 are linked to region R1. Changes of R1 would apply for both, Sample1\P1 and Sample2\P2:

- Sample1\P1 – R1
- Sample2\P2 – R1

Changing a region

Define the default behavior if a new region is moved by drag-and-drop in the plot.

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

2 Go to the **Change** dropdown list.

3 Select from the following options:

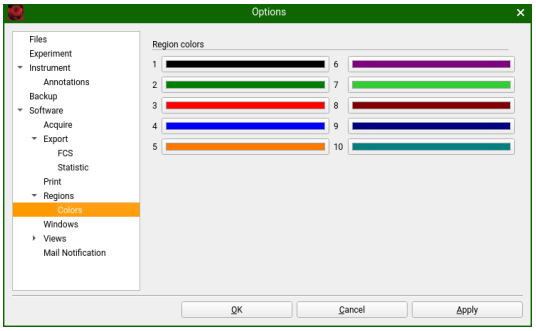
- **Current**: Any link is removed and only the current population is changed.
- **All**: All linked populations are adjusted.
- **Always ask** (default setting): A dialog box appears to select one of the options: **Current**, **All**, and **Cancel**.

Colors

Default settings for region colors can be modified.

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

2 Click the color panel button next to the according region number. The **Select color** dialog box opens.



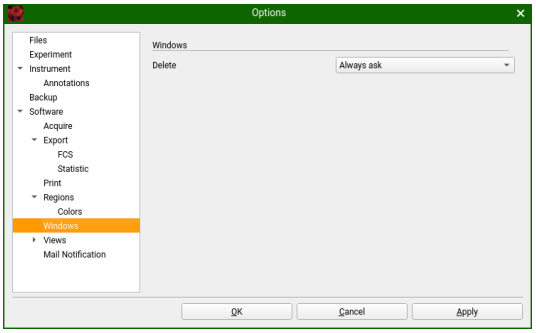
3 Select the desired color.

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

Windows

Define the default behavior when analysis windows are closed.

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



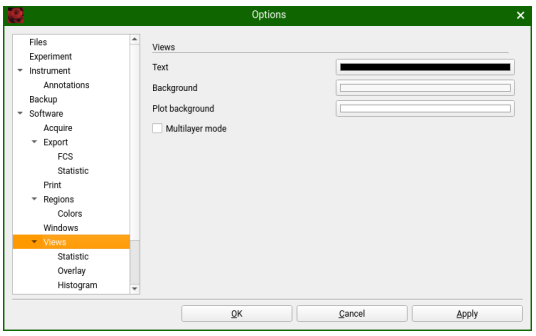
2 Select the desired option from the dropdown list:

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

Views

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

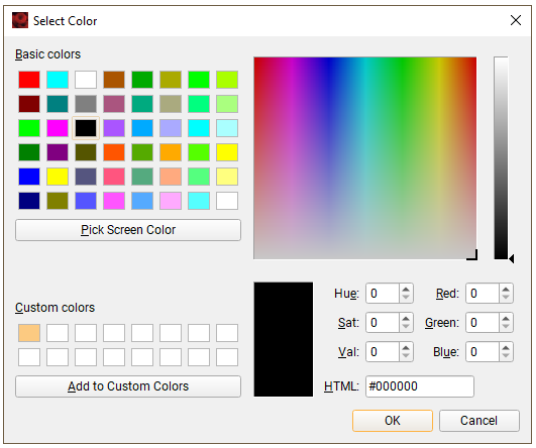
1 Go to **Edit** > **Options** > **Software** > **Views**.



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 Select the box **Multilayer Mode** to activate the multilayer mode for every plot. See **Multilayer mode display on page 131**.

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



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

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

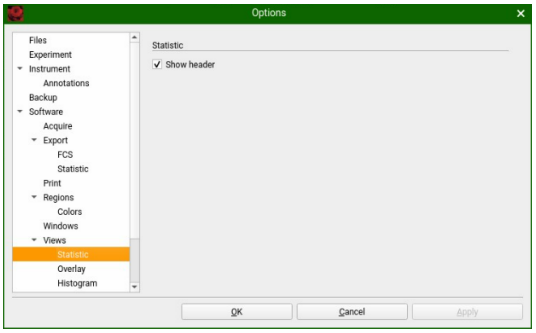
Statistic

Define the default settings for statistics tables.

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

2 Select from the following options:

- Select the box **Show header** to show a header for the statistics table.
- Clear the box **Show header** to hide the header for the statistics table.



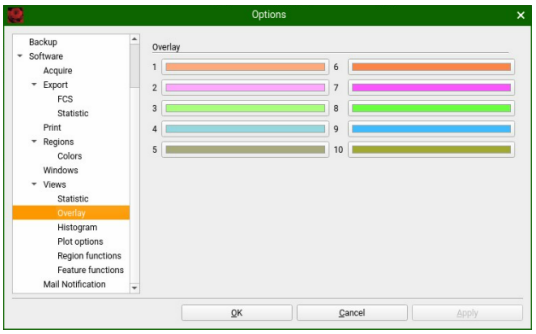
3 Click **Apply** to implement changes and **OK** to close the window.

Overlay

Define the default colors of histogram and dot plot overlays.

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

2 Click the color panel button next to the according overlay number. The **Select color** window opens.



3 Select the desired color.

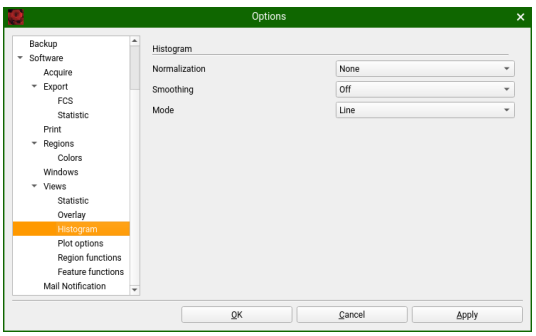
4 Click **OK** to close the **Select color** window.

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

Histogram

Define the display options for histograms.

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



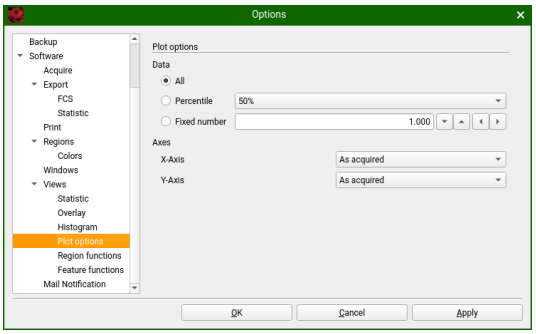
2 Select a value from the dropdown list for **Normalization**, **Smooting**, and **Mode**. For details, see **Options on page 117**.

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

Plot options

Define the default display options of plots.

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



2 Go to the **Data** section to define the number of shown events. Select from the options below:

- **All**: All acquired events are shown
- **Percentile**: Select **1%**, **2%**, **5%**, **10%**, **25%**, or **50%** from the dropdown list to define the shown events in a plot.
- **Fixed number**: Use arrows or enter a fixed number to define the shown events in a plot.

3 Go to the **Axes** section to define the default scaling for the x-axis and y-axis.

4 Select **As acquired**, **lin**, **log2**, **log3**, **log4**, **log5**, or **hlog** from the drow-down menu.

It is recommended to use the default setting **As acquired** and to change the axis scaling when performing data analysis.

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

Region functions

Region functions are parameters that can be shown in a plot. Define the region functions that are shown by default.

For a description of available region functions, see **Region functions on page 120**.

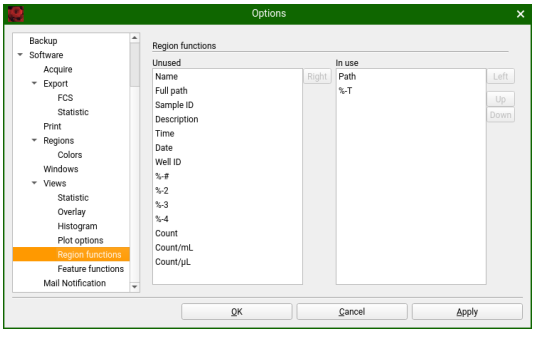
1 Go to **Edit > Options > Software > Views > Region functions**.

2 Select a region function from **Unused** and click **Right** to move it to **In use**.

3 Select a region function from **In use** and click **Left** to move it to **Unused**.

4 Change the order of region function **In use** by using the **Up** and **Down** buttons.

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



Feature functions

Feature functions are statistical values that can be shown in a plot. Define the feature functions that are shown by default.

For a description of available feature functions, see **Feature functions on page 121**.

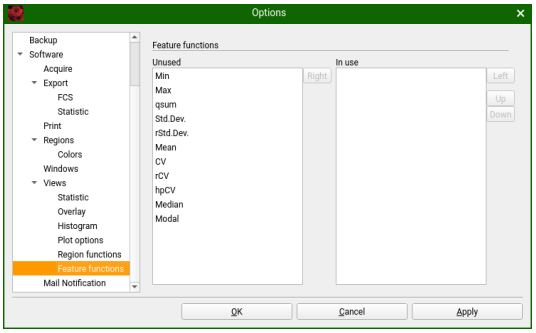
1 Go to **Edit** > **Options** > **Software** > **Views** > **Feature functions**.

2 Select a feature function from **Unused** and click **Right** to move it to **In use**.

3 Select a feature function from **In use** and click **Left** to move it to **Unused**.

4 Change the order of feature functions **In use** by using the **Up** and **Down** buttons.

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

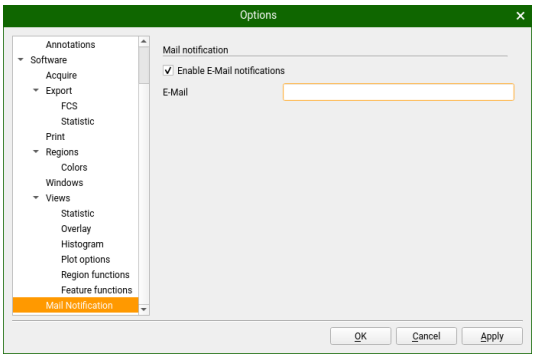


Mail notification

The MACSQuant Instrument can send mail notifications about error messages and the end of the experiment to an individual user. The MACSQuant Instrument must be connected to a network and the SMTP server must be set up. See **Network setup on page 184** and **SMTP Server on page 185**.

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

2 Select the box **Enable E-Mail notifications** to send notifications.



3 Enter one or more e-mail addresses separated by a comma without space.

4 Sign out.

5 Sign in.

Clear the box **Enable E-mail notifications** to deactivate notifications. It is not necessary to remove the mail address.

11.2 Mix parameters

11.2.1 Mixing modes of the MACSQuant Analyzer 10, VYB, and Analyzer 16

Mixing mode	Description
Off	No mixing
Mix gentle	Gentle mixing (two times aspiration). Can be modified by an MQ Administrator.
Mix medium	Moderate mixing (four times aspiration). Can be modified by an MQ Administrator.
Mix strong	Strong mixing (six times aspiration). Can be modified by an MQ Administrator.
User variable	Custom mixing mode, can be modified by any user. After sign out, all settings are eliminated.

Table 11.2: Available mixing modes and options for the MACSQuant Analyzer 10, VYB, and Analyzer 16

11.2.2 Mixing modes of the MACSQuant X

Mixing mode	Description
Off	No mixing, vibrating, or shaking
Vibrate gentle	Gentle vibrating of the uptake needle. Can be modified by an MQ Administrator.
Vibrate medium	Moderate vibrating of the uptake needle. Can be modified by an MQ Administrator.
Vibrate strong	Strong vibrating of the uptake needle. Can be modified by an MQ Administrator.
User variable (vibrating)	Custom vibrating mode, can be modified by any user. After sign out, all settings are eliminated.
Shake gentle	Shake plate gentle. Can be modified by an MQ Administrator.
Shake medium	Shake plate moderately. Can be modified by an MQ Administrator.
Shake strong	Shake plate strongly. Can be modified by an MQ Administrator.
User variable (shaking)	Custom shaking mode, can be modified by any user. After sign out, all settings are eliminated.

Table 11.3: Available mixing modes and options for the MACSQuant X

11.2.3 Changing the mix parameters

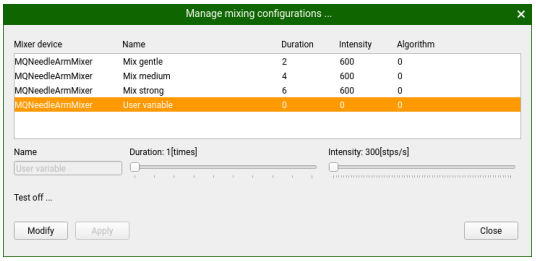
Mix parameters can be adjusted on the MACSQuant Instrument. Change of general settings for gentle, medium, and strong mixing require the MQ Administrator role.

The mix parameter **User variable** can be changed by any user. If user variable is activated, it becomes available in the **Mix sample** dropdown list in the **Experiment** tab. If the user logs out, the user variable is reset and not active anymore.

1 Go to the **Tools** tab.

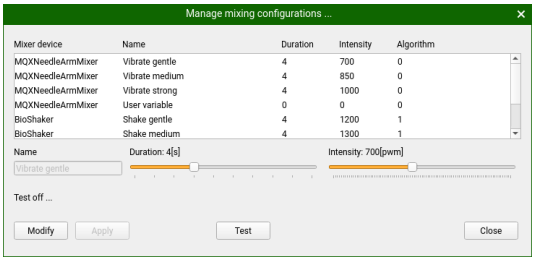
2 Select the **Mix Parameter** box.

3 Available modes depending on the instrument type are shown in the upper panel.



4 Select a mode.

5 Select **Modify**.



6 Move the slider to change the duration and intensity as required.

7 Click **Apply** to save the changes.

8 Parameters can be tested. If a vibration mode is selected, move the needle arm to a safe position. The needle will start vibrating with the selected intensity when the test is started.

9 Click **Test** to test the parameters.

10 Click **Test** again to stop the vibration or shaking. If a vibration mode was selected, the needle arm moves back to its home position.

Before closing the mix parameters, make sure that the needle is back home or the shaking has stopped completely.

11.3 Excess volume

The MACSQuant Instrument pick up some additional volume (excess volume) depending on the selected Mode in the Experiment tab.

11.3.1 Excess volume of the MACSQuant Analyzer 10, VYB, and Analyzer 16

The MACSQuant Analyzer 10, VYB, and Analyzer 16 pick up some additional volume (excess volume) depending on the selected **Mode** in the **Experiment** tab. Make sure to have enough sample available as the excess volume is additionally picked up to the programmed uptake volume.

Mode	Additionally picked up volume (excess volume)
Screen	19.92 µL
Fast	9.92 µL
Standard	9.92 µL
Extended	9.92 µL
Enrich	9.92 µL
EnrichS	9.92 µL
EnrichS2	9.92 µL

Table 11.4: Excess volume of the MACSQuant Analyzer 10, VYB, and Analyzer 16

11.3.2 Excess volume of the MACSQuant X

The MACSQuant X picks up some additional volume (excess volume) depending on the programmed uptake volume in the **Experiment** tab. Make sure to have enough sample available as the excess volume is additionally picked up to the programmed uptake volume.

Entered sample volume	Additionally picked up volume (excess volume)
≤5 µL	5 µL
6-14 µL	Same volume as uptake volume
≥ 15 µL	15 µL

Table 11.5: Excess volume of the MACSQuant X

11.4 Serial number

The serial number of the MACSQuant instrument can be looked up in the hardware monitor.

1 Go to **View > Hardware...**.

2 The serial number is shown in the header of the **Hardware Monitor** window.

The serial number is also printed on the type label on the rear side of the MACSQuant Instrument starting with SN.

11.5 Setup Express Modes

MQ Custom users and MQ Administrators have access to the Setup Express Modes listed in **Table 11.6**. The Express Modes are selectable in the **Experiment** tab.

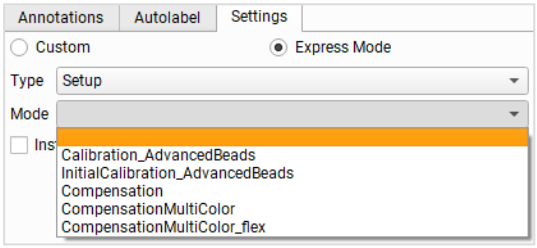


Figure 11.1: Setup Express Modes available for Custom users

Express Mode	Description	When to perform	Reference
InitialCalibration_ AdvancedBeads	Only available for MQ Administrator. Calibration required for Smart Gain technology to normalize the gains for fluorescence channels.	Twice a year by service during maintenance or after replacement of hardware parts of the optical bench.	**Initial calibration on page 194**
Calibration_AdvancedBeads	PMT calibration to adjust the gains for all channels.	Once per day when instrument is used.	**Photomultiplier tube calibration on page 41**
TubeLengthCalibration	MACSQuant X only. Corrects changes of sample tubing volume due to wearing.	Once per week.	**MACSQuant X user manual**
VolumeCalibration	MACSQuant X only. Determines the transported sample volume per time. Required for absolute cell count.	Once a month.	**MACSQuant X user manual**
Compensation	Automated multicolor compensation with single stained populations combined in one tube.	When changing a reagent lot. It is recommended to include compensation controls in every experiment.	**Materials required on page 56**
CompensationMultiColor	Automated multicolor compensation with one tube per fluorochrome plus optional blank sample(s).	When changing a reagent lot. It is recommended to include compensation controls for every experiment.	**Express Mode CompensationMultiColor on page 59**
CompensationMultiColor_ flex	Automated multicolor compensation with gating adjustment with one tube per fluorochrome plus optional blank sample(s).	When running compensation of both cells and beads simultaneously, in the same experiment or when changing a reagent lot. It is recommended to include compensation controls for every experiment.	**Express Mode CompensationMultiColor_ flex on page 64**

Table 11.6: Setup Express Modes

12 Express user

The Express user is designed to simplify the setup, running, and analysis of experiments. Users with minimal flow cytometry experience can perform complex flow cytometry experiments. When adding a new user account, the UM Administrator assigns user roles. Express users are allowed to perform only minimal alterations to settings. Managing different file types is restricted for Express users. See **Table 12.9** for more information. For a workflow protocol, see **Workflow on page 223**.

By default no Express Mode access rights are assigned to an Express user. If needed, additional roles with specific rights can be assigned to an Express user. See **Changing a user role on page 169**.

12.1 Structure

The MACSQuantify Software user interface for Express users includes the following elements.

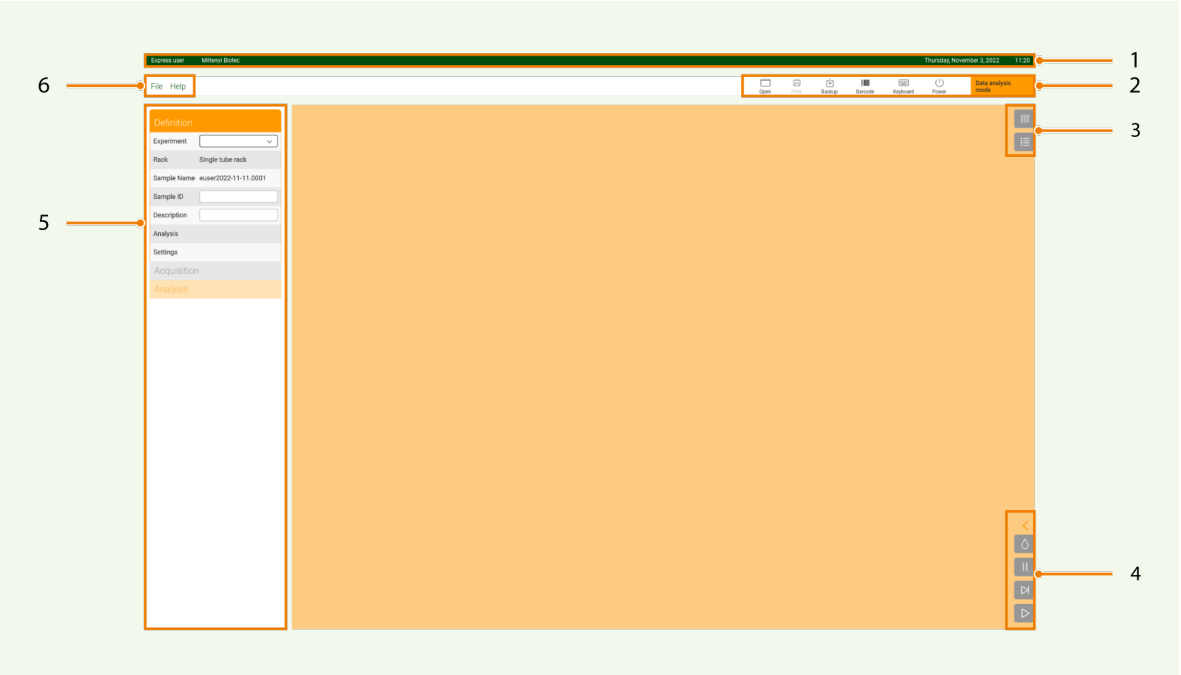


Figure 12.1: The MACSQuantify Software user interface for Express users

1	Title bar	**4**	Sample process and clean buttons
2	Toolbar	**5**	Side pane
3	Rack window and experiment table	**6**	Menu bar

12.1.1 Title bar



The title bar shows:

- the signed in user
- the owner hame
- the serial number of the MACSQuant Instrument
- the instrument name (if defined)
- date and time

12.1.2 Toolbar

Frequently used functions can be accessed in the toolbar. Click an icon to select a function.

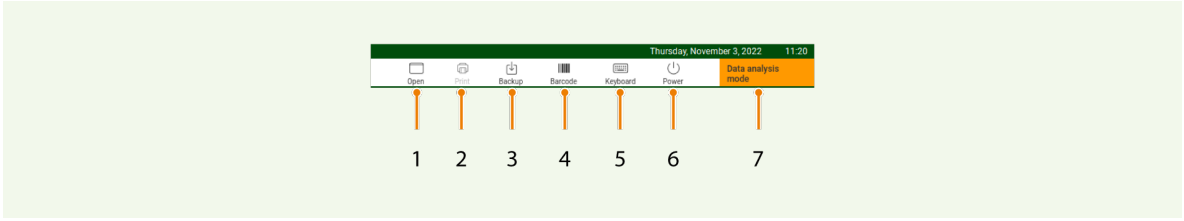


Figure 12.2: Icons in the toolbar

1	Open	**5**	On-screen keyboard
2	Print	**6**	Power
3	Backup	**7**	Instrument status
4	Barcode		

12.1.3 Toolbar functions

Open	Opens instrument settings, experiments, or data files.
Print	Prints data files. See **Printing files on page 225**.
Backup	Opens a backup dialog to save files to a remote storage location.
Barcode	Activates the 2D barcode reader to scan a reagent vial.
Keyboard	Opens or closes the on-screen keyboard of the MACSQuant Instrument.
Power	Switches from data analysis mode to acquisition mode or starts shut down procedure and switches off the instrument. See **Priming the instrument on page 41** and **Switching off the instrument on page 24**.

Table 12.1: Functions in the toolbar of the Express user interface

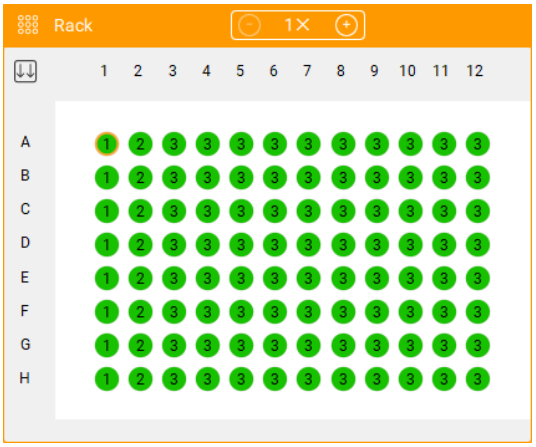
12.1.4 Rack window and experiment table

1 Click one of the buttons to expand the window.

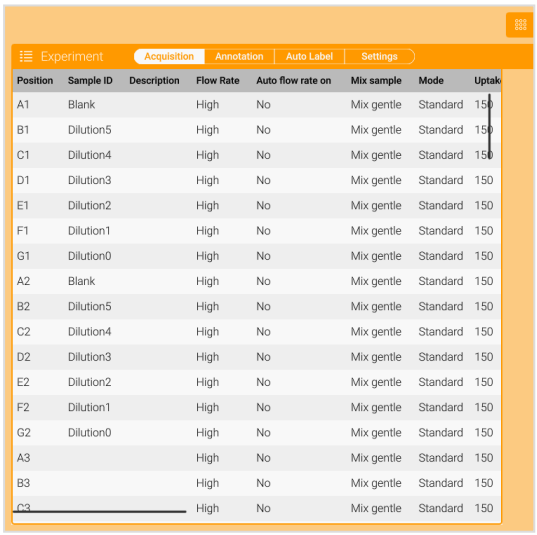
- Click the upper button for rack configuration.
- Click the lower button to open the experiment table.



2 Use the rack window to configure the sample positions on a rack. See **Table 12.2**.



3 Check the settings for **Acquisition** before starting the experiment.



Position	Sample ID	Description	Flow Rate	Auto flow rate on	Mix sample	Mode	Uptak
A1	Blank		High	No	Mix gentle	Standard	150
B1	Dilution5		High	No	Mix gentle	Standard	150
C1	Dilution4		High	No	Mix gentle	Standard	150
D1	Dilution3		High	No	Mix gentle	Standard	150
E1	Dilution2		High	No	Mix gentle	Standard	150
F1	Dilution1		High	No	Mix gentle	Standard	150
G1	Dilution0		High	No	Mix gentle	Standard	150
A2	Blank		High	No	Mix gentle	Standard	150
B2	Dilution5		High	No	Mix gentle	Standard	150
C2	Dilution4		High	No	Mix gentle	Standard	150
D2	Dilution3		High	No	Mix gentle	Standard	150
E2	Dilution2		High	No	Mix gentle	Standard	150
F2	Dilution1		High	No	Mix gentle	Standard	150
G2	Dilution0		High	No	Mix gentle	Standard	150
A3			High	No	Mix gentle	Standard	150
B3			High	No	Mix gentle	Standard	150
C3			High	No	Mix gentle	Standard	150

Sample position color code

Clear		**Default open circle indicates no operation.**
Closed green circle		**Sample used for measurement. Click circle to select for editing.**
Closed green circle with orange rim		**The orange rim indicates that the sample is selected and activated for editing.**
Closed yellow circle		**Processing of sample has started, for example, sample is diluted and incubated.**
Closed blue circle with orange rim		**Measurement is in progress.**
Closed gray circle		**Measurement has been finished.**

Table 12.2: Sample position color code

12.1.5 Sample process and cleaning buttons

Buttons for sample processing and cleaning of the MACSQuant Instrument are accessible in the lower right corner of the user interface. Use the orange arrow to hide or expand the buttons.

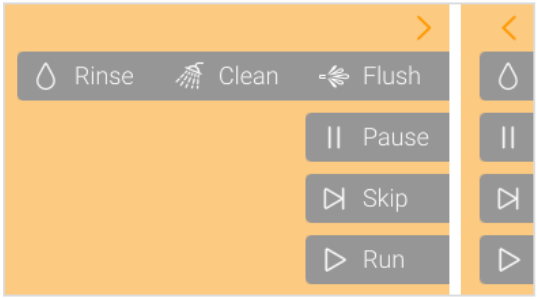


Figure 12.3: Sample processing and cleaning buttons

Button	Description
Rinse	Starts a needle rinse. Use the arrow to access the cleaning programs **Clean** and **Flush**. Refer to the MACSQuant Instrument user manual and MACSQuant X user manual.
Pause	Pauses the current sample measurement.
Skip	Stops the current measurement and continues with the next sample in line.
Run	Starts the sample measurement. While a sample is acquired, the **Run** button turns into a **Stop** button. Click the **Stop** button to stop the entire experiment.

Table 12.3: Sample process and cleaning buttons

12.1.6 Menu bar

The menu bar provides access to the **File** and **Help** menu.

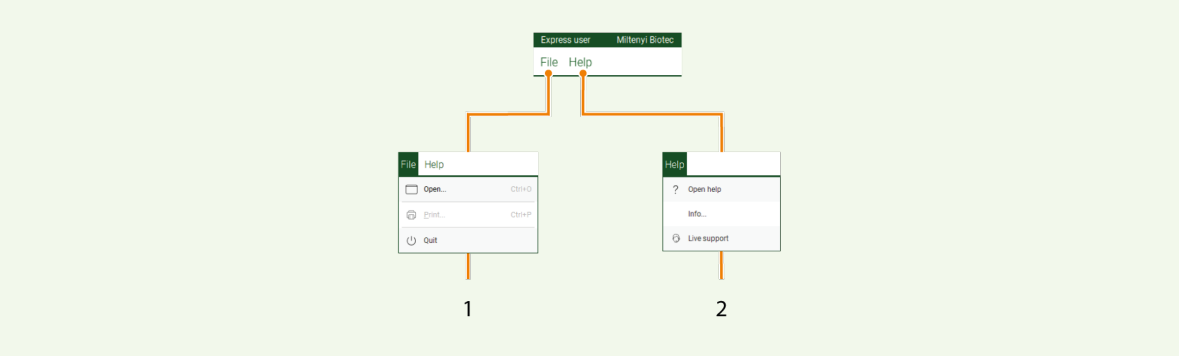


Figure 12.4: The menu bar

1	File menu	**2**	Help menu

File menu

Open...	Opens a window to select files including instrument settings, experiments, or data files (.mqd).
Print...	Prints active analysis windows. See **Printing analyzed data files on page 144**.

Table 12.4: Functions of the **File** menu

Help menu

Open Help	Opens the MACSQuantify Software user manual.
Info...	Displays information about currently installed MACSQuantify Software version and installed Express Mode version. It also indicates if the software is installed as 21 CFR part 11 setup.
Live support	Opens a window to connect to Miltenyi Biotec Technical Support for MACSQuant Live Support. Allows remote control during a live support session if required. See **MACSQuant Live Support on page 253**. Internet connection is required. The function is only available on the MACSQuant Instrument.

Table 12.5: Functions of the **Help** menu

12.1.7 Side pane

The side pane of the Express user interface has three tabs:

- **Definition**
- **Acquisition**
- **Analysis**

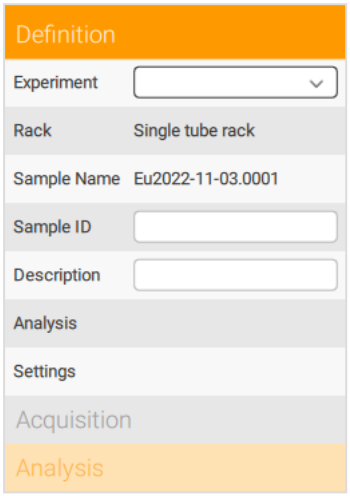


Figure 12.5: Side pane of the Express user interface with expanded **Definition** tab

Definition

Editable fields in the **Definition** tab are listed in the table below.

Option	Description
Experiment	Load an experiment file from the dropdown list. Experiment files saved in public are shown.
Rack	Shows the selected rack from the loaded experiment file.
Sample name	Shows the filename from the loaded experiment file.
Sample ID	Shows the sample ID from the loaded experiment file.
Description	Shows the description from the loaded experiment file.
Analysis	Shows the analysis template from the loaded experiment.
Setting	Shows the selected instrument setting from the loaded experiment.

Table 12.6: Options in the **Definition** tab

Programming sample positions

Program sample positions using the **Definition** tab. Selected samples are indicated by an orange rim and can be edited. All samples can be programmed at the same time, or each position or group of positions can be programmed individually.

1 Select the sample position(s).

2 To select multiple samples, do one of the following:

- Right-click the upper left circle for the entire rack.
- Right-click a column number to select the entire column.
- Right-click a row letter to select the entire row.

3 Optional: Select a well, row, column, or the entire plate to clear sample positions. See **Selecting a sample position on page 91**.

4 Use the **Definition** tab to specify **Sample ID** and **Description**.

Acquisition

The **Acquisition** tab opens when a measurement is started. It displays all information from the **Definition** tab about the currently processed sample.
During acquisition, time, volume, total count, and event rate per second are shown in the lower left corner of the software.

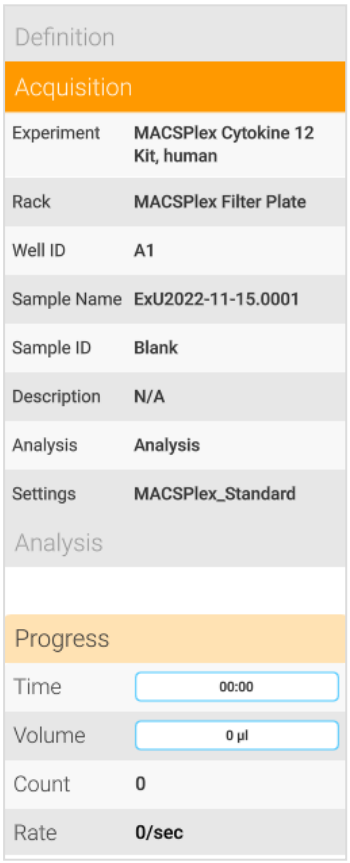


Figure 12.6: Side pane of the Express user interface with expanded **Acquisition** tab

Option	Description
Time	Time since starting the measurement
Volume	Remaining volume
Count	Total events of the current sample
Rate	Events per second

Table 12.7: Options in the **Acquisition** tab

Analysis

The **Analysis** tab opens when a measurement is finished or if a data file is opened via **File Open**. The acquired data are shown according to the analysis template defined in the experiment file. If no analysis template is linked to the experiment file, data are not shown automatically.

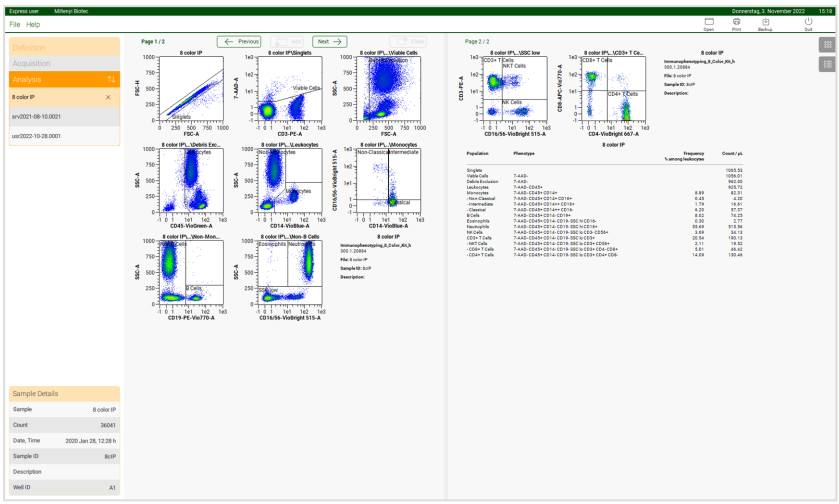


Figure 12.7: Example of an analysis template

See **Opening files on page 225** for details about how to show data.

12.1.8 Instrument status indicator

The current status of the instrument is shown on the right-hand side of the toolbar.

Initializing MACSQuant	The instrument is initializing and not available for any measurement.
Data analysis mode	The instrument is in data analysis mode. The fluidic system is not ready and lasers are switched off. Prime the instrument to switch to acquisition mode for measurement. See **Priming the instrument on page 41**.
Priming MACSQuant	Priming in progress. The instrument is not available for measurement.
Bottle level critical change a bottle	Shown if a bottle has reached a critical level. The affected bottle's LED will be blinking.
Acquisition mode Calibration ok	The instrument is in acquisition mode and ready for measurement. Calibration state can be ok, due, expired, or not calibrated. Calibration status is indicated. See the short instructions **PMT calibration**.
Processing rack Sample 1 of 3	The instrument is processing a sample. The processed sample is indicated.
Processing rack change a bottle	The instrument is processing a sample and a bottle level is critical.
Fatal error occured hardware disabled	Error alert.

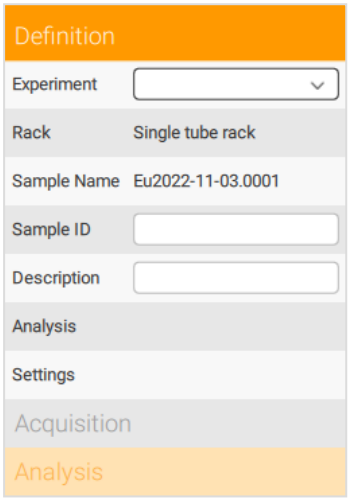
Table 12.8: States of the MACSQuant Instrument

12.2 Workflow

This chapter describes the workflow how to run a sample in the Express user interface. Express users can load experiment files which were created in a Custom user or MQ Administrator user account. The experiment files must be saved in a public location to be accessible for the Express user. The experiment file contains all information from the **Experiment** tab of the Custom user interface. The experiment file can also contain an analysis template for data analysis.

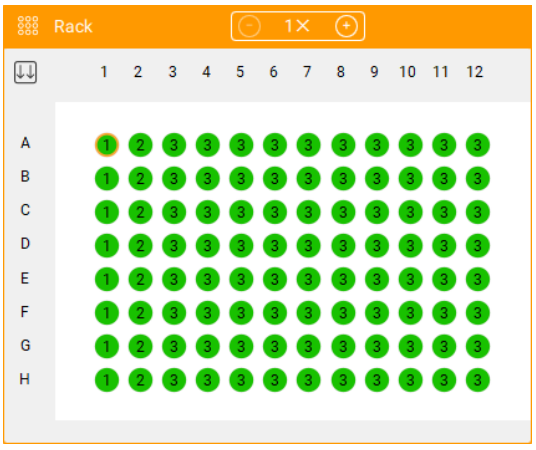
1 Sign in as Express user.

2 Go to the **Definition** tab.



3 Select an experiment file from the **Experiment** dropdown list. The **Rack** window opens on the right-hand side.

4 Optional: Click the plus symbol in the header of the window to enlarge the **Rack** window. Click the rack symbol in the upper left corner of the window header to hide the window.



Rack, **Sample name**, **Analysis**, and **Setting** cannot be changed by the Express user. They are defined in the experiment file. The other fields are optional.

5 Optional: Add or remove sample positions from the sample rack. See **Selecting a sample position on page 91**.

6 Optional: Select a rack position and change the sample ID. Use one of the following options:

- Use the **Sample ID** dropdown list.
- If no sample ID was defined in the loaded experiment file, enter the appropriate alphanumeric text. See **Using special characters on page 79** for a list of permitted characters.

7 Optional: Go to the **Description**field. Enter alphanumeric text.

8 Place samples and reagents on the assigned positions.

9 Remove caps and lids of samples and reagents.

10 Check the MACSQuant Running Buffer and waste bottle level.

11 Click the **Run** button in the lower right corner of the software. The **Acquisition** tab opens.

After finished sample acquisition, the MACSQuantify Software shows the data analysis as defined in the analysis template of the experiment.

12.3 Data management

Express users can open data files, experiment files, and instrument settings. Data files are automatically saved when starting a measurement. See **Table 12.9** for further details. This chapter describes how data files can be opened and backed up. Data files can be saved to and opened from a public, private, or external file location depending on the file access rights of the user defined by the UM Administrator.

- Public files are located on the local hard drive of the MACSQuant Instrument or PC and are accessible by all users.
- Private files are located on the local hard drive of the MACSQuant Instrument or PC and are only accessible by the signed in user.
- External files are located on an independent file storage device that is connected to the MACSQuant Instrument or PC.

12.3.1 Managing file types

The table lists which file types can be saved and opened by the Express user.

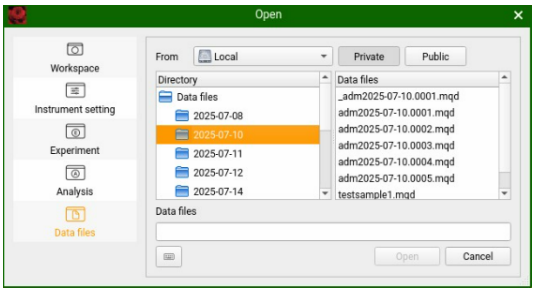
File type	Save	Open
Analysis template	No	No
Data files	saved automatically by software	Yes
Experiment	No	Yes
Instrument setting	No	Yes
Workspace	No	No

Table 12.9: Managing file types for the preconfigured role **MQ Express**

12.3.2 Opening files

1 Click the **Open** button in the toolbar.

2 Select **Instrument setting**, **Experiment**, or **Data file**.

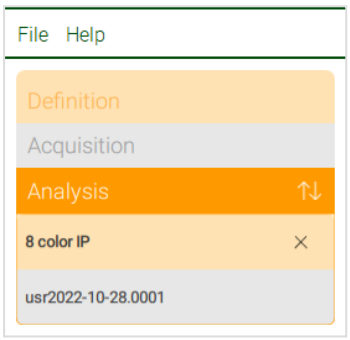


3 Go to the **From** dropdown list and select the source, for example, **Local** or an external USB flash drive.

4 Select the file location **Private** or **Public**.

5 Select the file(s).

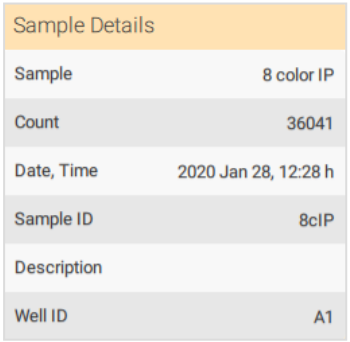
6 Click **Open**. The file is shown in the **Analysis** tab.



7 Optional: When several samples are opened, click a sample to show data of the selected file.

8 Use the arrows of the **Analysis** tab to sort the samples in an ascending or descending order.

9 See the sample details in the lower left corner of the software for an overview of the shown data file.



10 Click the cross behind the sample name to close the sample.

12.3.3 Printing files

1 Open a data file.

2 The **Analysis** tab opens.

3 Click the **Print** button.

4 Select a printer to print the current analysis window.

5 Click **Print**.

12.3.4 Data backup

It is recommended to back up data to a remote location. Data can be transferred from the MACSQuant Instrument to a remote storage location, such as network folders, USB flash drives, or external hard drives. See **Manual backup on page 155** for details.

The MQ Administrator must define a global backup path to enable the Express user to select the path as destination in the **Backup** dialog.

13 Pre-enrichment

The MACSQuant Technology permits the magnetic pre-enrichment of (rare) cells prior to fluorescence analysis. The enriched fraction is automatically analyzed by the MACSQuant Instrument. Pre-enrichment reduces the number of total analyzed cells required for reliable analysis. Use the pre-enrichment function for the analysis of cells present in low abundance, such as stem cells, dendritic cell subsets, or antigen-specific T cells.

The pre-enrichment function is only available on the MACSQuant Analyzer 10, VYB, and Analyzer 16.

Miltenyi Biotec offers many kits and reagents optimized for the efficient identification and enumeration of a number of cell types and their subsets, such as

- dendritic cells
- endothelial progenitor cells
- cancer stem cells
- cytokine-secreting cells
- antigen-specific T cells
- circulating tumor cells

13.1 Properties of the MACSQuant Column

- Capacity: retains up to 5×10^6 magnetically labeled target cells
- Life-span: 3 months
- Sample volume as defined in the **Experiment** tab (volume of sample to enrich on MACSQuant Column): maximum of 5 mL
- Uptake volume as defined in the **Experiment** tab (volume of eluted fraction to measure on MACSQuant Instrument): maximum of 450 µL

Refer to the MACSQuant Instrument user manual for instructions on how to use the MACS Cell Enrichment Unit and MACSQuant Column.

13.2 Pre-enrichment example

The **EPC Enrichment and Enumeration Kit, human**, (# 130-093-477) was developed for the enumeration of circulating endothelial progenitor cells (cEPCs) from human peripheral blood, cord blood, bone marrow, or leukapheresis products. EPCs were pre-enriched and analyzed by the MACSQuant Instrument using the

MACSQuant Column. In this example, the **Express Mode EPC_Enrichment_Enumeration_Kit_h** was used. For details of the staining protocol, manual separation, or manual data analysis see the data sheet of the kit on the website.

Protocol

1 Turn on the MACSQuant Instrument while preparing the samples.

2 Prime the instrument and wait for at least 30 minutes to warm up the optical bench.

3 Perform a PMT calibration.

4 Open or generate an appropriate instrument setting.

5 Prepare three samples according to the data sheet by red blood cell lysis and label with the following reagents from the EPC Enrichment and Enumeration Kit:

- CD133 control sample: 200 µL of lysed whole blood labeled with FcR Blocking Reagent and EPC Control Cocktail CD133
- CD309 control sample: 10 mL of lysed whole blood labeled with FcR Blocking Reagent, EPC Enrichment Cocktail, and EPC Control Cocktail CD309
- EPC sample: 10 mL of lysed whole blood labeled with FcR Blocking Reagent, EPC Enrichment Cocktail, and EPC Staining Cocktail

6 Resuspend each sample in 1 mL and transfer to a 12×75 mm tube.

7 Place the three samples in the following positions of the Chill 5 Rack:

- CD133 control sample: A1
- CD309 control sample: B1
- EPC sample: C1

8 Go to the **Experiment** tab and select Chill 5 rack.

9 Highlight A1, B1, C1.

10 Click the **Group** button.

11 Go to the **Settings** tab and select the **Express** radio button.

12 Go to the **Type** dropdown list and select **Analysis**.

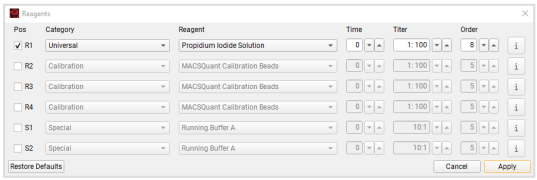
13 Go to the **Mode** dropdown list and select **EPC_Enrichment_Enumeration_Kit_h**. The software automatically defines the required settings in the **Experiment** tab.

14 Go to the **Sample ID** field and check if the positions of the samples in the rack match with the selection in the software.

15 In the **Autolabel** tab, click any **<add...>** button to open the reagent manager.

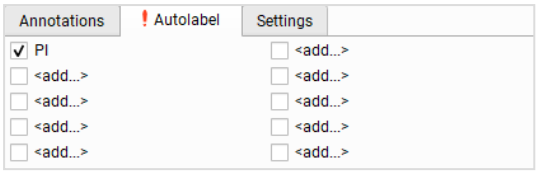
16 In position R1, select **Universal** from the **Category** dropdown list.

17 Select **Propidium Iodide Solution** from the **Reagent** dropdown list. The incubation time is set to zero and the titer set to 1:100.



18 Click **Apply** and close the **Reagents** window.

19 Select the box next to **PI** in the **Autolabel** tab.



Active autolabeling is indicated by a red exclamation mark.

20 Place the **Chill 5 Rack** on the MACS MiniSampler Plus with the Universal Reagent Rack.

21 Place an open vial of PI in **Universal Reagent Rack position 1**.

22 Check MACSQuant Running Buffer and waste bottle fluid level.

23 Go to **View > Experiment table...** to check all settings.

24 Start acquisition.

25 After acquisition, right-click the sample in the **Samples** tab and select **View with Analysis.EPC_ Enrichment_Enumeration_Kit_h**. The **Express Mode** automatically displays the analyzed data.

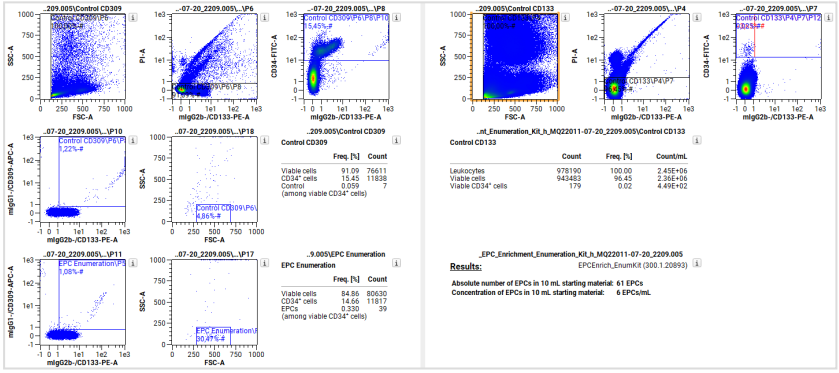


Figure 13.1: Analyzed data file with the **EPC_Enrichment_Enumeration_Kit_h** Express Mode

13.3 Pre-enrichment programs

The pre-enrichment programs are selectable on a MACSQuant Analyzer 10, VYB, and Analyzer 16 in the **Experiment** tab as **Mode** in the section **Pickup and measure**.

	Enrich	EnrichS	EnrichS2
Process description	The designated sample volume is loaded onto the MACSQuant Column. The negative fraction is discarded. The positive fraction is eluted and directed to the flow cell.		
Min/max sample volume	25 µL/5 mL	25 µL/5 mL	25 µL/5 mL
Cell separation rate	0.75 mL/min	0.5 mL/min	0.25 mL/min
Cell washing rate	1 mL/min	1 mL/min	0.5 mL/min
Volume of buffer	4.66 mL	4.66 mL	2.66 mL
Elution rate	37.5 mL/min	25 mL/min	37.5 mL/min
Elution volume	450 µL	450 µL	450 µL

Table 13.1: Pre-enrichment programs available on the MACSQuant Analyzer 10, VYB, and Analyzer 16

14 Instrument monitoring

This chapter describes how the status of the MACSQuant Instrument can be monitored. Users can find information about the fluidic system and lasers in the hardware monitor.

14.1 Monitoring the PMT calibration with Performance QC & tracking tool

The Performance QC & tracking tool tracks calibration parameters over time, to detect fluctuations that may impact signal accuracy and reproducibility. The tool is only available on MACSQuant Instrument.The erformance QC & tracking tool monitors the following parameters:

- coefficient of variation (CV) for each channel
- stain index (SI) for each channel
- voltage (V) for each channel
- temperature and power of the main laser
- temperature of the optical bench
- delay of side lasers

The Performance QC & tracking tool shows on a chart the calibration parameters against time, using a mean reference line. To detect performance variations, it sets control limits at +/-1, +/-2 and +/-3 standard deviation from the mean. The tool applies Westgard rules, a set of statistical quality control criteria that help identify systematic errors and random fluctuations.

The Performance QC & tracking tool applies the following Westgard rules:

- 1s3: A single calibration measurement exceeds the mean +/-3 standard deviation limit. This suggests a random error or a sudden system failure and requires immediate investigation.
- 2s2: Two consecutive calibration measurements on the same side of the mean exceed the +/- 2 standard deviation limit. This indicates a systematic error.
- 4s1: Four consecutive calibration measurements exceed the mean +/- 1 standard deviation limit on the same side. This suggests a small systematic error.

The Performance QC & tracking tool also tracks the date of each service visit. They are marked on the charts with an orange dashed line. The mean and standard deviation values are reset after each service visit.

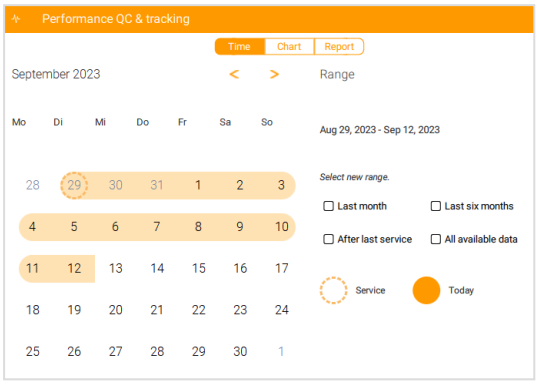
14.1.1 Generating Performance QC & tracking charts

Only the MQ Administrator user role has the rights to create and edit charts by default. If needed, additional user roles with Performance QC & tracking rights can be set up and assigned to users according **Table 10.5**

Before generating the Performance QC & tracking charts, make sure that at least seven PMT calibration took place. The charts are not generated until at least seven calibration took place.

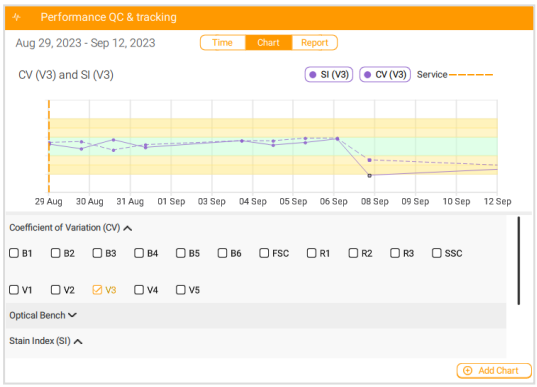
1 Click Performance QC & tracking button. The **Time** tab opens.

2 Select the time range for which to generate the chart. Select one of the four predefined ranges, or manually select the desired range.



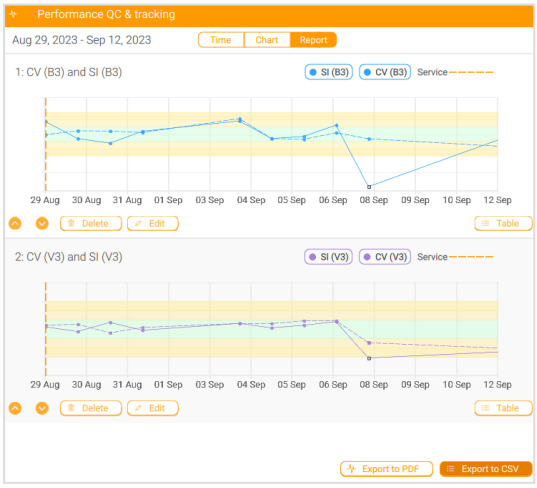
3 To select parameters for which to generate the chart, go to **Charts** tab.

4 Select the desired parameters and the channels for which to generate the chart. The charts are generated automatically.



5 To save the chart in **Report** tab, click **Add Chart** button.

6 Go to **Report** tab. All the saved charts are listed here.

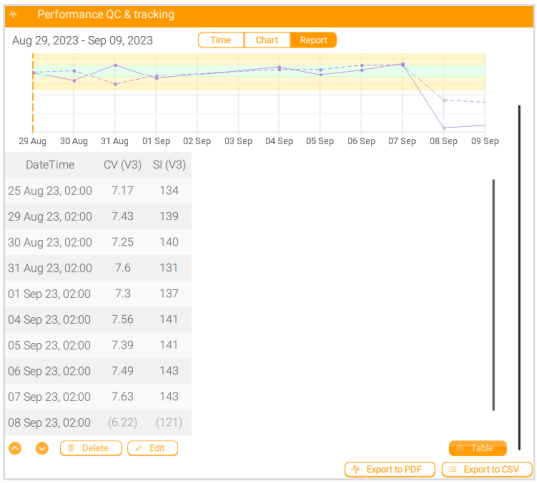


7 To move the reports up and down on the tab, click the corresponding arrow button. Last saved chart is shown on top of the list.

8 To delete a report, click the **Delete** button

9 To edit a report, click the **Edit** button. For details see **Editing Performance QC & tracking charts on the facing page**.

10 Click on **Table** button to show the parameter values as a table.



11 To close the table view of the parameters, click again on **Table** button.

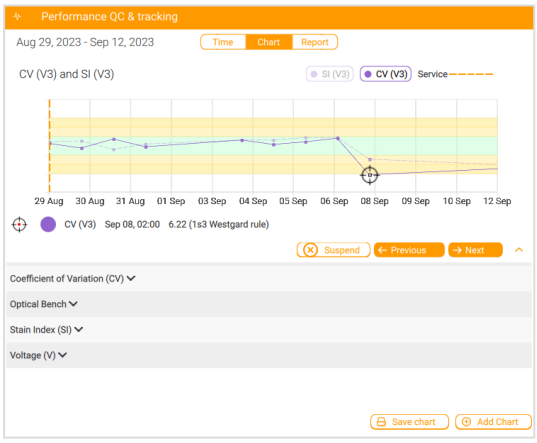
12 To export as .csv file, click on **Export to CSV** button. For 21 CFR Part 11 compliant setup, the reports can be exported as pdf via **Export to PDF** button.

14.1.2 Editing Performance QC & tracking charts

1 Go to **Chart** tab.

2 Click on a calibration parameter on the chart.

Parameter name, channel, date and time of calibration, and calibration value are shown.



3 To navigate between the parameters, click the **Previous** and **Next** arrows.

4 To exclude a calibration parameter from the Westgard rule evaluation, select the desired parameter.

5 Click on **Suspend** button.

The parameter is removed from evaluation, and the chart is recalculated with the new limits.

6 To save the updated chart and overwrite the old one, click **Save chart**.

7 To save the updated chart and also keep the old one, click **Add Chart**.

14.2 Hardware monitor

The hardware monitor can be accessed under the **View** menu. With the hardware monitor the real time status of the MACSQuant Instrument can be assessed, for example, to provide additional information in the case of error messages appearing on the screen. The serial number of the MACSQuant Instrument and photomultiplier tube (PMT) information can be found in the hardware monitor.

14.2.1 Fluidics

The **Fluidics** tab in the hardware monitor displays the pumps and valves as well as the status of the fluid bottles in live status.

The fluid bottles in the hardware monitor and on the instrument are illuminated in red when empty. The waste bottle is illuminated in red when full.

The waste (W), air (A), and fill pump symbols (F) are illuminated in green when active. Active valves 1-3 are indicated in green, defective valves in red. Valve status is indicated by o for open and c for closed.

The air pressure should be 300 mbar after priming when the instrument is in acquisition mode.

The reservoir indicates the level of MACSQuant Running Buffer in the reservoir. The level should be high or normal during acquisition mode.

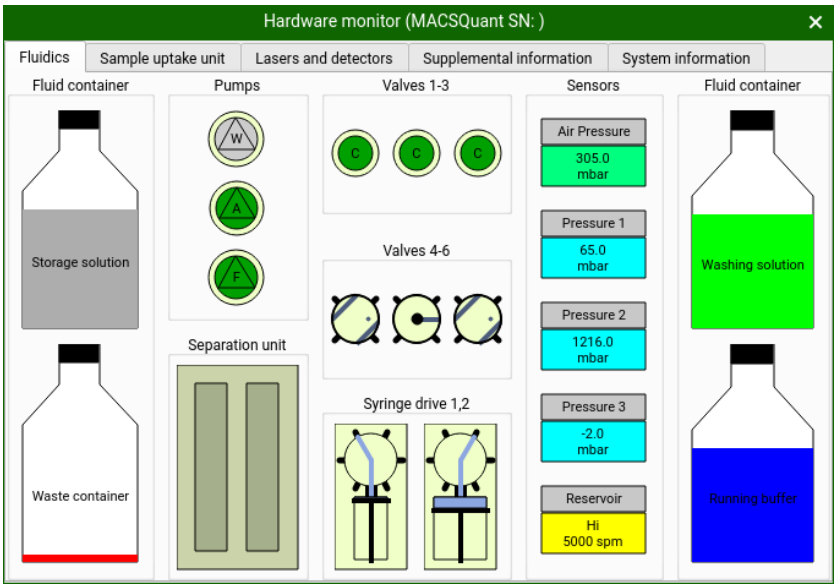


Figure 14.1: Real-time hardware monitor of the MACSQuant Instrument fluidic components

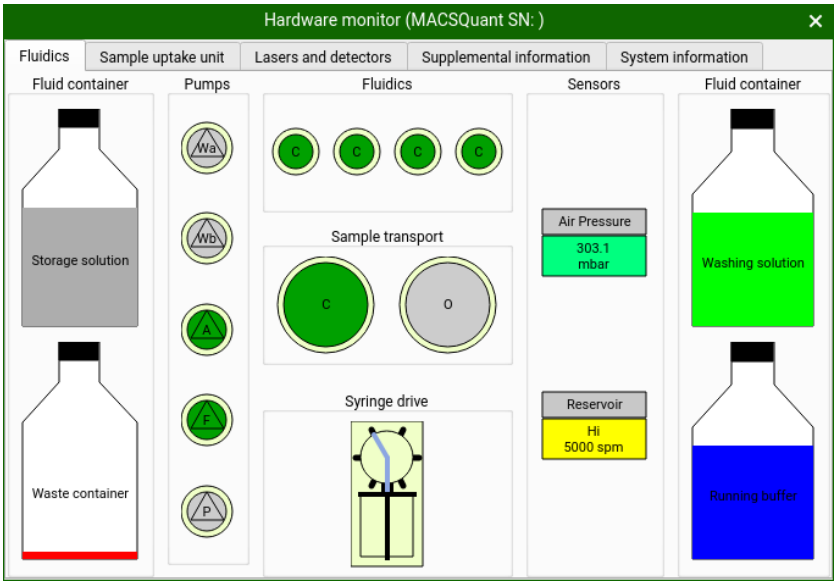


Figure 14.2: Real-time hardware monitor of the MACSQuant X fluidic components

Component	Further information
Fluid container	Displays liquid levels of bottles in real time (full/empty).
Pumps	Displays the status of the waste (W), air (A), and fill (F) pumps.
Separation unit	Displays the status of the MACSQuant Column and MACS Cell Enrichment Unit (MACSQuant Analyzer 10, VYB, and Analyzer 16 only).
Valves 1–3	Displays the position of the valves for the MACSQuant Column: C = closed, O = open, green = in use (MACSQuant Analyzer 10, VYB, and Analyzer 16 only).
Valves 4–6	Displays the position of the general fluidic system valves.
Syringe drive	Displays the position of the plunger of the syringes.
Sensors	Displays the general system pressure and level of the sheath fluid reservoir. Air pressure refers to pressure applied when instrument is in acquisition mode, for example, 300 mbar.

Table 14.1: Panels of the **Fluidics** tab

14.2.2 Sample uptake unit

The **Sample uptake unit** tab indicates the status of the robotic needle arm and if the MACS MiniSampler Plus is connected. The relative position of the robotic needle arm is listed in the lower left box and if the Single tube rack is calibrated.

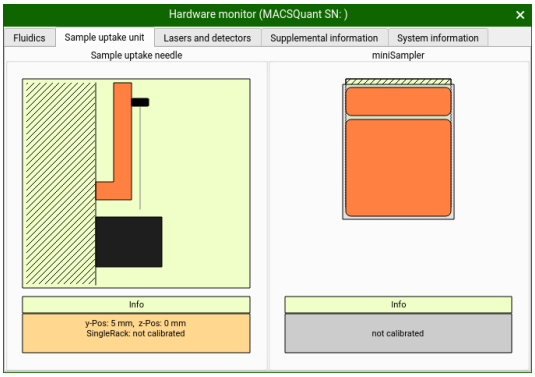


Figure 14.3: Real-time hardware monitor of the MACSQuant Instrument robotic needle arm

14.2.3 Lasers and detectors

The **Lasers and detectors** tab displays the status of the optical bench, including the temperature, fan speed, PMT voltage, and annotated path of each laser. The operating temperature for the main laser should be between 30 °C and 50 °C. The main laser is the blue laser for MACSQuant Analyzer 10, 16, and MACSQuant X, and the yellow laser for the MACSQuant VYB. The side lasers are kept at a temperature of 25 °C. When in acquisition mode, colored lines from the lasers to the detectors indicate that lasers are switched on. The bench temperature is kept between 31 °C and 37 °C. The fan speed is regulated automatically depending on the room temperature and internal temperature of the MACSQuant Instrument. See **Figure 14.4** for a schematic overview of the optical bench. In case of errors, contact Miltenyi Biotec Technical Support.

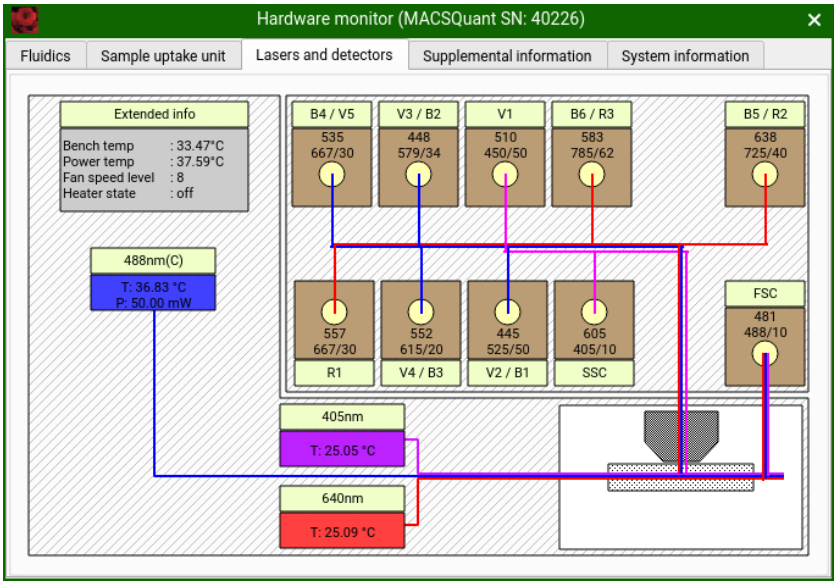
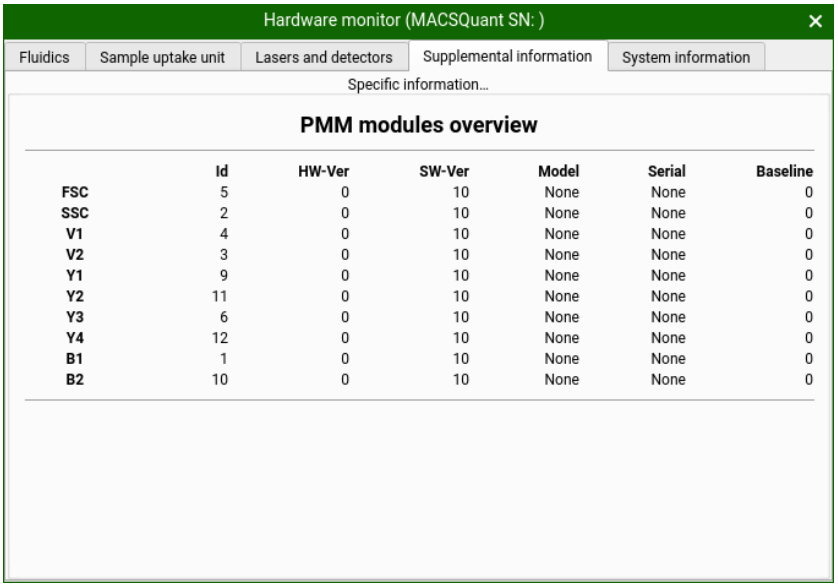


Figure 14.4: Real-time Hardware monitor of the MACSQuant 16 optical bench. T and P indicate the current temperature and power, respectively, of the main laser.

14.2.4 Supplemental information

The **Supplemental information** tab of the hardware monitor shows information about the photomultiplier modules.



	Id	HW-Ver	SW-Ver	Model	Serial	Baseline
FSC	5	0	10	None	None	0
SSC	2	0	10	None	None	0
V1	4	0	10	None	None	0
V2	3	0	10	None	None	0
Y1	9	0	10	None	None	0
Y2	11	0	10	None	None	0
Y3	6	0	10	None	None	0
Y4	12	0	10	None	None	0
B1	1	0	10	None	None	0
B2	10	0	10	None	None	0

Figure 14.5: Supplemental information tab of the hardware monitor

14.2.5 System information

The **System information** tab of the hardware monitor displays following information:

Device displays the name of the instrument, the serial number, and the name of the institution. See **Instrument on page 184**.

Counters displays available capacity of the hard drive, acquisition time (time after startup), number of finished measurements, and number of experiments (number of finished and running measurements).

Version displays the version of the installed MACSQuantify Software and of the installed Express Mode package.

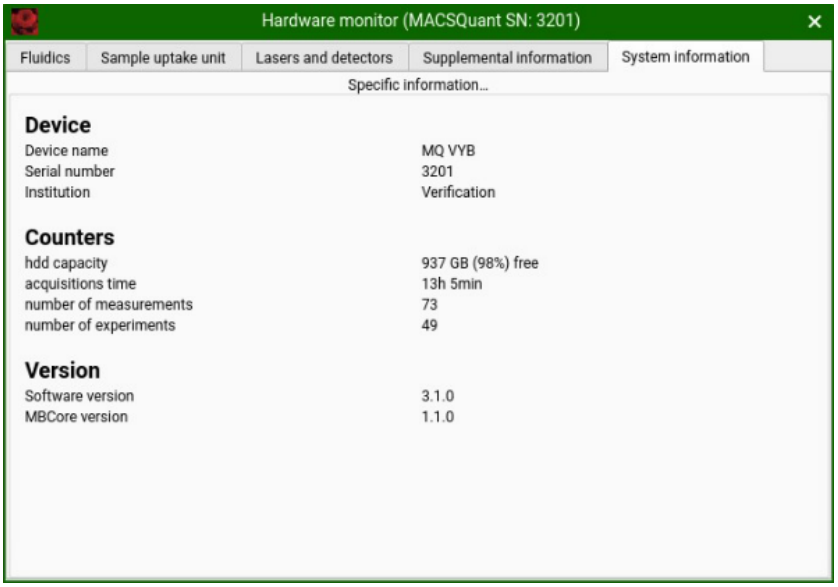


Figure 14.6: System information tab of the hardware monitor

14.3 Instrument status LEDs

The MACSQuant Instrument is equipped with LEDs, illuminating each bottle to indicate the status of the instrument in acquisition mode. If the fluid bottles are not illuminated, the MACSQuant Instrument is in data analysis mode and the lasers are off.

Bottle illumination	Description
Green	The MACSQuant Instrument is ready to measure, liquid levels are sufficient, and the instrument is primed.
Red	Liquid level error or general instrument error. The liquid levels are too low in a particular bottle or the waste bottle is full. The blinking red light indicates which bottle needs to be exchanged. Additionally, a message on the Instrument status bar specifies the instrument error. All bottles can be replaced during a measurement.
Blue	Indicates normal instrument function during sample processing, or that the instrument is busy.

Table 14.2: Color code of the fluid bottle illumination on the MACSQuant Instrument

15 Compliance with 21 CFR part 11

For compliance with 21 CFR Part 11 regulation, the MACSQuant 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 21 CFR Part 11 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 176**.
For setup, authentication, and modification of local user accounts, see **Adding a new local user account on page 173**.
For information on how to set up an LDAP connection, see **LDAP configuration on page 244**.
For information about user roles and permissions, see **User roles on page 20**.

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

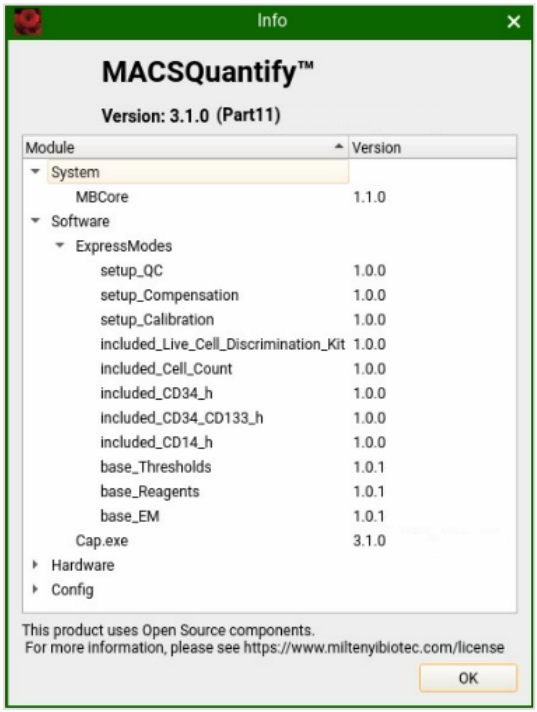
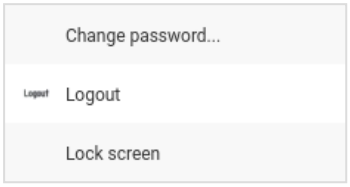


Figure 15.1: Help > Info window of a 21 CFR Part 11 compliant setup

15.1 Locking the screen

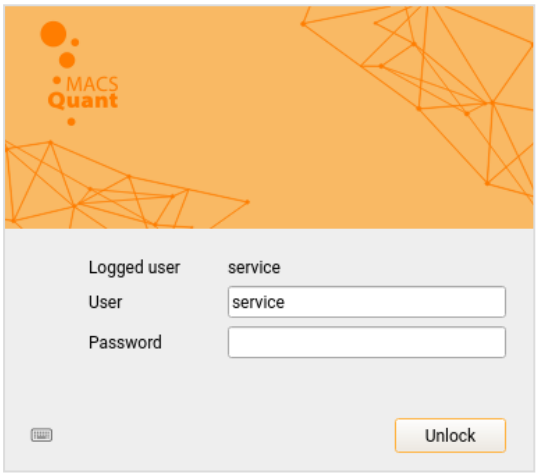
The current user can manually lock the screen to prevent other users from accessing the instrument. This function is only available in the 21 CFR Part 11 compliant setup. See **Timers on page 187** for setting up the automatic activation of the lock screen.

1 Click the username in the upper left corner of the screen.



2 Select **Lock screen**. The screen is locked.

3 To unlock, enter the password of the user account.



4 Click **Unlock**. The MACSQuantify Software is accessible again.

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

15.2 Continuous backup

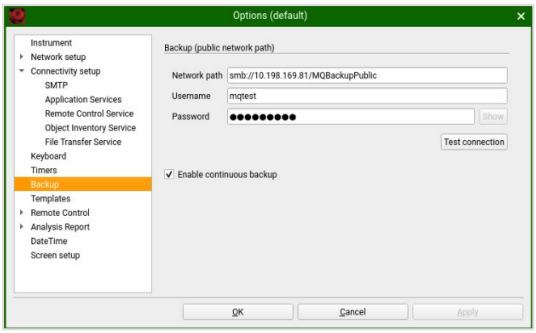
For the 21 CFR Part 11 compliant setup, a continuous backup is available to automatically save all MACSQuant Instrument data files from private and public folder on a dedicated network location. The continuous backup starts as soon as the **Enable continuous backup** checkbox is activated. Afterwards, the backup runs in the background, even if no user is signed in.
When the MACSQuant Instrument is doing a measurement, the continuous backup process is automatically paused. The backup process resumes automatically as soon as MACSQuant Instrument gets into an idle state.

The MQ Administrator can specify the backup network location and activate/deactivate the feature as needed. When the continuous backup is activated, an entry in audit trail is generated. See **Audit trail 21 CFR Part 11 on the facing page**.

1 Sign in as MQ Administrator.

2 Go to **Edit > Options (default) > Backup**.

3 To activate the continuous backup function, click **Enable continuous backup**.



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

The continuous backup is now active. The files are automatically backed up in the defined network path, in the folder **_cb_\MQ_SerialNumber**.

15.3 Audit trail 21 CFR Part 11

In an 21 CFR Part 11 compliant setup, actions are automatically tracked in the audit trail. An export can be created by a user with access to functions of the audit trail system, for example, an MQ Administrator. The user activities shown in **Table 15.1** are tracked 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.

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
Experiment started and finished	Create LDAP group including role assignments
Clean process	Copy LDAP certificate from USB
Manual and continuous backup	
Export system audit trail	
Activation, deactivation, and installation of Express Mode packages	
Disaster recovery image backup and restore	
Copy, delete and save files	
Creation of new plots	
Gate and plot changes	
Change date and time	
Sample export to CSV/ FCS	
Finished acquisition	
Device setup	
Sample recompensated	
Export, resample, group, ungroup samples	

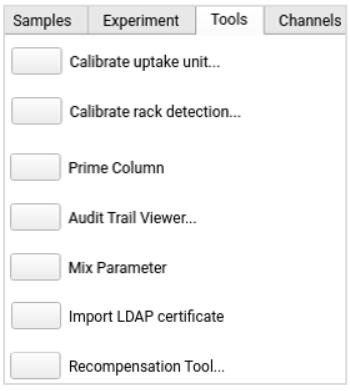
Table 15.1: Tracked activities in the audit trail of the 21 CFR Part 11 compliant software setup

15.3.1 Exporting an audit trail

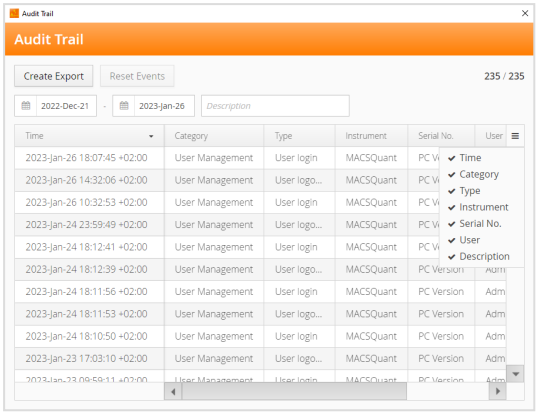
1 Sign in as user with access to the audit trail system.

2 Go to the **Tools** tab.

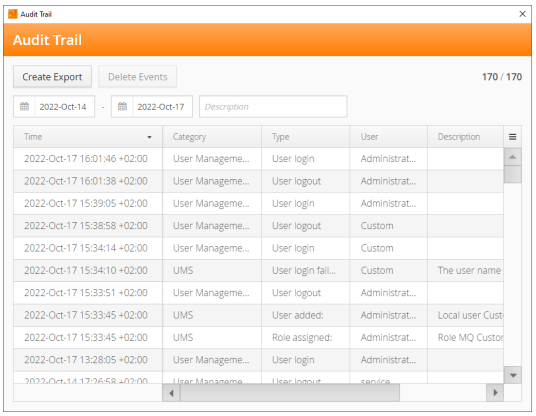
3 Select the checkbox **Audit Trail Viewer**.



4 To customize the shown columns, click on the symbol in the right-hand corner. Select the boxes to show the content of a column.

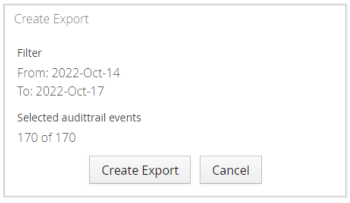


5 Define the time span in the **Audit Trail Viewer** window.



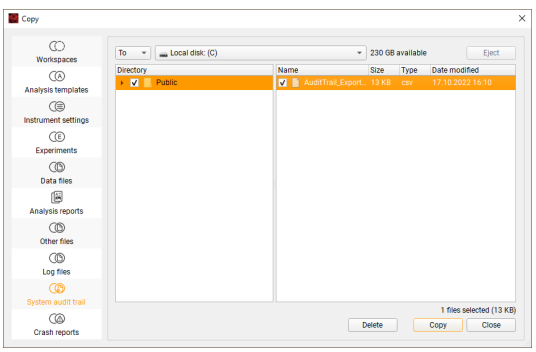
6 Optional: Type in search criteria in the **Description** field to limit the number of events. Filter will apply to the **Description** field.

7 Click **Create Export.**



PDF export of Audit Trail report is limited to 999 entries. In case more events are listed in the Audit Trail viewer, a compressed ZIP file is generated. Separate PDF exports of different time spans can otherwise be generated, each containing less than 999 events.

8 Go to **File > Copy** and select **System audit trail**.



9 Select the file.

10 Click **Copy**.

See **Copying files to an external location on page 150** for details how to copy files between instruments or a PC.

15.4 LDAP configuration

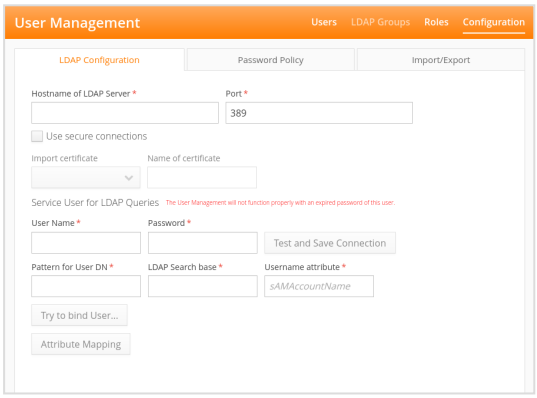
If a secure connection should be used, the certificate must be imported first before starting the LDAP configuration. See **Importing an LDAP certificate on page 248**.

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 address of the LDAP server.

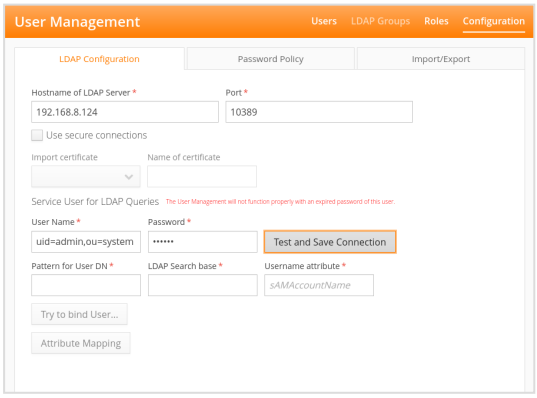
6 Select the box **Use secure connection** to use a secure connection via a LDAP protocol (recommended). Clear the box to use a LDAP protocol.

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

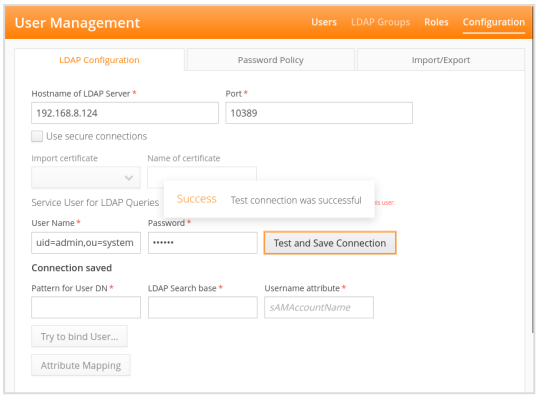
8 Select the certificate for import from the dropdown list.

9 Select **Import**.

10 Enter a username and password that can be used to send queries to the server. The username 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.

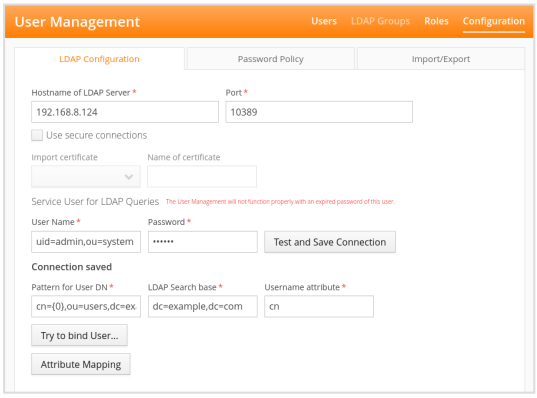
13 Enter a pattern to construct a distinguished name from a username (i.e. user id) to send the correct credentials as request for authentication to the LDAP system. The sequence '{0}' within the pattern is replaced with the user id for authentication requests.

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

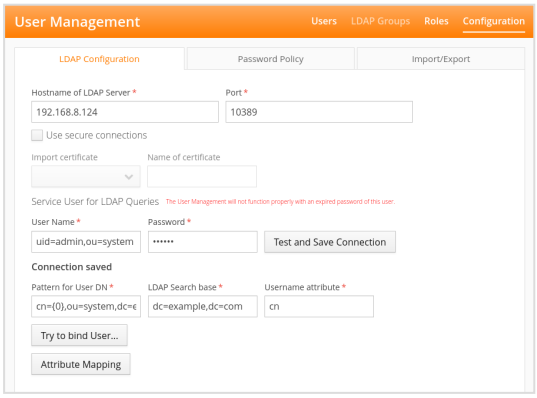
14 Go to the **LDAP Search base** field.

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.

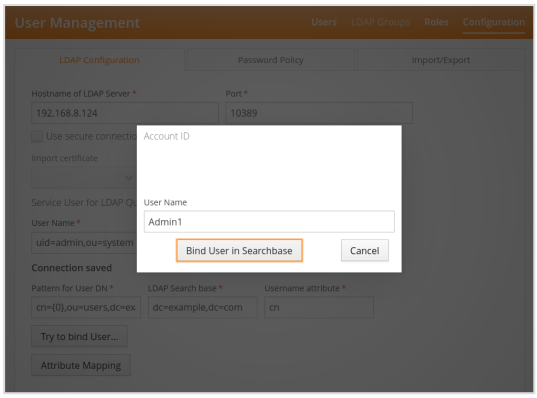


16 Select **Try to bind User...**.

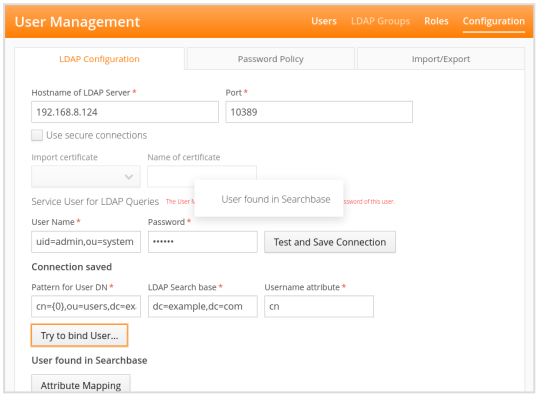


17 Enter the username of a regular account to test the configuration.

18 Select **Bind User in Searchbase**.



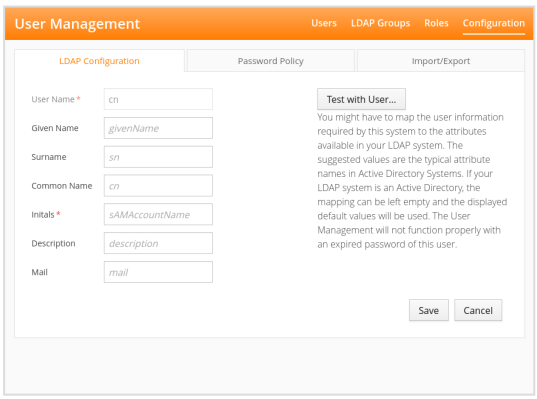
19 If the user is found in the searchbase, a confirmation message appears.



20 Go to **Username Attribute**.

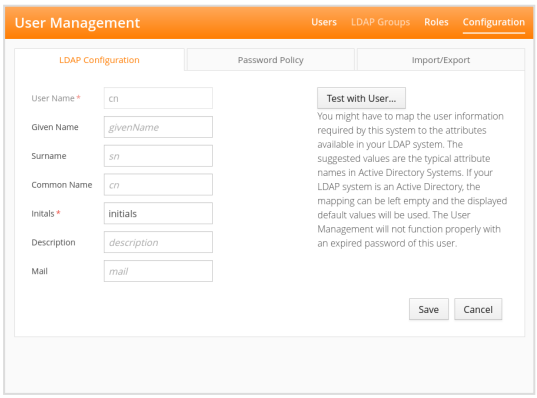
21 Enter the username attribute used by the LDAP system.

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



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

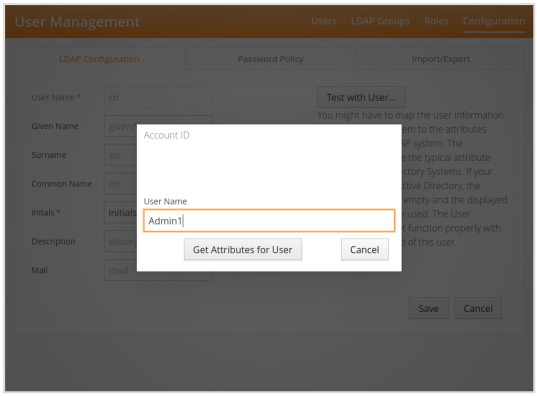
24 Enter "Initials" in the **Initials** field. For a Microsoft Active Directory setup, this must be "sAMAccountName".



25 Test the configuration with an LDAP user account.

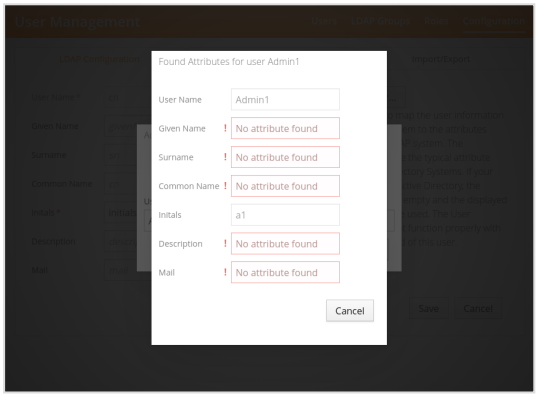
26 Select **Test with User...**.

27 Enter a username.



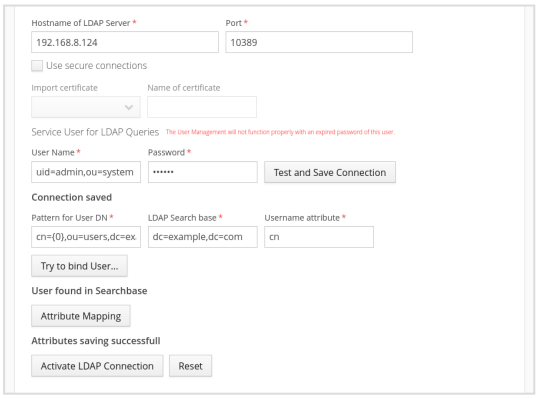
28 Select **Get Attributes for User**.

29 A window opens to show the attributes found for the user.

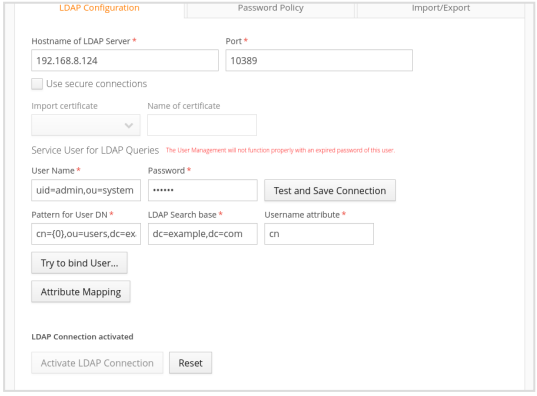


30 Select **Cancel**. The window closes.

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

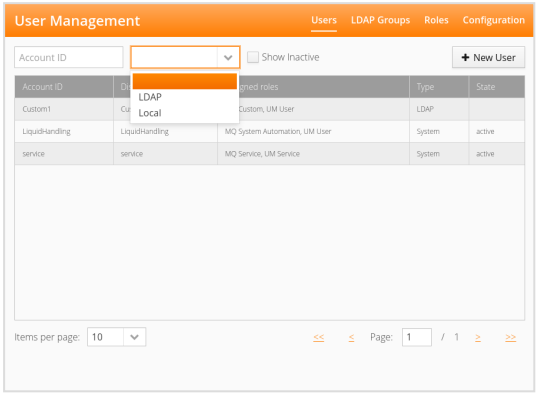


32 Select **Activate LDAP Connection** to save the LDAP configuration.



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

After activating the LDAP connection, restart the MACSQuant Instrument or PC. 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 171**.



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

15.4.1 Importing an LDAP certificate

If using a **secure** LDAP connection, the server's certificate is required.

1. Copy the certificate to a USB flash drive formatted as exFAT. The .cer file must be located in a folder called ldap_certificate on the top level of the USB flash drive.
2. Connect the USB flash drive to the MACSQuant Instrument.
3. Sign in as MQ Administrator.
4. Go to the **Tools** tab.
5. Select **Import LDAP certificate**.
6. Proceed with **LDAP configuration on page 244**.

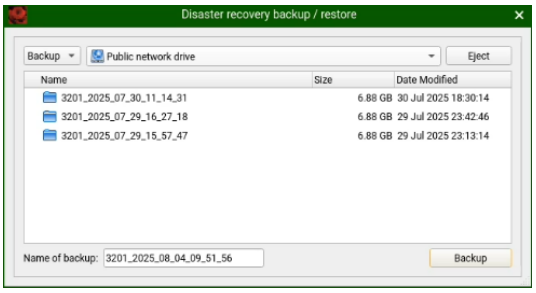
15.5 Disaster recovery

The disaster recovery function gives the possibility to create a complete backup of the entire system, including the operating system, software and hardware settings, all data, properties, and configuration files. The created system backup can be restored, when necessary, in order to bring the system back to that exact status. Disaster recovery creation and restoration is tracked in the Audit Trail. See **Audit trail 21 CFR Part 11 on page 241** for details.

Only the MQ Administrator user role has the disaster recovery rights by default. If needed, additional user roles with disaster recovery can be set up and assigned to users according **Table 10.5**

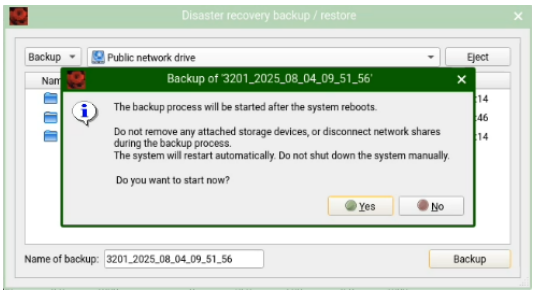
15.5.1 Creating disaster recovery backup

1. Sign in as user with access to disaster recovery function.
2. Make sure that MACSQuant Instrument is in data analysis mode.
3. Go to **Edit > Disaster Recovery...**. The disaster recovery backup / restore window opens.
4. On the upper left corner of the window, select **Backup**.
5. Select the private of public backup location or an external drive as a backup location.



The backup filename is generated automatically. It contains the serial number of the MACSQuant Instrument, the date and time of the backup.

6. To start the disaster recovery backup, click the **Backup** button. A warning message appears.



7. Click **Yes**. The MACSQuant Instrument restarts and the disaster recovery backup process begins when a blue screen is shown.

15.5.2 Restoring a disaster recovery backup

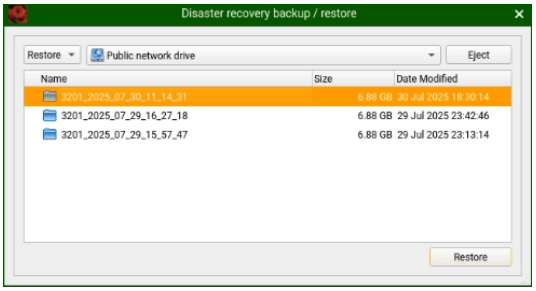
A disaster recovery restore will reset the instrument to the date and time of the disaster recovery backup creation. Everything stored on the instrument between the time of backup creation and restore will be lost. Save the relevant data before starting a disaster recovery restore.

1. Sign in as user with access to disaster recovery function.
2. Make sure that MACSQuant Instrument is in data analysis mode.

3 Go to **Edit > Disaster Recovery...**. The disaster recovery backup / restore window opens.

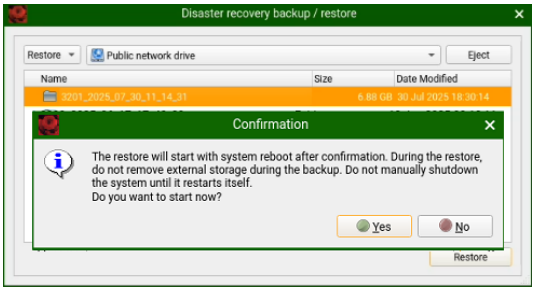
4 On the upper left corner of the window, select **Restore**.

5 Select the backup location from where to restore the disaster recovery backup file.



6 Select the disaster recovery backup file.

7 To start the disaster recovery restore, click the **Restore** button. A warning message appears.



8 Click **Yes**. The MACSQuant Instrument restarts and the disaster recovery restore process begins when a blue screen is shown.

15.6 Analysis reports

Analysis reports are only available in the 21 CFR part 11 enabled setup of the MACSQuantify Software. They allow electronic documentation of data analysis in a human-readable format. Analysis reports contain the analysis layout and information about the data files such as used instrument settings. The analysis reports are electronically signed by the user and can be exported as PDF/A. A checksum is applied to detect modifications of signed records. They can be created by MQ Administrators or MQ Custom users. Analysis reports are saved in the same folder where the original data file is located. The access rights of the user role defines where data files are stored during acquisition..

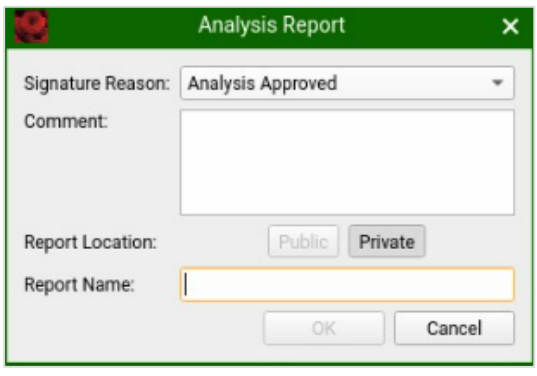
15.6.1 Creating analysis reports

1 Go to **File > Analysis report**.

2 Select a signature reason from the dropdown list.

3 Enter a comment for the signature reason.

4 Enter a name for the report.



5 Click **OK** 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. The analysis report can be saved in public or private, dependig of the user rights. The report includes following information:

- a front page including the electronic signature
- the current analysis
- a section with meta information about all samples used in the analysis

8 Go to **File > Copy** to copy the analysis report to an external network or an external drive.

15.7 Standard operating procedure (SOP)

15.7.1 General requirements

- The customer must check the identity of each created user (who can sign) prior to using the system. This shall be documented. Furthermore the customer must be able to provide a certificate for each user which states that he/she is using his/her electronic signature as a legally binding equivalent of a handwritten signature and that he/she is aware of that.
- The customer shall ensure that all employees, who are involved in 21 CFR Part 11 relevant processes, are trained in 21 CFR Part 11.
- The customer must inform the FDA as soon as electronic signatures are used (see example document in the SOPs)
- The customer must have relevant SOPs or Work instructions (WI), which regulate the responsibilities for electronically signed records in companies. (Note: We CANNOT pre-formulate this SOPs/WIs)
- The customer must comply with the legal archiving periods. Miltenyi Biotec DOES NOT provide an archiving system with their system.
- The customer must point out to its employees that the electronic data entry is an equivalent of the paper data entry and that employees will be held responsible for deliberate false entries or data manipulation. The same applies to electronic signatures. Each relevant employee must understand that the electronic signature is an equivalent of a handwritten signature and cannot be rejected (see SOPs-Corporate Compliance Policy). This is to prevent forgery of entries and signatures. This means the customer should have a company policy, which describes this in detail.

15.7.2 Training of employees (SOP)

- During employee training the customer must make it clear to each participant that the electronic data entry is an equivalent of paper data entry and that the employee may be held responsible for deliberate false entries or data manipulation. The same applies to electronic signatures. The relevant employee must understand, that the electronic signature is an equivalent of a handwritten signature and cannot be rejected.
- The customer has to ensure that the written sets of standards are established and followed. Individuals must be held responsible for actions which they cause with their data entry/signature. This is to prevent forgery

of entries and signatures. The customer should have a company policy for example which describes this in detail and outlines the consequences of deliberate or gross negligence actions.

15.7.3 Miscellaneous

- The customer must comply with the legal archiving periods.
- The customer must ensure that the date and time on the machines as well as the date and time on the corresponding PCs can only be changed by an MQ Administrator. Regular checks of the date and time as well as regular matching with the customers system date and time must occur. If this cannot be done functionally a regular manual task must be ensured.
- The customer should ensure that employee passwords contain at least 6 characters including special characters.
- The customer should ensure that passwords expire within a time period of 3-6 months and a new (different) password must be set by the user. If this is not executed by the employee it shall cause the account to be locked.
- The customer should ensure that the user manual gets replaced with the installation of the software. The Installation protocol of the software can also be used as an audit trail for the replacement of the user manual. Alternatively there should be a paper audit trail, which protocols the replacement of a printed user manual.

16 Technical Support

16.1 Technical support

16.1.1 Contact

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.

16.2 MACSQuant Live Support

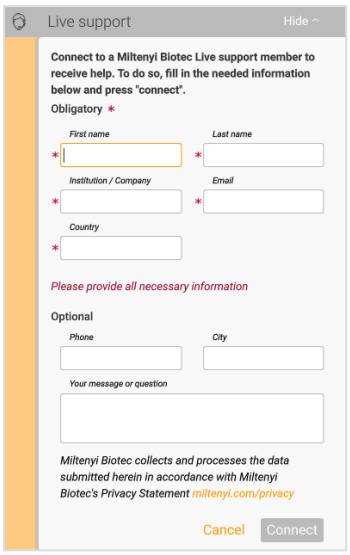
MACSQuant Live Support is a real-time diagnostic service provided by Miltenyi Biotec Technical Support. The Live support is available for all users without any costs 24 h a day from Monday through Friday. Highly trained MACSQuant specialists can be reached to assist with any queries. If required, the MACSQuant 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 Software. Disconnect the webcam during normal operation. The MACSQuant Instrument must have network access to the internet to use the MACSQuant Live Support. See **Network setup on page 184**.

The MACSQuant Live Support is not available in the PC version.

Use port 5938 for MACSQuant Live Support. Alternatively, ports 443 and 80 can be used. See **Proxy on page 185**.

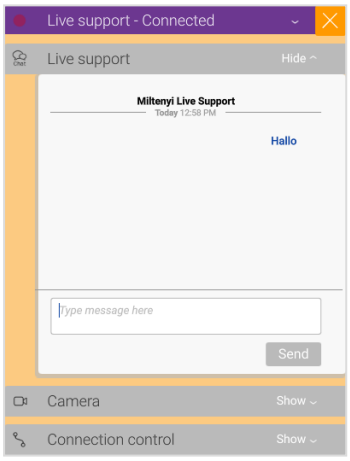
1 Go to **Help** > **Live Support**.

2 Complete all obligatory fields.



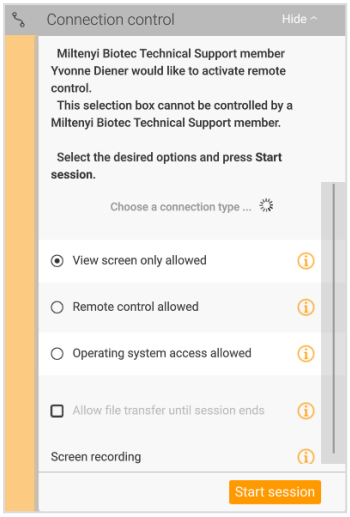
3 Select **Connect** to start the MACSQuant Live Support. A chat window opens.

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.

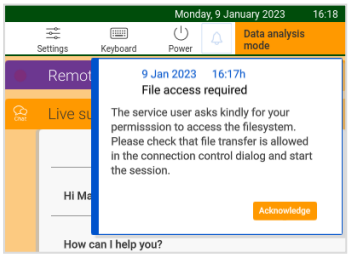


6 Select one of the available options. See **Table 16.1** for further details.

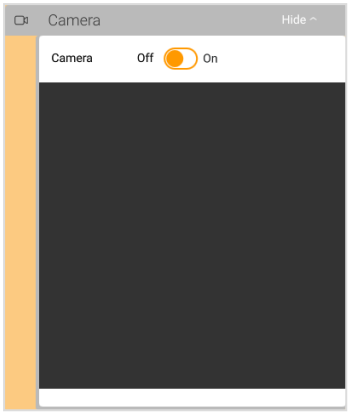
- **View screen only allowed**: Allows the Miltenyi Biotec Technical Support member to see the screen of the instrument but not to interact with it.
- **Remote control allowed**: Allows the Miltenyi Biotec Technical Support member to interact with the instrument like you do.
- **Operating system access 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 Instrument and record the screen based on the selection.

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

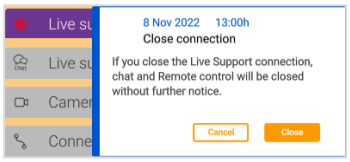


9 Activate the camera if a USB webcam is attached and it is required to show parts of the instrument during MACSQuant Live Support.



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

11 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 16.1: Connection control options during MACSQuant Live Support

17 Legal notes

17.1 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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Visit **www.miltenyibiotec.com/license** to access information.

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19 Abbreviations

21 CFR part 11	Title 21 of the Code of Federal Regulations part 11
ATS	audit trail system
CD	cluster of differentiation
CSV	comma-separated values
CV	coefficient of variation
DHCP	dynamic host configuration protocol
EPC	endothelial progenitor cell
exFAT	extended file allocation table
FCS	flow cytometry standard
FSC	forward scatter
HDMI	high definition multimedia interface
IP	internet protocol
LAN	local area network
LDAP	lightweight directory access protocol
LHS	liquid handling system
MC	MACS Control
MFI	mean fluorescence intensity
MQ	MACSQuant
MQD	MACSQuant data
NTP	network time protocol
PI	propidium iodide
PMT	photomultiplier tube
RUO	research use only
SI	stain index
SMTP	simple mail transfer protocol
SSC	side scatter
SOP	standard operating procedure

UM	user management
USB	universal serial bus
VGA	video graphics array
VYB	violet yellow blue
-A	area
-H	height
-W	width



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