MACSQuantify™ Software 2.8

User manual

MACSQuantify™ Software 2.8

User Manual

Read the user manual before using the instrument

Before using the MACSQuantify™ Software, read the chapter **Important safety information** and all other information contained in the MACSQuant™ 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, please contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Technical Support.

Content

1
Introduction

This MACSQuantify™ Software guide applies to the MACSQuant Analyzer 10 (# 130-092-197) and the MACSQuant VYB (# 130-096-116) running MACSQuantify™ Software version 2.8.

The MACSQuant Instruments are bench-top flow cytometers equipped with three lasers for measurement of up to eight fluorescence channels and two scatter channels (FSC, SSC). They facilitate the absolute quantification of cell populations from single samples, multiple tubes or 96-well plates. The instruments are designed to use with MACS® Flow Cytometry Reagents, but common fluorochrome-conjugated antibodies and fluorescent reagents from other suppliers could also be used.

The MACSQuant Analyzer 10 and the MACSQuant VYB are equipped with the MACS Enrichment Unit to provide a fast and easy way to analyze rare cell populations using MACS Technology. For more details about the key accessible components and technical specifications of the MACSQuant Instruments, refer to the MACSQuant Instrument user manual.

Flow cytometry is the measurement of chemical and physical properties of cells as they "flow" one by one through an integration point, most commonly a laser. As cells scatter the laser light in different directions (forward or to the side), intrinsic cellular properties, such as relative cell size and cytoplasmic complexity, can be measured. In human whole blood, for example, lymphocytes, monocytes, and granulocytes can be distinguished simply because they scatter laser light differently.
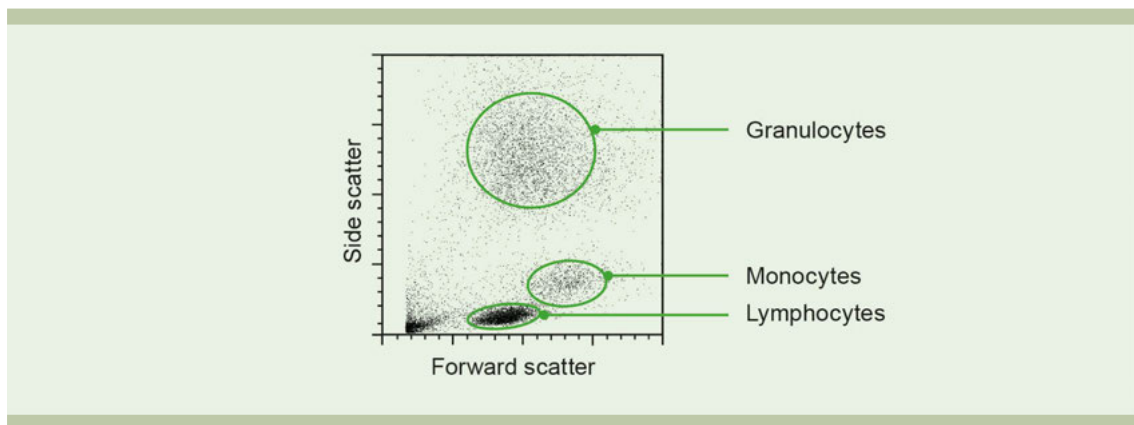
Figure 1.1: Typical light scatter profile of lysed human whole blood.

Most modern flow cytometers can further measure extrinsic cellular properties: cells can be characterized by the expression of cell surface or intracellular markers, their nucleic acid content, enzyme activity, and much more. To probe these cellular properties, fluorescent reagents are used, such as antibodies conjugated to fluorochromes. These fluorescent reagents have very unique light emission qualities so that they can be detected separately in distinct fluorescent channels.

A unique feature of flow cytometry is that fluorescence on a cellular or particle level can be measured very rapidly. As cells or particles travel through the light path, the fluorescent probes are excited, resulting in the specific detection of these cellular properties at the rate of 10,000 events/second.
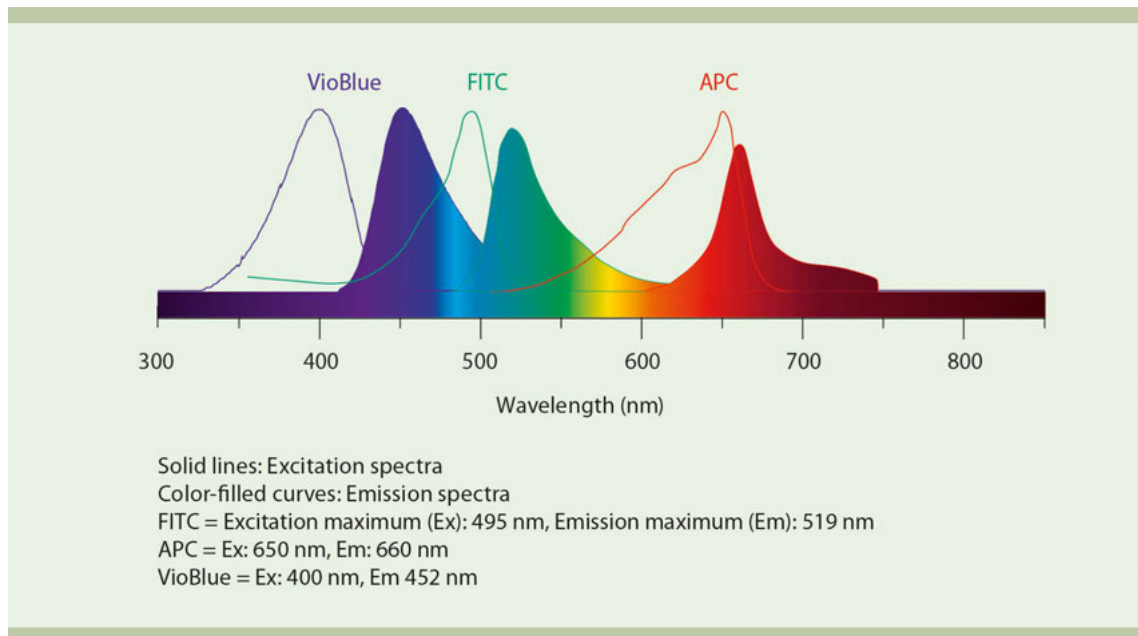
Figure 1.2: Excitation and emission spectra for VioBlue, FITC, and APC.

Flow cytometry is used in both clinical and basic research. Several research fields benefit from this technology, including immunology, transplantation, hematology, neuroscience, stem cell research, cancer research, cell biology, molecular biology, drug discovery, systems biology, marine biology, microbiology, virology, and bioprocessing. Clinical applications are, among others, flow diagnosis, clinical trials, or monitoring patients after treatment. Some clinics also use flow cytometry to characterize donor and recipient cells prior to transplant to lower the risk of adverse effects following transplantation.

Flow cytometry applications

- Eight-color immunophenotyping
- Cell viability staining and apoptosis detection with PI and Annexin-V conjugates
- Intracellular staining for cytokines and transcription factors
- MACS® Cytokine Secretion Assays
- Cell cycle analysis using PI and or BrdU
- Microvesicle analysis
- Cell enumeration
- Calcium flux using Fluo4
- Detection of oxygen reactive (H2DCFDA) and NO reactive (DAF-FM diacetate) species
- Cell proliferation with CFSE or CMFDA
- Reporter fluorescent protein detection – GFP, CYP, YFP, tdTomato, mCherry, RFP
- Analysis of virus particles, bacteria, mitochondria

1.1 Components of a flow cytometer

To enable the light scatter and fluorescent measurements of cells and particles, MACSQuant® Instruments utilize a combined system of fluidics, optics and electronics.

- **Fluidics** – a liquid flow system to align and move cells or particles through a laser path.
- **Optics** – a series of optical filters and mirrors to direct light of specific wavelengths emitted by cells or particles towards detectors, which amplify the light.
- **Electronics** – interface between the optics and computer. It converts the amplified light signals proportionally into voltage and plots these values.

1.1.1 The fluidic system

Hydrodynamic focusing is used to align and move the cells or particles through the laser path. The sheath fluid is flowing through the flow cell with constant pressure. The cells are injected into the middle of the sheath fluid flow. By principles of laminar flow, the cell sample and sheath fluid flow in the same direction but stay separate. The pressure differential between the sheath and the cell sample determines how wide the sample stream is as it flows through the flow cell. The lasers excite the cells or particles individually and the scatter and fluorescent light are measured.
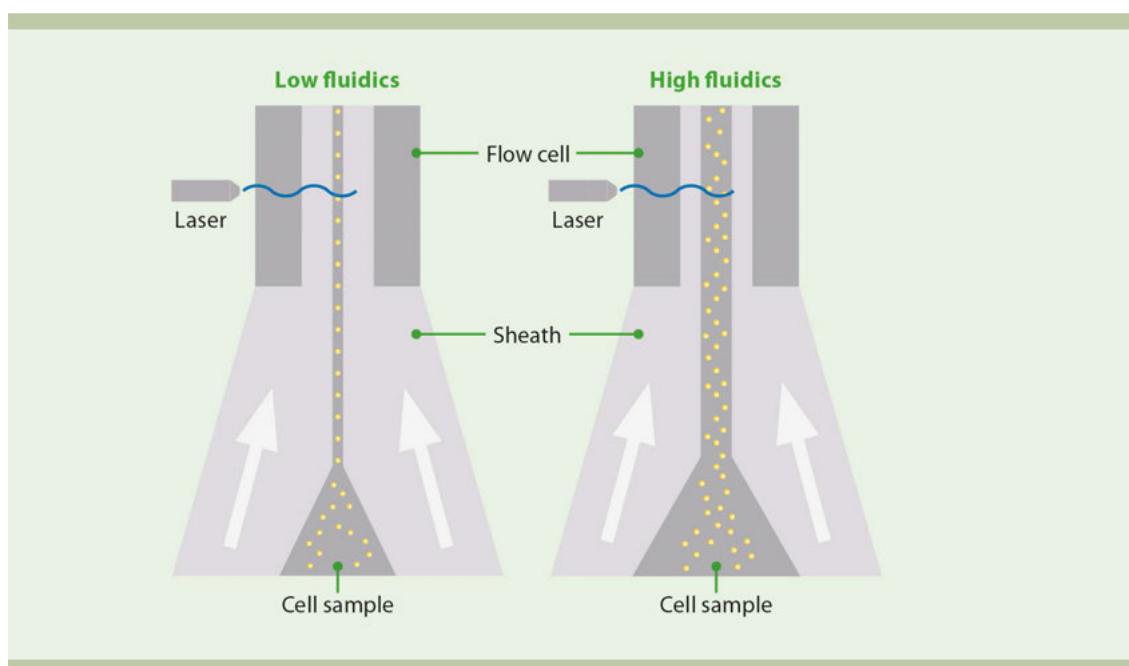
Figure 1.3: Illustration of hydrodynamic focusing of the MACSQuant10 Instrument

MACSQuant Instruments utilize a 0.5 mL pump syringe for volumetric uptake and subsequent sample injection to the flow cell. The syringe-pump driven fluidics will draw the specific volume into the uptake needle. The uptake needle will then align with the flow cell injection port (unless running an enrichment protocol) and inject the specific volume at the programmed fluidic speed.

For flow applications that require very tight population resolution and CV of the measured peaks, e.g. cell cycle analysis, low fluidic speed is recommended, as the sample stream is the narrowest. For general immunophenotyping applications, any fluidic speed is sufficient. It is important that the instrument event rate does not exceed 10,000 events/second for any of the fluidic speeds.

1.1.2   The optical system

After excited by a laser, fluorescent reagents bound to cells or particles emit light, which is detected according to their specific wavelength range. When using a combination of fluorescent reagents, they should have distinct emission spectra. MACSQuant Instruments utilize various optical filters and dichroic mirrors to direct light of specific wavelengths to the according fluorescent detectors. This arrangement creates "fluorescent channels".

Optical filters and mirrors

There are different types of filters that are used to delineate what wavelengths can go into the fluorescent channels. They are designated longpass (LP), shortpass (SP) and bandpass (BP). The MACSQuant Instruments utilize LP and BP filters.
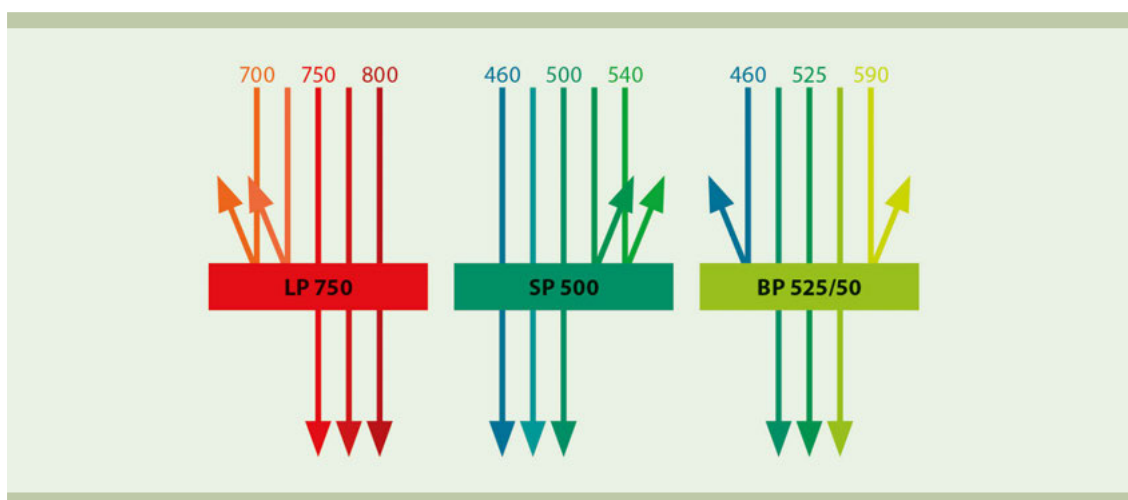
Figure 1.4: Illustration of light of different wavelength passing through LP, SP and BP filters

Longpass filters are designated by the letters LP and a specific number, e.g. LP 750. That is, light of a wavelength of 750 nanometers (nm) and longer can pass through the filter. Light of all other wavelengths will be absorbed. A shortpass filter has a similar designation, e.g. SP 500. Here, light of wavelengths of 500 nm and shorter can pass through, while the remaining light will be absorbed. Bandpass filters allow light of a specific wavelength range to pass through. Bandpass filters are designated by BP followed by two numbers. The first number represents the midpoint of the wavelength range. The second number is the wavelength range in nm that can pass. Thus, light that can pass through a BP 525/50 filter ranges from 500 to 550 nm.

Mirrors have essentially the same function as filters, but are oriented at a 45° angle to the light path, whereas filters are oriented perpendicular to the light path. The light which is not able to pass through is deflected at a 90° angle towards another light detector. With this optical setup, eight distinct fluorescence channels and two scatter channels are created by combinations of the light emitted after excitation by the three lasers, as well as various filters and mirrors.

Photon detectors – Photomultiplier tubes (PMTs)

The specific wavelength light paths, or fluorescence channels, are monitored by photo-multiplier tubes (PMTs). The PMT will amplify the signal of the detected wavelengths of light emitted from the cell or particle. The amount of amplification is dependent on the voltage applied to each PMT. As the voltage is increased the amplification of the detectable fluorescence is increased, resulting in increased mean fluorescence intensity (MFI). As the voltage is decreased, the amplification is decreased, resulting in lower MFI.

As each channel's PMT is detecting and amplifying the light emission of a specific fluorescent reagent, it is recommended to assign the correct nomenclature to these channels. The PMT not only amplifies the detectable light, but converts the optical signal into a voltage pulse that is relayed to the electronics for signal conversion and display.

Analog to digital conversion

Analog signals are converted to digital signals for display of data according to the bit depth for the analog-to-digital (ADC) of the flow cytometer. The MACSQuant Instruments have a 16 bit ADC for all parameters, but for the pulse area, an additional calculation allows for 18 bit processing. This means that for the pulse area measurements, the signals are resolved into over 253,000 bins. This allows for a five-decade logarithmic scale for data display.

1.1.3   The electronics

The electronics of a flow cytometer converts the light signals into proportional electronic signals (voltage pulses), digitizes the signal according to the ADC bit depth, and interfaces with the computer for data transfer. MACSQuant Instruments are digital flow cytometers, meaning that the conversion from analog signals to digital signals happens at the point of signal detection and amplification. Advantages of digital flow cytometers include:

- Voltage pulse height, width and area can all be a measureable parameter.
- Compensation of spectral overlap is applied after digital conversion of signal, meaning compensation can be re-adjusted any time post acquisition.
- Detection thresholds or triggers can be set on any of the detection channels.
- Fluorescence values detected as zero or negatives will be measured as such and can be displayed in the plot choices.

Voltage pulses

As a cell is passing through the laser path, the attached fluorochrome will begin to fluoresce. This light is detected by the PMT and starts the generation of a voltage pulse. As the cell fully enters the laser path, the fluorescence reaches peak emission, resulting in the highest peak of the voltage pulse. Finally, as the cell leaves the laser path, this detectable fluorescence decreases, finishing the voltage pulse.
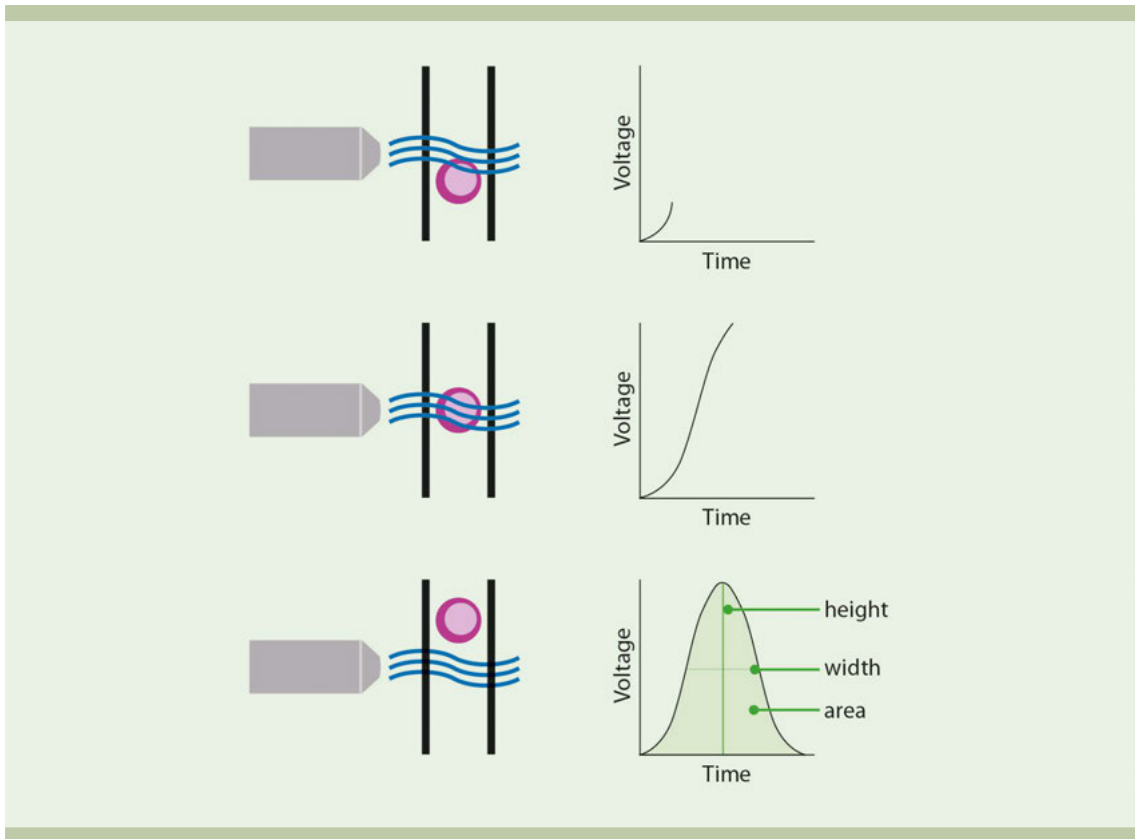
Figure 1.5: A Voltage pulse can becharacterized by its height (signal intensity), width (time of flight), and area (emitted fluorescence while the cell passes through the laser path).

1.2 Data visualization

For data visualization, values of each measured parameter can be plotted in various ways. These plots can be univariate (histograms) or bivariate (dot plots).

1.2.1 Univariate plots – histograms

Univariate plots allow the visualization of one optical parameter versus event number. The measured values for each event are stored in a data file, comprised of a table of stored signal intensities (of fluorescence and scatter signals) for each measured parameter. As events start to accumulate, a "curve" is generated that describes the distribution of the measured events according to their signal brightness. Looking at the scale from left to right, the intensity of the plotted signals increase. Populations on the left side of the scale have lower signal intensity than the populations on the right side of the scale.
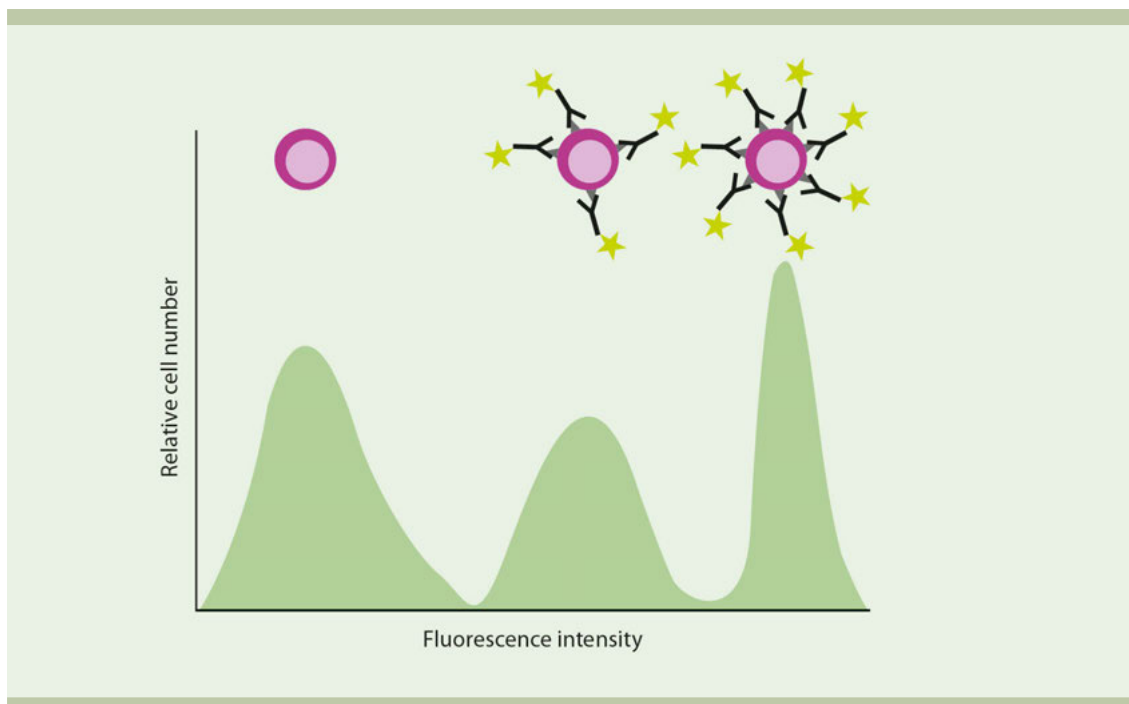
Figure 1.6: A univariate histogram plot describes distribution of intensity.

1.2.2 Bivariate plots – Dot plots

Dot plots allow the display of two optical parameters in one graph. The position of an event is determined by two values, one for the x- and one for the y-axis. When all events are plotted, events with similar intensity values accumulate into clouds or populations.
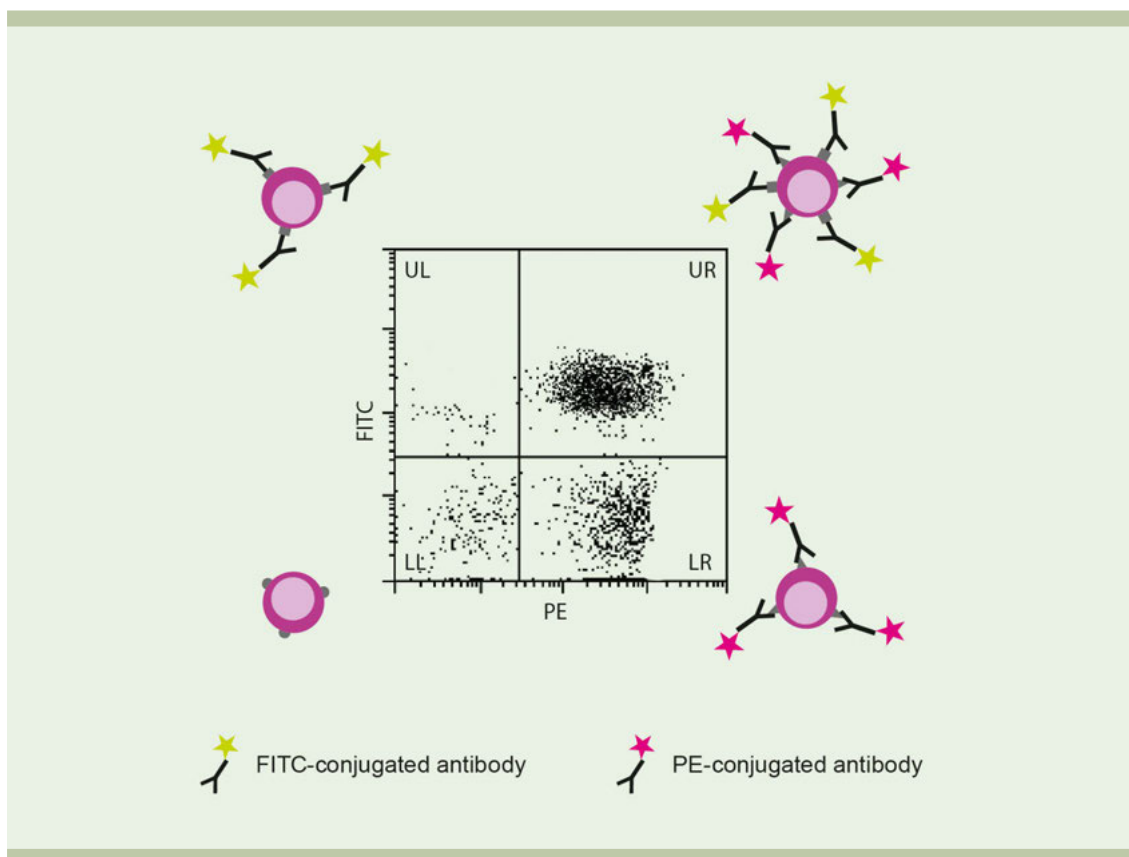
Figure 1.7: Bivariate dot plots allow visualization of two optical parameters simultaneously.

1.2.3 Bivariate plots – Density plots

Density plots display two parameters as a frequency distribution similar to a dot plot (each cell is represented as a dot). Additionally, they depict the distribution of cells within a population with a very high density of events. In the figure below, the red color represents the highest density within a population. With decreasing density, the color transitions from yellow over green to blue.
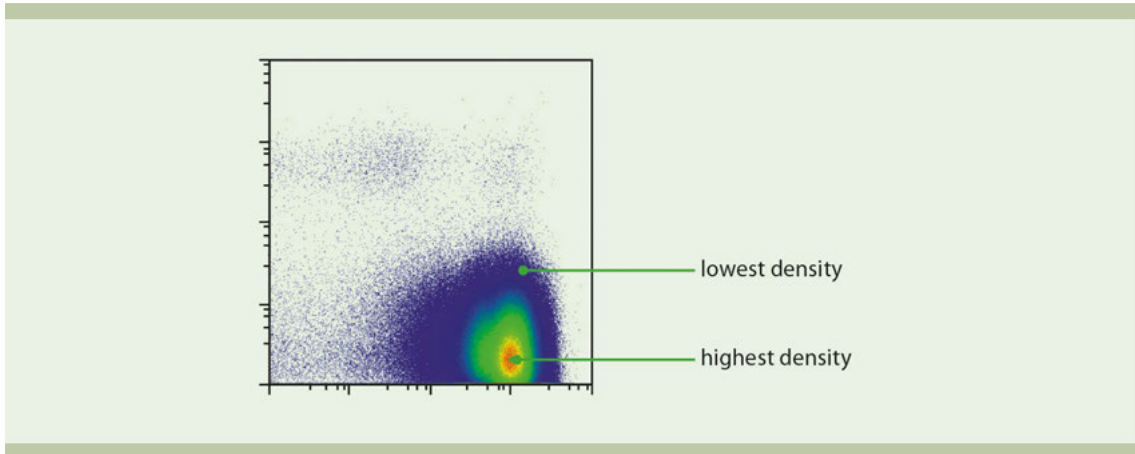
Figure 1.8: A density plot depicts the distribution of cells within a population.

2

How to use this instrument

⚠WARNING Read the chapter **Important Safety Information** in the MACSQuant™ user manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

2.1 Switch on the instrument

1 Switch on the instrument to turn it into stand-by mode.

2 Touch the touchscreen to power up the MACSQuantify Software.

3 A login window will appear.

2.2 Login

1 If in standby mode, touch the touchscreen to launch the MACSQuantify Software.

2 Enter user name and password.

3 Click **Login**.
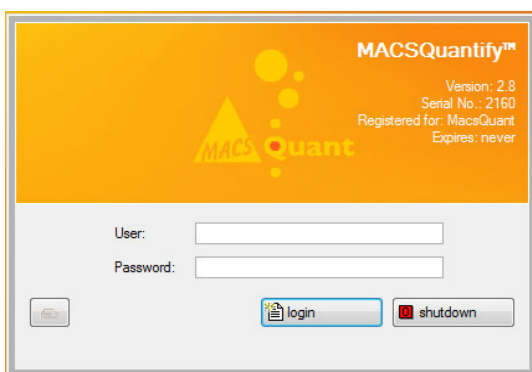
Note: After login, the instrument is in analysis mode.s

2.3 Prime the instrument

1 To prime the instrument (power up the lasers, activate fluidics), click on the **Main instrument control button** at the upper right of the window and choose **Acquisition mode**.

2 Wait for the optical bench to warm up, which requires at least 30 minutes.

Note: While the instrument warms up, it is recommended to perform a flush cycle followed by a clean program. Refer to the MACSQuant Instrument user manual for details.

2.4　Lock the screen

1　To lock the screen, click on the user name at the upper right hand side.

2　From the drop-down menu, chose **Lock screen**.

3　To unlock, enter your password.
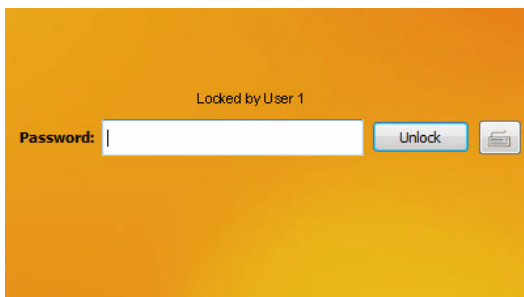
2.5　Logout

To log out, do one of the following:

- Click the **Logout button**.
- Click the user name at the upper right hand of the screen. From the drop-down menu, chose **Logout**.

2.6　Change passwords

1　To change the password, click on the user name at the upper right hand of the screen.

2　From the drop-down menu, chose **Change password...**.

3　Enter your old password.

4　Enter a new password and confirm it by entering it again.

5　Click **Change password** to proceed or **Cancel** to abort.

2.7 Shut down the instrument

When you finished your experiments, shut down the system before switching off the instrument. By default, automatic shutdown after a certain time is enabled to maximize diode lifetime. To change the automatic shutdown settings, refer to section **Timer**.

1 To shut down manually, click the **Main instrument control button** at the upper right of the window.

2 Select **Instrument off**. This will automatically start a washing procedure that requires about seven minutes.

3 The instrument is now in stand by mode. Touch the touchscreen to start the instrument again.

4 To shut down completely, switch off the main switch at the lower right side of the instrument.

2.8 The user interface
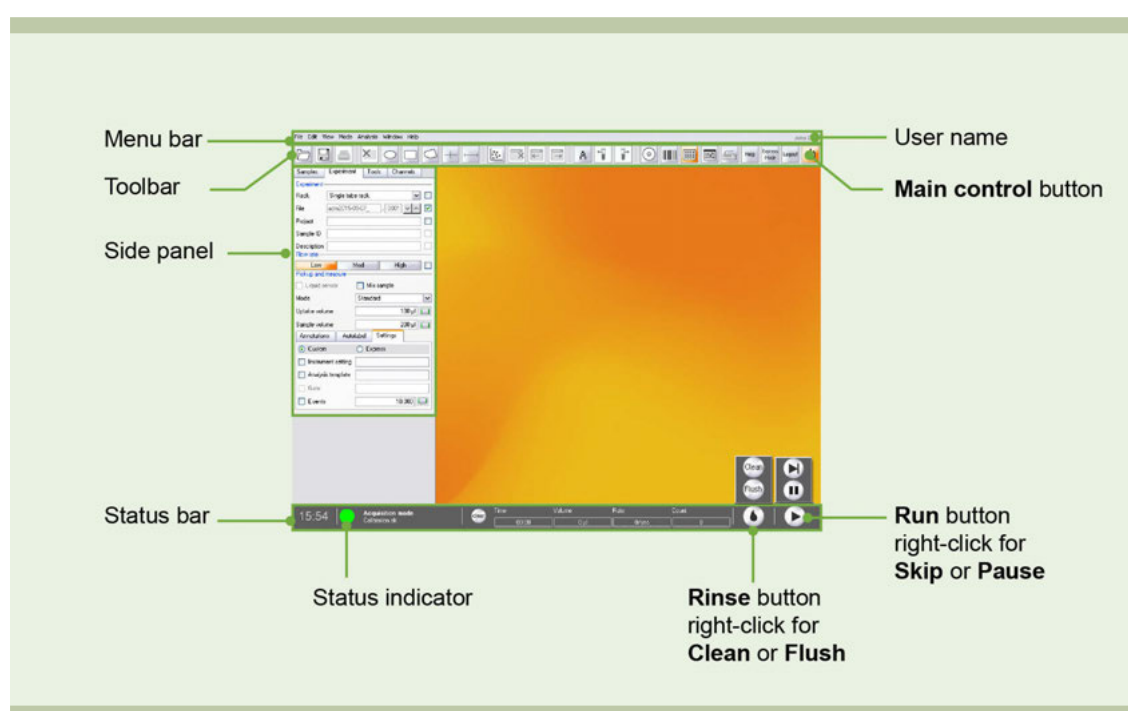
Figure 2.1: The MACSQuantify user interface.

2.8.1 The menu bar

Access most MACSQuantify functions via the menus **File**, **Edit**, **View**, **Mode**, **Analysis**, **Window** and **Help**. Some frequently used functions can also be accessed directly from the toolbar (see section **The toolbar**). Click on the user name to change the password, logout or to lock the screen.

Figure 2.2: The menu bar.

The File menu

▤	**New workspace**	Create a new workspace. The new workspace is initialized with the default instrument settings.
📂	**Open**	Open workspaces,instrument settings, experiments, analysis templates, or data files.
💾	**Save**	Save workspaces, instrument settings, experiments, or analysis templates.
	Import FCS file…	Import data files of FCS format.
	Copy…	Transfer workspaces, instrument settings, experiments, analysis templates, data files (.mqd or .fcs files), log files, or other files (bitmap or excel files) to and from the MACSQuant Instrument.
	Print…	Print active analysis windows. Opens a **Print dialog box** (see section **Printing data files**). The default page range will include all active analysis windows. Using the option **Pages** allows for the print of specific analysis windows. The number of printed analysis windows per sheet can be selected under **Edit > Option > Software > Print** (see section **Print**
	Print selected...	Print selected samples in the sample list. For more information, please refer to section **Print selected windows**.
	Print all...	Print all samples in the sample list. For more information, please refer to section **Print all windows**.
	Analysis report	Generate a report of your analysis (PDF-A format).

The Edit menu

←	**Undo**	Undo the last action.
→	**Redo**	Redo the last action.

	Copy page	Copy the entire content of an analysis window to the clipboard.
	Copy plot	Copy a single selected plot/histogram.
	Delete region	Delete a selected region of interest.
	Ellipse	Draw an elliptical gate.
	Rectangle	Draw a rectangular gate.
	Polygon	Draw a polygonal gate.
	Quadrant	Define a quadrant.
	Interval	Define an interval.
	User settings…	Define user setting. Only available for administrators, see chapter **The Administrator**.
	Instrument settings…	Modify instrument settings.
	Rack...	Edit sample rack settings.
	Reagents...	Modify reagent settings.
	Options (default)	Customize global settings that apply to all users. Only accessible for administrators, see chapter **The Administrator**.
	Options...	Customize user-specific settings. Refer to chapter **The Custom mode user** for detail.
	Configuration…	When using MACSQuantify Software on a PC, use this option to designate the optical configuration that data files were or shall be collected.
	Calibration…	Define calibration settings.

The View menu

Hardware...	To view hardware settings. View setting for **Fluidics**, **Sample uptake unit**, **Lasers and detectors**, **Camera**, **Supplemental information**, or **System information**. The System information tab includes the following information:

Device: displaying the name of the instrument, the serial number, and the name of the institution. Refer to section **Instrument name**.

Counters: displaying available capacity of the hard drive, acquisition time (time after startup), number of measurements (number of finished measurements), and number of experiments (number of finished and running measurements).

Version: displays the number of the installed MACSQuantify Software version and the version of the installed Express mode packages.

Experiment table...	Provides a tabulated overview of experimental details. Overview of **Acquisition**, **Annotations**, **Autolabel** and **Settings**

The Mode menu

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

The Analysis menu

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

The Window menu

	New analysis window	Open a new analysis window using predefined templates. The currently visible window is the active window. The total number of opened windows and the active window are indicated in the upper left corner of the screen.
	Clone window	Create an exact copy of the analysis window.

	Close	Close an analysis window.
	Close all	Close all currently opened analysis windows.
	Previous window/ Next window	Scroll through open analysis windows.
	Window list	List all currently open windows. If multiple windows are opened, an individual window can be chosen for viewing.

The Help menu

	Open Help	Opens the MACSQuantify Help.
	Info…	Displays information about currently installed MACSQuantify Software version.

2.8.2   The toolbar

Frequently used buttons are located in the toolbar.

	1	Open, save, or print files by clicking the respective button.
	2	Create or delete gates.
	3	Open and close an analysis windows and flip through several analysis window pages.
	4	Activate or deactivate the **Analysis button** to toggle between acquisition mode (Analysis button is grey) and data analysis mode (Analysis button is orange).
	5	If several sample files are open, flip through the files by clicking the previous and next sample button.
	6	Click on the **Backup button** to save your data to a network location or to a (rewritable) DVD.
	7	Enter a reagent for autolabeling. Click the **barcode button** to activate the 2D code reader.

	8	Click the **Rack button** to edit Rack settings or the **Instrument settings button** to edit Instrument settings
	9	Opens n on-screen keyboard.
Help Express Mode Logout	10	Access the MACSQuantify Help, switch to Express mode, logout or shut down the instrument by clicking on the respective button at the far right of the toolbar.

2.8.3 The side panel

The side panel has four tabs: **Samples**, **Experiment**, **Tools** and **Channels**.

The Samples tab

Go to the Samples tab to view a list of open data files available for analysis. If a gating hierarchy was applied, it is also displayed.
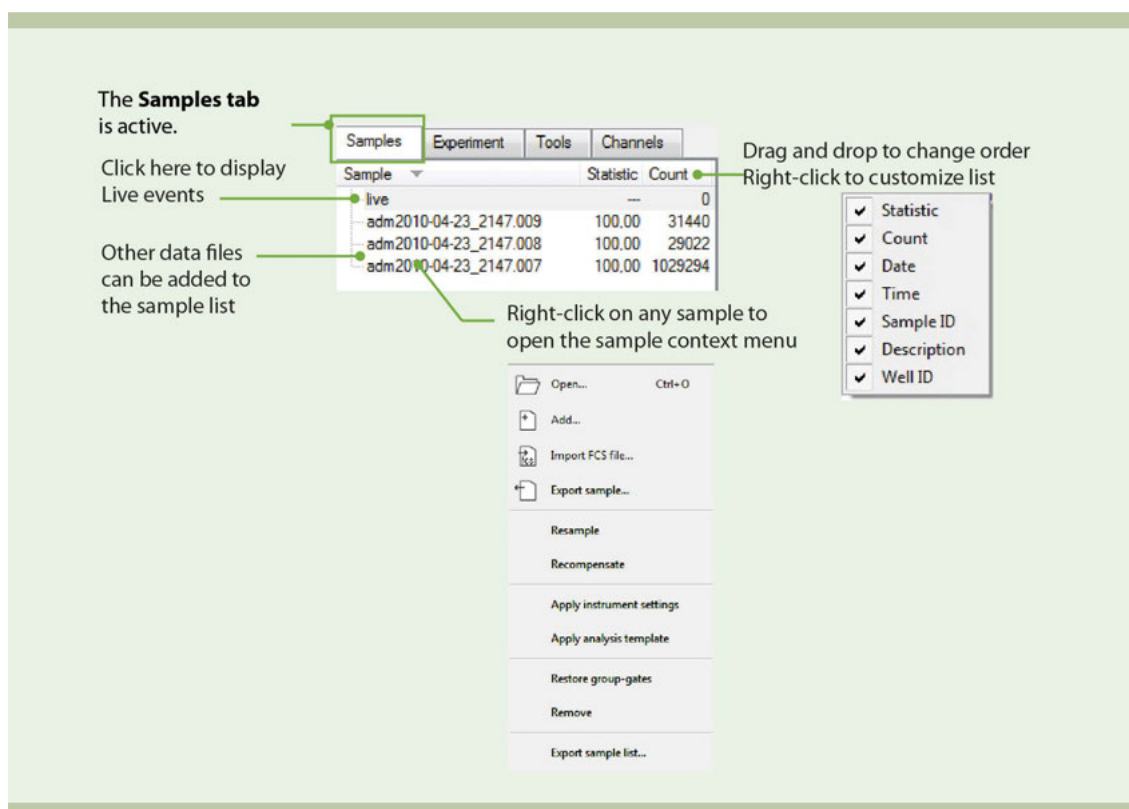
Figure 2.3: The side panel: Samples tab options.

The list contains the following columns: **Sample**, **Statistic**, **Count**, **Time**, **Sample ID**, **Description** and **Well ID**. Move the scrollbar at the bottom of the sample list to view all categories. Mouse-over to open a pop-up balloon containing the information of all categories.

Sample	name of the data file
Statistic	%-T

Count	number of events
Date	date of data acquisition
Time	time of data acquisition
Sample ID	as defined in the **Experiment tab**.
Description	as defined in the **Experiment tab**.
Well ID	as defined in the **Experiment tab**.

Customizing the sample list

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

The Samples tab

Samples tab context menu

Right-click on the samples list to access the context menu. The number and type of functions available in the samples tab context menu may vary. It depends on the samples included in the samples list and the number of samples selected. Refer to the table below for all samples tab context menu functions.

Open...	Ctrl+O
Add...	
Import FCS file...	
Export sample...	
Resample	
Recompensate	
Apply instrument settings	
Apply analysis template	
Restore group-gates	
Remove	
Export sample list...	

Open…	Open files such as Workspaces, Instrument settings, Experiments, Analysis templates, or Data files. For more information about opening files, see section **Open files**.
Add...	Only available if using MACSQuantify on a PC. Add samples to the samples list before open data files stored in an external storage location.
Import FCS…	Only available if using MACSQuantify on a PC. Import .fcs data files for analysis.
Export sample …	Export samples as .csv and .fcs format using the export sample function. For more information about exporting samples, see section **Export FCS files**.
Print all...	Batch print option only accessible in **Analysis mode**. Choose **Print all…** from the **Print dialog box** to print the current analysis template for all samples in the sample list. The current analysis has to be applied to all samples in the sample list prior to print.
Print selected...	Batch print option only accessible in **Analysis mode**. Choose **Print selected…** from the **Print dialog box** to print the current analysis template for the selected samples in the sample list. Ensure that the current analysis is applied to all selected samples in the sample list prior to print.
Group	Available if two or more individual samples are selected. Generates a group that contains all previously selected samples.
Ungroup	Available if grouped samples are selected. Ungroups a set of grouped samples and generates a separate file for each sample.
Resample	Display data on a scale that differs from the acquisition scale. This feature also allows the user to modify annotations if there were mistakes during sample acquisition. For more information about this feature, see section **Resampling: Change annotations**.
Recompensate	Change the compensation matrix in data that were already acquired. The results can be reanalyzed using a different compensation matrix, eg. if data were acquired using incorrect instrument settings. For further information, see section **Recompensation**.
Apply instrument settings	Apply the instrument settings that were run with a selected data file. For more information about using instrument settings, see section **Apply PMT voltage and compensation settings**.

Apply analysis template	Only available for files acquired with a MACSQuant Instrument. Right-click on a data file or files within the **Samples tab**. Select **Apply analysis template** to display the analysis template used during acquisition. The **Analysis Mode** is automatically activated when opening the analysis template. To modify the gating strategy or the display window of the analysis template, deselect the **Analysis Mode** and then modify as needed. For more information about analysis templates, see section **Apply analysis templates**.
View with Express Mode	Available if a sample that was measured using an express mode is selected. Starts the Express mode analysis.
Restore group gates	Restores original group gates from files. Existing gates will be maintained, added gates will be linked to all group members. Not available in **Analysis mode**.
Clear subgates	Available if a sample with associated gates is selected. Clears all assciated gates and reagions.
Remove	Remove selected samples from the sample list.
Export sample list	Export the sample list and associated statistics to Microsoft Excel. For more information, see section **Exporting the sample list to Microsoft® Excel**.

The Experiment tab

Under the Experiment tab, experimental parameters for sample acquisition can be defined. For further details on experimental setup, see chapter **Set up an experiment** .
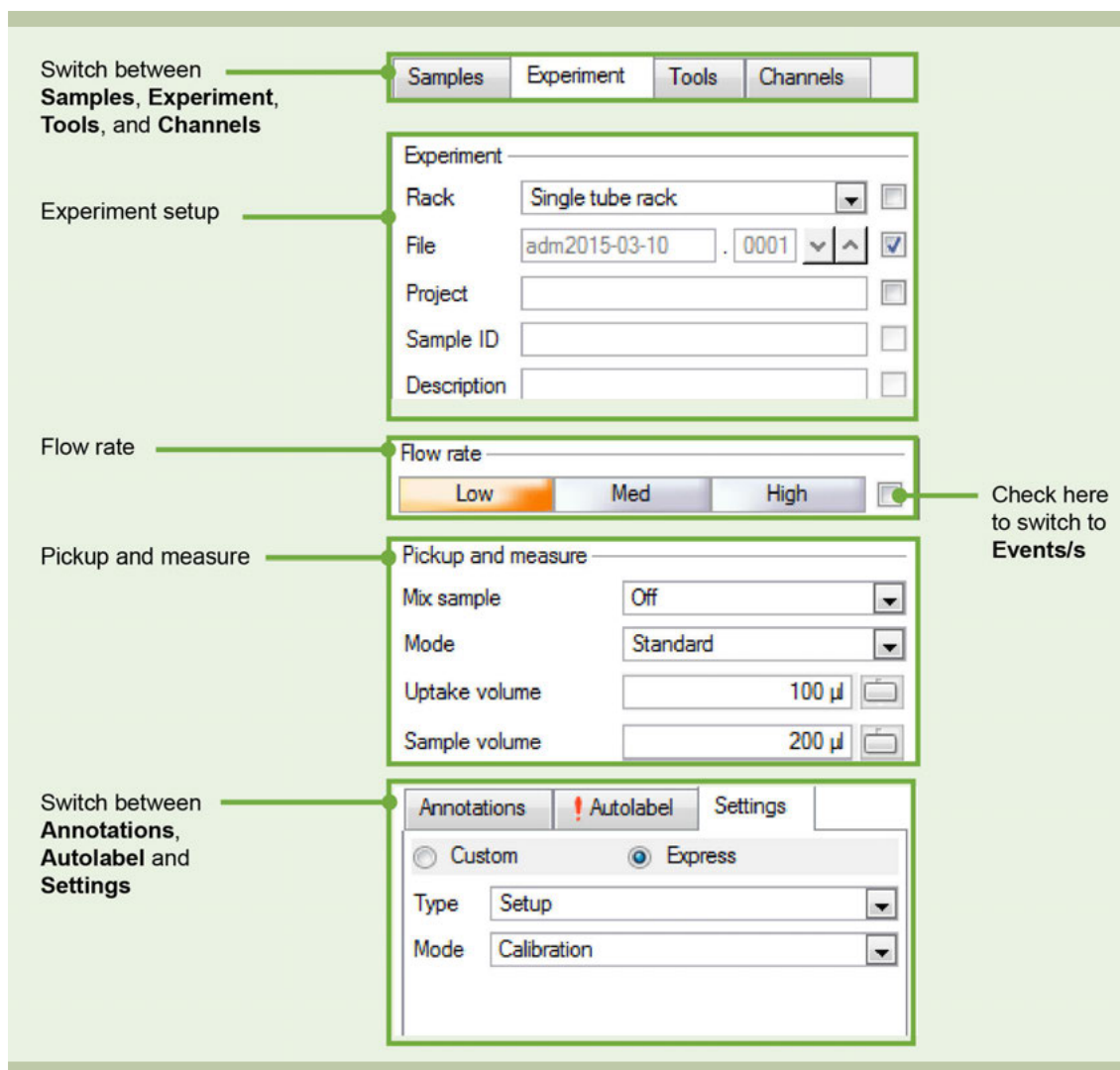
Figure 2.4: The side panel: Experiment tab options.

Experiment setup

Rack	Select a sample rack. Several racks are available for use with the MACSQuant Instrument. Choose the appropriate rack for your experiment from the drop-down list. Check/uncheck the checkbox on the right to enable/disable automated rack detection. For details, see the MACSQuant Instrument user manual.
File	Name of the current file. To generate the file name automatically, check the box on the right. To change the file name, uncheck the box and enter a new name. Use this naming field only when using the Single tube rack. The number of the file can also be changed; existing files with the same number will be overwritten. Avoid the forbidden Windows characters: ? / \ < > : * | ", plus special characters typed with the ctrl key. The following characters can be included in the file name: . - , ml ( ) &. The period . can only be used at the beginning of the file name, i.e. ".temp".
Project	Select a project folder where to save files. Check the checkbox to choose an existing project folder from the drop-down list. To create a new project folder, uncheck the box and enter a new name.
Sample ID	Enter alphanumeric name for the sample ID if desired.
Description	If desired, additional information can be added into this field.

Flow rate

Click on the respective mode button to choose the desired flow rate. MACSQuantify Software adjusts the flow rate depending on the number of acquired events per second. Activate the check box on the right and choose the desired number of events per second. A flow rate closest to the specified value is chosen automatically.
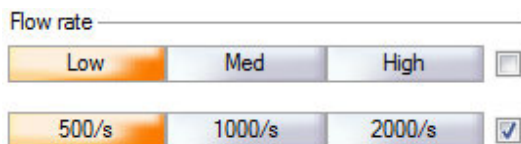
Pickup and measure

Mix sample	Activates sample mixing prior to analysis by pipetting up and down. **Uptake** and **Sample volume** need to be specified if mixing is activated.
Mode	Select the desired washing or enrichment mode from the drop-down list. For more information about the modes **Enrich.Measure Pos**, **EnrichS.Measure Pos**, and **EnrichS2.Measure Pos**, see section **Pre-enrichment programs**.
Uptake volume	Uptake volume defines the volume to be measured. Note that the MACS Quant Instrument picks up additional 9,92 μL of the sample if using Standard-, Extended-, Fast-Mode, Enrich.Measure Pos, EnrichS.Measure Pos, and EnrichS2.Measure Pos, and additional 19,92 μL if using the Screen-Mode.
Sample volume	Sample volume defines the volume in the vial, tube, or well.

Annotations, Autolabel and Settings

The Annotations tab – Set default channel annotations.

To set the default annotation values for the different channels, go to **Edit > Options > Instrument > Annotations**. Annotations can also be changed under the Experiment tab.

> **Note:** Annotations are automatically set when using selected MACS Reagents. Clearing the entries will set the annotations back to the default values.

The Autolabel tab – To activate automated Sample labeling.

Clicking on any **<add...>** will open the Reagents dialog box. In the Reagents dialog box, up to four reagents can be assigned to the Reagent Rack positions. If a reagent has been properly assigned to a sample, a red exclamation mark on the Autolabel tab indicates that autolabeling was activated.

For details on how to activate autolabeling, see section **The Reagents window**.
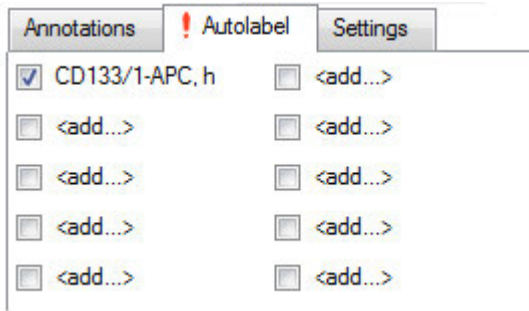
The Settings tab – Define Custom or Express instrument settings.

Chose between **Custom** or **Express** by activating the appropriate radio button.

- The Custom settings enable the user to combine predefined instrument settings, analysis templates and gates, and set event thresholds.

- Express settings can be used to select predefined express mode applications for either **Analysis** or **Setup**. If Setup was selected, the user can access Express modes for PMT calibration and compensation. If Analysis was selected, predefined Kits (e.g. MACSPlex Kits) can be selected from the **Mode** drop-down menu.

For further details on Calibration, see section **Automated calibration**

For further details on Compensation, see section **Automated compensation using the Express mode "Compensation"**.

For further details on CompensationMultiColor, section **Automated multicolor compensation**.

The Tools tab

Some tools in the tools tab are accessible for administrators, only. In the list below, asterisks mark administrator-only tools.
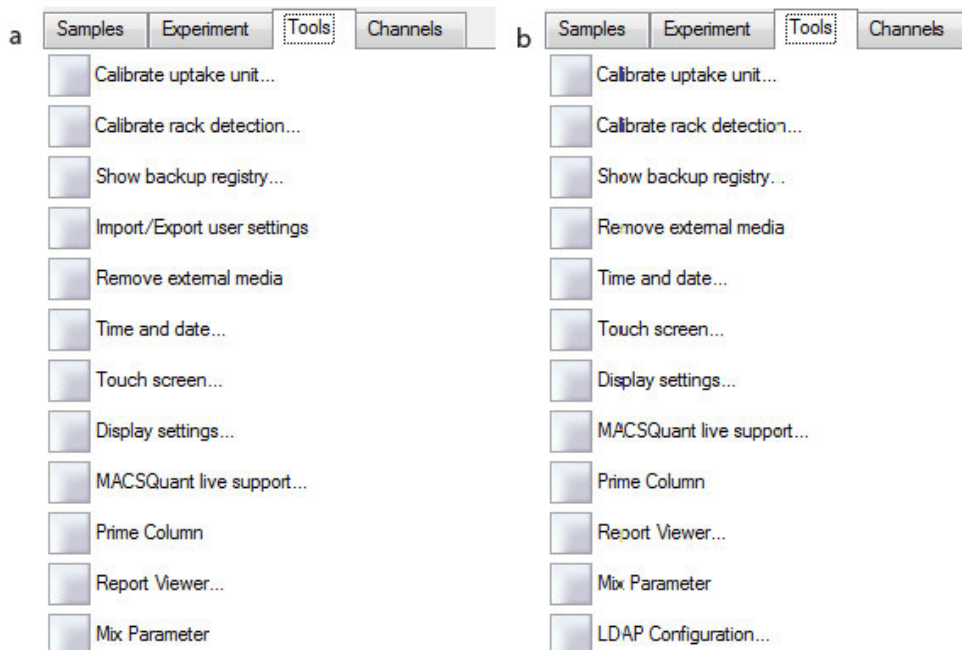
Figure 2.5: Tools available for administrators. Functions available for standard setup (a) or for CFR21 Part 11 compliant setup (b, refer to chapter **21 CFR Part 11 compliance** for details).

Calibrate uptake unit*

For details on uptake unit calibration, refer to the MACSQuant Instrument user manual.

Calibrate rack detection*

For details on rack detection calibration, refer to the MACSQuant Instrument user manual.

Show backup registry

To display backed up data. Information about previously backed up files is stored in a file called the backup registry. For details on data backup, refer to section **Designate a backup location** and section **Data Backup**.

Import/Export user settings*

To synchronize users when using multiple MACSQuant Instruments. Refer to section **Import or export user settings** for details. If the MACSQuantify software is installed and set up for 21 CFR Part 11 compliant use, this option is not available. Refer to chapter **21 CFR Part 11 compliance** for details.

Remove external media

To safely eject removable media from a MACSQuant Instrument to prevent data loss or corrupted data after writing to a USB stick.

> **Note:** The DVD drive, if ejected, will remain disconnected until the MACSQuant Instrument is restarted.

Time and date*

To set time and date. Refer to section **Set the time and date** for details.

Touch screen*

To adjust the touch screen. Refer to section **Touchscreen calibration**.

Display settings*

Adjust display properties of an external monitor. Seesection **Installation of an external monitor** for details.

MACSQuant live support

Opens a dialog box to start a MACSQuant Live Support session. Complete all fields and detail any queries using the Message/Questions box. Refer to chapter **MACSQuant® Live Support**.

Prime Column

Check the box to prime a MACSQuant Column for rare cell enrichment. For details, see chapter **Pre-enrichment** and also the MACSQuant Instrument user manual.

Report Viewer*

To track individual users. For details, see section **Report user activities**.

Mix Parameter

To configure sample mixing and change the number of times the sample is pipetted up and down.

> **Note:** Do not change this parameter while experiments are running.

LDAP Configuration*

Only available if the MACSQuantify Software is installed and set up for 21 CFR Part 11 compliance. To use an external LDAP system for user management, which is required for 21 CFR Part 11 compliance.

The Channels tab

The Channels tab can be used to view and modify optical channel settings. For further details, see section **Adjusting FSC and SSC gains** and section **Adjusting fluorescence channel gains**.

> **Note:** Channel settings can be also modified by clicking on the **Instrument settings button** on the toolbar.

2.8.4 The Instrument status bar

The current status (**1**) of the instrument is displayed in the **Instrument Status bar**. The Instrument status indicator changes its color depending on the current status. The **Clear button** (**2**) deletes the current data from the plot. The status of the current acquisition is also shown in detail (**3**). At the far right, the **Rinse button** (**4**) and the **Start button** (**5**) are located. Status bar buttons may be inactive (grayed out) during certain processes.

- Right-click on **Rinse** for the options **Clean** or **Flush**. Refer to the MACSQuant Instrument User manual for details on those programs.

- Right-click on **Start** to access the options **Skip** (skip to the next sample in line) or **Pause**.

2.8.5 The Instrument status indicator

🟢 **Green**		The MACSQuant Instrument is in **Acquisition mode** and ready for measurement.
🟡 **Yellow**		Cleaning and priming in progress. The MACSQuant Instrument is not available for measurement. If buffer or waste bottle needs to be changed, the respective information will appear in the status bar.
🟠 **Orange**		The MACSQuant Instrument is in **Data analysis mode**. Set the instrument to **Acquisition** for measurement.
⚪ **Grey**		The MACSQuant Instrument is initializing and not available for any measurement.
🔵 **Blue**		The MACSQuant Instrument is processing a sample.

3
Set up the instrument

⚠️ **WARNING** Read the chapter **Important Safety Information** in the MACSQuant™ user manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

Before running an experiment, be sure to set up the instrument properly. This chapter describes how to calibrate the instrument and adjust all necessary parameters for an optimal outcome of your experiments.

- Calibration of the photomultiplyer tubes (PMTs)
- Adjusting of forward and side scatter PMT gains
- Compensation for spectral overlap
- Setting primary and secondary triggers to exclude unwanted signals
- Addition of height and/or width (pulse processing)

All settings and adjustments can be stored in an **Instrument Settings file** or in a **bank**. Refer to section **Save Instrument settings** for details.

3.1 Photomultiplier tube calibration

The reproducibility and stability of the fluorescence signal over time is of vital importance. In order to ensure a stable measurement that is independent of time and instrument settings, the instrument needs to be calibrated. Fluorescence calibration curves are calculated based on the measurements of standardized fluorescence MACSQuant Calibration Beads with pre-defined size and fluorescence intensity. A linear regression equation is calculated from the instrument's response in mean or modal histogram channel values to these pre-defined values.

Use MACSQuant Calibration Beads (# 130-093-607) to calibrate the MACSQuant Instrument. MACSQuant Calibration Beads are a mixture of 2 µm-sized unstained beads and 3 µm-sized beads stained with fluorochromes emitting fluorescence in all channels. For further details, please refer to the data sheet of the MACSQuant Calibration Beads. As a quality control, the MACSQuant Instrument automatically adjusts voltage gains when performing photomultiplier tube (PMT) calibration with MACSQuant Calibration Beads to ensure that known fluorescent intensities are always set to the same channel.

> **Note:** It is recommended to calibrate the instrument every other day.

3.1.1   Automated calibration

1   Ensure that the Single tube rack is correctly attached and that the MACSQuant Instrument has been in the acquisition mode for at least 30 minutes.

2   Click the **Barcode button** (**1**) in the toolbar to activate the 2D code reader.

3   Scan the 2D barcode printed on the vial label of the MACSQuant Calibration Beads and follow the dialog box instructions.

4   Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates, dispense one drop into an empty tube, and place it in the Single tube rack.

5   Click **OK** to start calibration. The calibration beads are automatically diluted to a total volume of 500 µL. 150 µL of the diluted calibration beads are injected into the sample injection port. During calibration, the gain for each respective channel is automatically adjusted.

6   The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on a two-page (two-screen) report. Click the **Next window button** or **Previous window button** (**2**) to switch between the screens. Successful calibration for each channel is indicated by a green check mark. When the process is successfully completed, the MACSQuant Instrument Status bar reports **MACSQuant ready: Calibration OK**. All settings will be automatically saved as default settings.
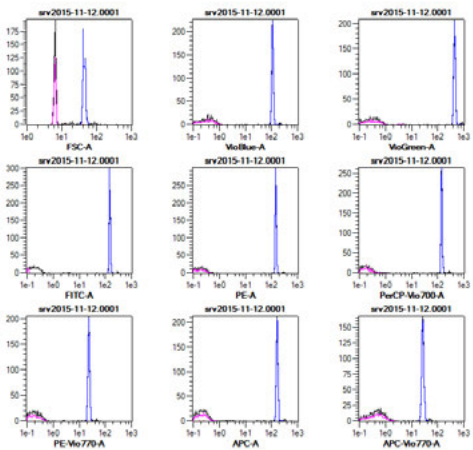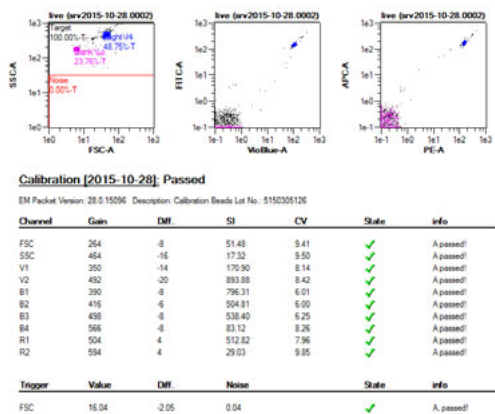
Calibration [2015-10-28]: Passed

EM Packet Version: 28.0.15096 Description: Calibration Beads Lot No.: 5150305126

Channel	Gain	Diff.	SI	CV	State	Info
FSC	264	-8	51.48	9.41	✓	A.passed!
SSC	464	-16	17.32	9.50	✓	A.passed!
V1	350	-14	170.90	8.14	✓	A.passed!
V2	452	-20	893.88	8.42	✓	A.passed!
B1	390	-8	796.31	6.01	✓	A.passed!
B2	415	-6	504.81	6.00	✓	A.passed!
B3	438	-8	538.40	6.25	✓	A.passed!
B4	566	-8	83.12	8.26	✓	A.passed!
R1	504	4	512.82	7.96	✓	A.passed!
R2	594	4	29.03	9.85	✓	A.passed!

Trigger	Value	Diff.	Noise	State	Info
FSC	16.04	-2.05	0.04	✓	A. passed!

7 The notification **Passed** in the statistic shows that the MACSQuant Instrument is well adjusted. If the MACSQuant Instrument displays **Incomplete** in the statistic and **Calibration recommended** in the status bar after performing the fully automated calibration, please check whether the number of Live events was set to at least 10,000 under **Edit > Options > Software > Acquire**.

3.1.2 Manual calibration
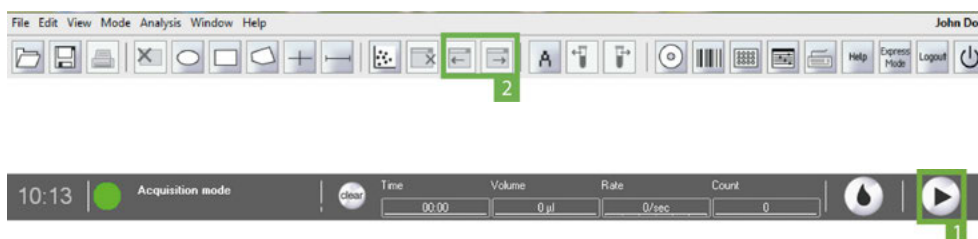
1 Ensure that the Single tube rack is correctly attached and that the MACSQuant Instrument has been in the acquisition mode for at least 30 minutes.

2 Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates, dispense one drop into an empty tube, and place it in the Single tube rack.

3 In the side panel, go to the **Experiment tab**. Under **Settings**, select **Custom**.

4 Go to the **Autolabel tab** to set the dilution and mixing of the calibration beads prior to calibration.

5 Click **<add…>** to open the Reagent dialog box.

6 Select **S1** Special and Running Buffer A, B or C and adjust the dilution appropriately.
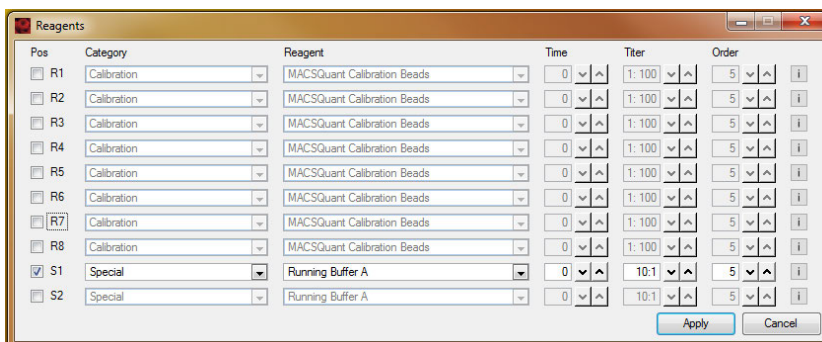
7 Set Time to 0 and Titer to 10:1, corresponding to a 10:1 dilution with no incubation time.

8 Close the Reagents box and check the box next to S1 Running Buffer A, B, or C.
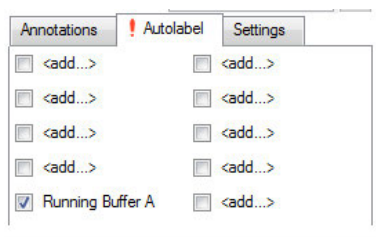
9 Select the **Settings** tab and select **Express**.

10 Under **Type**, select Setup. Under **Mode**, choose **Calibration**.
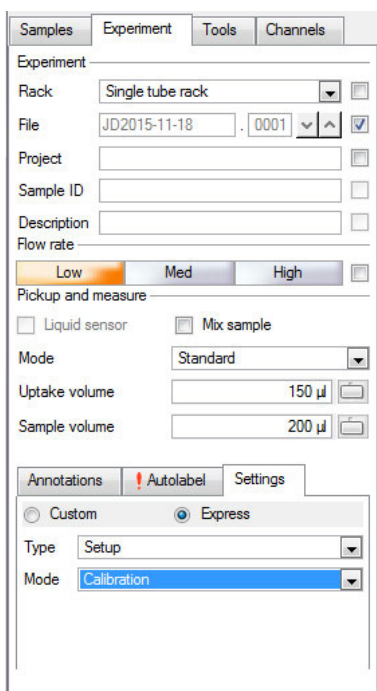
11 Enter a sample volume of 50 µL.

12 Click the **Start Measurement button** (1) in the instrument status bar to start calibration. The calibration beads are automatically diluted to a total volume of 500 µL. 150 µL of the diluted calibration beads are injected into the sample injection port. During calibration, the gain for each respective channel is automatically adjusted.

13 Upon completion, an analysis template will indicate that the calibration has passed. Voltage gain, staining index, and fluorescence histogram plots are displayed.

14 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on a two-page (two-screen) report. Browse between the different screens by clicking the **Next window or Previous window buttons** (2). Successful calibration for each channel is indicated by a green checkmark. When the process is successfully completed, the MACSQuant Instrument Status bar reports MACSQuant ready: Calibration **OK**. All settings will be automatically saved as the default settings.

15 The notification **Passed** in the statistic shows that the MACSQuant Instrument is well adjusted. If the MACSQuant Instrument displays **Incomplete** in the statistic and **Calibration recommended** in the status bar after performing the fully automated calibration, please check whether the number of Live events was set to at least 10,000 under **Edit > Options > Software > Acquire**.

3.2 Adjusting the PMT gains

After PMT calibration, it might be necessary to adjust the PMT gains so that they are appropriate for the cells or particles used and the expected signal intensities of the fluorescent positive populations. For analysis of most primary cells (e.g., human blood cells, mouse splenocytes), only FSC and SSC need adjustment, whereas the PMT values for the fluorescence channels will be appropriate for these cell types. An exception to this rule accounts for the autofluorescence of cells and resultant positive signals. However, when down regulating PMT gains from the calibrated values, there is the possibility of loosing the sensitivity for weakly stained antigens. Therefore, it is not recommended to adjust the PMT solely due to autofluorescence. Only adjust the PMT to bring the brightly positive events onto the display scale.
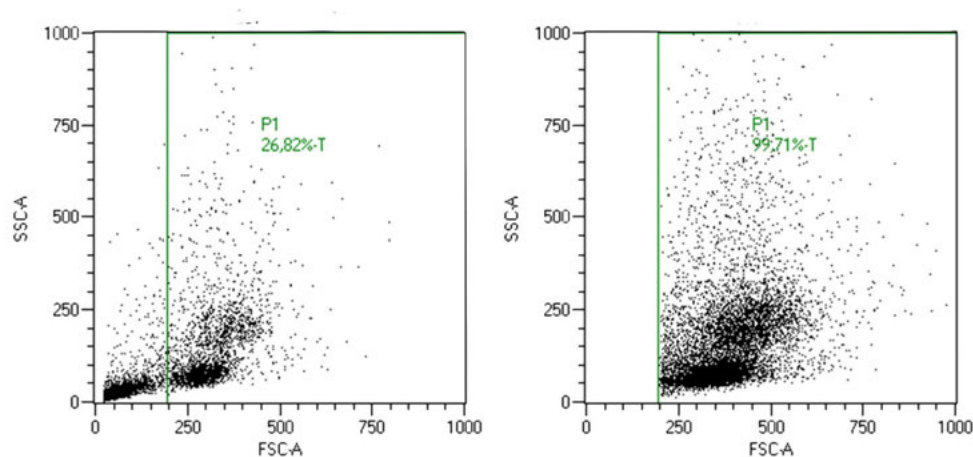
Figure 3.1: Adjustment of FSC and SSC PMT gains appropriate for PBMCs. Left: PBMCs prior to adjustment. Right: PBMCs after adjustment. A trigger has been set to exclude the debris.

3.2.1 Adjusting FSC and SSC gains
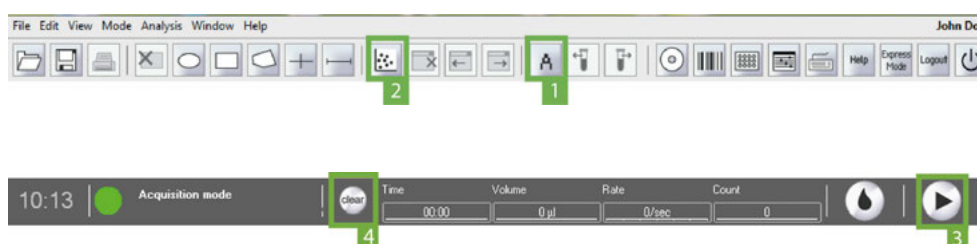
1 Perform PMT calibration if necessary.

2 Deselect the **Analysis mode button** (1) in the toolbar and close the PMT calibration template by clicking **Window > Close all**.

3 Go to the **Samples tab** and right-click on the file "live". Select **Clear subgates** in the context menu.

4 Click the **New analysis window button** (2) to open a new display window,.

> **Note:** The default plots display FSC-A and SSC-A.

5 In the **Experiment tab**, select **Single tube rack** from the **Rack drop-down list**.

6 Set an uptake volume.

7 Optional: select a project, add a sample ID, and chose a mixing level.

8 Place an unstained sample in the Single Tube Holder and start acquisition by clicking the **Start Measurement button** (3) in the instrument status bar. As events begin to accumulate, open the **Channels tab**.

9 Depending on where the desired population of cells is located on the FSC versus SSC plot, adjust the voltage gains for FSC and SSC to bring the population of events well into the dot plot for proper gating. Adjust gains by either:

- sliding the scroll bar up or down to increase or decrease the gains, respectively,
- activating the toggle bar by clicking on it and adjust the gains in increments of 10 V by clicking on the line above or below the scroll bar (recommended), or
- double-clicking the value box, typing in a value and pressing **Enter**.

10 After an adjustment has been made it is recommended to clear the previous events collected with the older voltage gains and only visualize events with the new settings. Click the **Clear button** (4) on the instrument status bar twice to refresh the events.

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

> **Tip:** If the desired population of events is unknown and FSC and SSC cannot be easily adjusted, it is recommended to use a cell sample that is labeled with a fluorescent marker to identify the cells of interest. This marker can be used for backgating to help determine the proper FSC and SSC adjustment. See section **Apply back gating**.

3.2.2 Adjusting fluorescence channel gains

During adjustment of the fluorescence channel gains, it is recommended to use a sample with both a negative and the positive population. It is necessary to keep both populations on scale and only make adjustment if one population is not on scale. In general, PMT adjustments for the fluorescence channels might be necessary if:

- using CFSE or other bright amine-binding dye,
- looking at high GFP or other fluorescent protein expression,
- brightly expressed antigens are stained a for detection in the V1, V2, or B1 channels in which autofluorescence is highest (i.e., for dendritic cells).

1 Following adjustment of FSC and SSC on an unstained sample, place a sample that contains a negative and positive population for each of the utilized fluorescent channels for the experiment (i.e., compensation controls like MACS Comp Bead Kit, anti-mouse Igκ # 130-097-900).

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

3 Under the Experiment tab, choose **Single tube rack** from the Rack drop-down list.

4 Select an uptake volume. Optional: select a project, add a sample ID, choose to mix sample.

5 Place a stained sample in the Single Tube Holder and start acquisition by clicking the **Start Measurement button** (1) in the instrument status bar. As events begin to accumulate, open the Channels tab.

6 Determine the position of the positive and negative events. Optionally, draw a gate around the appropriate population in the FSC versus SSC dot plot and display only these events in the plot with the fluorescence display. Choose the gate in the drop-down menu of the plot header.

7	In the **Channels tab**, adjust the gains up or down for the specific fluorescence channel as needed to get both the positive and negative population on scale. Adjust gains by either:

- sliding the scroll bar up or down to increase or decrease the gains, respectively,

- activating the toggle bar by clicking on it and adjust the gains in increments of 10 V by clicking on the line above or below the scroll bar (recommended), or

- double-clicking the value box, typing in a value and pressing **Enter**.

8	It is not recommended to only adjust the PMT down to have the negative population appear in the left hand most area of the plot. Making these adjustments may decrease sensitivity for weakly expressed antigens.

9	Continue with other fluorescence channels if necessary.

Note:	Adjustment to fluorescence channel PMTs must be made prior to applying any compensation.

3.3 Compensation for spectral overlap

Compensation essentially accounts for the inherent overlap in emission spectra observed between different fluorochromes. Due to fluorescence spectral overlap, or spillover, individual fluorochromes will be detected in more than one fluorescence channel. To compensate for this overlap is especially important when performing multicolor analysis. Without proper compensation, results may be misinterpreted.

For multicolor analysis, compensation of spectral overlap is necessary to produce accurate and consistent results. Compensation can be done automatically on the MACSQuant Instrument. The MACSQuant Instrument and MACSQuantify Software allow the user to set compensation adjustments in four different ways:

- Automated compensation using the **Compensation express** program

- Automated compensation using the **CompensationMultiColors** express program

- Manual compensation using the compensation matrix

- Offline recompensation after data acquisition

Multiparameter cell analysis allows the examination of multiple cellular properties by simultaneously detecting different fluorochromes, which identify these properties. The configuration of the MACSQuant Instrument allows the excitation and detection of up to eight different fluorochromes in individual detection channels. for the filter detection ranges of each fluorescence channel. If the combination of chosen fluorochromes have emission spectra in overlapping wavelength ranges, it is possible that a certain percentage of detectable light from one fluorochrome could be detected (or spill over) in an incorrect detection channel (e.g., FITC fluorescence should be detected in the B1 channel, but is also detected to a certain degree in the B2 (PE) channel).
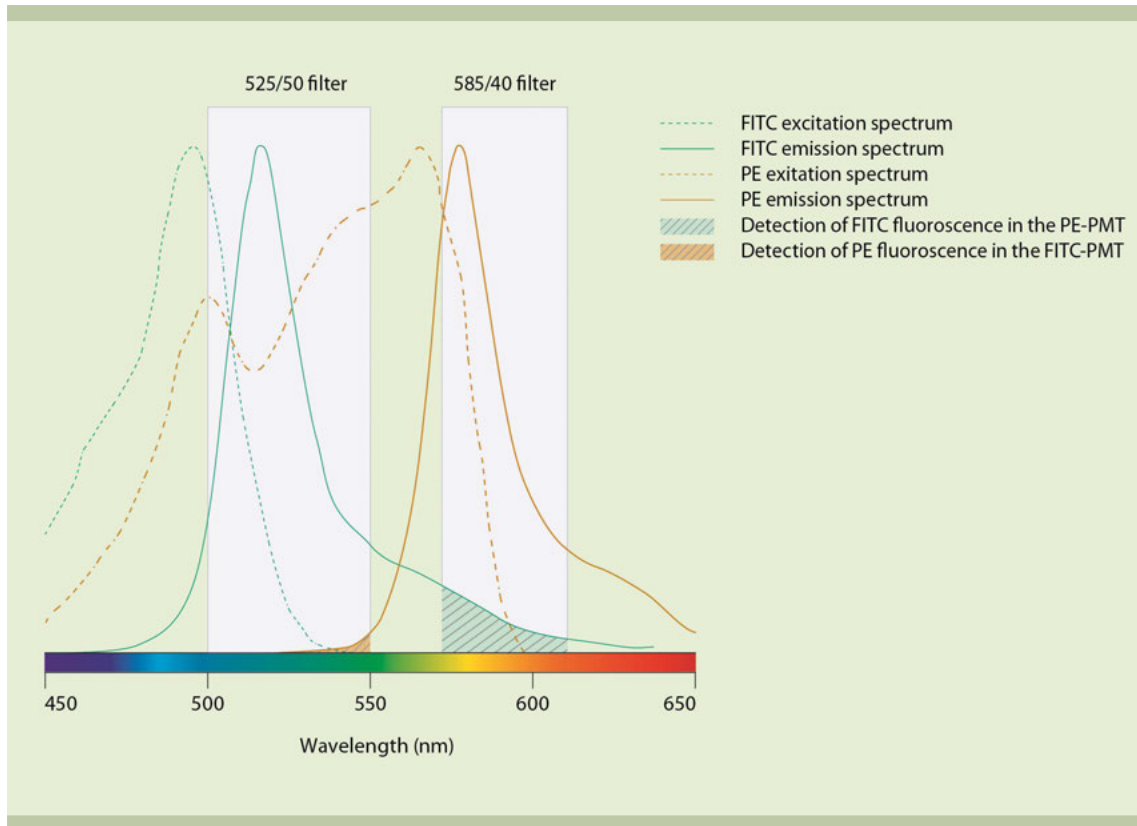
Figure 3.2: Emission spectra of FITC (green solid line) and PE (red solid line). The green hashed area represents the amount of FITC signal detected by B2. The red-hashed area represents the amount of PE signal detected by B1.

3.3.1   Example

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

With properly compensated instrument settings, the median fluorescence intensity detected in the spillover channel of the positive and negative population is equal.
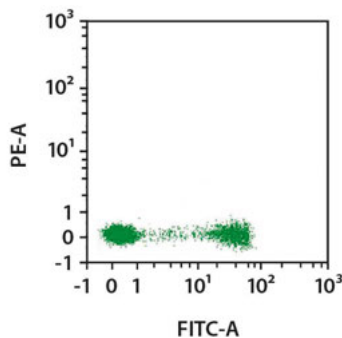
Figure 3.3: Compensated dot plot

When the instrument settings are undercompensated, the median fluorescence intensity detected in the spillover channel of the positive population is greater than that of the negative population.
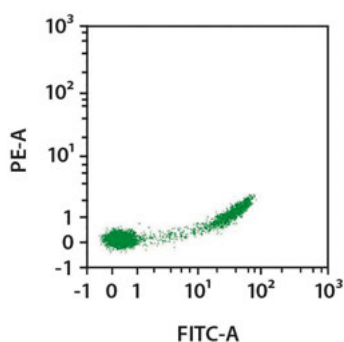
Figure 3.4: Undercompensated dot plot.

When the instrument settings are overcompensated, the median fluorescence intensity detected in the spillover channel of the positive population is less than that of the negative population.
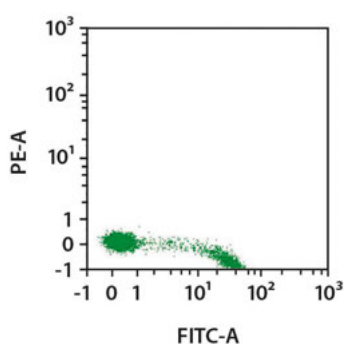
Figure 3.5: Overcompensated dot plot.

3.3.2   Compensation guidelines

For proper compensation, the use of single-stained controls is absolutely necessary. These controls can be cells, stained compensation beads, or antibody-capture compensation beads (e.g., MACS Comp Bead Kit, anti-mouse Igκ, (# 130-097-900)). When setting up and choosing single-stained controls for compensation, consider the following:

- Compensation should be performed using the same fluorochrome that will be detected in the experimental panel. For example, if FITC is used in the experimental staining panel, FITC must be used to set the compensation. Do not substitute with other fluorochromes that are detected in the same channel, such as GFP or AlexaFluor 488.

- The positive and negative populations within the sample must have the same autofluorescence or level of background fluorescence in the spillover channel.

- If antibodies used in an experiment vary from antibodies used for compensation, consider that fluorochrome-conjugated antibodies used for setting the compensation adjustments should display fluorescence intensity levels equal or brighter than the antibody used in the experiment.

- All changes to voltages of fluorescence PMT detectors must be made prior to adding compensation adjustments. Changing PMT voltages after compensation will change the spillover detected and will require reevaluation of compensation values.

- Compensation can be set using antibody-capture compensation beads. However, remember to not change PMT voltages after setting the compensation.

- The single-stained compensation controls must include a positive and negative population within the sample.

- It is best to aim for a positive population frequency of >10%. If using an antibody that detects a rare cell population, it might be possible to use compensation beads, a control cell line of similar autofluorescence

(meaning positive and negative signal remain on scale) or another antibody coupled to the same fluorochrome that labels a higher frequency of cells in the sample.

- If using tandem fluorochromes, the exact same lot from the same manufacturer must be used to set compensation adjustments. In these instances, compensation beads (e.g., MACS Comp Bead Kit, anti-mouse Igκ, (# 130-097-900)) or control cell lines can be useful. Do not mix lots of tandem fluorochromes.

- Proper compensation should not be performed using visual inspection of fluorescence intensity alone. Statistical analysis of the median fluorescence intensity for the spillover channel must be used to set the adjustments.

- Ideally, only compensate for the channels used in the experimental panels.

- Using hlog scaling for data display is preferred when visualizing compensated data.

3.3.3 Automated compensation using the Express mode "Compensation"

Materials required

- 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 tubes or 1.5 mL tubes

- Single tube rack

> **Note:** Mix the single stained cells into the tube shortly before starting the compensation. Do not store the cells before starting the compensation.

Protocol

1 Ensure that the MACSQuant Instrument has been properly calibrated.

2 Adjust all PMT voltages for the cell sample, e.g., FSC versus SSC.

3 Select **Edit > Calibration** from the menu bar.

4 Under the default tab, designate the channels that require compensation by choosing the proper lab from the drop-down list:

- **No** = No compensation required

- **Yes** = Compensate spillover out of this channel, but no fluorochrome is present in the compensation sample

- **Yes(P)** = compensate spillover out of this channel and compensate this fluorochromes signal from other channels

5 In the **Experiment tab**, choose **Single tube rack**.

6   Under the **Settings tab**, click the **Express radio button**.

7   Select **Setup** from the **Type** drop-down list.

8   Select **Compensation** from the **Mode** drop-down list.

9   (Optional) Enter sample ID, project folder, uptake volume etc.

10 Click the **Start Measurement button** (▶) in the instrument status bar.

11 The compensation program will use the current instrument settings from the MACSQuant Instrument for compensation. If the current instrument settings on the instrument are different than the default from that day, an informational dialog box will appear stating that the live settings are not the default set after calibration. Choose the default setting from that day or continue with the current settings.

12 When prompted, draw a region around the scatter population that defines the cells or beads used for compensation.

13 When compensation is completed, a dialog box will ask to save the instrument settings.

14 Choose either a **Public** or **Private** saving option and name the instrument settings.

3.3.4   Automated multicolor compensation

Materials required

- Single-stained controls representing all fluorochromes to be used in the experimental staining panel. Ensure that there is a comparable positive and negative population for setting compensation.

- One unstained sample for use as blank sample and another unstained sample for compensation against PI staining (optional).

- 12×75 mm round bottom tubes or 96-well plate

- MACS MiniSampler

- Chill 5 or Chill 96 Rack

Protocol

1 Ensure the MACSQuant Instrument has been properly calibrated.

2 Adjust all PMT voltages for the cell sample, e.g., FSC versus SSC.

3 (Optional) Open the instrument setting to start with the compensation.

4 Select **Chill 5 rack** from drop-down list in the **Experiment tab**.
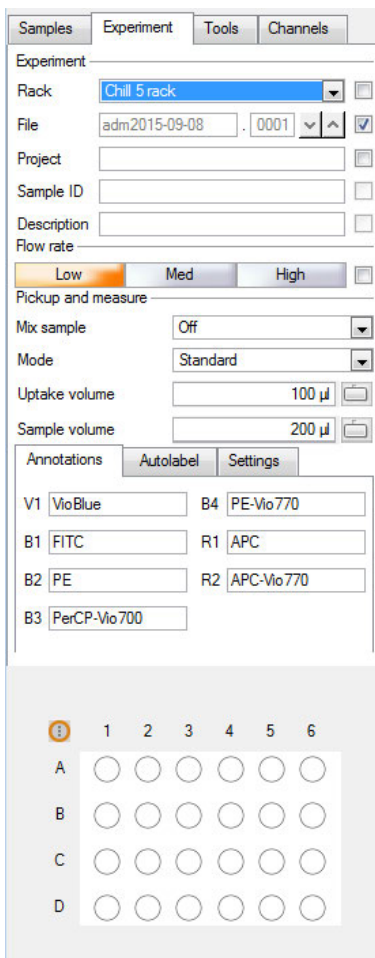
5 Click on the **Rack button** (1) in the toolbar.

6 In the Racks dialog box, the Chill 5 rack will be displayed. Select the appropriate number of sample positions to match the number of samples that will be used for compensation. See also section **Sample rack configuration**.
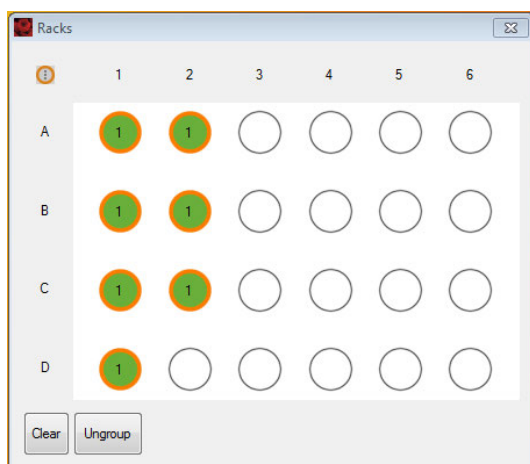
7 Click on the **Group button** at the bottom of the window. Each of the selected rack positions should now be labeled with the number 1.

Note: Only rack positions that are adjacent and in columns or rows can be grouped. See also section **Sample grouping**.

8 Choose **Express** from the Settings tab within the Experiment tab.

9 Select **Setup** from the Type drop-down list and **CompensationMultiColor** from the Mode drop-down list.
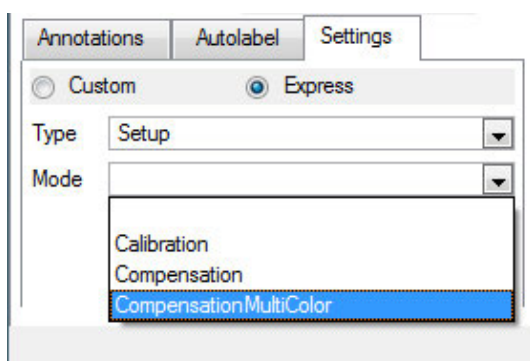
10 To set the fluorochrome for a rack position, highlight the respective circle in the rack window. Only one circle must be highlighted at a time. In the Sample ID field, a drop-down list will be available from which the representative fluorochrome can be chosen.

11 Each fluorochrome that appears in the drop-down list represents a specific detection channel. For example, PerCP-Vio770 represents the channel B3. Also when using another B3–compatible fluorochrome, e.g., PE-Cy5, PerCP-Vio770 must be selected. See also section **Optical channel annotations**.

12 The position **Blank** is selected for a sample of unstained cells. This is the reference for negative cells, which should always be included.

13 The position PI is selected, if propidium iodide (PI) will be used for dead cell exclusion in later experiments. It is used for compensating all fluorescence channels against the channel B3. This ensures that there is no fluorescence spillover into the B3 channel.

Note: For compensation, use unstained cells and do not add PI.

14 Place samples in proper rack positions and start the acquisition.

15 When prompted by the software, draw a region around the cells of interest (e.g. lymphocytes) in the scatter plot and click on the **Continue button**.

16 At the end of the process enter the desired name for the instrument setting file in the **Save window** and click **Save**.

Compensation MultiColor ExpressMode

EM Packet version: 28.0.15047

Compensation:

Name	VioBlue	VioGreen	FITC_COMP	PE	PerCP-Vio700	PE-Vio770	APC	APC-Vio770
V1:	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
V2:	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
B1:	0.000	0.000	0.903	0.016	0.000	0.000	0.000	0.000
B2:	0.000	0.000	0.080	0.797	0.000	0.000	0.000	0.000
B3:	0.000	0.000	0.017	0.186	1.000	0.000	0.012	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	0.987	0.000
R2:	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

### 3.3.5 Manual compensation using the 8×8 compensation matrix

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 tubes or 1.5 mL tubes

- Single tube rack

Protocol
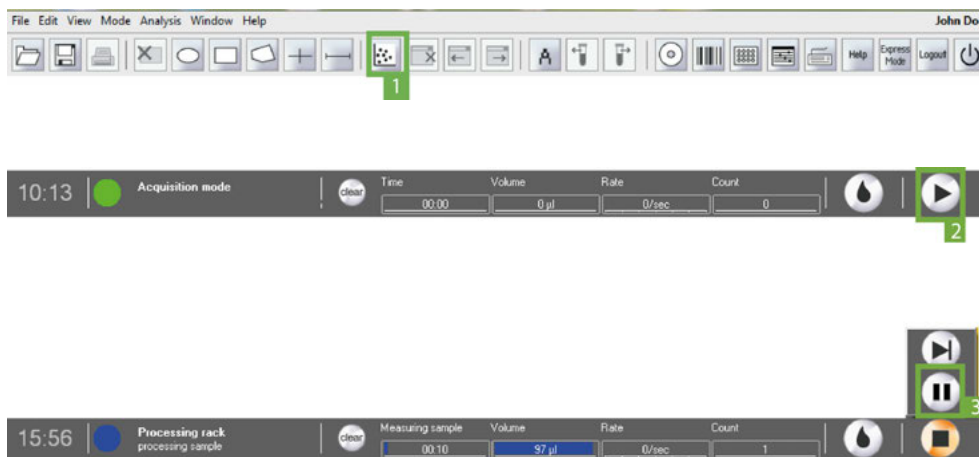
1 Ensure the MACSQuant Instrument has been properly calibrated.

2 Adjust all PMT voltages for the cell sample, e.g., FSC versus SSC.

3 Choose an analysis template (e.g., four dot plots) by clicking on the **New analysis window button** (1) in the toolbar. To change one of the dot plots into a statistics table, click on the **'i' button** next to of the plots and select **Statistic**.

4 In the left dot plot, set up to visualize FSC on the x-axis and SSC on the y-axis.

5 Set up one plot to display the channel of the stained particles (e.g., for FITC use B1) versus the channel where the spillover fluorescence should be corrected (e.g., B2).

6 In the **Experiment** tab, set all necessary parameters, e.g. Sample ID and Uptake volume.

7 Open the 8×8 (7×7) compensation matrix by clicking the Instrument settings button in the toolbar or under **Edit > Instrument settings** and selecting the **Compensation** tab (**Figure 3.6**).

8 Click the **Matrix** checkbox.

9 Place the first single-stained tube (e.g., FITC-stained cells) into the single tube holder.

10 Click the **Start Measurement button** (2) in the instrument status bar.

11 When events start to appear on the plots pause the measurement by right-clicking the stop button in the instrument status bar and choosing pause.

12 Draw a scatter region around the population of interest within the FSC versus SSC dot plot. This will be P1.

13 Display only events in P1 in the FITC versus B2 dot plot, by selecting P1 from the drop-down menu of the plot header.

14 Draw a region around the positive (P2) and negative (P3) populations (**Figure 3.6** B).

15 (Optional) To rename P2 and P3 regions to positive and negative, respectively, right-click on the region and select region properties.

16 In the statistics table, format properties under the button. Select to display P2 and P3 regions under the . Select to display the median of all the spillover fluorescence channels (e.g., for FITC spillover, display V1, V2, B2, B3, B4, R1, and R2… although not all require compensation).

17 Resume measurement by clicking on the **Pause button** (3) in the instrument status bar.

18 To add compensation to the combination of channels in the plot, choose the appropriate cell in the matrix (**Figure 3.6**). The columns represent the measured fluorochrome, the rows represent the detection channels, where the spillover fluorescence should be corrected. For example, to compensate a FITC-stained sample against the PE channel, go to the cell FITC/B2 and adjust the value to achieve equal intensities for the positive and negative populations.

19 Adjust the matrix values choosing one of the following options:

- adjust the scroll bar up or down to increase or decrease the gains, respectively, or,

- activate the toggle bar, by clicking on it and adjust the gains in increments of 0.01 by clicking on the line above or below the scroll bar (recommended), or

- double-click the value box, type in a value and click **Enter**.
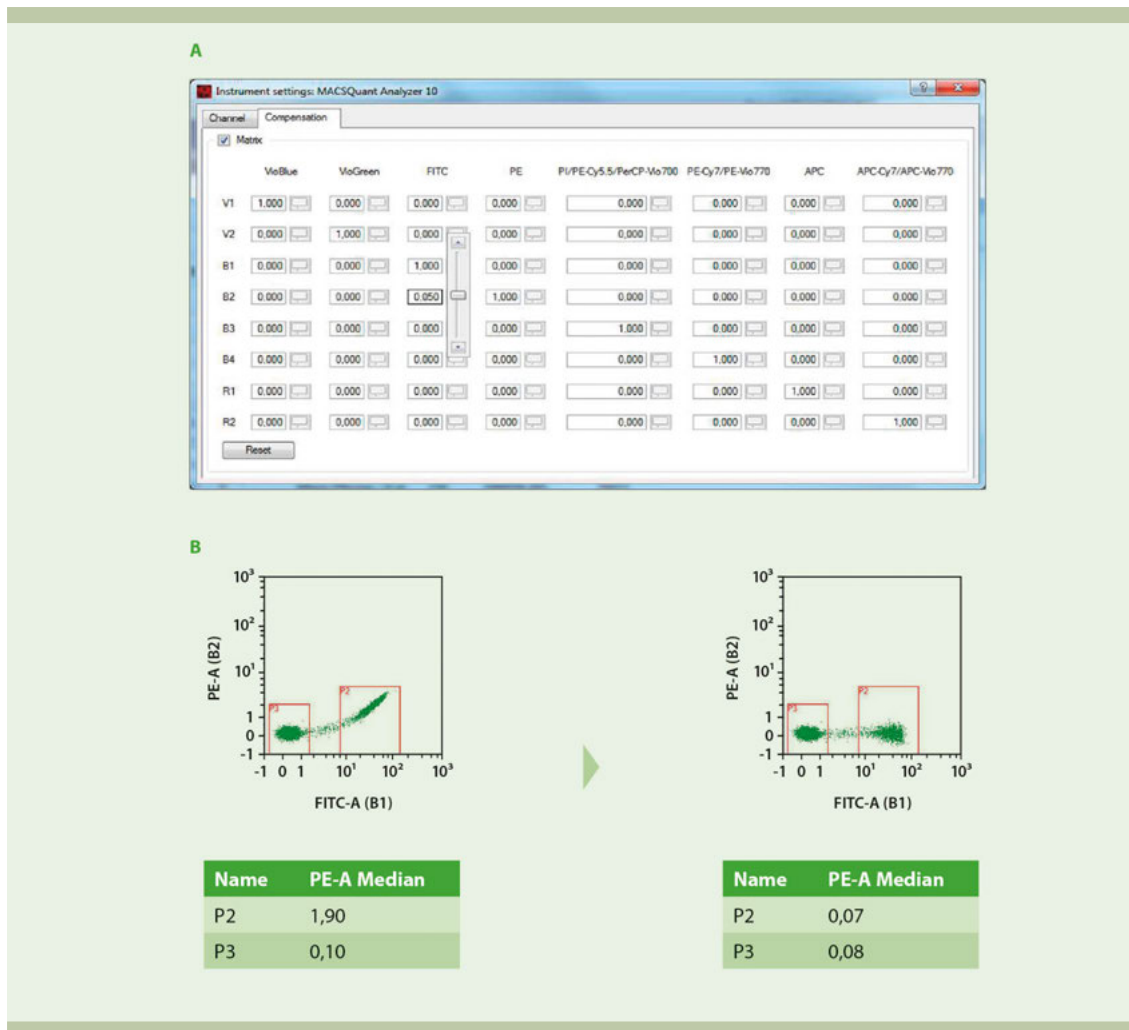
Figure 3.6: Manual compensation of the FITC spillover signal in the B2 channel. (A) Adjustment of compensation values in the compensation matrix. Here, the value of the cell FITC/B2 is lowered to diminish spillover of the FITC signal into the B2 channel. (B) Left column: Dot plot of a FITC-stained sample prior to compensation. The higher PE Median for region P2 in the statistics table indicates a spillover of the FITC signal into the PE channel. Right column: Dot plot of the FITC-stained sample after compensation. Equal PE Median values in the statistics table indicate that there is no signal spillover.

20 Values should continue to be adjusted until the median fluorescence values for the positive and negative populations are equal for the spillover channel (**Figure 3.6** B, right plot).

21 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.

22 Adjust values for other spillover channels, if necessary.

23 Once compensation is adjusted for this fluorochrome, repeat compensation for all additional fluorochromes.

3.3.6   Compensation matrix

MACSQuantify Software uses a compensation matrix, depending on the number of fluorescence channels of the respective MACSQuant Instrument. This matrix organizes the detection of individual fluorescence signals by columns. Column headers identify representative fluorochromes detected on the MACSQuant Instrument. The rows indicate the fluorescence channels. The default value is set to 1.0 in the diagonal (column versus row). This position within the matrix indicates the primary detection channel for the respective fluorescence. The default value for all other positions is 0.000.
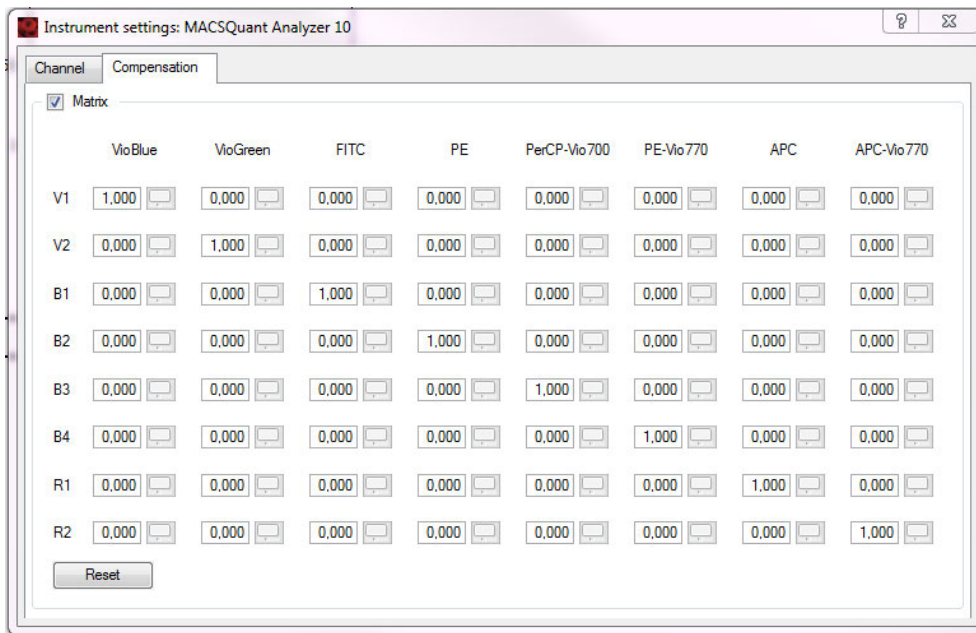
Figure 3.7: The 8×8 compensation matrix of the MACSQuant Instrument.

When a level of spillover is detected with single-stained samples, values between 0 and 1 are inserted into the spillover detection channel to set the compensation adjustments. As values are added into the various spillover channels, the same value should be subtracted from the primary detection channel, thus the entire value within the column should equal 1.0 or 100% of detected signal. To adjust values in the compensation matrix choose the following options:

- adjust the scroll bar up or down to increase or decrease the gains, respectively, or
- click on the toggle bar and adjust the gains in increments of 0.01 by clicking on the line above or below the scroll bar (recommended), or
- double-click the value box and type in a value followed by pressing **Enter**.
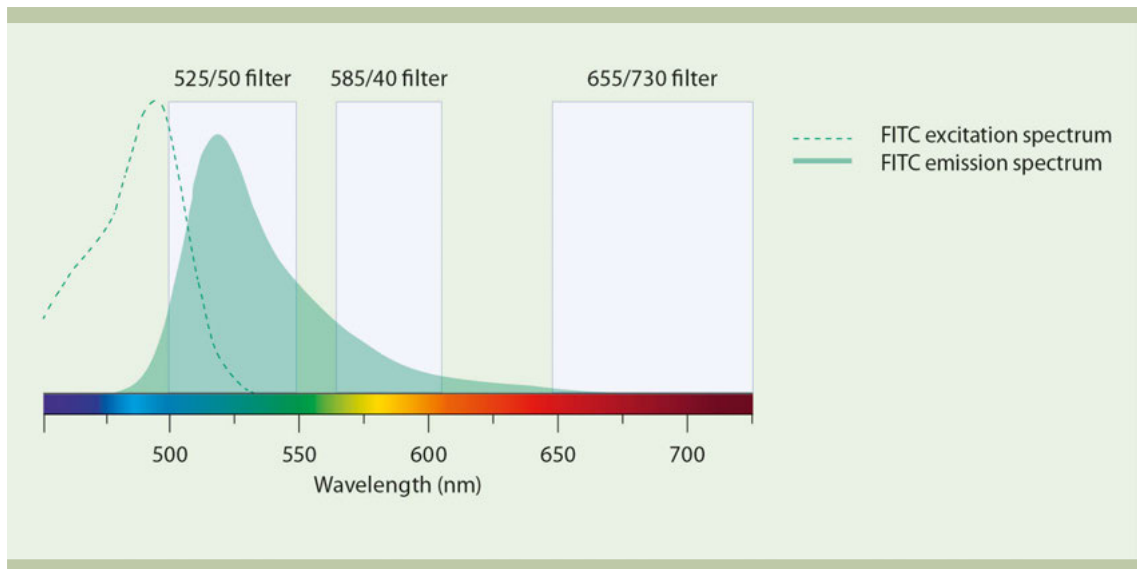
Figure 3.8: Spectrum of FITC single-stained cells. The green area corresponds to the B1 (525/50) channel; the yellow area corresponds to the B2 (585/40) channel, and the red area corresponds to B3 (655–730) channel. In this example, spillover into the B2 (PE) channel of FITC single-stained cells was determined with 15%. In other words, 15% of the detectable FITC signal was seen in the B2 (PE) channel. To compensate this spillover, a value of 0.150 would be added to the according box (FITC-B2) of the compensation matrix, and the box FITC-B1 would be adjusted from 1.000 to 0.850. If additional spillover of the FITC signal would also be found in the B3 (PE-Cy5) channel, values would be adjusted accordingly in box FITC-B3.

3.3.7 Offline compensation

Recompensation can be performed on any file acquired on the MACSQuant Instrument using the MACSQuantify Software. To start recompensation, ensure that the instrument settings used with the file of interest are loaded into the software.

1 Choose the file from the sample list in the **Samples tab** of the side panel.

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

3 Click the **New analysis window button** (1) and open an analysis window (e.g.**'Plot3'**). Open the file.

4 In the left dot plot, set up to visualize FSC on the x-axis and SSC on the y-axis.

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

6 Set up to visualize the first fluorochrome requiring compensation (e.g., FITC) in the histogram.

7 Open the compensation matrix by clicking the **Instrument settings button** in the toolbar and selecting the **Compensation tab**.

8 Draw a scatter gate around the population of interest within the FSC versus SSC dot plot. This will be P1.

9 Display only events in P1 in the histogram plot, by selecting P1 from the drop-down menu of the plot header.

10 Draw an interval around the positive (P2) and negative (P3) populations.

11 (Optional) Rename P2 and P3 regions to positive and negative, respectively, by right-clicking on the region and selecting **Region properties**.

12 Click the **'i' button** to format statistics table properties . Go to the **Region functions tab** to display P2 and P3 regions (✔). Select to display the median of all the spillover fluorescence channels (e.g., for FITC spillover, display V1, V2, B2, B3, B4, R1, and R2… although not all require compensation).

13 Enter values in the box within the compensation matrix by using the associated slider. For example, compensating FITC signal from the PE detection channel, the value in the FITC/B2 cell should be adjusted. Once the value has been entered into the matrix, select the file to be recompensated from the sample list.

14 Right-click on the file and choose **Recompensate**. A new file will be created with an underscore (_) in front of the file name. To view the newly created file, click the **Analysis mode button** (2) and then double-click on the recompensated file. All gates and statistics will be applied to the new file.

15 Values should continue to be adjusted until the median fluorescence values for the positive and negative populations are equal for the spillover channel.

16 To avoid creation of multiple files, continue to apply the recompensation to the original file (file without underscore). This will rewrite the second file (with an underscore in front of the file name), and changes will be immediately visible on the analysis page.

3.4   Setting a trigger

Triggers, or thresholds, are used to instruct the instrument as to what to consider a cell or particle of interest. When a trigger value is used on a specific detection channel, the instrument will ignore particles that generate smaller voltage pulses and only collect particles that generate voltage pulses greater than the selected trigger value. Commonly, a value for FSC or SSC is set as a trigger to exclude debris from the sample.
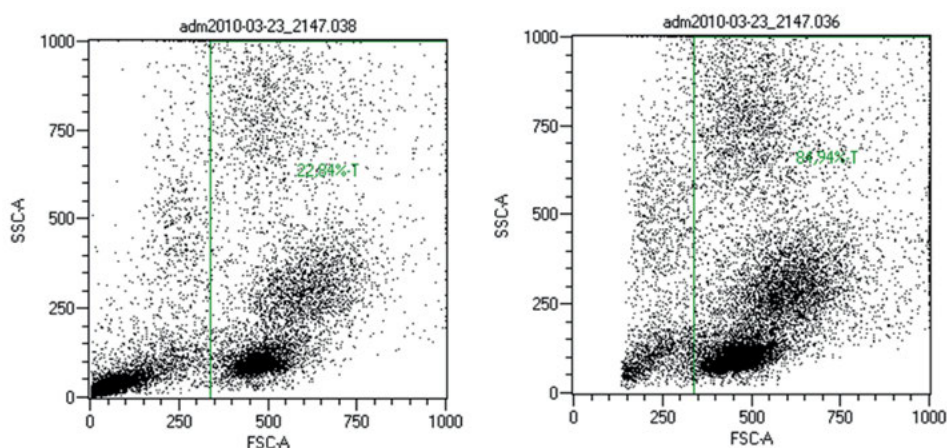
Figure 3.9: Trigger to exclude debris. Left: Debris represents 77% of sample. Right: After setting a trigger, debris represents 15% of sample.

3.4.1   Selecting and adjusting a primary trigger
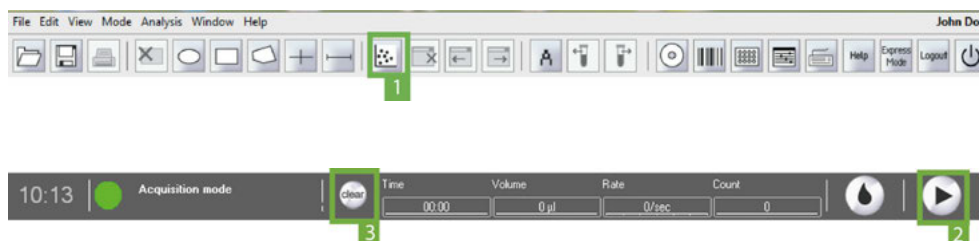
Any optical parameter (FSC, SSC or fluorescence) can be used for a primary trigger. Using a fluorescence channel as a trigger can be very helpful in applications where a large number of undesirable events can be distinguished by the lack of a fluorescence marker, i.e., triggering on CD45 expression to exclude red blood cells and platelets.

1 Go to the **Channels tab**.

2 Activate the radio button to the right of the desired channel (FSC, SSC or fluorescence).

3 Click the **New analysis window button** (**1**) to open an analysis window and format a plot to view the desired parameter, e.g., FSC versus SSC, if setting an FSC trigger.
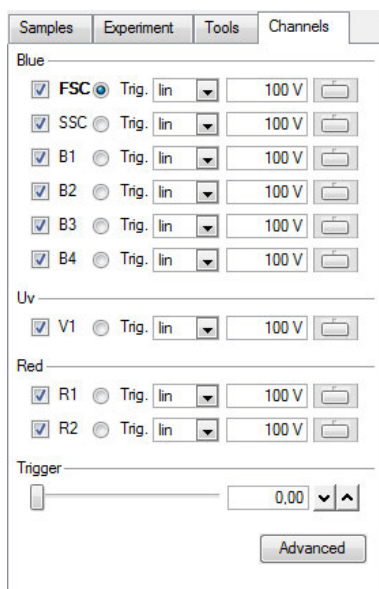
4 Click the **Start Measurement button** (**2**) to start data acquisition. As data become visible on the plot, adjust the trigger value in the **Channels tab**.

5 The trigger can be adjusted by:

- sliding the Trigger toggle bar right and left to increase or decrease the value, respectively, or,

- clicking on either side of the toggle bar to adjust the value in increments of 5, or,

- double-clicking inside the value box. (type in the desired value, then click **Enter** to apply), or

- use the up and down arrows to increase or decrease the value by increments of 1.

6 Click the **Clear button** (**3**) in the instrument status bar to remove all events acquired with the old trigger and refresh the plot to visualize only the newly triggered events.

7 By default, the voltage parameter **Height** is used for triggering. If desired, switch to **Area** before data acquisition by clicking the **Advanced button** in the Channels tab and checking the box **Use area for triggering**.

3.4.2 Selecting and adjusting a secondary trigger

If a fluorescence parameter is chosen as a primary trigger, FSC or SSC can be designated as a secondary trigger. The secondary trigger, however, cannot be adjusted during an acquisition, so the correct value must be pre-determined and entered.

1 To set the FSC or SSC trigger value, follow the steps described in section **Selecting and adjusting a primary trigger**.

2 Once the value has been determined for FSC or SSC, stop the acquisition and open the Channels tab. Click on the **Advanced button**.

3 Under **Trigger settings**, check the box **Secondary trigger**. From the drop-down list, select either **FSC** or **SSC** and enter the pre-determined trigger value.

4 Click **OK**. The secondary trigger appears in bold in the Channels tab.
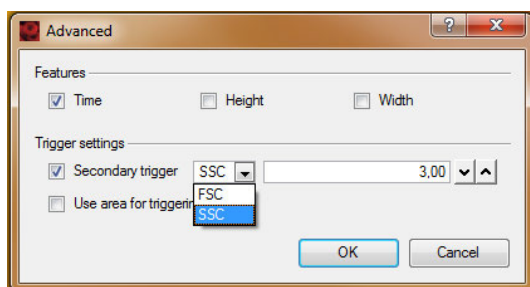
5 Now that the secondary trigger has been set, the primary trigger can be selected and adjusted as previously described.

3.5 Pulse processing

As cells pass the laser beam, their fluorescence signal generates a voltage pulse by the PMT. 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. In the MACSQuant Instruments, three voltage measurements are available for all optical channels.

- **Height** is the peak voltage intensity.
- **Width** equals the time of a particle traveling through the laser path. It is also called "time of flight" and can represent the size of the particle.
- **the Area under the curve** represents the light scatter emitted by the particle.

For digital instrumentation, like the MACSQuant Instruments, the pulse area is the default parameter for fluorescence 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, these parameters must be selected prior to measurements to be saved in the data file.
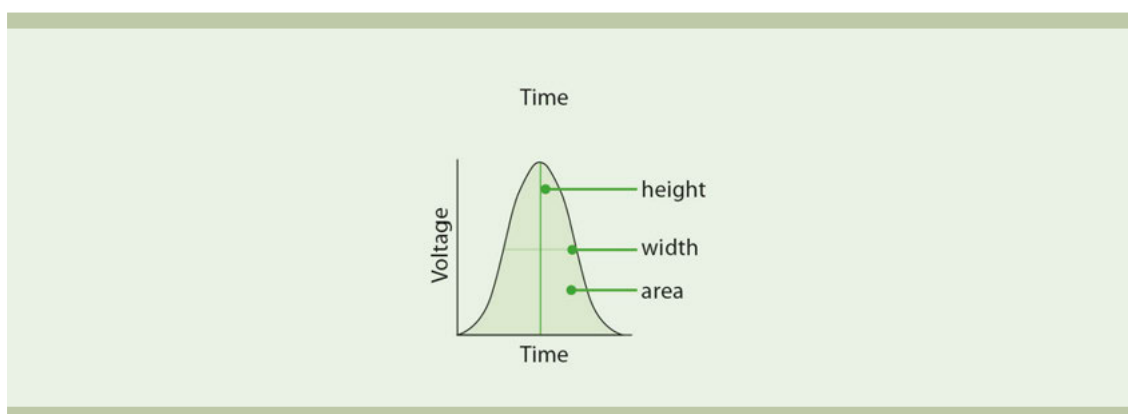
Figure 3.10: Three different parameters can be used to measure a voltage pulse: Height, width or area.

3.5.1 Selection of height and width as part of an instrument setting

1 Go to the **Channels tab** in the side panel. Alternatively, click on the **Instrument settings button** in the toolbar or go to **Edit > Instrument Settings**.

2 Click on the **Advanced button**.
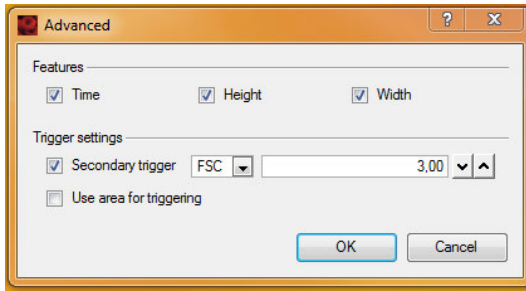
3   Select **Height** or **Width** by checking the box next to the desired parameter.

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

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

Note: If a saved instrument setting or bank-dependent instrument setting is used, it is advised to first open this setting as the active setting on the instrument (see section **Open Instrument settings**) and then activate height or width as additional parameters.

3.6   Adjusting data display scales

The MACSQuantify Software offers various options to scale data to properly visualize data.MACSQuant Instruments can display data in a variety of formats. FSC and SSC can be displayed in lin, log2, log3, and hlog scale. All fluorescent channels (V1–2, B1–4 and R1–2) can be displayed in lin, log2, log3, log4, log5, and hlog scale.

lin – Linear scale
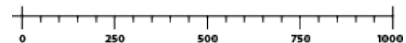
log2 – Logarithmic scale of two decades
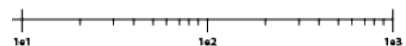
log3 – Logarithmic scale of three decades

log4 – Logarithmic scale of four decades
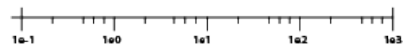
log5 – Logarithmic scale of five decades

hlog – Biexponential scale

The choice of scale to visualize data depends on the nature of the fluorescent signal, the difference between positive and negative signals, and user preferences. Standard dot plots showing side scatter and forward scatter are typically displayed using linear scales, as different cell populations often do not vary a lot in their light scatter signal intensity. In some instances, a logarithmic scale is useful, for example, when analyzing cell lines or samples that include very large particles (e.g., CHO cells, compensation beads) as well as small particles (e.g., bacteria, microvesicles) that need to be analyzed simultaneously.

Linear scaling is often not appropriate when displaying fluorescence intensity of different cell populations. Fluorescence signal intensities that extend over several orders of magnitude have to be displayed in a logarithmic scale.
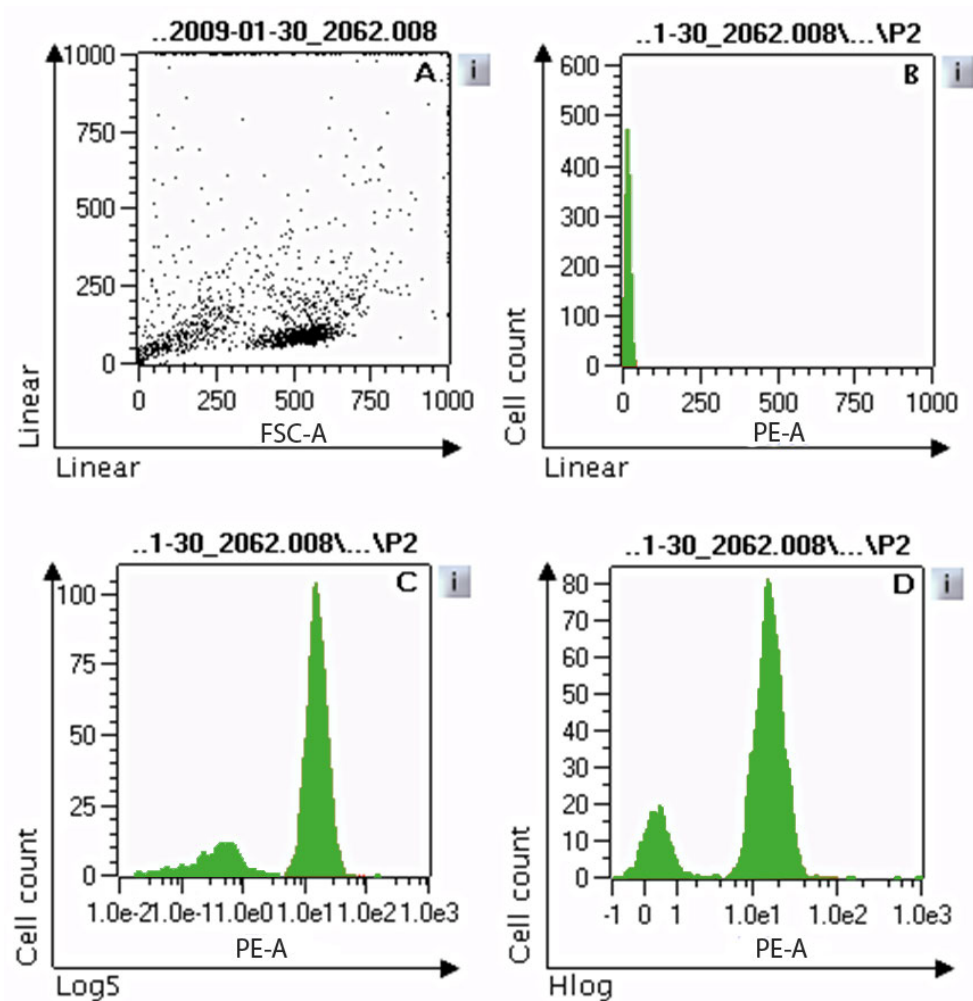
Figure 3.11: Comparing log and linear scales when displaying fluorescence intensities of fluorochrome-conjugated antibody binding. (A) Light scatter dot plot in linear scale. (B) PE fluorescence intensity in log5 scale. (D) PE fluorescence intensity in Hlog scale.

A sample containing a population of white blood cells was labeled with an antibody conjugated to the fluorochrome PE. A linear scale was used to display a FSC versus SSC dot plot (**Figure 3.11** A). PE fluorescence intensity was plotted against the cell count and depicted as a histogram with a linear scale (**Figure 3.11** B). The signal intensities of non-fluorescent cells and PE-labeled cells are not separated. To separate the signals, a log5 scale was used (**Figure 3.11** C), revealing two peaks. The left peak is attributable to background fluorescence, whereas the right peak is due to cells labeled with PE.

Because the MACSQuant Instrument acquires data in a digital format, some fluorescence intensities may be below zero. As digital processing allows the assignment of true zeros and negative fluo-rescence values, data values below zero may not be displayed properly using a conventional logarith-mic 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 (**Figure 3.11** D).

Occasionally, the difference between fluorescence values in a dataset can be relatively small, e.g. 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 (**Figure 3.12**). However, in most cases fluorescence intensities of stained populations within a sample span over several orders of magnitude, and therefore a logarithmic or hlog scale is recommended.
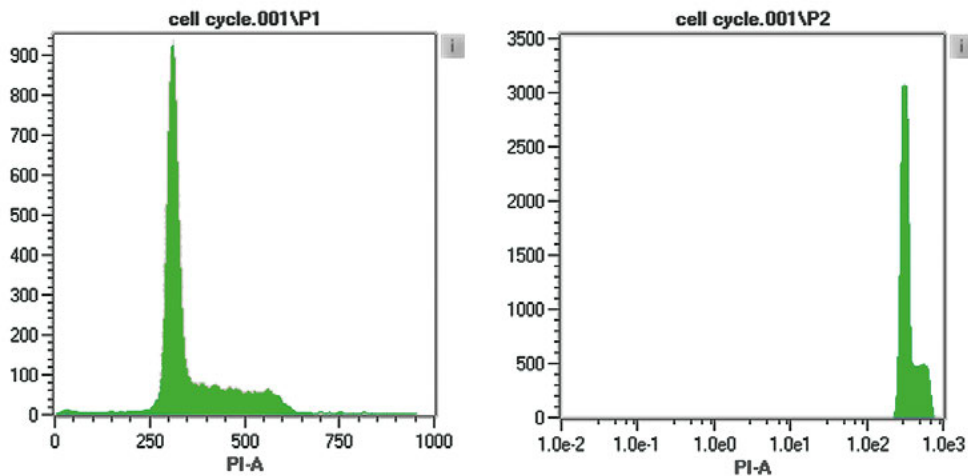
Figure 3.12: Visualizating quantitative changes to cellular DNA during cell cycle. The DNA was labeled with propidium iodide (PI). A linear scale (left) and a log5 scale (right) are used to display the fluorescence intensity. The subtle changes can only be visualized with a linear scale.

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

Note: Scales can be also adjusted after acquisition. For details, refer to section **Resampling: Change scales**.

1   To change the data scaling for the specific channel, go to the **Channels tab**.

2   Change the display scale by selecting the respective scale from the drop-down menu next to the specific channel.
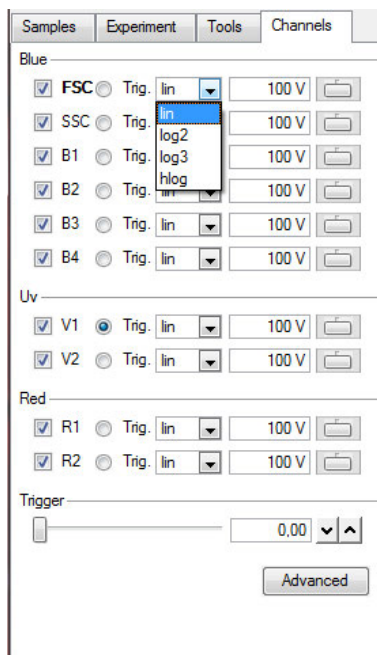
Alternatively, use the Calibration settings dialog box:

1 Click **Edit > Calibration** to display the dialog box.

2 Go to the **Default tab** and choose the default scale for each parameter.
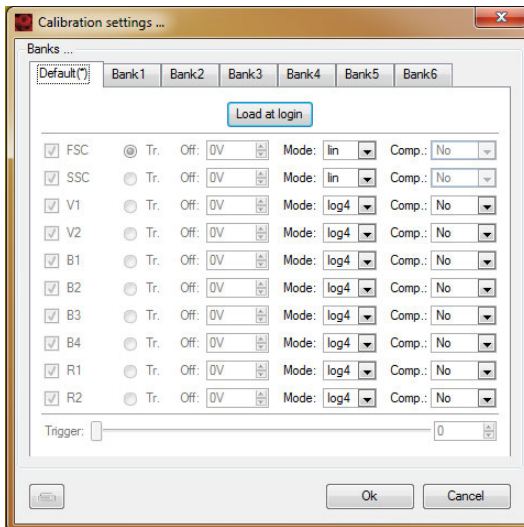
3 Click **Load at login**.

4 During each PMT calibration process, the scales will be changed to these default scales.

3.7 Reduce the number of channels

For every particle, a lot of information is stored in the data files. To analyze a very large data file can be cumbersome. 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.

> **Note:** If a saved instrument or bank-dependent instrument setting will be used for data acquisition, this setting must be opened as an active setting on the instrument prior to deselecting the unused channels.

1 Click on the **Channels tab**.

2 Uncheck the boxes next to the unused detection channels.

3 Upon data acquisition, only the checked detection channels will be saved and available for analysis. In this example, V1, B4, and R2 are deselected and no longer available for analysis.
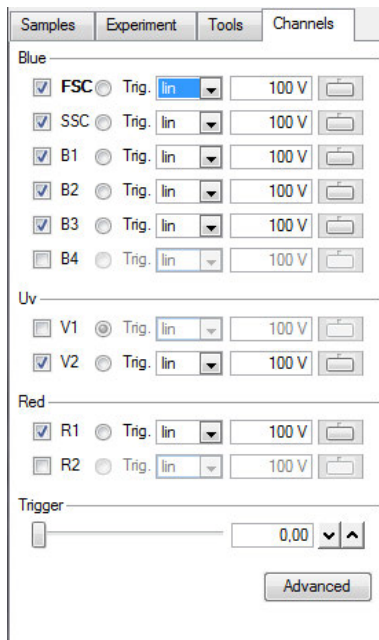
3.8   Manage Instrument settings

3.8.1   Save Instrument settings

1   Click the **Save button** (1) in the toolbar and select **Instrument settings**.

2   Select **Public** or **Private** for save location, if necessary.

3   Enter a name for the Instrument setting that is easily associated with the necessary experiment. For example, if this setting contains a compensation matrix appropriate for PBMCs labeled with VioBlue, FITC, PE, PerCP, APC, and APC-Vio770, then a suggested name could be PBMC_VB_F_PE_PerCP_ APC_APC-Vio770.

4   This saved Instrument setting can be used for a few days. Long-term use of an Instrument setting (i.e., 1– 2 month) is not advisable. Use a bank setting instead.

Note: It is recommended to include unstained and single-stained controls for each experiment to test if the saved Instrument settings are still appropriate, or to perform recompensation after analysis.

3.8.2   Open Instrument settings

Opening an instrument settings file

1   Click the **Open button** (1) in the toolbar and select **Instrument settings**.

2   Find the appropriate settings file in the **Public** or **Private** directory.

3   Highlight the setting from the options on the right and click **Open**.

4   This setting will now be an active setting on the MACSQuant Instrument, and all data will be acquired using these settings.

5   Adjustments to the settings can be made if necessary for the specific experiment.

Opening an instrument setting from a data file

1   Click the **Open button** (1) in the toolbar and open the data file linked to the desired instrument settings into the Samples tab.

2   In the **Samples tab**, right-click on the data file and select **Apply Instrument Settings**.

3   The instrument settings that the data file was acquired with will now be opened as the active setting on the MACSQuant Instrument. All subsequently acquired samples will be run with these instrument settings.

3.8.3   Deleting Instrument settings

1   Click the **Open button** (1) or the **Save button** (2) in the toolbar and select **Instrument settings**.

2   Find the appropriate setting in the **Public** or **Private** directory.

3   Highlight the setting from the options on the right and click **Delete**. The setting is permanently deleted.

3.8.4   Store Instrument settings in a bank

To facilitate sample measurement, PMT and compensation settings can be saved in an Instrument settings file and reloaded the next time the same or very similar experiment is performed. However, since lasers decrease in intensity over time, the long-term usage of defined instrument settings is not advisable. A decline of laser intensity results in weaker scatter and fluorescence signals. The original signal strength can only be restored by adjustment of PMT sensitivity, which means that new instrument settings have to be created. The MACSQuant Instruments offer an alternative way in saving instrument settings, called bank-dependent instrument settings, which comprise changes in laser intensity.

Banks are designed to save changes to PMT calibration settings, such as increase or decrease in FSC or SSC voltages, which are appropriate for various cell types. A bank does not contain an absolute value for PMT voltage setting but offset values, e.g., -16 V or -108 V from the initial PMT calibration setting. If the PMT voltage is increased (to achieve the optimal sensitivity) during calibration, application of a bank to a PMT calibration will then further adjust the PMT voltage by the saved offset values. Thus the cell populations (when using the same type of cells) will be always in the same location in the plot, even if the laser power has declined. Banks are set up and controlled under **Edit > Calibration**.

3.8.5   Generate a bank

1   Perform a PMT calibration (see section **Automated calibration**).

2   Adjust PMT voltages, compensation matrix, and triggers to be appropriate for the cell type for the bank setting.

3   Keep this setting active on the instrument or ensure that the desired instrument settings are loaded and active on the instrument.

4   Click **Edit > Calibration**. The Calibration settings window opens.
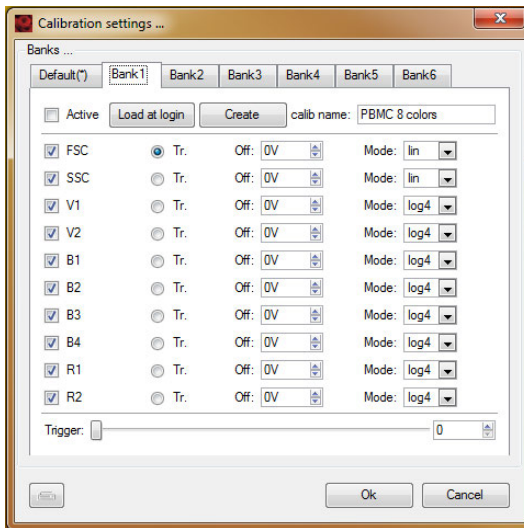
5 Select one bank (1–6), not the Default(*) bank.

Note: The asterisk marks the bank loaded upon log-in. Click **Load at login** to change.

6 Enter the desired bank name in the field calib name, e.g., PBMC 8 colors, and click **Create**. A pop-up window confirms the generation of the bank-dependent instrument setting files. Click **Close**.

7 In the **Calibration settings window**, check the **Active box** to enable the update of the bank-dependent instrument settings with each PMT calibration.
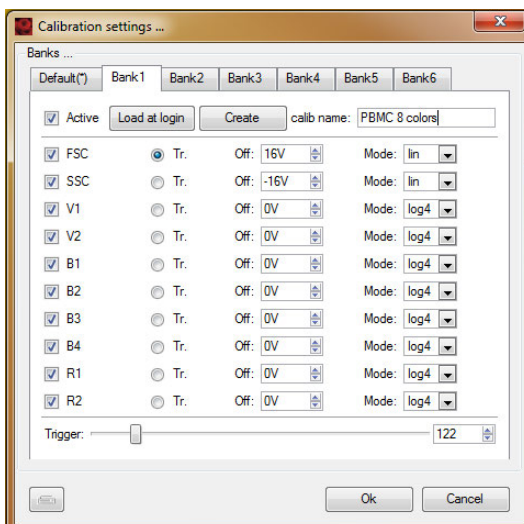
8 Selecting the **Load at login button** will set this bank as the default setting loaded at login into the software and when creating a new workspace.

3.8.6 Open a bank

1 Click on the **Open button** (**1**) in the toolbar and choose **Instrument settings**.
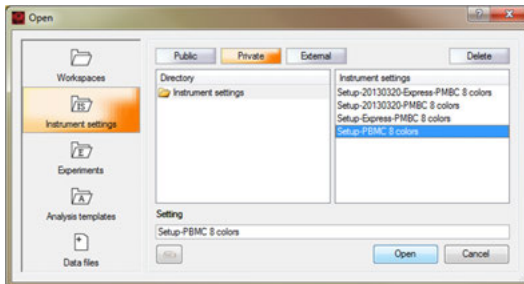
2 Four files are created for each bank. For this example, four files were created.

3 To open a file, select the file Setup-'name of the bank', in this example Setup-PBMC 8 colors, and click **Open**.

3.8.7 Delete a bank

1 Click the **Open button** (**1**) in the toolbar and select **Instrument settings**.

2 To delete files, select the respective files and click **Delete**.

3 Click **OK** to confirm.

> **Note:** When **Active** is selected for a bank in the Calibration settings window, new bank-dependent instrument setting files will be generated with every PMT calibration. Deselect to avoid the regeneration of bank-dependent instrument setting files.

3.8.8 Example: Generate a bank

Day 1

1 Perform a PMT calibration.
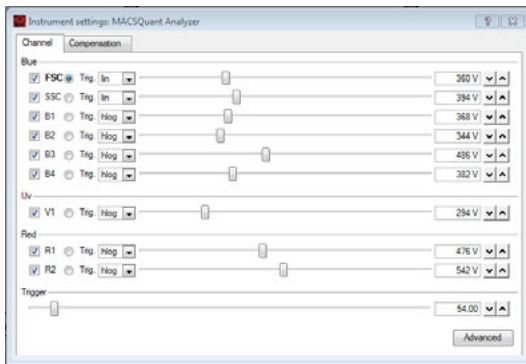
2   Set up the MACSQuant Instrument for the specific experiment.
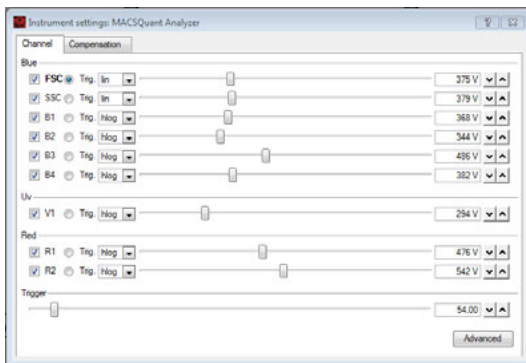
3   Note that the voltage values for FSC and SSC differ to the default instrument setting by +15 V and -15 V, respectively.

4   Open the Calibration setting dialog box and follow steps 4–9 in <u>section **Generate a bank**</u>.
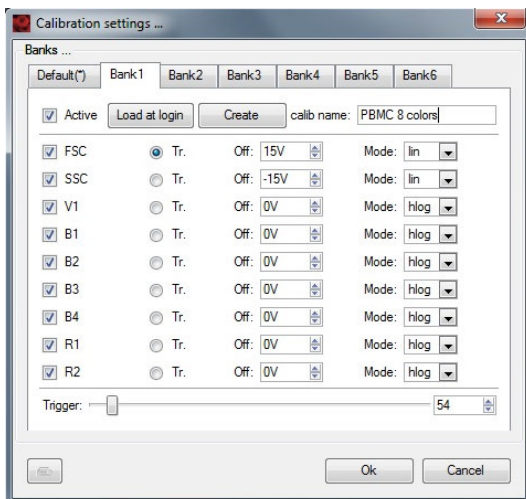
5   Note the voltage offset values of +15 V and -15 V for the FSC and SSC channels, respectively, that are specific for this bank.

6   If the bank was created as Active (Active box checked in the Calibration settings dialog box), the bank-dependent instrument settings will be updated during subsequent calibrations, i.e., the PMT voltage values of a calibration will be adjusted according to the specific offset voltage values of the bank.

Day 2

7   Perform a PMT calibration.
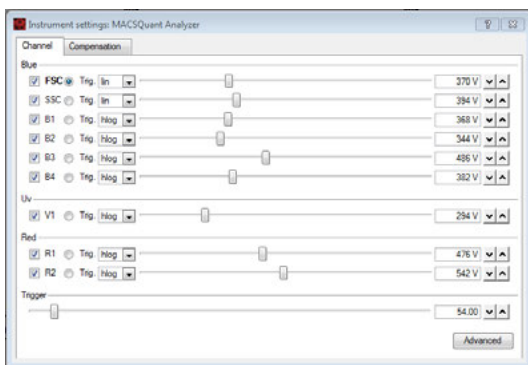
8 Note the voltage values of the FSC and SSC channels have changed compared to day 1 to 370 V and 394 V, respectively.

9 Open the bank PBMC 8 colors by clicking **Open > Instrument settings** in the toolbar. If the bank was created with **Load at login** selected, it will already be open.
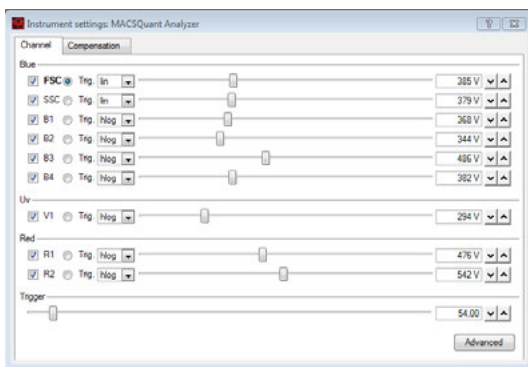
10 Note that the voltage values for the FSC and SSC channels have been adjusted according to the offset values saved in the bank by +15 V and -15 V, respectively. Moreover, the voltage value for the FSC channel increased by 10 V from day 1 to day 2 during PMT calibration, causing the same increase for the FSC channel in the bank-dependent instrument settings at day 2.

4

Set up an experiment

⚠ **WARNING** Read the chapter **Important Safety Information** in the MACSQuant™ user manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

This chapter describes experiment setup for advanced flow cytometry users (Custom mode users and administrators). Administrators and custom mode users can create customized experiments with sample autolabeling and uptake, data acquisition, gating, data analysis, and generation of print-ready results.

Less advanced users can run experiments from the Express mode. Please refer to chapter **The Express mode user** for details.

4.1   What to consider before setting up an experiment

How many samples will be analyzed and what is the sample volume?

Single samples are processed using the single tube sample rack.

Multiple samples (up to 96) are processed by using the MACS MiniSampler in combination with MACS Chill Racks. Choose the appropriate rack and configure it accordingly.

Is the autolabeling function required?

Yes, autolabeling is required. Up to four MACS Reagents can be used in combination with the MACS Reagent Rack 4 to label samples:

- Fluorochrome autolabeling of specific cell populations
- Magnetic autolabeling of rare cell populations for subsequent pre-enrichment and analysis
- Autolabeling with dyes such as propidium iodide.

No, autolabeling is not required. If samples are manually labeled no MACS Reagent Rack is required. For manual labeling follow the labeling instructions in the corresponding reagent data sheets.

Are rare cells being analyzed?

Yes, rare cell analysis is required. Rare cells can be automatically labeled magnetically and enriched using the MACS Cell Enrichment Unit. Depending on the selected analysis mode, the enriched and non-enriched fractions can be subsequently analyzed by flow cytometry. Refer to chapter **Pre-enrichment** for

details. A MACSQuant Column must be installed for pre-enrichment. Refer to the MACSQuant Instrument user manual for instructions on how to use the MACS Enrichment Unit and MACSQuant Column. MACS Control Antibody Cocktails are available for automated and reliable rare cell analysis.

4.1.1 When running a multisample experiment

If running a multisample experiment, it is advisable to use the MACS MiniSampler in combination with one of the Chill Racks. Five different kinds of sample tube racks are available. If autolabeling is to be performed, use the MACS Reagent Rack 4 for labeling several samples with up to four different reagents. The MACS MiniSampler can be configured to perform autolabeling and measurements from any sample position. Reagents can be entered automatically using the 2D code reader.

Rack	Slots	Maximum number of samples
Single Tube Rack	1 x 5 mL	1 (5 mL tube)
Chill 5 Rack	24 x 5 mL	24 (5 mL tubes)
Chill 15	15 x 15 mL 5 x 5 mL	15 (15 mL tubes)
Chill 50	6 x 50 mL 3 x 15 mL 3 x 5 mL	6 (50 mL tubes)
Chill 96	96 wells	96 (96-well microtiter plate)

Table 4.1: Racks available for single- and multisample processing.

Figure 4.1: The Reagent Rack.

Sample grouping

The maximum sample volume that can be acquired in a single step by the MACSQuant Instrument is 450 µL. If the sample size exceeds this volume, aliquots of the sample must therefore be spanned over two or more tubes.

By grouping these samples, the acquired data will be consolidated into a single file on the hard drive, which can also be analyzed in a single data file or analysis plot. When a grouped data set is opened, group gates are created automatically.

Further, samples belonging to one experiment can be grouped, e.g., original, positive, and negative fractions of a sample separated using MACS MicroBeads can be grouped and analyzed together.

Samples can be grouped prior to data acquisition. If grouping was not performed prior to data acquisition, it is still possible to group samples post-acquisition.
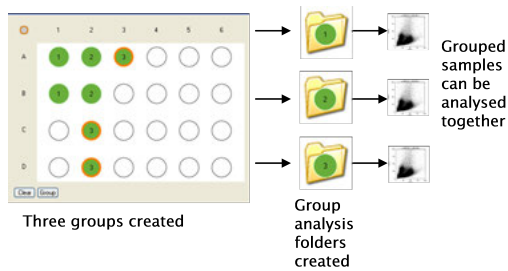
Figure 4.2: Sample grouping prior to acquisition.
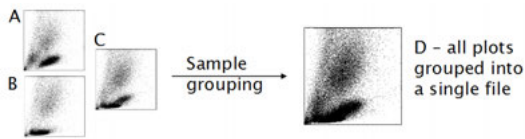
Figure 4.3: Sample grouping after acquisition.

Autolabeling

The MACS Reagent Rack 4 is used in combination with the MACS MiniSampler for autolabeling of cells. There are four positions available on the MACS Reagent Rack 4 for reagent vials. Reagents can be entered using the 2D code reader, or via the Reagents window.

Figure 4.4: Enter reagents using the 2D code reader.

4.1 Define experiment parameters

4.1.1 General experiment settings

In this section, general settings for a single-sample experiment are described. For settings specific for multisample experiments, see section **Process multiple samples**, page **1**.

1 Go to the **Experiment** tab.

2 From the **Rack drop-down list**, select the single tube rack. If the checkbox to the right of the field is activated, the instrument will automatically detect the used rack.
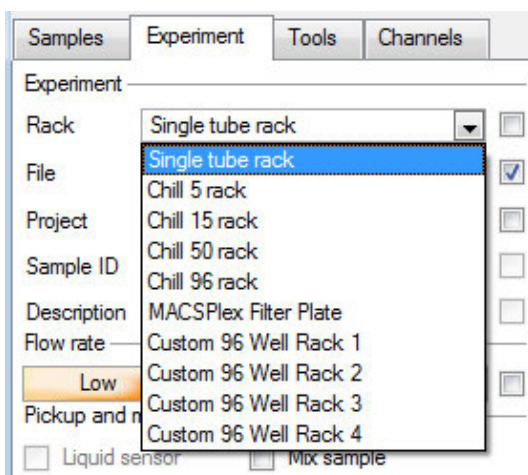
3 (Optional) Change the file name: uncheck the **File** checkbox to the right of the file name and enter a new file name. The file number in the field to the right of the file name will automatically increase with each file acquired. If desired, use the arrow keys to change the file number manually. If the file number has already been used in a previously acquired file, a warning will appear upon acquisition that the respective file will be overwritten.

Note: See section **Modify the default file name** to learn more about file names and how to change the default file name. By default, the MACSQuantify Software uses the logged in user's initials and date to construct the file name.
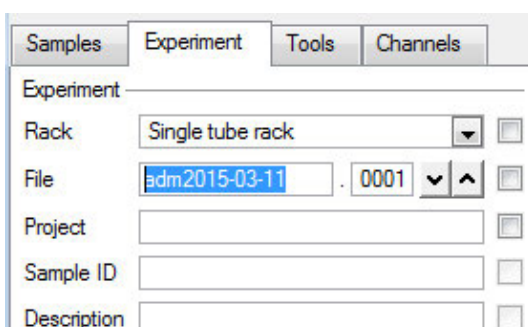
4 Enter alphanumeric text for the **Sample ID** and **Description** if desired.
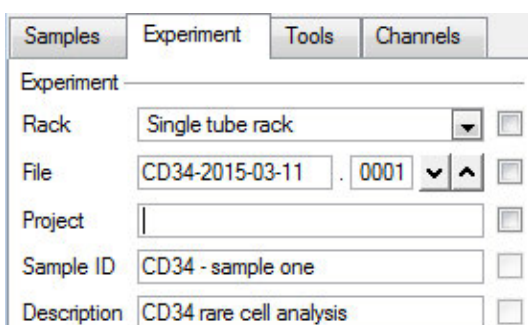
4.1.2 Flow rate

Select a flow rate: **Low**, **Medium**, or **High**. Alternatively, check the box to the right to switch to events/s. The flow rate is adjusted to approximate the selected value.

Flow rate (μL/min)	Sheath buffer consumption during measurement cycle (μL)	Total time (s)
Low: 25	18,000	240
Med: 50	13,000	120
High: 100	8,600	60

Table 4.2: Flow rate and sheath buffer consumption for a 100 μL sample, run in standard mode.

4.1.3 Pickup and measure

1 Go to the **Experiment tab**.

2 From the drop-down menu **Mix sample**, select a mixing mode if it is desired to premix the sample before sample pickup, data acquisition and analysis.

3 Select an analysis mode from the **Mode** drop-down list.

4 Enter an **Uptake volume** and a **Sample volume**. The maximum uptake volume and sample volume is 450 μL and 5 mL, respectively.

Note: The MACSQuant Instrument uses a defined percentage of the sample volume when mixing. Therefore, it is necessary to define the sample volume to avoid the uptake of air bubbles.

4.1.4   Annotation

1 Go to the **Experiment tab** and select the **Annotations tab**.

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

4.1.5   Settings

Custom mode analysis

1 Go to the **Experiment tab** and select the **Settings tab**.

2 Activate the radio button next to **Custom**.

3 For Custom mode analysis, check the checkbox associated with the **Instrument settings**, **Analysis template**, **Live gate**, and **Events options** to activate these features. When unchecked, the instrument will run with the current active Instrument settings.

- **Instrument setting:** previously saved instrument calibration and compensation settings can be loaded and applied by checking the adjacent box.
- **Analysis template:** previously saved cell analysis templates can be loaded and applied by checking the adjacent box.
- **Gate:** check the adjacent box to activate live gating – a live gating strategy can be saved as an analysis template for future use.
- **Events:** check the adjacent box to stop data acquisition after a defined number of events is obtained, in this example 10,000 events.

> **Note:** It is recommended to limit measurement by volume, because volumetric measurements allow for absolute cell counting. If using an event rate to limit measurement, ensure that sufficient uptake volume has been selected to acquire the desired number of events.

Express mode analysis

1 Go to the **Experiment tab** and select the **Settings tab**.
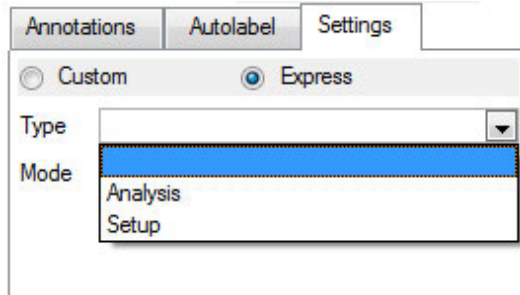
2 Activate the radio button next to **Express**.

3 Express mode can be used for either calibration or compensation processes using the **Setup** option from the drop-down list, or with MACS Antibody Cocktails for rapid and easy analysis of cells using the **Analysis option** from the drop-down list.

4.1 Process multiple samples

Process multiple samples by using the MACS MiniSampler in combination with the MACS Reagent Rack 4 and Chill Racks (sample tube racks). Besides the Single tube rack, four different kinds of sample tube racks are available. The MACS MiniSampler can be configured to perform autolabeling and measurements from any sample position. Refer to for all available rack types.

4.1.1 Chose a rack
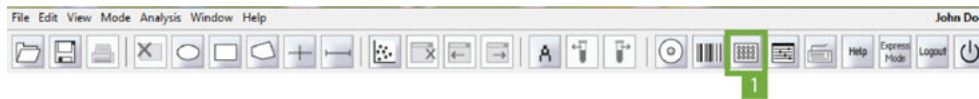
1 Go to the **Experiment tab**.

2 Select the required rack type from the **Rack** drop-down list.
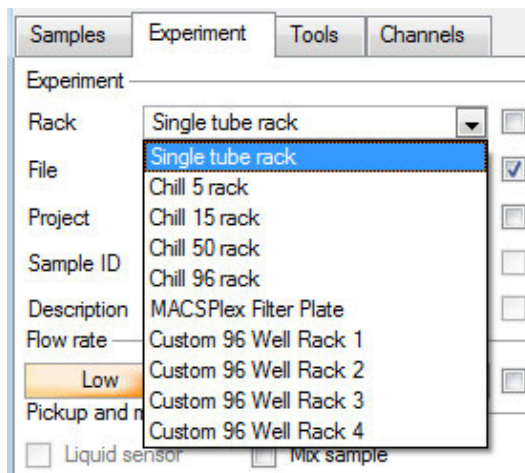
3 The corresponding rack template will pop up in an independent window and will also appear in the lower section of the **Experiment tab window**. Alternatively, open the Racks dialog box by clicking the **Rack button** (1) in the toolbar.
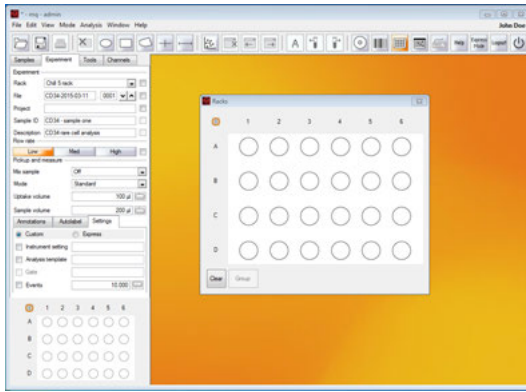
4.1.2 Sample racks

Sample racks are represented graphically by the MACSQuantify Software. All rack positions are given by coordinates: columns are identified by numbers; rows by letters. To activate a single rack position, left-click (single-click) on the desired position. Alternatively, the MACSQuant Instrument touch screen may be used. Click again on the same position to select it for measurement. Positions are marked with colors depending on their status.

Note: MACSQuantify Software offers a second color scheme in order to support users with color discrimination deficiency (see section **Adjust rack colors for red-green colorblind users** for details).

Sample rack color scheme

|		
○	**Clear**	Default open circle indicates no operation.
●	**Closed green circle**	Sample selected for measurement. Double-click on circle to select.
●	**Closed green circle with orange rim**	Sample selected and activated for editing. Single click on circle to activate. The orange circle indicates that the sample is activated for editing.
●	**Closed yellow circle**	Processing of sample has commenced, e.g., sample has been labeled and incubation is underway.
●	**Closed blue circle**	Measurement in progress.
●	**Closed gray circle**	Measurement finished.

Select a sample position

Several rack positions or even an entire rack, row or column can be selected or deselected. Furthermore, samples can be grouped together using the Group function. After sample acquisition, grouped samples can be analyzed together in a single dataset/analysis window.

Sample menu button – To change settings of the entire rack or all selected positions. Right-click on the Sample menu button on the upper left corner of the rack to **Select All**, **Select Used**, **Deselect All**, **Clear All**, **Clear selected** or **Change processing order** (not for Chill 50 Rack). The sample menu button indicates the current processing order (row or column).

In order to set all rack positions to allow measurement and modification of the experiment strategy (e.g., labeling): Click **Select All**, followed by **Deselect All**.
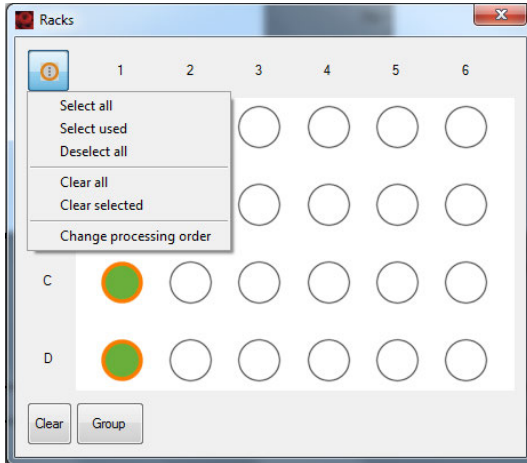
Column header – To change settings of the entire column. Right-click on the desired column number, e.g. 1, to **Select col**, **Select Used in col**, **Deselect col**, **Clear col** or **Clear selected in col**.
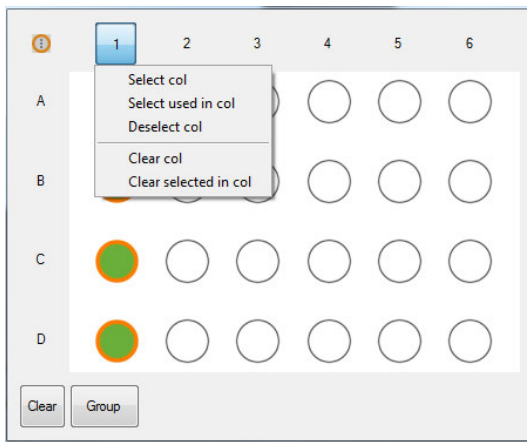
Row header – To change settings of the entire row. Right-click on the desired column letter, e.g. A, to **Select row**, **Select Used in row**, **Deselect row**, **Clear row** or **Clear selected in row**. Row A is selected for sample labeling and measuring. Row B is selected for sample measurement only.
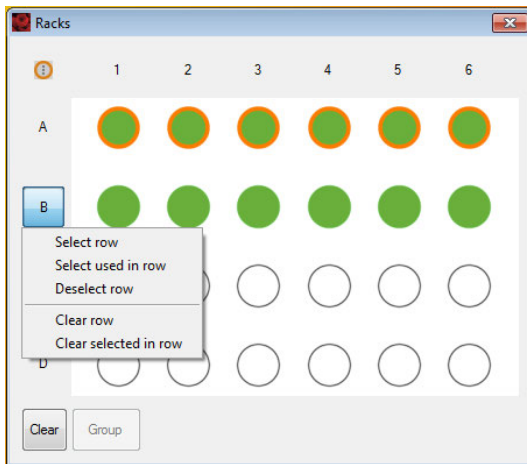
Single rack position – Right click on a single rack position to completely clear this position. In this example, position A2 will be cleared.
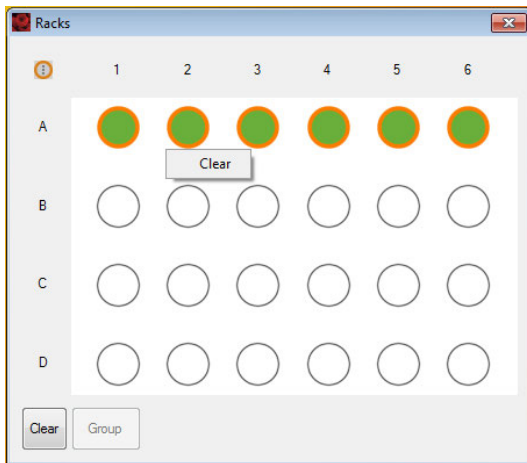
Group – To group several adjacent rack positions. Only rack positions that are adjacent in either a column or row (not for Chill 50 Rack) can be grouped. Click on the sample menu button in the upper left corner to chose the desired processing order (horizontal or vertical). The default processing order is vertical. To change the default processing order, go to **Edit > Options > Experiment**. To group selected positions, click the **Group button** at the lower left corner. The entire group can be selected concurrently by double-clicking one sample position of the respective group.
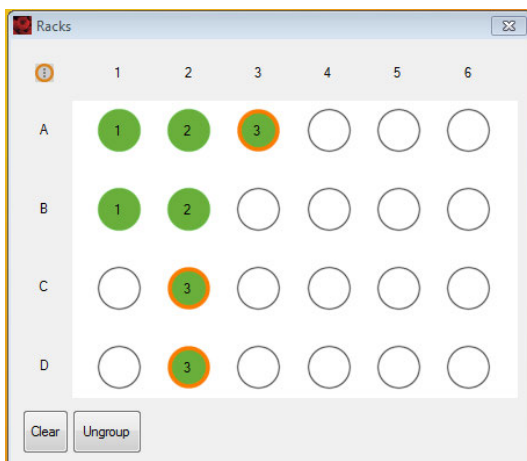
4.1.3   Sample rack configuration

1   Click on a sample position(s) using the left mouse button or touch screen. An entire row, column, or table can be selected.

2   To program sample positions, ensure that all positions to be programmed by a given strategy are activated (⬤). All samples can be programmed at the same time, or each position or group of positions can be programmed individually.

3   Use the **Experiment tab** to specify the **Experiment**, **Flow rate**, and **Pickup and measure** options. Verify proper annotations as well as autolabeling reagents and designations.

4   Use left mouse button or touch screen to switch between ⬤ and ⬤ .

- designated sample will be measured and associated "Experiment" definitions for this sample can also be modified using the Experiment tab (e.g., sample name, labeling strategy, uptake volume, etc.).
- designated sample will be measured and associated "Experiment" definitions are locked.

5   Ensure all sample positions have the correct associated programming. To view the experiment settings for all sample positions, click **View > Experiment table**.

6   Before starting the run check that:

- Experiment definitions are correctly assigned to each rack coordinate and that each sample is correctly positioned on the Chill Rack.
- Sufficient quantities of reagents and buffers are provided. Ensure that the waste bottle is empty.
- The reagents have been imported and assigned to a position on the MACS Reagent Rack 4(section **Automated reagent entry using the 2D code reader**).
- The instrument is correctly calibrated and compensated (see section **Automated calibration** and section **Automated compensation using the Express mode "Compensation"**).

4.1.4   Sample grouping

Sample grouping can be made before acquisition, or afterwards during data analysis. Refer to section **Group data after acquisition** for information about sample grouping after data analysis.

1   In the **Experiment tab**, select the desired rack from the drop-down list.

2   Click the **Rack button** (1) in the toolbar to open the corresponding rack window.

3   Select sample positions for grouping. Selected samples must be in a column or in a row.
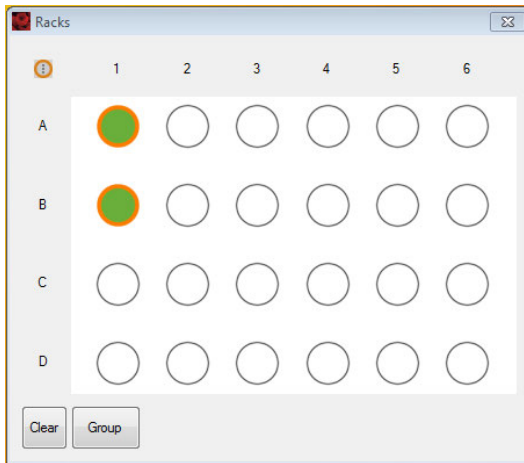
4 Click **Group**.

5 Enter the sample information using the **Experiment** tab.
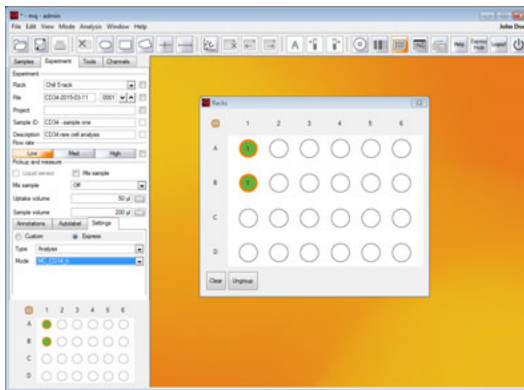
6 Click on additional desired rack positions to perform further grouping.
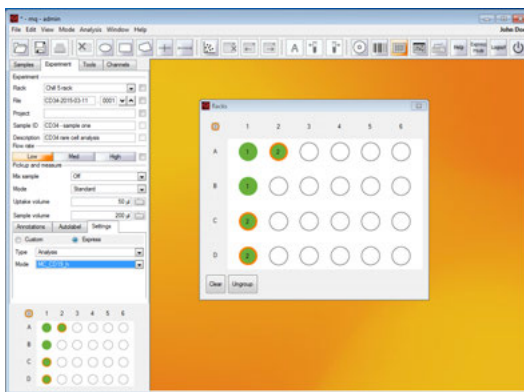
7 Close the rack window.

4.1 Reagents

For subsequent autolabeling of samples, reagent specifications can be entered manually via the Reagents window. Alternatively, reagents can be scanned with the 2D code reader.

4.1.1 The Reagents window

The MACSQuant Instrument reagent management window allows users to select reagents from a drop-down list and assign reagents to a position on the reagent rack. Click the **'i' button** for more information about the reagent. An info text is displayed in a modal dialog above the Reagent dialog.

> **Note:** The MACS MiniSampler must be correctly installed to view these options.

Pos – Assign reagents to rack positions R1, R2, R3, R4, S1, or S2. R1-R4 positions are located on the MACS Reagent Rack 4. S1 and S2 positions denote "Special" positions: Running Buffer is taken directly from the buffer bottle to dilute the sample as defined by the user.

Category – Assign reagents categories (species, conjugated fluorochrome or purpose).

- Calibration: MACSQuant Calibration Beads for the calibration of the instrument settings.
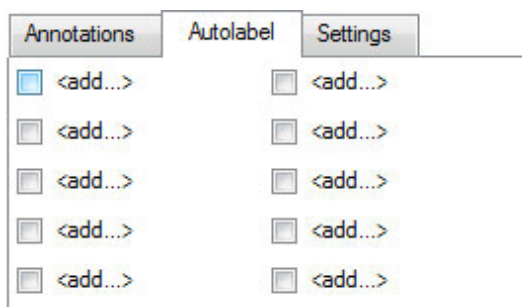- Species and conjugated fluorochrome, e.g., Human – APC, Mouse – PE.
- Isotype control: isotype control antibodies are raised against non-mammalian epitopes and can be therefore used as a negative control for non-specific binding.
- MACS Comp Reag. (MACS Compensation Reagents): These reagents are used to correct the inherent spectral overlap between excitation and emission wavelengths of fluorochromes.
- 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)

Reagents – A drop-down list of available reagents is displayed in accordance with the selected category.

Time – For autolabeling an incubation time is provided and displayed in a black font type. Experienced users may change the incubation time using the adjacent arrows.

Titer – For autolabeling, a sample titer is recommended and displayed in a black font type. Experienced users can change titers using the adjacent arrows.

Order – Signifies the order in which this reagent will be used during cell processing. Lower numbers will be added first.

> **Note:** Non-recommended or changed titers or times will appear in red font.

4.1.2 Automated reagent entry using the 2D code reader

CAUTION! Read the **Important safety information** in the MACSQuant Instrument user manual before using the 2D code reader.

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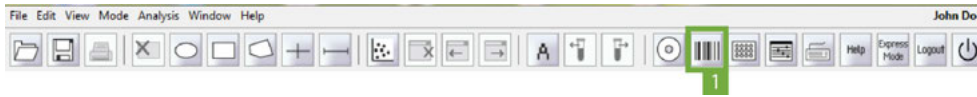

1   Click the **Barcode button** (**1**) in the toolbar.

2   Tilt the reagent vial slightly and present it in front of the 2D code reader, with the 2D code facing the blinking code reader light. The optimal reading distance is 0.5–2.5 cm.

3   Scanned reagents are reported to the **Reagent** dialog box.

4   After scanning the MACS Reagents, the MACSQuantify Software will prompt the user to place the vial(s) on the MACS Reagent Rack. Be sure to place the reagent onto the corresponding position (1-4) on the reagent rack.

5   If the code reader fails to recognize the 2D code, enter the information directly into the MACSQuantify Software **Reagents dialog box**.

Note: Scanning MACSQuant Calibration Beads will prompt the MACSQuant Instrument to initiate the calibration procedure. For more information on calibration, see section **Automated calibration**.

4.1.3   Select and assign reagents manually

It is recommended to use the 2D code reader to scan reagents, as this prevents incorrect reagent entries. However, if the reagent label or data sheet insert is damaged, it may be necessary to select reagents manually using the **Reagents dialog box** .

1   Place a reagent onto the reagent rack, noting its position.

2   Click **Edit > Reagents…** Alternatively, open the **Reagents** window by checking any **<add…>** box located in the **Autolabel** tab within the **Experiment** tab.
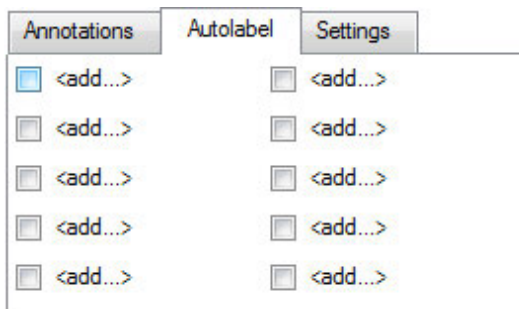
3   The **Reagent window** opens. Check the desired reagent position (**R2, R3, R4, S1** or **S2**).
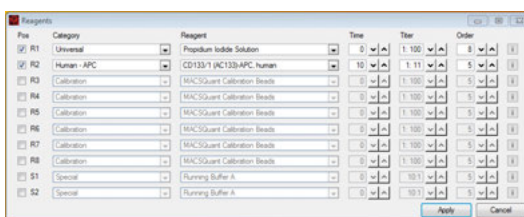
4   From the **Category drop-down list**, select a reagent.

5   From the **Reagent drop-down list**, select a reagent.

6   Modify the incubation time (**Time**), label, sample titer (**Titer**), and order if required.

4.1.4 Autolabeling

1 If autolabeling is required, add the relevant reagents by clicking on an **<add…>** checkbox. Alternatively, the **Reagents** menu can be found under **Edit >Reagents**.
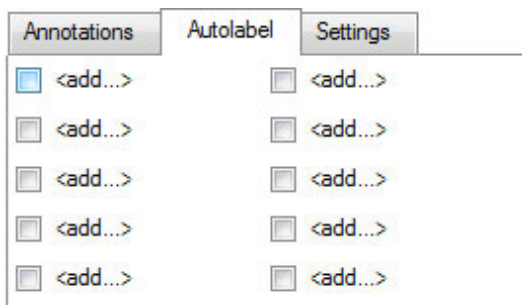
2 Select the reagent and reagent rack position from the drop-down list. Check and adjust the incubation time, titer, and order as needed.
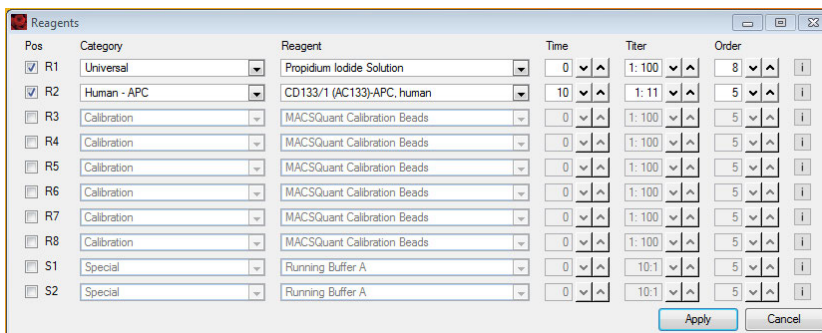
3 Depending on the selection and assignment of reagents for autolabeling of a well, the checkbox on the left to **<add...>** indicates:

☐ No wells were selected for reagent assignment

☑ All wells were selected for reagent assignment

◼ Some wells were selected for reagent assignment

4.1 Review experiment settings

1 All experiment settings can be reviewed in an experiment table.

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

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

Acquisition | Annotations | Autolabel | Settings

	Sample ID	Description	Flow rate	Auto flow rate on	Mix sample	Mode	Uptake volume	Sample volume
A1		Donor1	Med	No	yes	Mix gentle	100	500
B1		Donor2	Med	No	yes	Mix gentle	100	500
C1		Donor3	Med	No	yes	Mix gentle	100	500
A2		Donor1	Med	No	no	Mix gentle	100	500
B2		Donor2	Med	No	no	Mix gentle	100	500
C2		Donor3	Med	No	no	Off	100	500

Acquisition | **Annotations** | Autolabel | Settings

	V1	B1	B2	B3	B4	R1	R2
A1	CD45-VioBlue	CD3-FITC	CD4-PE	PI	PE-Vio770	CD8-APC	CD45RA-APC-Vio77
B1	CD45-VioBlue	CD3-FITC	CD4-PE	PI	PE-Vio770	CD8-APC	CD45RA-APC-Vio77
C1	CD45-VioBlue	CD3-FITC	CD4-PE	PI	PE-Vio770	CD8-APC	CD45RA-APC-Vio77
A2	CD45-VioBlue	GFP	CD4-PE	PI	PE-Vio770	CD14-APC	APC-Vio770
B2	CD45-VioBlue	GFP	CD4-PE	PI	PE-Vio770	CD14-APC	APC-Vio770
C2	CD45-VioBlue	GFP	CD4-PE	PI	PE-Vio770	CD14-APC	APC-Vio770

Acquisition | Annotations | **Autolabel** | Settings

	R1	R2	R3	R4	R5	R6	R7	R8	S1
A1	PI								
B1	PI								
C1	PI								
A2	PI								
B2	PI								
C2	PI								

Acquisition | Annotations | Autolabel | **Settings**

	Category	Instrument setting	Analysis template	Gate	Events	Type	Mode
A1	Custom		*T cells	stop: P1	10000		
B1	Custom		*T cells	stop: P1	10000		
C1	Custom		*T cells	stop: P1	10000		
A2	Express					Analysis	MC_CD4_h
B2	Express					Analysis	MC_CD4_h
C2	Express					Analysis	MC_CD4_h

5

Data analysis

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

5.1   Plot properties

Users can change the properties of each individual plot using the **'i' button**. To modify these analysis templates, either during live data acquisition or on already acquired, saved data, make sure the analysis mode is not selected.

Analysis mode selected   display settings are locked

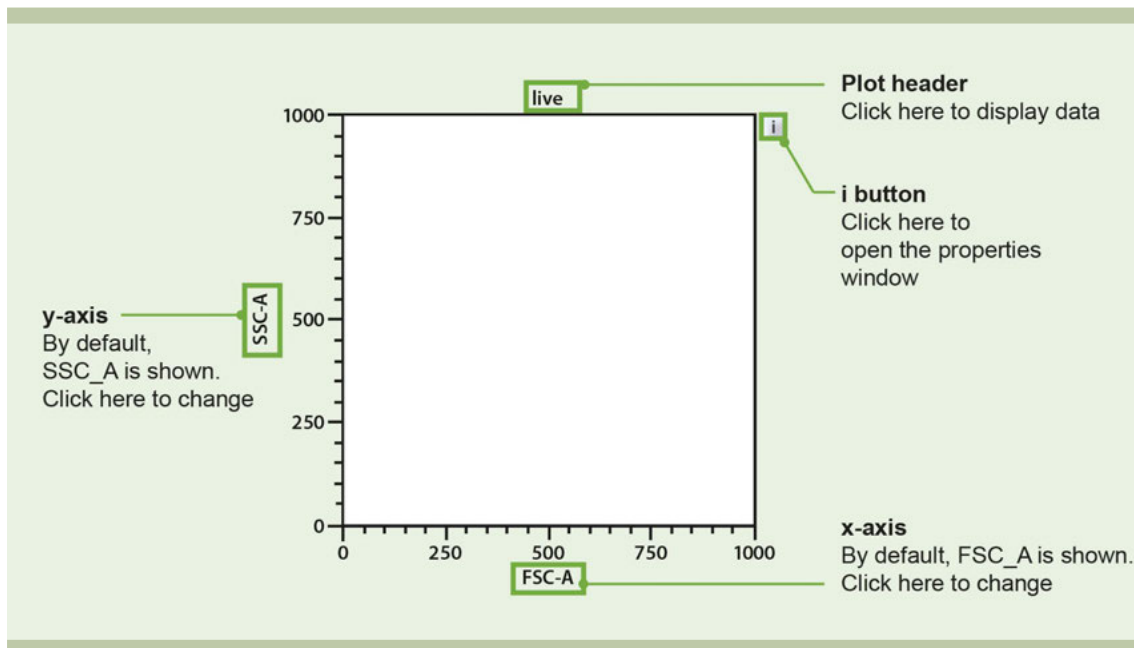**Analysis mode deselected**   display settings and analysis template can be modified.

Figure 5.1: Features of the MACS Quantify plot. To modify its appearance, click on the **'i' button** at the upper right corner of the respective plot.

5.2 Plot types

Flow cytometry data can be displayed in four different formats by MACSQuantify Software: As a **Dot plot**, a **Density plot**, a **Histogram**, as a **Statistics table** or as a **Heat map**. Additionally, a **Text box** can be used to display additional information. Click on the **'i' button** next to a plot to open the following properties window (see section **Plot properties**). Switch between the plot types by clicking on the respective button.
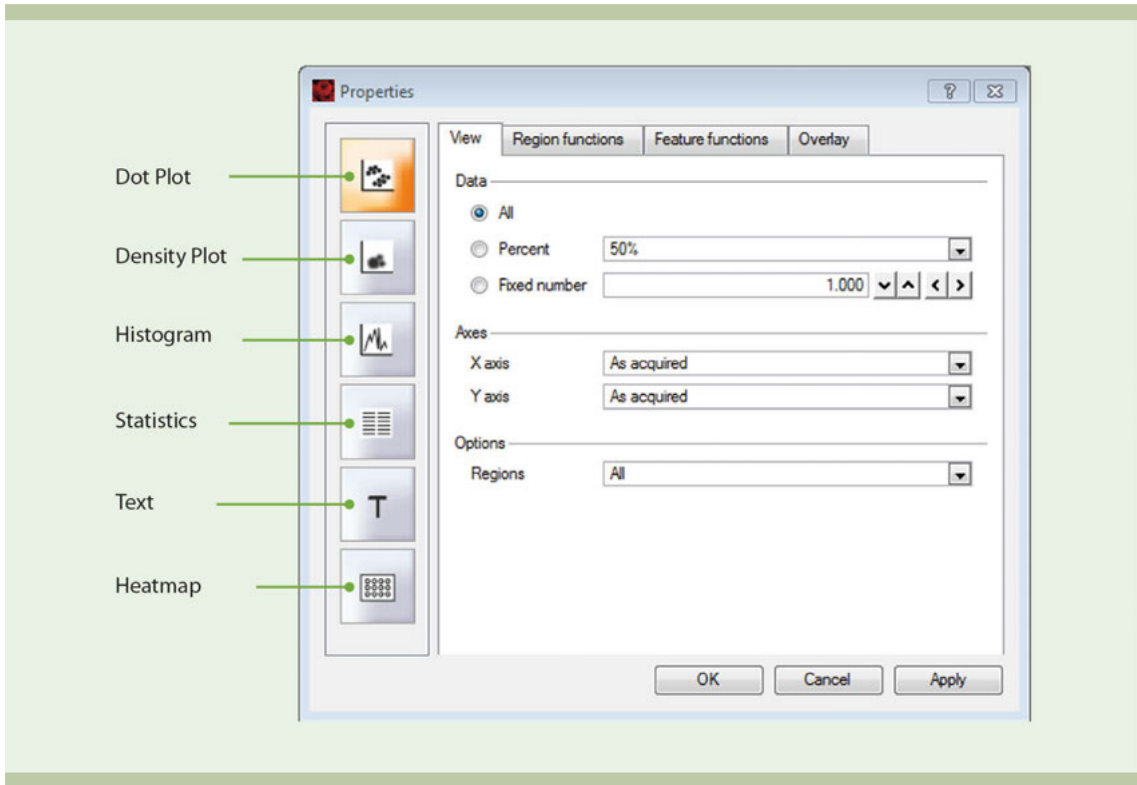
Figure 5.2: Switch beween plot types or modify their appearance.

5.3 Displaying data

5.3.1 Open an analysis window

Analysis templates consist of a plot template and, if required, a gating strategy. Analysis templates can be created post-acquisition or during acquisition in Live mode.

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

2 Select the desired analysis template from the plot options.

3 (Optional) Multiple analysis windows can be opened. These can be comprised of single or multiple experiments.
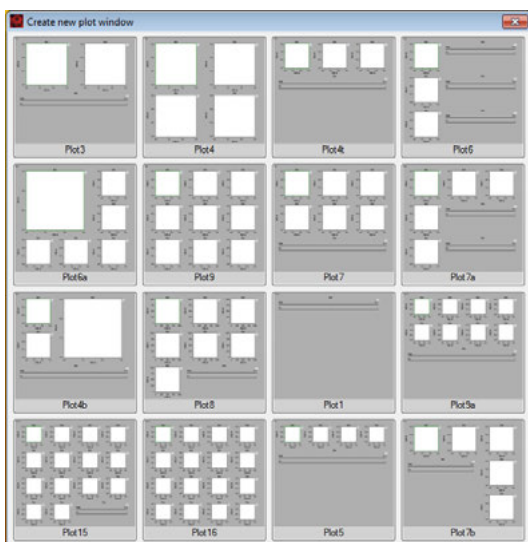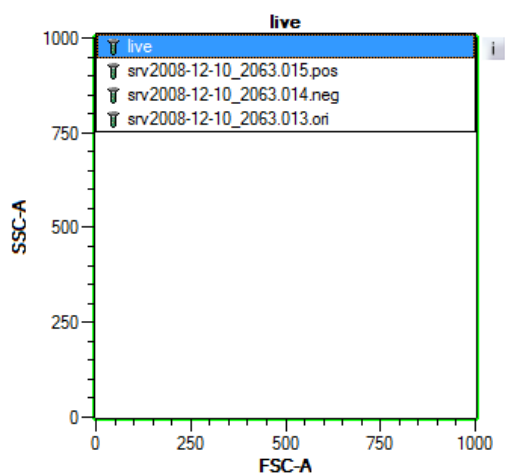
4 If multiple analysis windows are open, use the **previous and next analysis window buttons** (**2**) in the toolbar to access hidden windows.

5.3.2 Displaying data using the plot header drop-down menu

1 Click on the plot title and choose a data file from the drop-down list.

2 The selected data file will be displayed in the plot. The dot plot defaults to display FSC versus SSC.

3 Click on the **'i' button** in the upper right corner to change the plot type. See section **Plot types** for details.

4 Parameters displayed on the axes can be chosen by clicking on the axis label and choosing one of the saved parameters in the drop-down list.
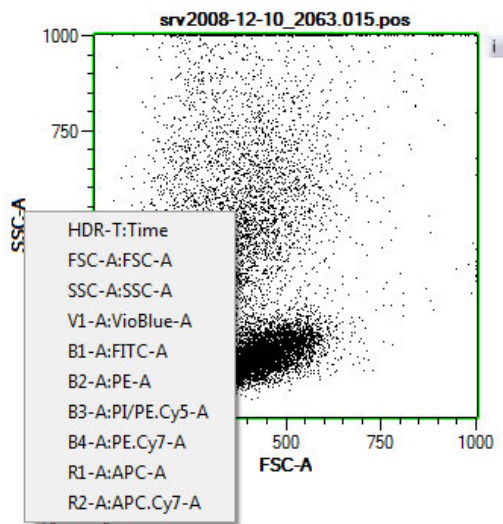
5.3.3 Displaying data by double-clicking on data file in sample list

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

2 From the samples list, double click the file for analysis. It will populate the active plot in the layout.
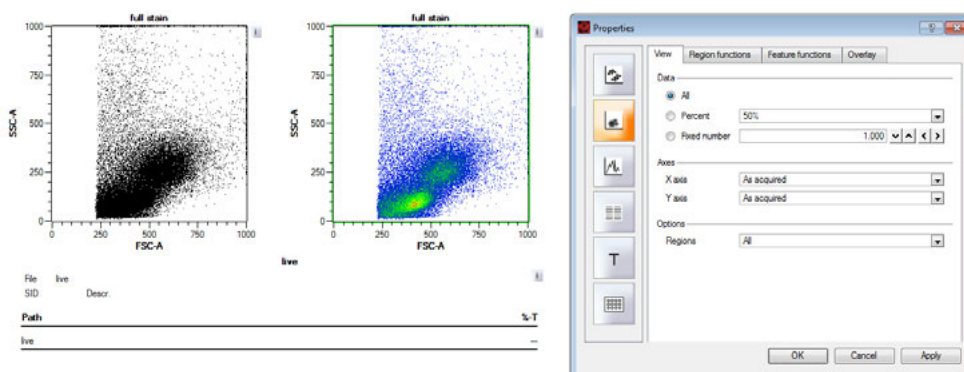
3 The plot defaults to FSC-A on the x-axis and SSC-A on the y-axis.

4 Click on the **'i' button** in the upper right corner to change the plot type.

5 Parameters displayed on the axes can be chosen by clicking on the axis label and choosing one of the saved parameters in the drop-down list.

6 If desired, the selected plot can be viewed full screen by double-clicking in the plot area. Double-click again to return to the original view.

5.4 Modify data display

5.4.1 Dot plots and density plots
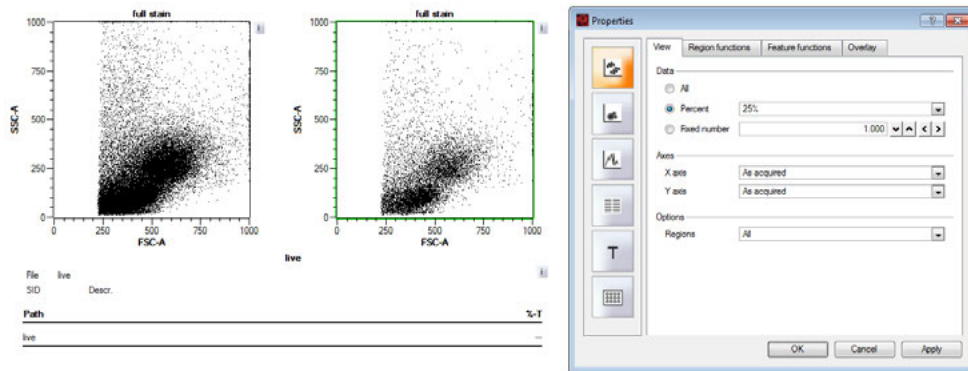
Dot plots and density plots are bivariate plots, as they both display two parameters. In addition, density plots depict the distribution of cells as a color-coded gradient. Displaying data as density plots is useful if the number of cells is large.

Number of events shown

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

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

2. Go to the **View tab**.

3. From the Data section, choose from the following:

   - **All** – All events are displayed on a dot plot or density plot.

   - **Percentile** – 50%, 25%, 5%, 2%, or 1% percentile values of the total events are displayed on a dot plot or density plot.

   - **Fixed number** – A fixed number of events can be displayed on a dot plot or density plot. Numbers can be entered directly into the field or by using the arrows.

Plot axes scales

1. To change the y- and x-axes scales of a dot plot or density plot, click on the **'i' button** next to the plot. A Properties window opens.

2. Go to the **View tab**.

3 From the Axis section, choose 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** – biexponential scale

Region options for dot plots and density plots

All – All regions ("gates") are shown on the plot.

This – Only a selected region ("gate") is shown on the plot.

None – No regions ("gates") are shown on the chart.

Region functions

1 To change the display of Region functions, click on the **'i' button** next to the plot. A **Properties window** opens.

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

3 Select (✔) or deselect (✘) the function using the left mouse button or by touching the display.

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

> **Note:** By default, the region functions **Path** and **%-T** are shown. .
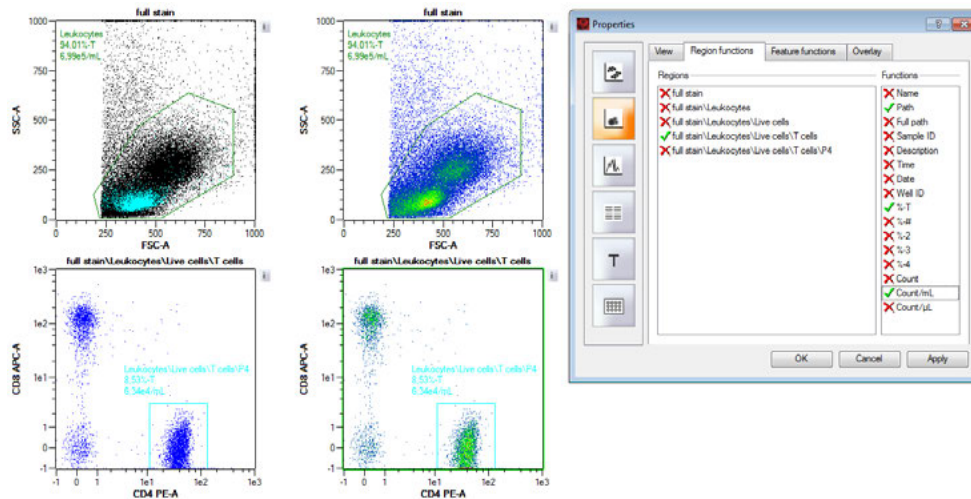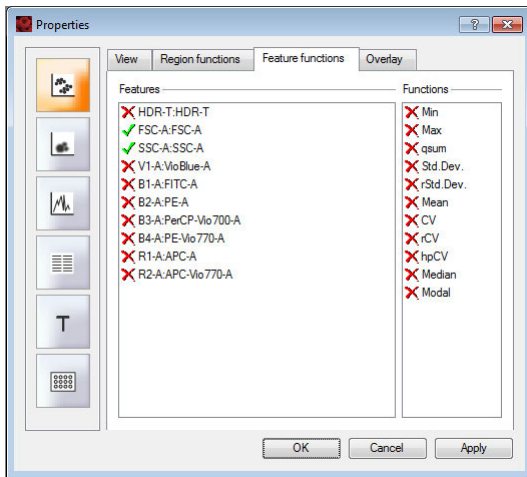
Figure 5.3: In this example, the **Region function Count/mL** was selected for display. **Path** and **%-T are shown by default.**

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-Month-DD
Well ID	Well ID in the format RC; R represents row, C represents column
%-T	Percent total
%-#	Percent gated / percent parent
%-2	Percent grand parent
%-3	Percent great grand parent
%-4	Percent great-great grand parent
Count	Number of events
Count/ml	Number of events per mL
Count/µl	Number of events per µL

Table 5.1: Region functions

Feature functions

1 To change the display of Feature functions, click on the **'i' button** next to the plot. A **Properties window** opens.

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

3 Select (✔) or deselect (✘) the function using the left mouse button or by touching the display.

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

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
hpCV	Coefficient of variation calculated as full width at half maximum (FWHM)/Mean*0.423*100%
Mean	Average value (addition 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 the value is easily influenced by outliers.
CV	Coefficient of variation calculated as StdDev/Mean
rCV	Robust coefficient of variation calculated as rSD/Median
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 5.2: Feature functions

Overlay

1 To display several cell populations in one density or dot plot, click on the **'i' button** next to the plot. A **Properties window** opens.

2 Go to the **Overlay tab**.

3 From the drop-down menu, choose the desired population.

4 Click on the colored box to the right to change the color of the population as desired.

5 Check the box to the left to mark the population as active. Only active populations are shown in the Overlay plot.
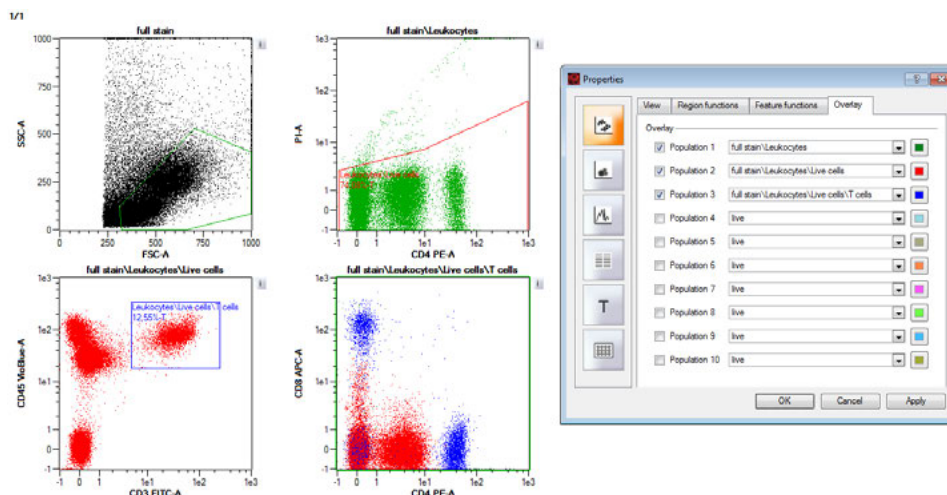
5.4.2   Histograms

Number of events shown

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

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

2   Go to the **View tab**.

3   From the Data section, choose from the following:

- **All** – All events are displayed on a dot plot or density plot.

- **Percentile** – 50%, 25%, 5%, 2%, or 1% percentile values of the total events are displayed on a dot plot or density plot.

- **Fixed number** – A fixed number of events can be displayed on a dot plot or density plot. Numbers can be entered directly into the field or by using the arrows.

Plot axes scales

1   To change the y- and x-axes scales of a dot plot or density plot, click on the **'i' button** next to the plot. A **Properties window** opens.

2   Go to the **View tab**.

3   From the **Axis section**, choose 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** – biexponential scale

Region options for histograms

All – All regions ("gates") are shown on the plot.

This – Only a selected region ("gate") is shown on the plot.
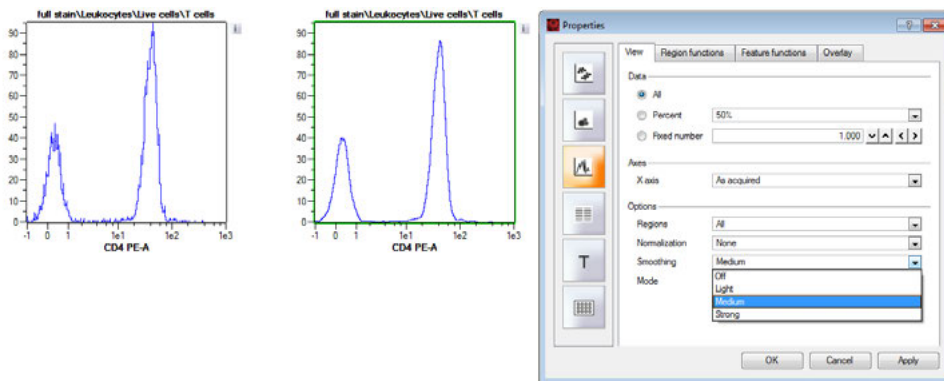
None – No regions ("gates") are shown on the chart.

Normalization

The histogram graphically summarizes the distribution of a univariate data set. Data can be normalized by area (integral of total area under the curve) or by height.
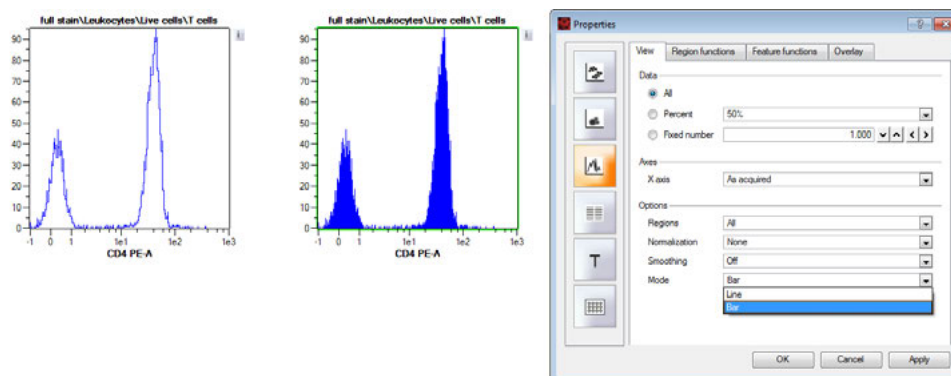
Smoothing

Algorithms can be used to smooth the histogram. Light, medium, or strong smoothing is available.

Mode

Histograms can be displayed as a line chart or bar chart.

5.4.3   Statistics tables

1   To change the format of the statistics table, click on the **'i' button** next to the plot. A **Properties window** opens.

2   Go to the **View tab**.

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

- **Header** – Check the header box to include a table header.

- **Table** – Check the table box to include a statistics table.

- Column headers of the table can be displayed in two formats: "shown using annotations" and "shown using channel names". Annotations are defined by the user in the Experiment tab.
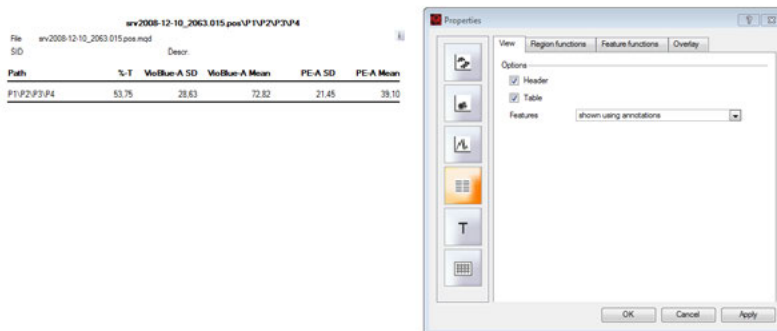
Region and Feature function

1   To change the display of Region functions, click on the **'i' button** next to the plot. A **Properties window** opens.

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

3   Select (✔) or deselect (✘) the function using the left mouse button or by touching the display.

Overlay

1   To display several populations in the **Statistics** table, click on the **'i' button** next to the plot. A **Properties** window opens.

2   Go to the **Overlay tab**.

3   From the drop-down menu, chose the desired population.

4   Check the box to the left to mark the population as active. Only active populations are shown in the Statistics table.
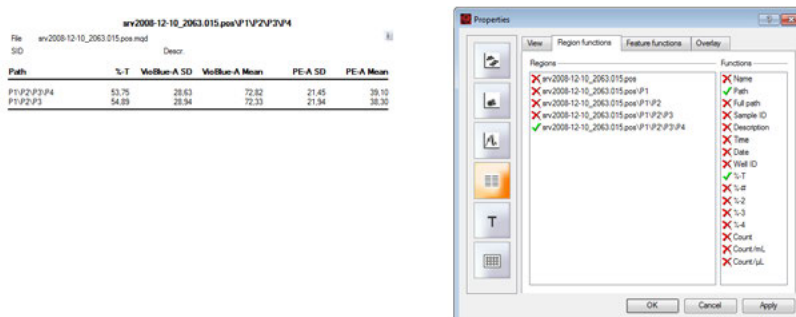
5.4.4   Text boxes

Script text

Enter free text or chose one of the predefined scripts to dilpay additional information.Chose one of the following options from the drop-down menu.

Free… – Free text or a MACSQuantify Software script can be entered into the "Script Text" field. Scripts are based on HTML (hypertext mark-up language) and can be written to automatically display statistics about each region or gate. Specific regions can be removed from or added to a text table by using the overlay tab. A script was used to create the following text table.

Compensation matrix – Values from the compensation matrix will be displayed as a table.

Experiment info from sample – Experimental settings such as uptake and sample volumes, sample ID, and description will be displayed as a table.

General info from sample – Displays information about the sample such as User or Software Version.

Instrument settings – The current instrument settings for each channel (PMT voltages and scales) will be displayed as a table.

Overlay

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

5.4.5 Heatmaps

Only grouped data (manual grouped or grouped measured data) can be displayed. If data sets are grouped manually and sample position overlap, or two plates are grouped, a chill 96 template is used and filled according to the sample list.

1 To set the heat map parameter, click on the **'i' button** next to the plot. A **Properties window** opens.
2 Go to the **View tab**.
3 From the **Display** drop-down menu, choose a sub-population.

4 In the **Function** section, activate the radio button next to **Feature** or **Region** to display the respective data in the heatmap:

- **Feature**: choose the feature (e.g. FSC-A) and a numeric feature function
- **Region**: choosea numeric region function (e.g. count)

5 From the **Options** section, choose a display mode. Three modes are available for display: color gradient, 2 color heat map or 3 color heat map. Pick colors and set thresholds accordingly.
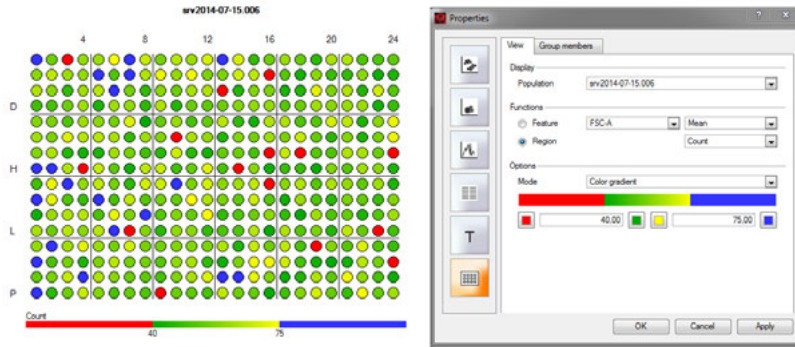
5.5 Analysis templates

Sixteen analysis templates are available to choose from. Analysis templates consist of a plot template and, if required, a gating strategy. The size and layout of the dot plot windows are predefined.

Depending on which **New plot window** is applied, the analysis window may contain dot plots, density plots, histograms, statistics, and text tables.
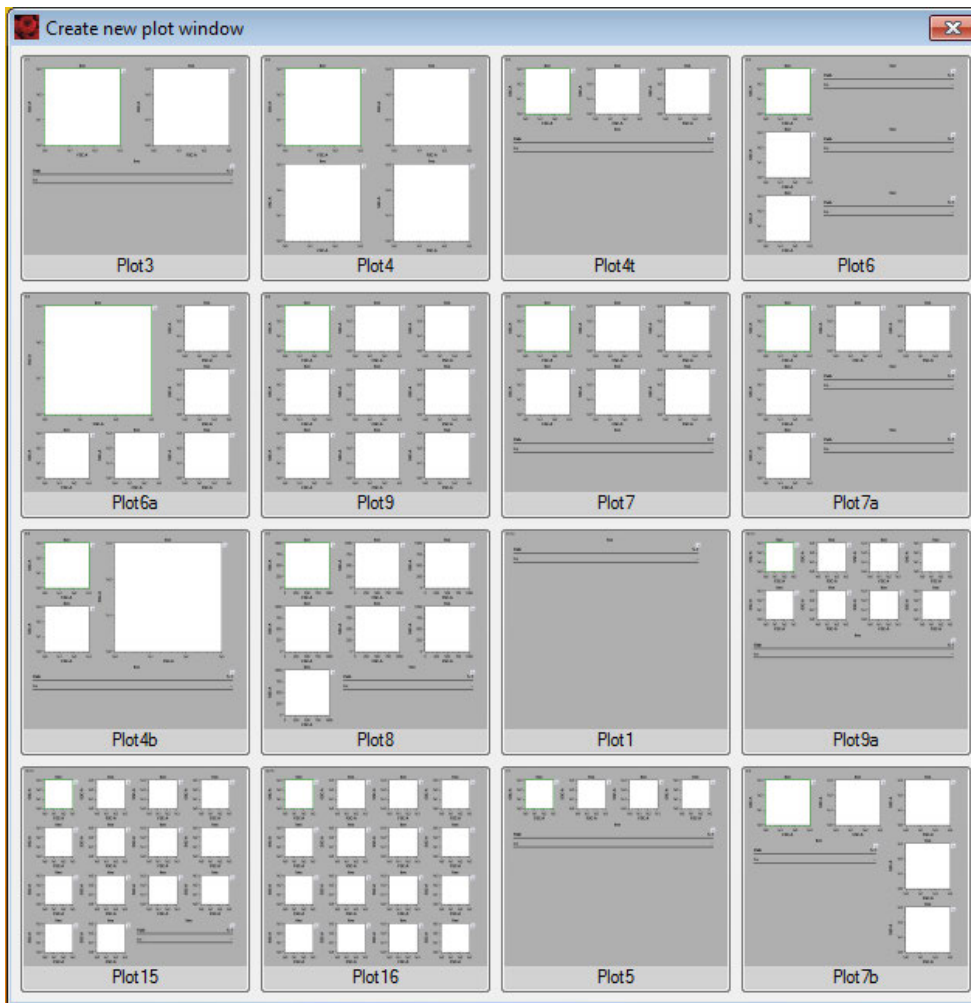
Figure 5.4: Predefined Analysis templates.

5.6 Gating tools

1. To create a gate or region, click on one of the **tool buttons** (**1**) from the toolbar or choose the tool from the **Edit** menu.

2. Left-click on a plot and start dragging a gate.

3. Release the left button to finish drawing.

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

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

Note: It is not possible to add additional points/manipulators to a polygon.

5.6.1   Available gating tools

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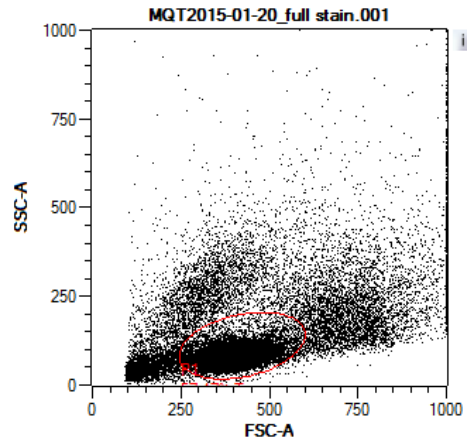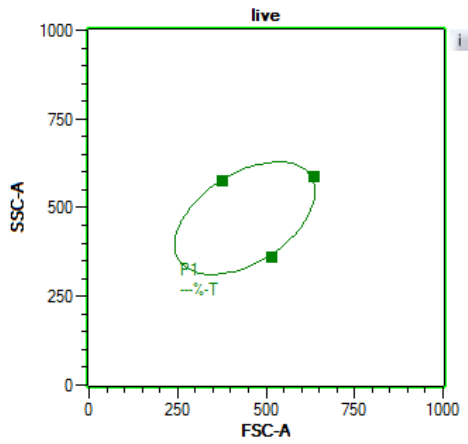
Rectangle – Click on the **Rectangle** in the toolbar or click **Edit > Rectangle**.

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

Quadrant – Click on the **Quadrant button** in the toolbar or click **Edit > Quadrant**.
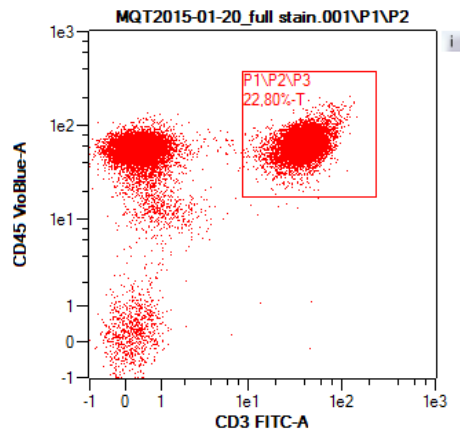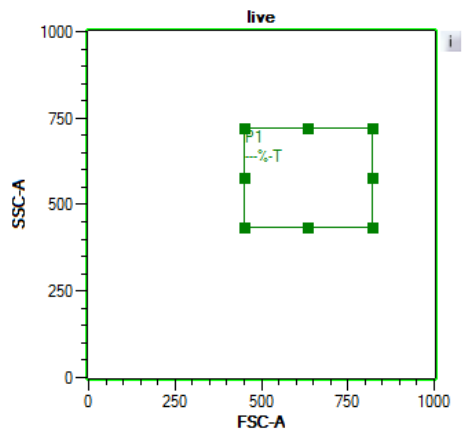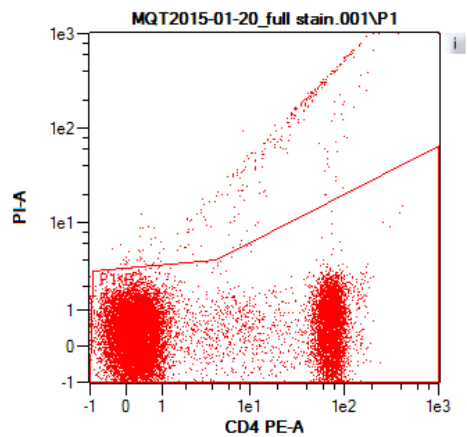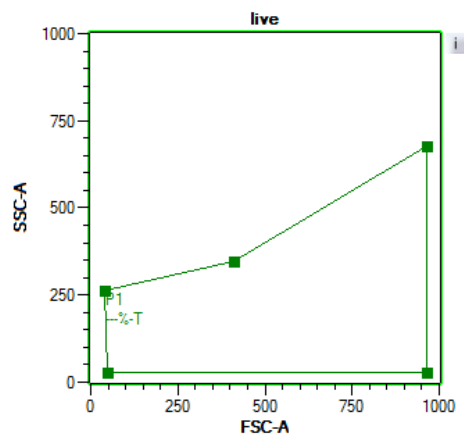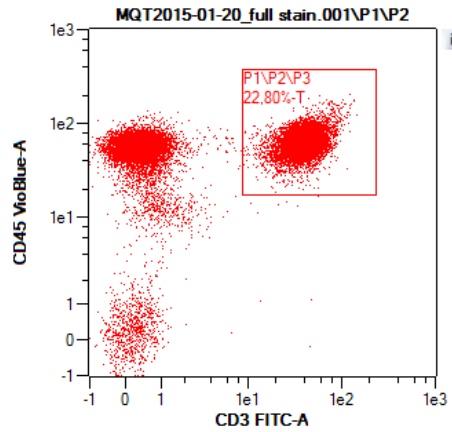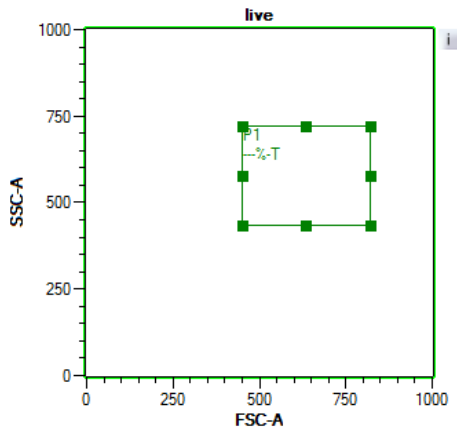
Interval – Click on the **Interval button** in the toolbar or click **Edit > Interval** .
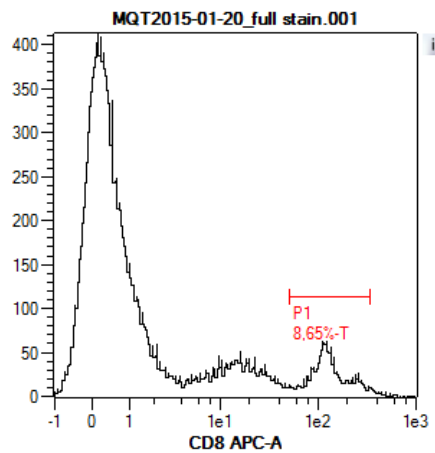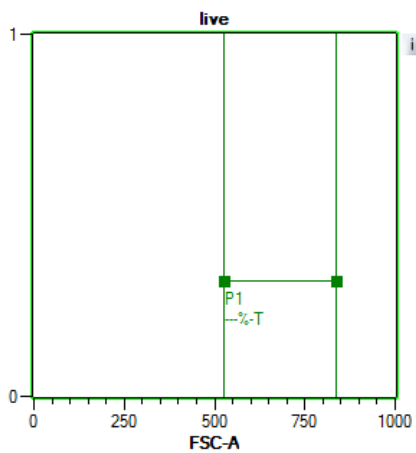
5.6.2   Copy gates

Copy gates via drag and drop

Regions or entire gating strategies can be copied to other files by a drag and drop functionality. Additionally, regions can be moved to other areas of the gating strategy by drag and drop functionality.

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

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

Copy/paste regions

Regions can be copied from one plot and pasted to the same or different but compatible plot; i.e., a region from a dot plot can be pasted to another dot plot or density plot, but not a histogram. Upon pasting, the same physical coordinates are used for the region, even if the plot has different scales.

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

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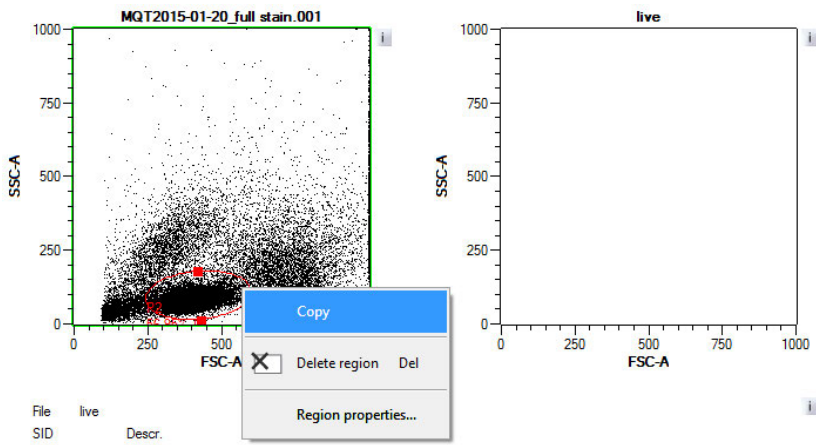
3 Right click on a compatible plot and choose **Paste**. This region will be identified as P2.This new region is completely modifiable using the edit points and **Region** properties.
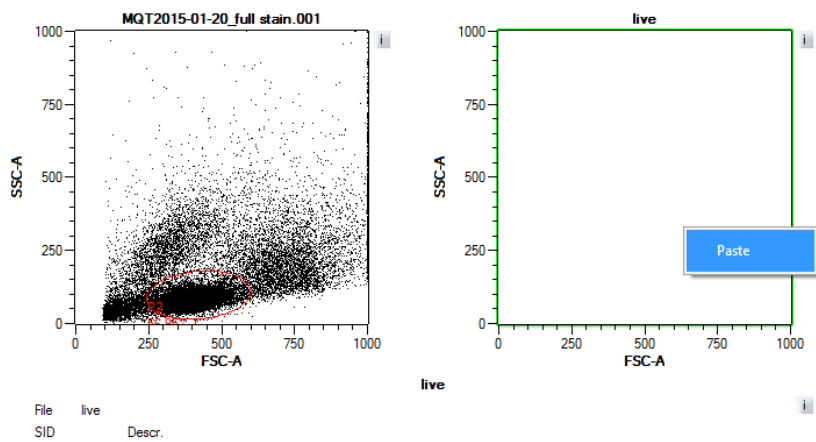
5.6.3   Delete gates

To delete a gate, do one of the following:

- Select the gate and press the delete key, or click the **Delete region button** (**1**) in the toolbar.
- All regions under a file or under other regions can be deleted by right clicking on the file or the top region in the hierarchy and choosing **Clear subgates**.
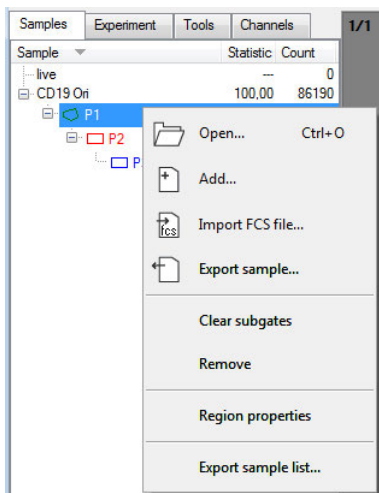
5.6.4 Move a region within a gating hierarchy

After establishing a gating strategy within a data file, click on a region that you would like to move under another region within the strategy. The region will then be copied under the chosen region and will become a new incremental region.

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

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

It is also possible to create two different regions from one parental gate. Regions drawn in a single plot will 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.
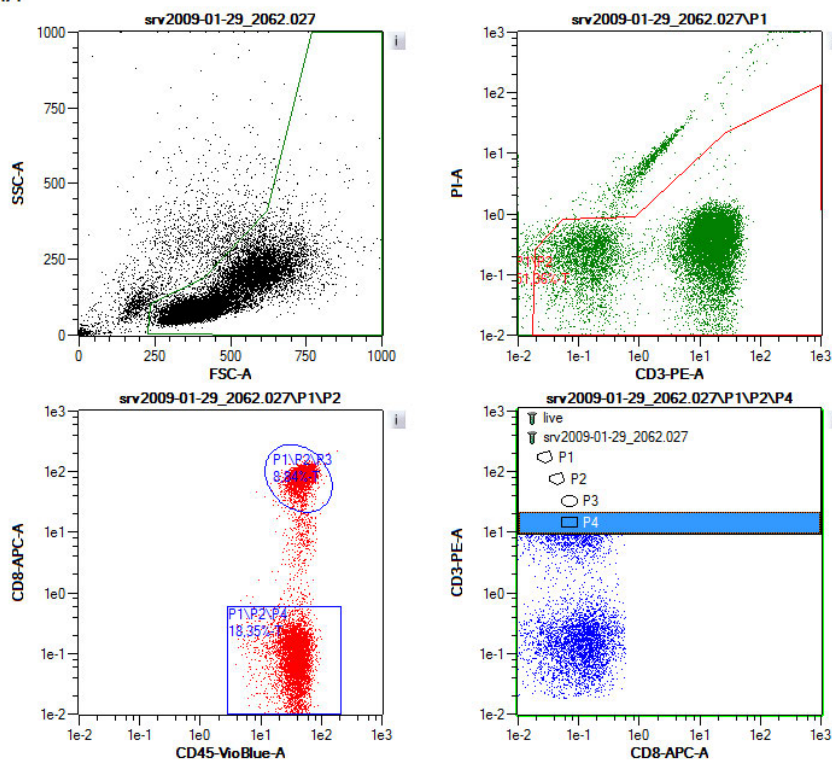
5.6.6   Change region properties

1 To change color, region name, and/or define the region as a **Not gate**, right-click on the region name displayed in the sample list or right-click on the activated gate in the dot plot.
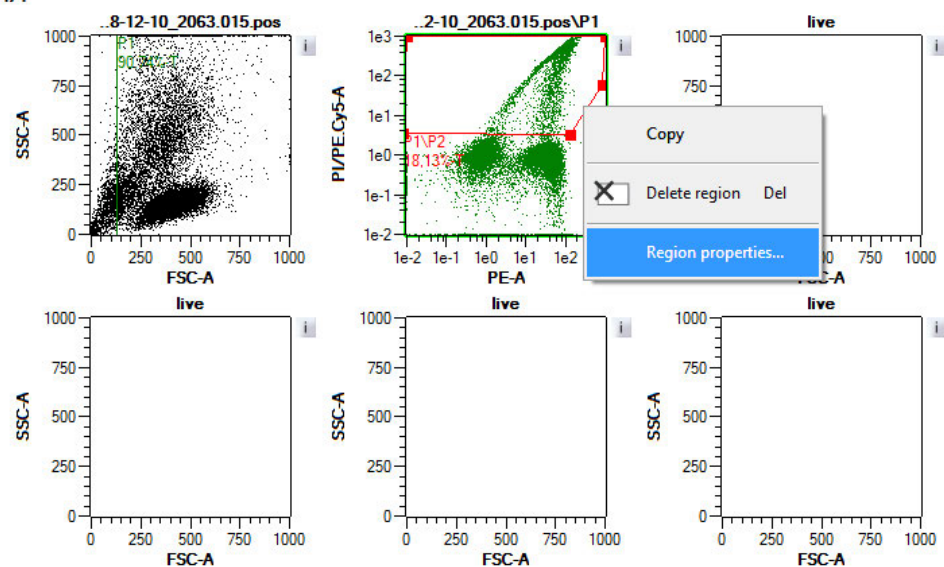
2 Select **Region properties**. Select color, enter name, and/or set the gate as a **Not gate**. Click **OK**.

3 To change other Region properties, click the **'i' button** next to the plot of interest. Adjust as desired. Refer to section **Plot properties** for details.

5.7 Gating strategies

5.7.1 Live gates

Only events within a live gate are acquired and saved by the MACSQuantify Software. Live gates are useful when large data sets are being acquired. Since only data in a gate is saved, the data file is smaller. Experienced users can, for example, exclude debris by live gating. However, it is recommended to acquire and save all data to avoid unintended loss of data. Live gating strategies can be saved for future use as Analysis templates.

5.7.2 Stop gates

Unlike with a live gate, all data are acquired and saved by the so called **stop gate**. However, a stop gate used in combination with the Events option instructs MACSQuantify Software to acquire data from the entire analysis window until a pre-defined number of events are acquired within the stop gate (i.e. a gate or region that is defined as the stop gate). Stop gating strategies can be saved for future use as Analysis templates.
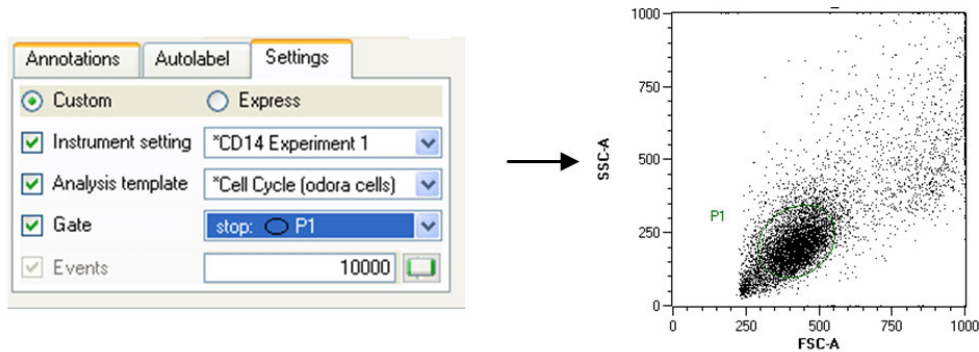
Figure 5.5: Region P1 is defined as a stop gate. At 1000 events, data acquisition stops automatically.

5.7.3 Not gates

So-called **Not gates** are used to eliminate a cell population from analysis. **Not gates** are surrounded by a dashed line.
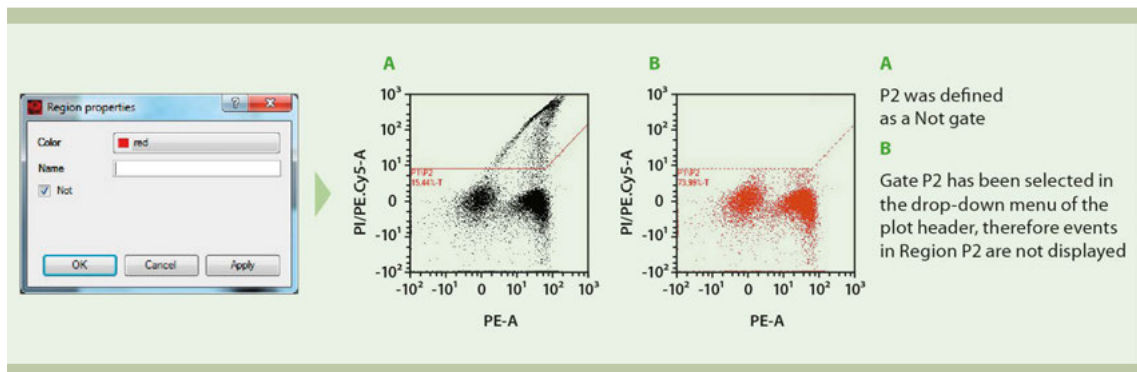
Figure 5.6: Region P2 (red) is defined as a **Not gate**.

5.7.4 Multilayer mode display

The MACSQuantify Software allows the user to display gated events within dot plots by their gated colors. While establishing a gating strategy, each subsequent plot is formatted to display only events of the parent gate. By using the multilayer mode, all events with parent populations can be displayed and are identified by their region color.
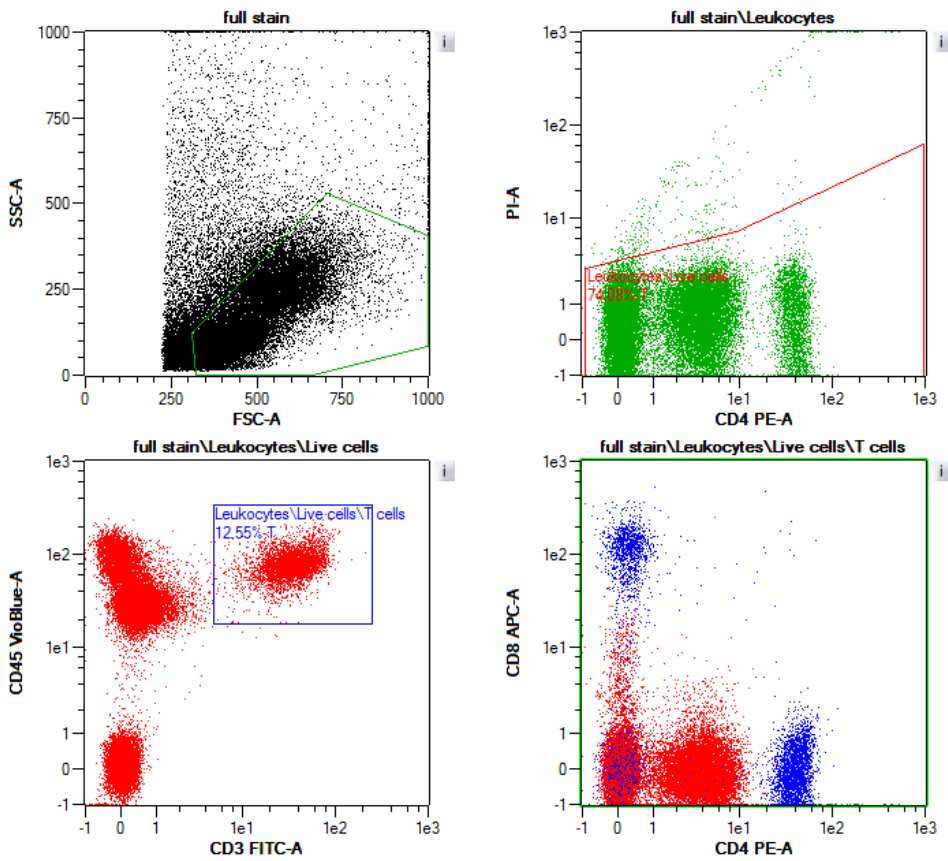
Figure 5.7: Multilayer mode example gating strategy.

5.7.5 Back gating

Back gating means that a specific subpopulation of cells defined by a particular gating strategy can be viewed on a previous plot. This can be useful to help the user identify where within the previous plot the cells of interest are displayed.
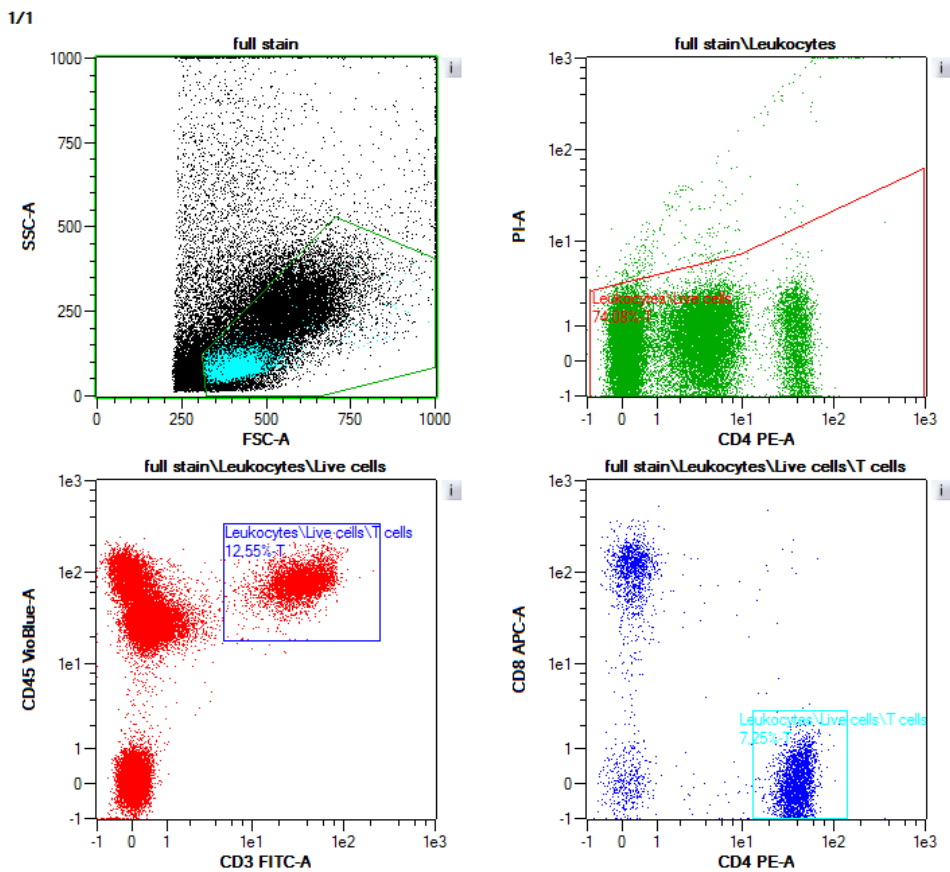
Figure 5.8: The region "T helper cells" was chosen for display in the upper left plot.

5.7.6   Set a live gate
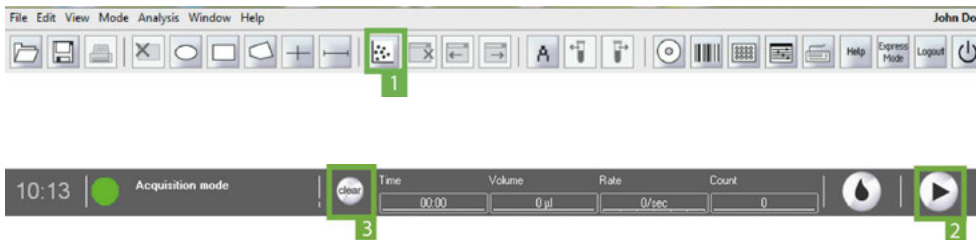
1   Click the **New analysis window** (1) button in the toolbar to open a new analysis window. Choose a plot design.

2   Define the Experiment settings.

3   Ensure that the live gate option for the appropriate gate is selected in the **Experiment** and **Settings tab**. Check the **Gate** checkbox and choose population live.

Data analysis

5
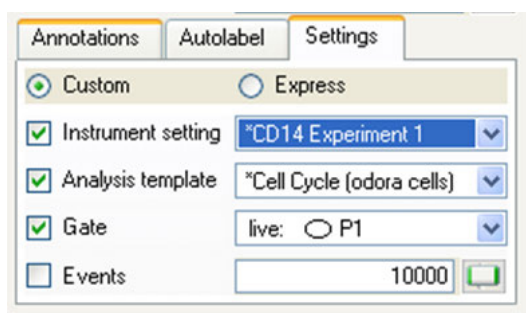
4   Ensure that the correct instrument settings are loaded and that compensation is correctly performed.

5   Ensure that enough sample, reagents, and buffers are provided, and the waste is empty.

6   Click on the **Start Acquisition** (**2**) button in the instrument status bar. The MACSQuant Instrument will commence sample uptake and measurement.

7   Set plot axes appropriately and draw regions on the plots.

8   Click **Clear** (**3**) to delete all events displayed on a dot plot, i.e., to refresh the plot.

9   Save the **Analysis template** for future use.

5.7.7   Set a stop gate

1   Click the **New analysis window button** (**1**) in the toolbar to open a new analysis window. Choose the required plot design. See section **Plot types** for more information about plots.

2   Define the Experiment settings as described in section.

3   Enter the required number of events for the associated stop gate.

4   Check the **Gate checkbox** and select the desired stop gate from the drop-down list.
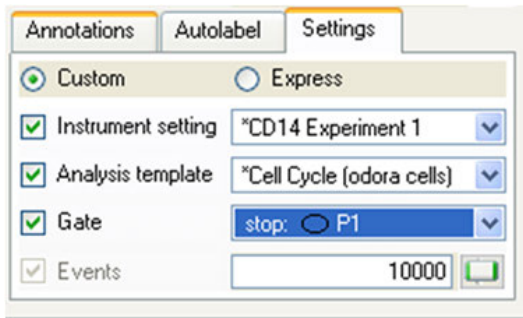
5   Ensure that the correct instrument settings are loaded and that compensation is performed correctly.

5.7.8   Set a not gate

1   Draw a region or gate around the population to be excluded from analysis.
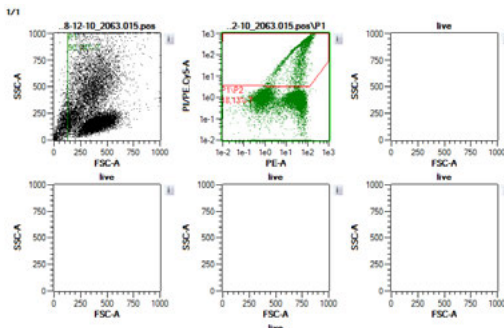
2   In the Samples menu, right-click on the region of interest.
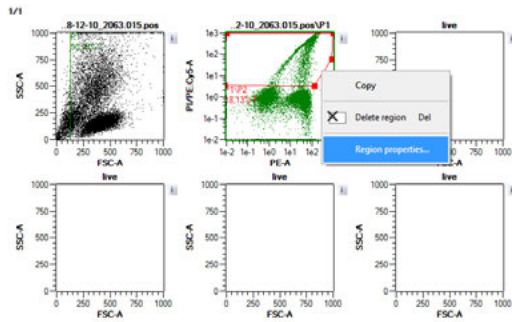
3   Select **Region properties** from the drop-down list.
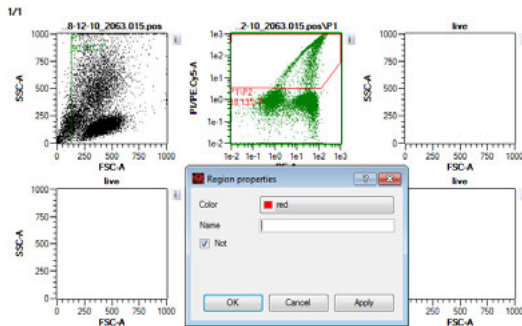
4   Check the box **Not**. This region is now excluded from analysis. (Optional) Select a color and/or name the Not gate as desired.

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

6   In subsequent plots, gated cells are excluded.
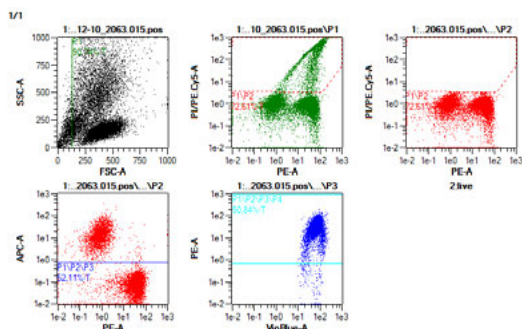
5.7.9   Multilayer mode

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

2   Define a Boolean gating strategy, such as a scatter region (P1), dead cell exclusion region (P2), and various subpopulations (P3, P4…Pn). Also see example in figure below.

3   To create a multilayer mode plot where gated populations are displayed by their region colors, choose **Multilayer mode** from the Mode menu.

4   The plot will now display all events with their gated region color.
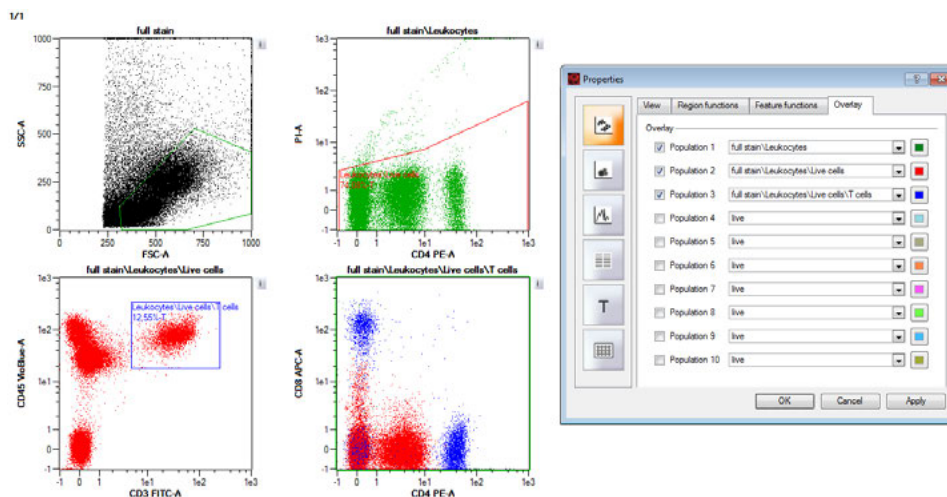
Figure 5.9: Multilayer mode example gating strategy and display. PBMCs were stained with a T cell panel (CD45-VioBlue, CD3-FITC, CD4-PE, and CD8-APC). Cells were gated on leukocytes within the scatter plot (upper left), then gated for all live cells (upper right). The live leukocytes were gated on all T cells (lower left) and the CD8+ and CD4+ T cells were displayed in a final plot (lower right). The CD4/CD8 plot was formatted for multilayer mode to show all cells with their specific region colors (Green – all leukocytes; Red – all live, leukocytes; Blue – all live, T cell leukocytes). Green cannot be seen, as Red designates almost 99% of all green events.

5.7.10 Apply back gating

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

2 Define a Boolean gating strategy, such as a scatter region (P1), dead cell exclusion region (P2), and various subpopulations (P3, P4…Pn).

3 Identify the gated region to back gate onto the FSC versus SSC plot (e.g., T helper cells).

4 Click the button of the FSC versus SSC plot. Within the **Region Properties** dialog box, select region for back gating, e.g., T helper cells.
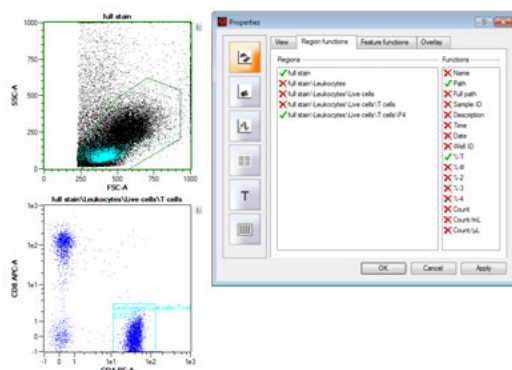
5 Gated events with their associated region color will be displayed on top of all other events within the FSC versus SSC plot.
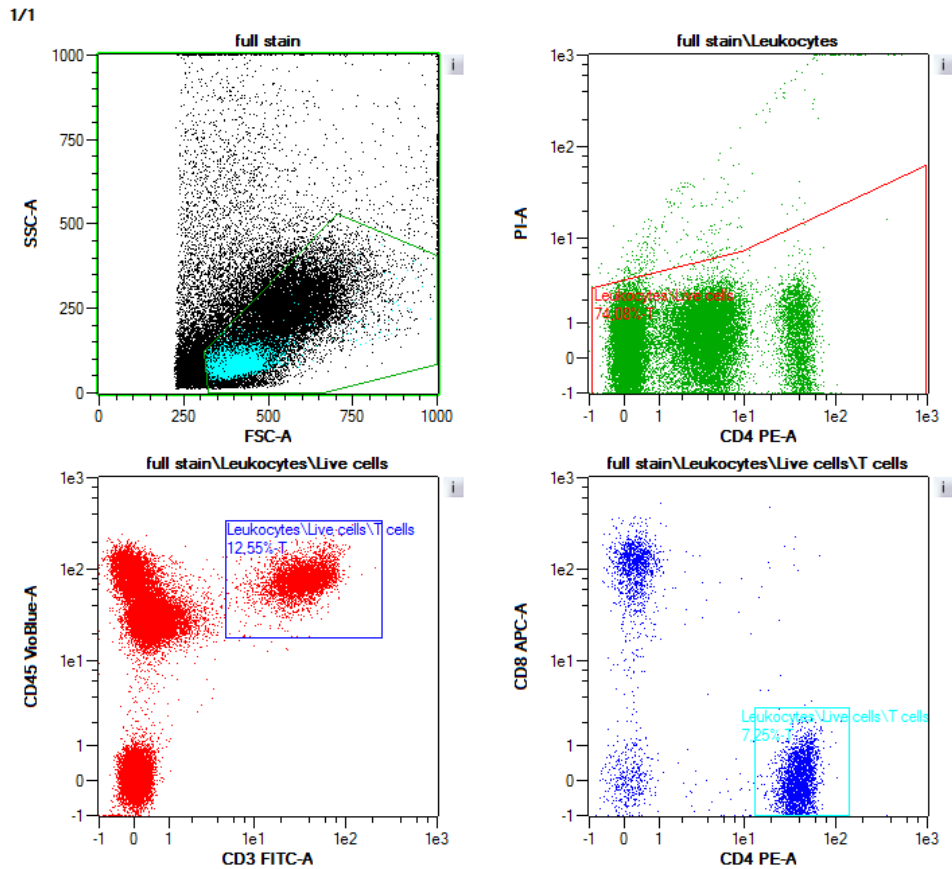
5.7.11   Classic hierarchical gating – a walk-through example

In the following example, the IL-17 Secretion Assay – Cell Enrichment and Detection Kit (# 130-094-542) was used for the analysis of viable human IL-17 secreting leukocytes from PBMCs. Enriched human IL-17 secreting cells were labeled with the following fluorochrome-conjugated antibodies: IL-17 – PE, CD4-FITC, and CD154-APC.

1   Open a data file or simply draw a region around events that are being acquired by the MACSQuant Instrument in real-time. To open a file, click **File > Open** and highlight the data file(s) to be analyzed. Click **Open**. Alternatively, select the Samples tab, right-click in the sample window, and select **Open**: Highlight the data file(s) to be analyzed. Click **Open**.

2   Click the **New analysis window button** in the toolbar to open an analysis window. Select the desired plot layout. In this case, a Plot4 layout was chosen.

3   Select the plot of interest. A green border indicates the chosen plot.

4   Double-click on the opened data file (not necessary when acquiring data in real-time).

5   Use the appropriate button to select a geometrical shape for gating:

6   An elliptical region (P1) was drawn to select lymphocytes and exclude unwanted debris.
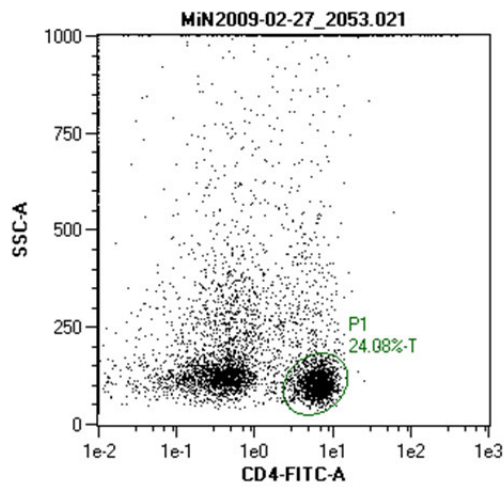
7   A polygonal region (P2) was drawn using the polygon tool to exclude unwanted dead cells on the P1 population.
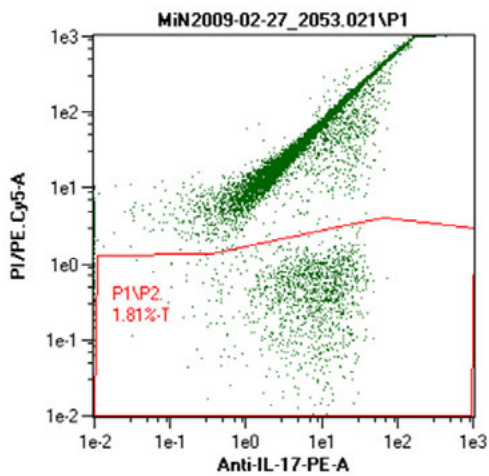
8   A rectangle region (P3) was drawn using the rectangle tool to select for IL-17+ CD4-viable lymphocytes.
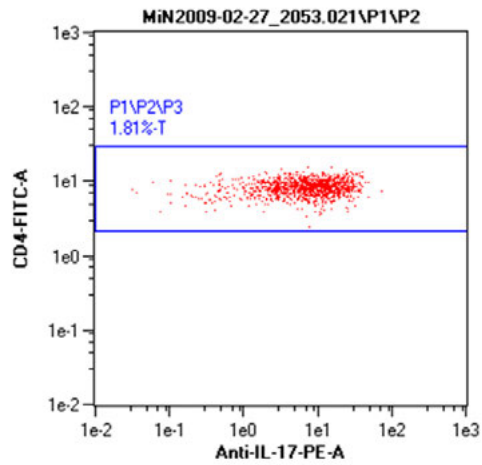
9   The region P3, i.e., P1/P2/P3, was displayed in the Anti-IL-17-PE versus CD154-APC dot plot.
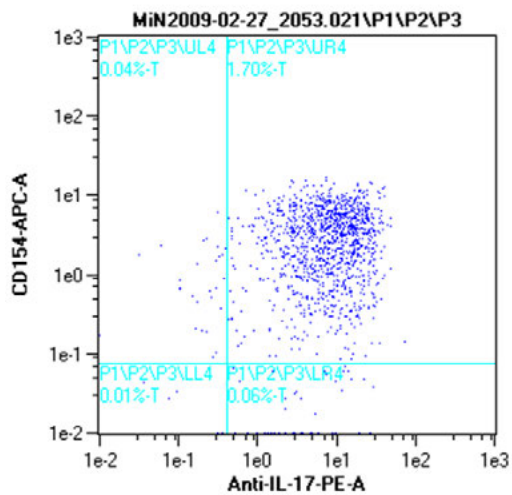
10 Click the **+ button** to expand the gating strategy in the **Samples menu**. The regions in this gating strategy
    are not on the same level in hierarchy. Regions are created within regions, creating children,
    grandchildren and great-grandchildren of the primary gated region.
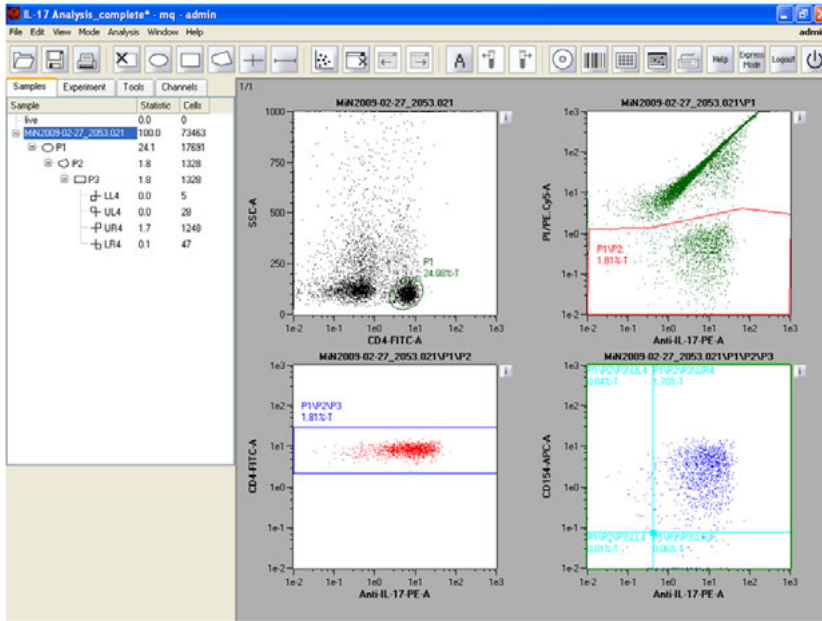
11 To save the gating strategy as an analysis template, ensure that MACSQuantify Software is in Analysis mode, i.e., the **Analysis mode button** in the toolbar should be highlighted.

12 Click the **Save button** in the toolbar and select **Analysis templates**.

13 Choose the file location **Public**, **Private**, or **External**.

14 Name the file. Click **Save**.

5.8 Post-aquisition data analysis

5.8.1 Apply analysis templates

It is possible to apply or view an analysis template that was used in a previous experiment, i.e., the gating strategy and corresponding plots that are associated with acquired data files can be reapplied and viewed.

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

2 Right-click on the data file and select **Apply analysis template**.
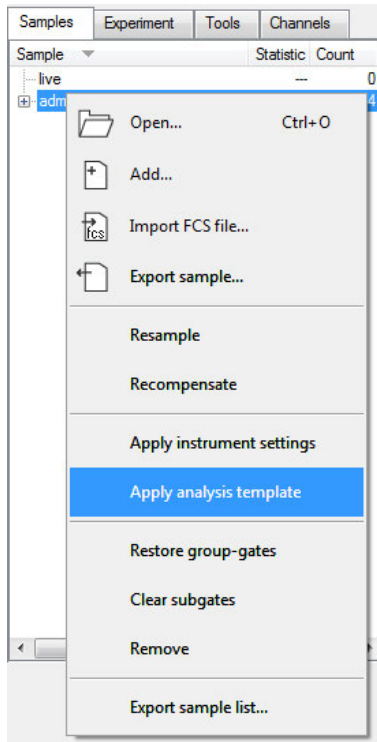
3 The data file and corresponding analysis template will be loaded in analysis mode.
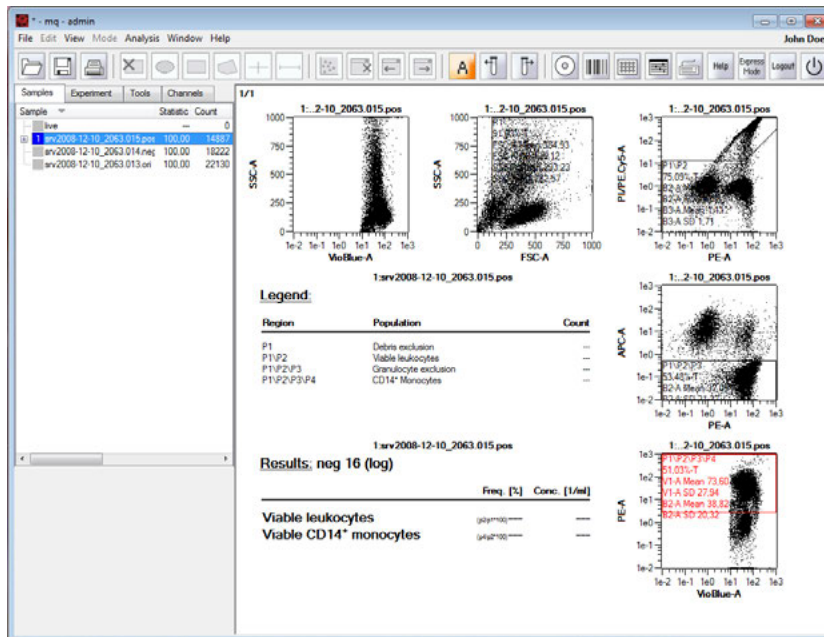
4 To view analyzed results of the previously defined analysis, right-click on the data file and select **View with analysis<name of analysis>**.
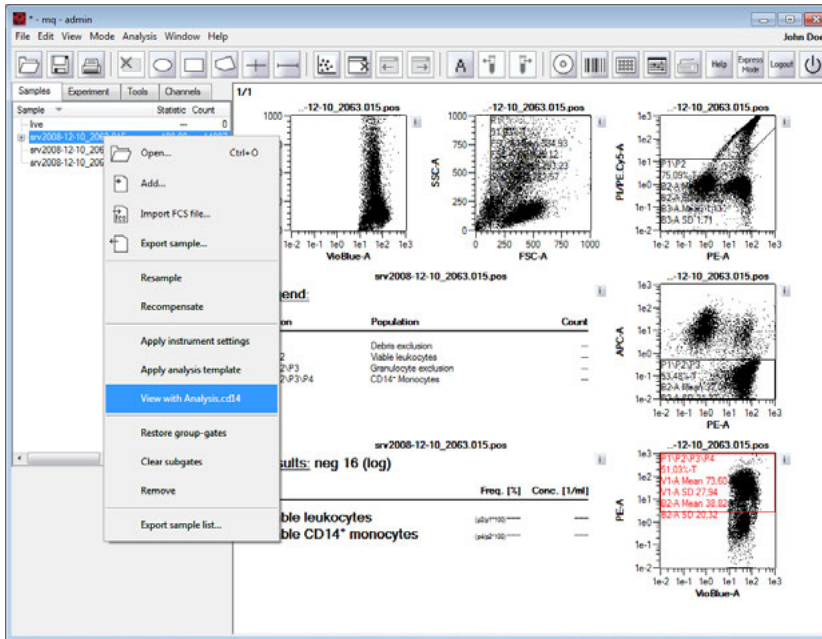
5 The analyzed results of a previously defined analysis are shown.
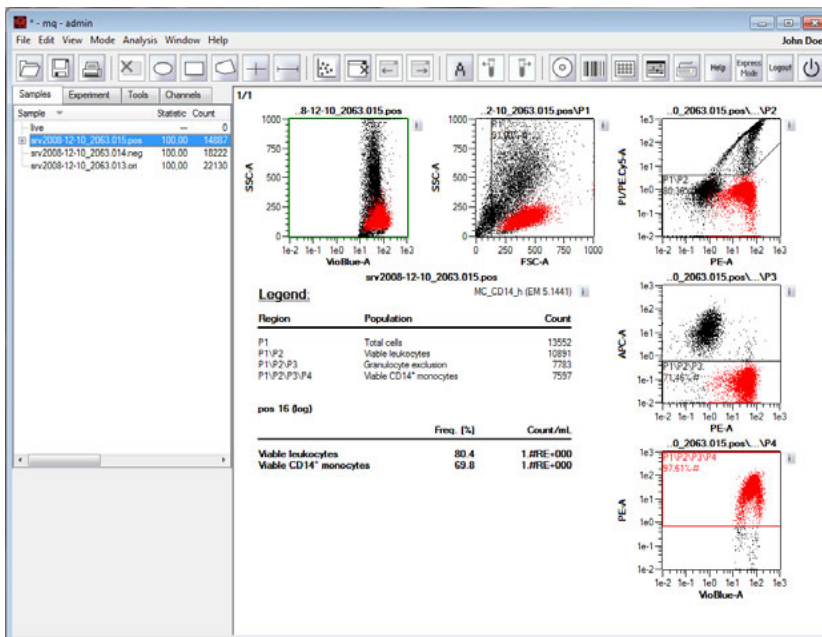
5.8.2 Apply PMT voltage and compensation settings

It is possible to apply instrument settings associated with a data file.

1 Go to **File > Open** or click the **Open button** in the toolbar.

2 Select **Instrument settings**.

3 Highlight the desired instrument setting from a **Public**, **Private**, or **External source**.
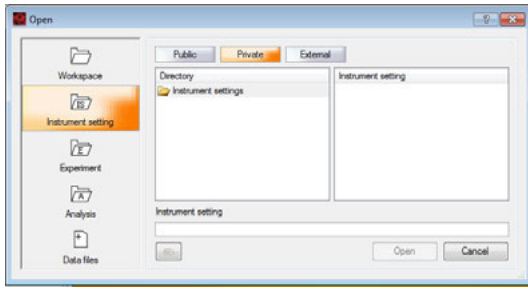
4 Click **Open**. The file settings will be loaded.

Alternatively:

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

2 Click on the **Samples tab**.

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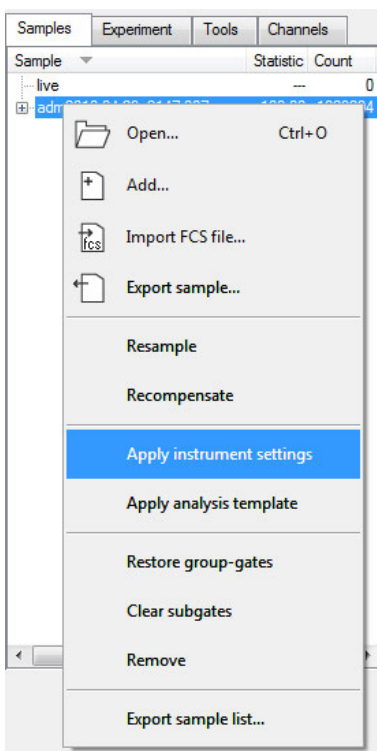
5.8.3 Recompensation

It is possible to recompensate data that was already acquired. This is useful if data was acquired using incorrect instrument settings. The results can be reanalyzed using a different compensation matrix.

1 Open the desired data file(s).

2 Go to the **Samples tab**.

3 Apply instrument settings and analysis template.

4 Click the **Instrument settings button** in the toolbar to view the instrument settings. Experienced users can make adjustments to the compensation values in the **Compensation tab** if required.

5 Right-click on the data file in the sample list and select **Recompensate**. A new file is created with the adjusted compensation values applied. These files will be prefixed with an underscore, i.e., **_srv2008-12-10_2063.018.pos**. The original data file is retained.

5.8.4 Group data after acquisition

The maximum sample volume that can be acquired in a single step by the MACSQuant Instrument is 450 µL. However, there are occasions when the sample size exceeds this volume and aliquots of the sample must therefore be spanned over two or more tubes. By grouping these samples, the acquired data will be consolidated into a single file on the hard drive, which can also be analyzed in a single data file or analysis plot. This can be easily accomplished by grouping sample prior to data acquisition. Refer to section **Sample grouping** for more details. If grouping was not performed prior to data acquisition, it is still possible to group samples post-acquisition.
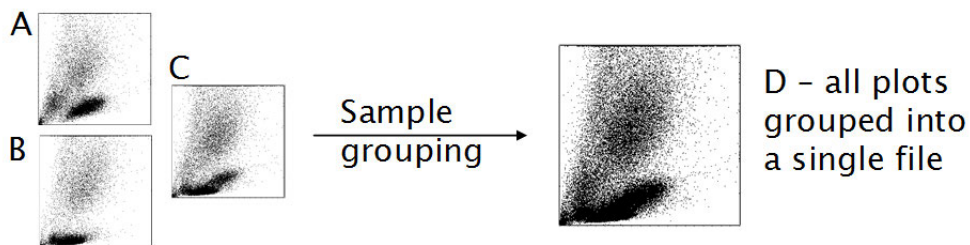
1   Click **File > Open** and highlight the files. Click **Open**.
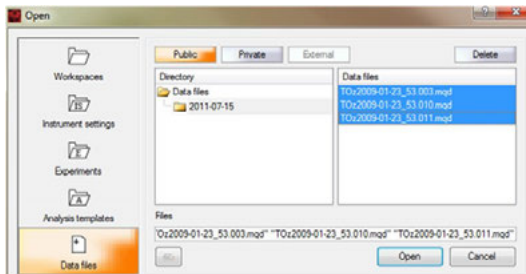
2   In the Samples tab, highlight each file that must be grouped. Right-click and select **Group**. The resulting grouped file is highlighted, and preceded by an underscore (_).

3 The resulting grouped file is highlighted.

5.8.5 Resampling: Change annotations

1 Open/Add data files into the MACSQuantify Software.

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

3 Choose **Apply instrument settings**.

4 In the **Annotations tab** of the **Experiment tab**, type in all the correct annotations.

Note:	Annotations are not transferred from the file, so that all annotation will need to be added.

5 Right-click on the data file and choose **Resample**.

6 A new file with an underscore (_) at the beginning of the file name is created. The underscore file will contain the new annotations, whereas the original data file is unaltered.

7 If subsequent data files require the same annotation changes and were run using the same instrument settings, they can also be updated in a batch format.

8 Highlight all files requiring the changed annotations.

9 Right-click on the highlighted files and select **Resample**.

10 New versions of all files will be created with an underscore in front of the file name. These underscore files can be analyzed the same as any other .mqd files.

5.8.6　Resampling: Change scales

1　Open/Add data files into the MACSQuantify Software.

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

3　Choose **Apply instrument settings**.

4　In the **Annotations tab** of the **Experiment tab**, type in all the correct annotations.

Note:　Annotations are not transferred from the file, so that all annotation will need to be added.

5　Right-click on the data file and choose **Resample**.

6　A new file with an underscore (_) at the beginning of the file name is created. The underscore file will contain the new annotations, whereas the original data file is unaltered.

7　If subsequent data files require the same annotation changes and were run using the same instrument settings, they can also be updated in a batch format.

8　Highlight all files requiring the changed annotations.

9　Right-click on the highlighted files and select **Resample**.

10　New versions of all files will be created with an underscore in front of the file name. These underscore files can be analyzed the same as any other .mqd files.

6

Report your data

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

6.1 Copy pages or plots

6.1.1 Copy an entire page

1   Display and properly analyze data.
2   Click **Edit > Copy page**. The page is saved onto a clipboard.
3   Open program for pasting, e.g., Word or PowerPoint.
4   Right-click and select **Paste** within this program.
5   The page including all the plots and/or statistic tables will be copied.

6.1.2 Copy a single plot

1   Display and properly analyze data.
2   Click on the specific plot or statistic table for copying. The plot should is highlighted (light green rim).
3   Click **Edit > Copy plot**. The plot is saved onto a clipboard.
4   Open program for pasting, e.g., Word or PowerPoint.
5   Rightclick and select **Paste** within this program.
6   The plot including any gating and annotations will be copied.

6.2 Print

6.2.1 Printing data files

1   To print active workspaces, open the desired workspace or analysis window.
2   Click the **Print button** (1) in the toolbar.
3   Select the desired printer. Click **Print**. The printer can be connected directly to the MACSQuant Instrument or to the PC running the MACSQuantify Software, or via a network connection. The active workspace is printed as shown below.
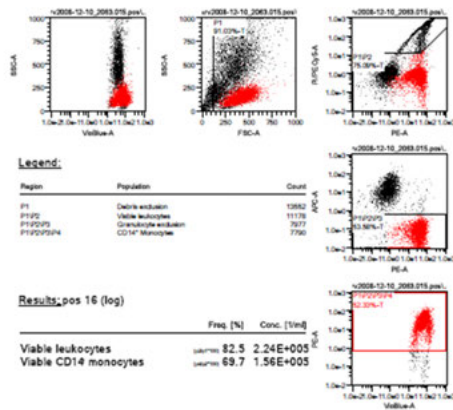
6.2.2   Print all windows

1   To print the current analysis template for all samples in the sample list, do one of the following:

- Go to **File > Print all…**.

- Alternatively, right-click on the **Samples list** to open the **Samples tab context menu** and chose **Print all…**.
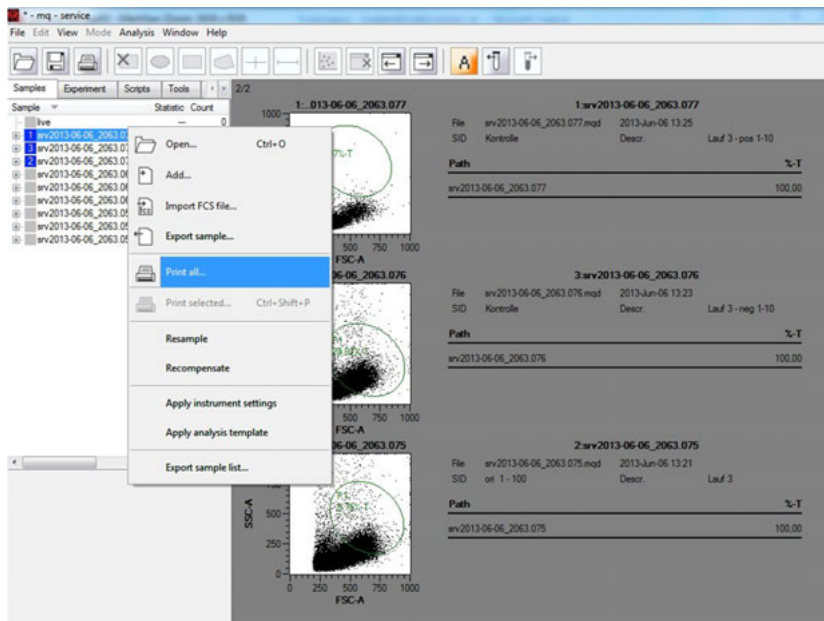
2   Ensure that the current analysis is applied to all samples in the sample list prior to print. By default, the radio button next to **All** is checked, i.e., all analysis windows will be printed. By checking the radio button next to **Pages**, analysis windows can be selected for print. When performing multiple analyses in one analysis window, the MACSQuantify Software will assign a number for the sample and its respective analysis. This number will be displayed in the sample list.
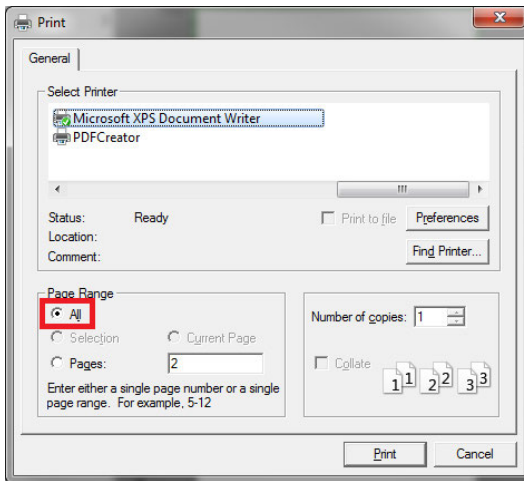
To use the **Print all…** command, several parameters have to considered and accomplished. If a prerequisite for the Print all… command is not fulfilled, the command will be disabled.

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

6.2.3   Print selected windows

Note: The Print selected... batch-print option is only accessible in Analysis mode.

1   Choose **Print selected…** to print the current analysis template for all selected samples. Ensure that the current analysis is applied to all selected samples in the sample list prior to printing. By default, Page Range the radio button next to **All** is checked and all analysis windows will be printed. Checking the radio button next to **Pages** to select analysis windows for printing.
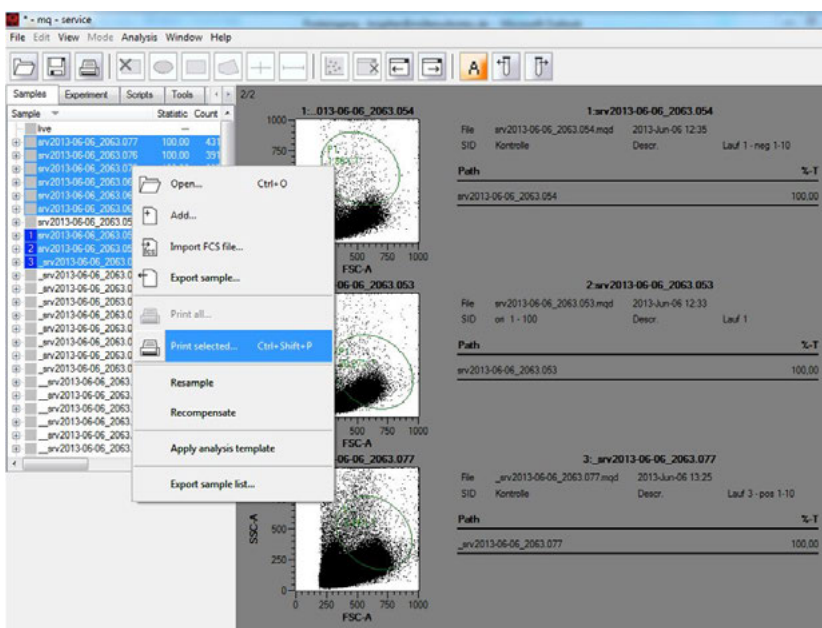
2 When performing multiple analyses in one analysis window, the MACSQuantify Software will assign a number for the sample and its respective analysis. This number will be displayed in the sample list. For using the Print selected… command, several parameters have to considered and accomplished. If a prerequisite for the Print selected… command is not fulfilled, the command will be disabled.

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

6.3 Exporting the sample list to Microsoft® Excel

1 Generate an appropriate gating strategy for the data files and apply the gates to all data files within the experiment.

2 Right-click within the **Samples tab** and choose **Export sample list**.

3 A dialog box will open to select the parameters to export.
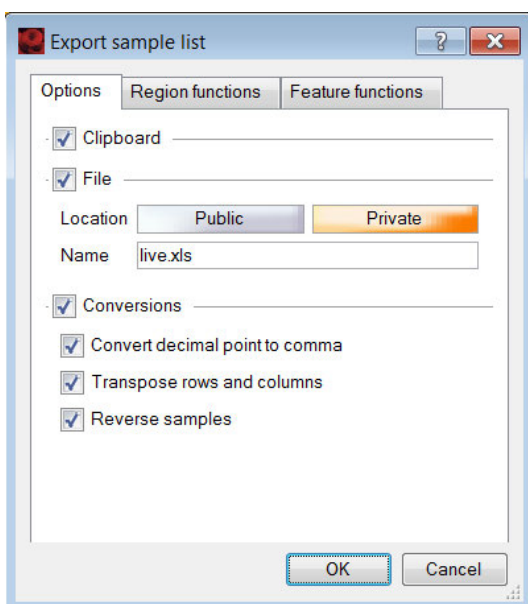
4 Select one or both of the following:

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

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

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

> **Note:** Several regions can be easily selected or deselected at once by right clicking on any region and choosing the desired region from the context menu.

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

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

7

Data management

This chapter describes data management for custom mode users and administrators. For information about data management if using MACSQuantify as an express mode user, refer to chapter **The Express mode user**.

7.1 Data backup

In order to backup data from the MACSQuant Instrument, a mass storage drive must be connected or a network location designated.

Note: Only administrators can assign a public backup folder.

7.1.1 Designate a backup location

1 Login as an administrator to designate a network location for data backup.

2 Click **Edit > Options (default) > Files**.
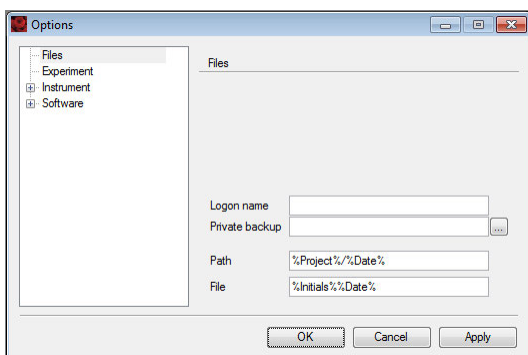
3 Chose a location on the network that can be accessed by all users of the MACSQuant Instrument. Individual users can format their own personal network folder within the designated backup folder by clicking **Edit > Options > Files**.

4 Enter the full path of the network location, starting with \\, or select the destination folder via browsing. Depending on your specific network structure, access of the backup folder might require a logon name and a password, which can differ from the user login for the MACSQuant Instrument. Therefore you can also set the logon name for your network.

7.1.2 Data backup to a Network location

1 To backup data files, click on the **Backup button** from the toolbar.

2 If requested, enter password when prompted.

3 Select all files or all data files when prompted.

4 All previously backed up .mqd files will be deleted, if you selected delete cloned files. However, all .fcs files will need to be deleted manually.

5 When the backup is complete, a dialog box confirmation appears.

> **Note:** If a Private backup option has been defined, it will also appear as a source or destination in the Copy dialog.

7.2 Files

7.2.1 Open files

Workspaces, Instrument settings, Experiments, Analysis templates, and Data files can be opened into the according side panel tab of MACSQuantify Software.

Data files saved in a network location or on a USB stick first have to be added to the MACSQuant Instrument by right-clicking within the Samples tab and choosing Add.... from the context menu.

1 To open files, do one of the following:

- Clicking **File > Open...**,
- Clicking the **Open button** in the toolbar, or
- Right-clicking within the **Samples tab** and choosing **Open**.

2 Select **Workspaces**, **Instrument settings**, **Experiments**, **Analysis templates**, or **Data files** on the left-hand side of the dialog box.

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

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

5 Confirm your selection with **Open**.

7.2.2 Add files to the samples list

If the MACSQuant Software is installed on a PC, it can be used to view and analyze previously generated .mqd files. If files are located on an external storage device, they need to be added to the sample list prior to open them. This function is only available when using MACSQuantify Software on a PC.

1 Rightclick on the **Samples tab** in the side panel.

2 Click **Add...**, select files and click **Open**.

7.2.3 Save files

Workspaces, **Instrument settings**, **Experiment**, and **Analysis files** can be saved to the **Public** or **Private** locations as defined under **User settings**, or to external locations. Data files are saved automatically.

> **Note:** Saving Workspaces during acquisition is not possible. Analysis templates can only be saved in Analysis mode when the button is highlighted orange.

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

2 Choose **Workspaces**, **Instrument settings**, **Experiments**, or **Analysis** to save the file type.

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

4 Assign an appropriate file name.

5 Click **Save**.

7.2.4   Import FCS files

Flow cytometry data generated with other instruments can be imported in order to be analyzed with MACSQuantify Software.

1   Rightclick on the Samples tab in the side panel.

2   Chose **Import FCS file...** from the context menu.
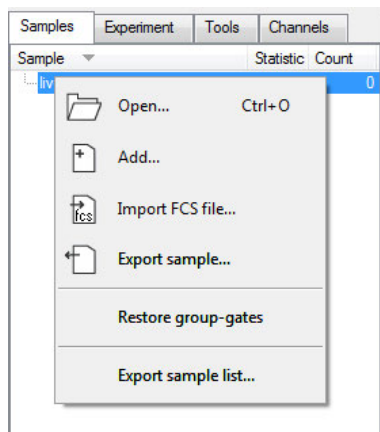
7.2.5   Export FCS files

The MACSQuant Instrument will store all acquisition data in the .mqd file format.

MQD files can be exported as FCS or CSV files at any time by right-clicking on opened or added data files in the Samples tab and selecting Export sample. Select the desired file type (.fcs or .csv) from the drop-down list. If a gating strategy was applied to any of the data, MACSQuantify Software will create a separate FCS file for each gated population. If only one FCS file is required for the original data file, check the box **Skip subpopulations**. The FCS files will be stored to the same folder as the original MQD data files were opened or added from.

Note: Go to **Edit > Options > Software > Acquire** to generate FCS files automatically after data acquisition (see section **Acquisition**).

7.2.6   Copy files

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

7
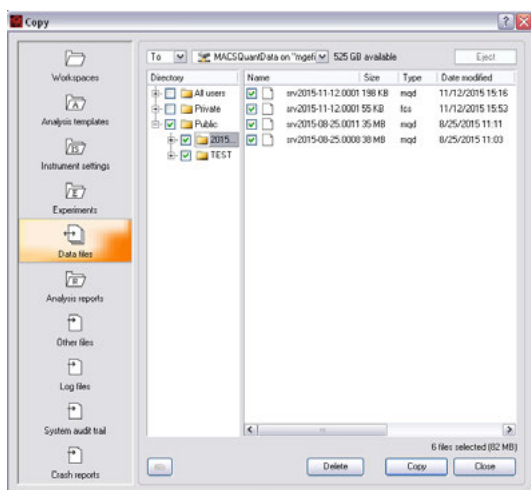
Custom mode users can only copy their own files (Private folder) and files in the Public folder (depending on their access rights).
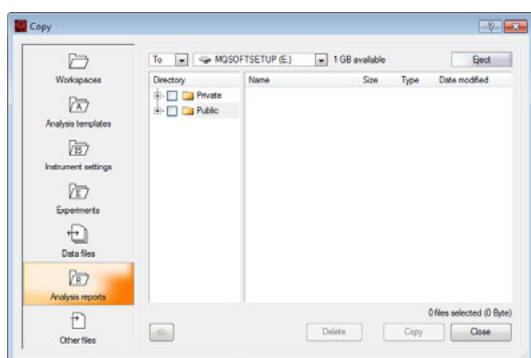
1. To transfer data from the MACSQuant Instrument to a remote storage location, either click on the **Backup button** in the toolbar or go to **File > Copy...**. Choosing **File > Copy...** also allows the deletion of files from the MACSQuant Instrument.

2. To transfer data from the MACSQuant Instrument to a USB stick, insert it into an USB port of the MACSQuant Instrument. Wait for the device to be recognized.

3. Choosing **File > Copy...**, data files as well as other files, e.g., instrument settings and templates, can be copied.

4. Choose the destination location from the available drop-down menu in the dialog box. If you wish to transfer data to a network location, enter the password when prompted.
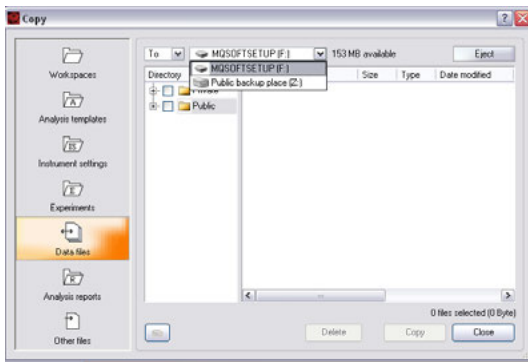
5 From the drop-down list, select:

- **To**: the selected files in the folder structure of MACSQuantify Software are copied to the selected destination. Please note that the according folder structure is also preserved at the destination location.

- **From**: selected files will be imported. It is necessary to have the files organized in the same folder structure as on the destination location of MACSQuantify Software. Otherwise the files will not show up.

6 Check the box next to the desired files or folders .
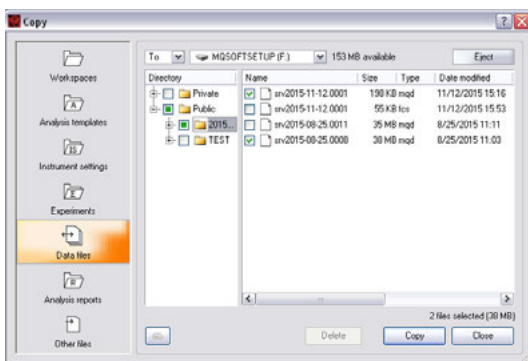
Tip: Multiple files can be selected by pressing the **Ctrl key**. To select a list of files, select the first and the last file while holding the **Shift key** of the keyboard. Selected multiple files by drawing a rectangular using a mouse while pressing the space bar of the keyboard. The checkbox displays a green check mark if all files of a folder are selected, while it shows a green square if only a part of files within a folder are selected.

7 Confirm your selection by clicking **Copy**. To remove folders or files that have been copied, click **Delete**.

8 When all files are copied, a report dialog box appears. Close the box to execute another copy or deletion command.

9 When using a USB stick, the option **Close and eject** is available to allow safe removal of the USB stick. Alternatively, you can select **Remove external media** from the toolbar to remove the USB stick safely.

7.3 Data storage

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

- **Public files** are located on the local hard drive of the MACSQuant Instrument and are accessible by any user.

- **Private files** are located on the local hard drive of the MACSQuant Instrument and are only accessible by the logged-in user.

- **External files** that are located on an independent file storage device e.g., an USB stick, can be accessed when the device is connected to the MACSQuant Instrument.

Files are saved within the cap folder structure on the MACSQuant Instrument hard drive:

- in a Public location in the folder global,

- in a Private location in folders bearing the name of the user (e.g., admin, John Doe, service).
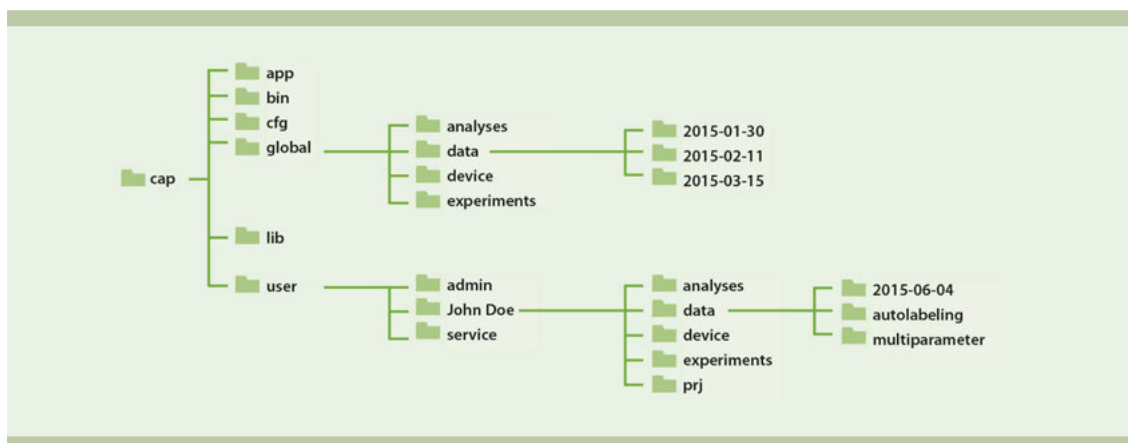
Figure 7.1: File structure of the cap folder on the hard drive of the MACSQuant Instrument.

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, any user can store and load files to and from this location.

The following file types are supported by MACSQuantify Software and can be accessed using commands to open or save files. The file types **Workspace**, **Instrument Settings**, **Experiment** and **Analysis template** can be stored and loaded with the **Open** and **Save** dialogs. Upon completion of a measurement, MQD data files are automatically stored in the location defined for the user. MQD data files can be loaded into the sample list by clicking **File > Open...**, or by clicking the **Open button** in the toolbar. In addition, MQD files can be exported as FCS and CSV files. FCS files can also be imported for viewing and analysis. MQD files can be copied / backed up to an external location with the **Copy** and **Backup** dialog.

7.3.1   Workspaces

A Workspace file is all encompassing, and represents a master file including information for the other file types. It contains the following information:

- Sample tab: samples currently displayed in the sample list

- Experiment tab: All experiment parameters

- Instrument settings: Current instrument settings including compensation and calibration

- Analysis: current analysis view and template, if selected

Loading a Workspace file will reopen the MACSQuant Software with the settings valid at the point of storing the workspace, i.e. it will load the same data files to the sample list, will enter the same parameters in the experiment tab and instrument settings, and will display the same analysis. Workspaces are most useful on a PC, due to the fact that instrument settings and sample lists will change from day to day on the MACSQuant Instrument.

Workspaces can be stored in the **Private** or **External** location but not in the **Public** location. **Workspaces** are saved in the **Private** or **External** location in the prj. folder.

7.3.2 Instrument settings

Instrument settings comprise compensation and calibration parameters for the MACSQuant Instrument. These parameters are important for data analysis and are vital to maintain standardized results over time. They will be saved within each .mqd file during data acquisition and can be used after data acquisition for recompensation. Default instrument settings during calibration as well as bank settings are saved in the Public location in the folder called device. Manually modified instrument settings are saved in the Private location in the folder called device.

To facilitate measurement of samples, the PMT calibration and compensation can be saved in an **Instrument settings** file. This file can be loaded the next time an experiment is performed. Default instrument settings during calibration as well as bank settings are saved in the **Public** location in the folder called **device**. Manually modified instrument settings are saved in the **Private** location in the folder called **device**.

Compensation and calibration parameters are important for data analysis and are vital to maintain standardized results over time. They will be saved within each .mqd file during data acquisition and can be used after data acquisition for recompensation.

7.3.3 Experiments
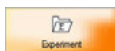
Experiments can be defined for repeated use. Files comprise all data that were defined for a particular experiment in the Experiment tab. Experiment settings are stored in any location in the folder called experiments.

Data management

7

135

8

The Administrator

8.1 User management

This section describes user management for standard installation. If the MACSQuantify Software is installed and set up for 21 CFR Part 11 compliant use, refer to chapter **21 CFR Part 11 compliance**

The MACSQuantify Software allows three types of user accounts with different permission levels: **administrator**, **custom mode user** and **express mode user**.

Administrators and **custom mode users** can both customize certain software and instrument settings, such as file name, experiment settings, instrument settings, and software settings. They can both perform calibration and compensation.

In addition to that, the **administrator** can specify global settings for the MACSQuant Instrument that apply to all users. Only the administrator can create user accounts and set permission levels for each user. Miltenyi Biotec recommends to assign only one administrator.

Express mode users have only limited access to the MACSQuantify Software functions. The Express mode is designed to simplify the setup, running, and analysis of experiments. With only a few actions, users with minimal flow cytometry experience can perform complex flow cytometry experiments. Express mode users are allowed to perform only minimal alterations to settings. They do not have permission to perform calibration.

8.1.1 Create new user accounts

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

2   Go to **Edit > User Settings**.
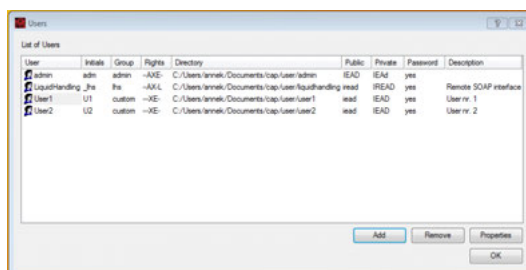
3   Click **Add** in the dialog box.
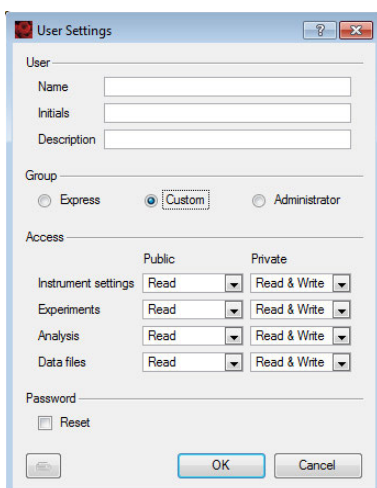
4 Enter a name, initials and designate the user to be **Custom** or **Express**.

- **Administrator users** can specify global settings for the MACSQuant Instrument that apply to all users. Administrator users have the right to manage all user accounts (i.e. create and delete user account, set permission levels for each user, reset passwords. You should assign only one Administrator user per Instrument.

- **Custom mode users** have access to full access to software capabilities (except administrator privileges) such as establishing instrument settings.

- **Express mode users** can only access the Express mode interface, which is intended for simplified use of the instrument. Express users can open public instrument settings but cannot create new instrument settings.

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

6 Set up file access for Instrument settings, Experiments, Analysis and Data files. Instrument settings, Experiments, Analysis and Data files can be saved to either **Public** or **Private**. The **Public** location is a shared location for every user. The **Private** location is individual for each user.

- **Read** means that the user can only open files from the specified location, but cannot save files to that location.

- **Read & Write** means that the user can open and save files from the specified location.

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

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

8.1.2 Delete user accounts

> **Note:** You need administrator rights to delete user accounts.

1 Go to **Edit > User Settings**.

2 Select user to delete from list and click **Remove**.

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

4 Click **OK** to proceed with deleting the user account and associated data files.

8.1.3 Change access rights of an existing user account

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

1 Login as user with administrator rights.

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

3 Select the user to modify.

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

5 Modify the users type of access or the users file access rights.

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

8.1.4 Resetting passwords

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

1 Login as an **administrator**.

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

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

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

5 The user can log in without a password and set a new password as described in section **Change passwords**.

8.1.5 Import or export user settings

1 Login as a user with administrator rights.

2 Connect a USB stick called "MQSOFTSETUP" to the instrument.

3 Go to **Tools > Import / Export user settings**.

4 Choose whether you want to import or export the user settings from the dialog box:

- Click import to import the user configuration from a connected USB stick.

- Click export to copy the system's user configuration to a connected USB stick.
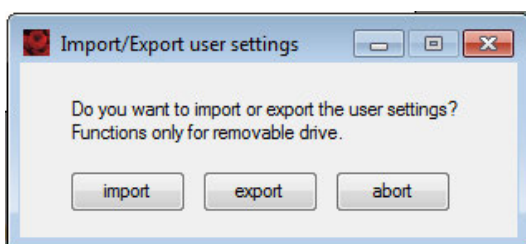
5 If no USB stick is attached to the system, or the configuration is not available from the USB stick, an error message appears.

6 When the user settings are successfully imported / exported, a dialog box is displayed.
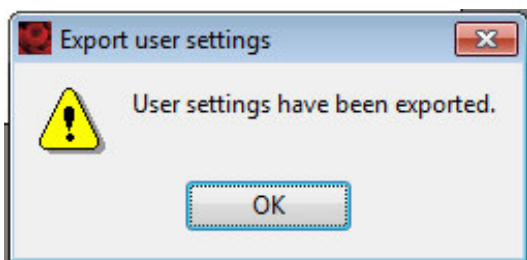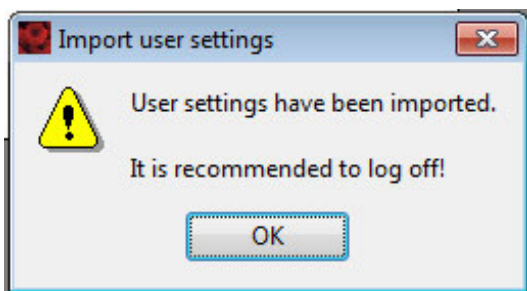
7 At the end of the process a dialog box is displayed. Remove the USB stick safely.

8.2 Tracking users

8.2 Audit trail

Refer to section **Report user activities**. The following user activities are tracked:

- User login (including failed logins)
- User logout
- Experiment started (with details about experiment setup)
- Sample creation during data acquisition
- "Manual" sample creation (group / ungroup / resample / recompensate)
- File transfers (copy dialog / backup)
- File save (save dialog / automatic creation)
- File delete (copy dialog / backup / save dialog)
- FCS import / export
- Save report viewer report ("system audit trail")
- Create analysis report
- User management activities: roles created / modified, role assignments

8.2.1 Report user activities

The duration that individual users are logged on to the MACSQuant Instrument can be reported for billing or other tracking purposes.

Reports can be sorted by Time, Type of activity (**log on, log off, auto shutdown**, ...), Version, serial number, user, user description or role. The fields to be viewed can also be selected on the left hand side of the dialog box.

1 Go to the **Tools tab**.

2 Click the grey button next to **Report Viewer**.

- If the MACSQuant Instrument is connected to a printer, the report can be printed directly from the Report Viewer dialog box by clicking **Print**.

- Export the report in .xls or .html format by clicking **Save**. Select the desired file format, name the file, and then click **OK**.

- To transfer the report to a USB stick or network location, click **File > Copy**. Select **System audit trail** and select the desired report, designate the destination and click **Copy**.
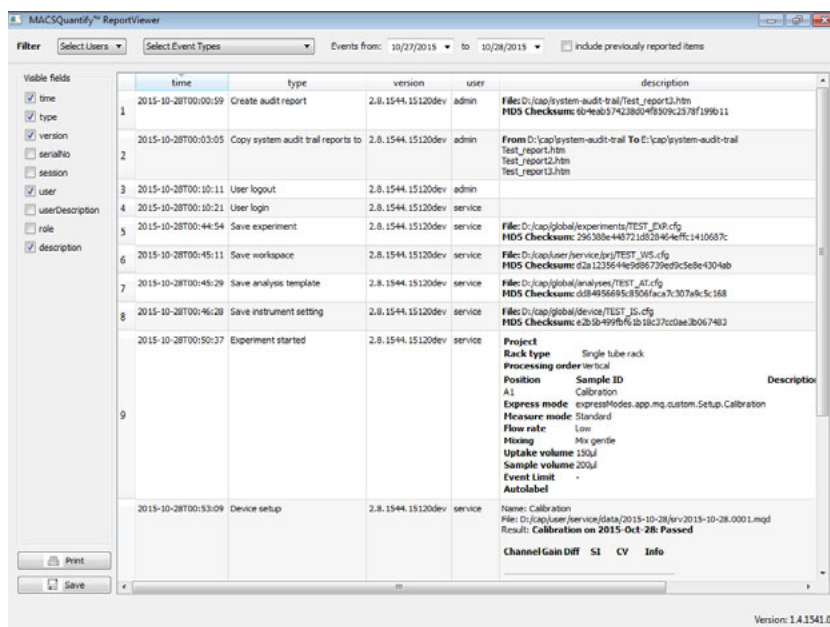
8.3 Hardware setup

The MACSQuant Instrument runs a Windows XP® based operating system image (OSI), iEi KINO-QM57a image. This iEi KINO-QM57a image includes service pack 3 and every security update until 12/2011. The OSI of the MACSQuant Instrument is physically stored on a CF-card. The enhanced writing filter (EWF) acts as virtual hard disk layer to this CF-card that allows read only. Thus, the MACSQuant Instrument is a self-contained system, which prevents any changes to the OSI. Any changes made to the OSI persist only in the random access memory (RAM) and are lost after rebooting the MACSQuant Instrument.

In order to maintain a clean and untouched OSI, disconnect the MACSQuant Instrument from the network connection and reboot the MACSQuant Instrument each time before you do any of the following procedures:

- Install an external monitor
- Calibrate the touch screen
- Change the time or date
- Perform an update.

8.3.1 Touchscreen calibration

1 Disconnect the MACSQuant Instrument from the network connection.

2 Start the MACSQuant Instrument and log in as administrator.

3 Go to **Tools > Touch screen** in the side panel of MACSQuantify Software to start the touch screen calibration.

4 Select the **Calibration tab**.

5 Click on the crosshair cursor to calibrate the touch screen.

6 Follow instructions to touch, hold and release the crosshairs, which appears on different positions of the screen.

7 If you are content with the setup, save the calibration by pressing **Accept** which appears on the lower left corner of the screen. Close the control panel by clicking **OK**.

8 A dialog box will appear. Click **Yes** to save this change and restart the system.
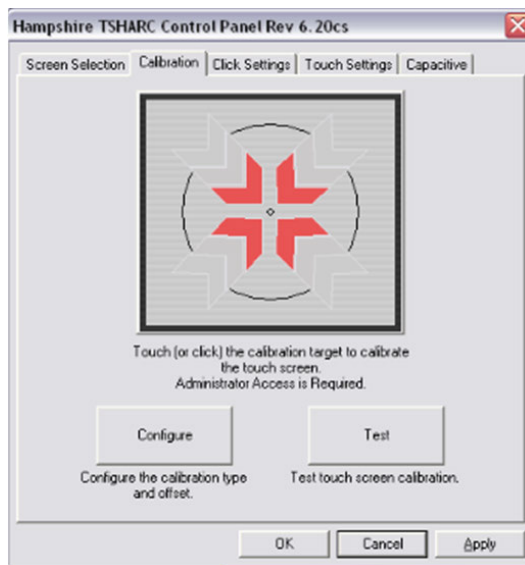
8.3.2   Installation of an external monitor

1 Disconnect the MACSQuant Instrument from the network connection.

2 Connect the second monitor to the VGA or HDMI interface of the MACSQuant Instrument.

3 Start the MACSQuant Instrument and log in as administrator.

4 Go to **Tools > Display settings** to adjust the display properties.
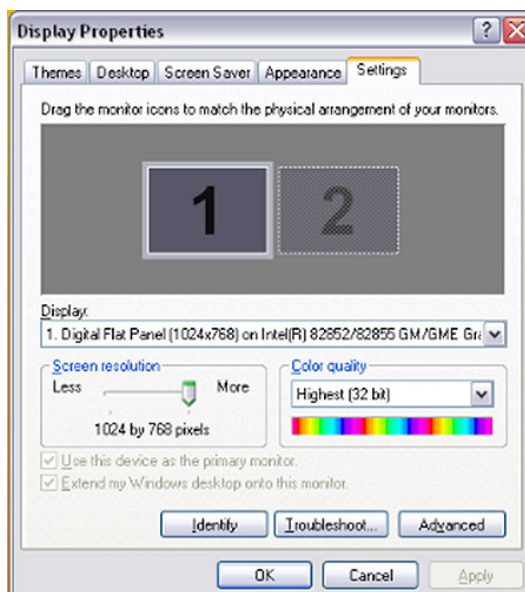
5 Go to the Settings tab, select the 2nd (default) monitor, and check **Extend my Windows desktop onto this monitor**.

6 Click **Apply**. Confirm that you would like to keep these settings with **Yes**.

7 Click on **Advanced** to configure the monitor.

8 Go to the Intel® Graphics and Media Control Panel tab and click on Graphic Properties.

9 Go to the Multiple Displays menu to configure the Operating Mode. If you select Clone Displays, the resolution of the built-in display is set to the maximum (1024×768), which can be changed only if you use the second monitor as single display or primary extended desktop. If you select Extended Desktop the primary display will show the MACSQuantify Software main screen. Assign the Primary Display as desired. If you decide for Single Display, select Monitor as Primary Display.

10 Adjust the monitor resolution under **General Settings > Display > Monitor**.

11 Confirm any changes with **OK**. Verify that you want to keep these settings with **OK**.

12 Ensure that the displays are configured correctly and confirm that you would like to save this setting permanently with **Yes**.

13 After system restart, recalibrate your touch screen (see section **Touchscreen calibration**).

8.3.3   Set the time and date

**1** Disconnect the MACSQuant Instrument from the network connection.

2 Start the MACSQuant Instrument and log in as administrator.

3 Click on **Tools > Time and Date** in the side panel of the MACSQuantify Software.
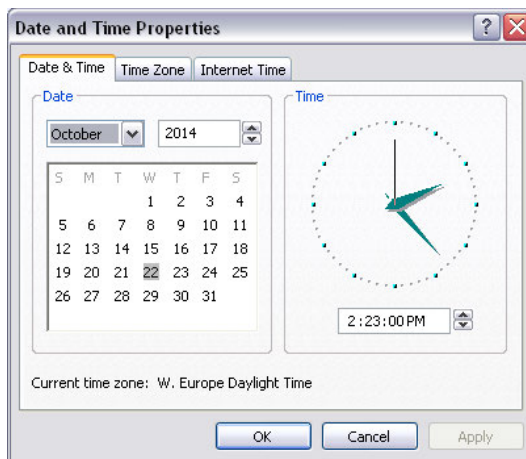
4 Adjust Date & Time and Time Zone.

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

8.4   Global customization options

8.4.1   Files

1 Click **Edit > Options (default) > Files** to access and assign default file management values.
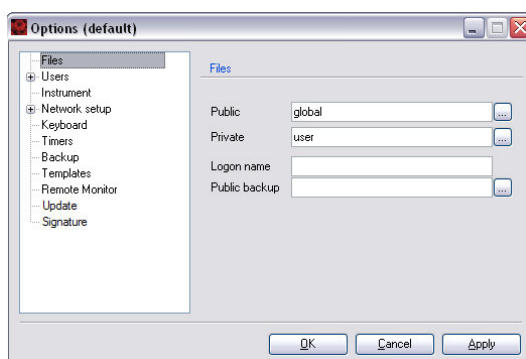
2   Assign a folder for **Public** and **Private** locations on a network.

3   Enter a logon name to be used when connecting to the public backup location.

4   Assign folder for Public backup on a network, if desired. For details on data backup.

5   Enter the full path network location, starting with \\. You can also select the destination folder via browsing. Depending on your specific network structure, access of the backup folder might require a logon name and a password, which can differ from the user login for the MACSQuant Instrument.

6   Public data is stored in **Public** locations. **Private** locations are used for user-specific data.

8.4.2   Users

1   Click **Edit > Options (default) > Users** to adjusts **User settings**.
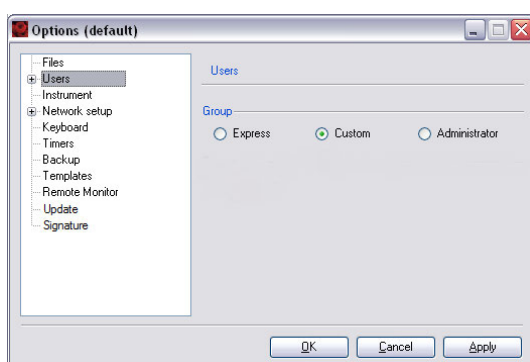
2   Assign users to a **Group** (Express, Custom, or Administrator) by checking the respective radio button.

3   For password protected user accounts, check the box next to **Required**.

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

8.4.3   Access

1   Click **Edit > Options (default) > Users > Access** to assign default access permissions for user settings. Please note that these settings can be changed when creating a new user account.
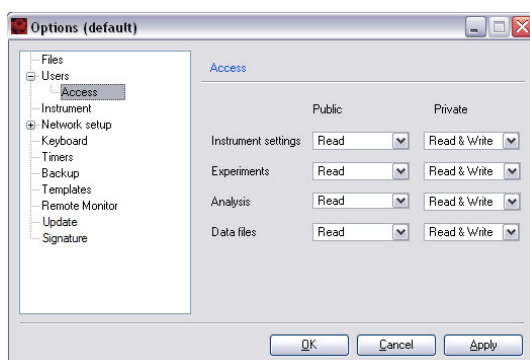
2   Define rights concerning the file management of **Instrument settings**, **Experiments**, **Analysis**, and **Data files**. Use the drop-down lists to select the access parameters as desired.

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

8.4.4   Instrument name

By default, a MACSQuant Instrument is unnamed. Assigning an instrument name might be useful if several MACSQuant Instruments are run in an institution, or if the data is analyzed on a PC. The instrument's name is displayed in the login dialog and in the right corner of the menu bar, next to the user name.

1   Go to **Edit > Options (default) > Instrument** to enter the instrument/institution name.
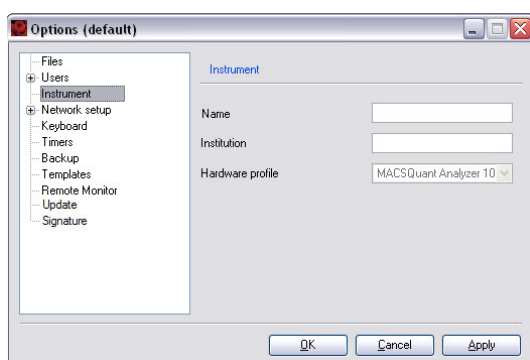
2   The institution name can be displayed by clicking **View > Hardware**.

8.4.5   Network

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.

If your DHCP server does not provide the MACSQuant Instrument with an IP address, please set it manually under **Edit > Options (default) > Network setup > IP address**. If one needs a domain user authentication to access the network, MACSQuantify Software will prompt for your credentials as you open a network location. If necessary, specify a particular proxy server in order to access to the internet under **Network setup > Proxy**. For further information contact Miltenyi Biotec Technical Support.

Since antivirus software might influence the reliability of the data acquisition processes, antivirus software is not provided and not intended to be used with the MACSQuant Instrument. The Enhanced Write Filter (EWF) protects the OSI from any permanent virus, because with every reboot of the MACSQuant Instrument, the OSI returns to its clean and untouched state. In addition, the auto execution for external devices is switched off by default, which minimizes the risk of virus spreading via USB sticks.

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

8.4.6 Keyboard

1 Click **Edit > Options (default) > Keyboard** to access and assign the keyboard settings of the MACSQuant Instrument and to adjust the keyboard layout.
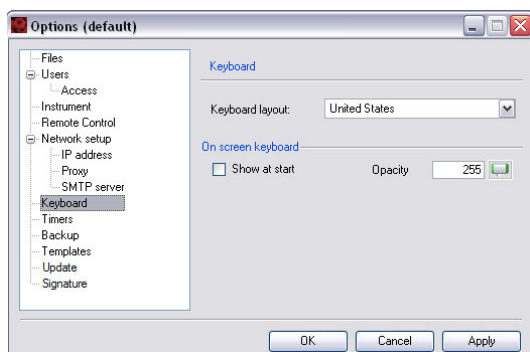
2 Use the drop-down list to select an appropriate Keyboard layout.

3 Check the box **Show at start** to automatically activate the touch screen keyboard during the startup of the MACSQuant Instrument.

4 Adjust opacity if desired.

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

8.4.7 Timer

By default, MACSQuant Instruments perform an automatic shutdown procedure to prevent non-essential illumination of lasers, which can shorten the diode lifespan. Refer to the MACSQuant Instrument user manual for instructions on manual shutdown of the instrument.

1 Go to **Edit > Options (default) > Timers**.
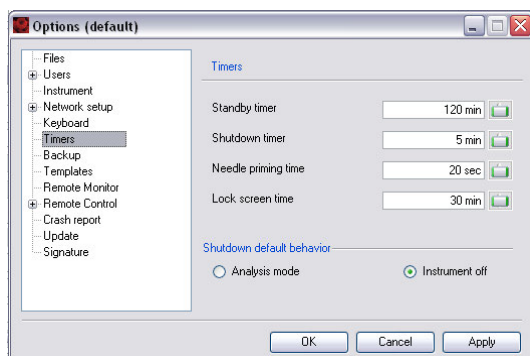
2 Modify timers as needed.

- The **Standby timer** defines the idle time (no fluidic operation) before automated shutdown begins.

- The **Shutdown timer** 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.

- The **Needle priming time** is 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.

- The Shutdown default behavior offers two options: **Analysis mode** or **Instrument off**.

- The Lock screen time defines the idle time (no user input) before the screen is locked automatically.

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

The Administrator

8

8.4.8 Backup

1 To change the default backup parameters, go to **Edit > Options (default) > Backup**.
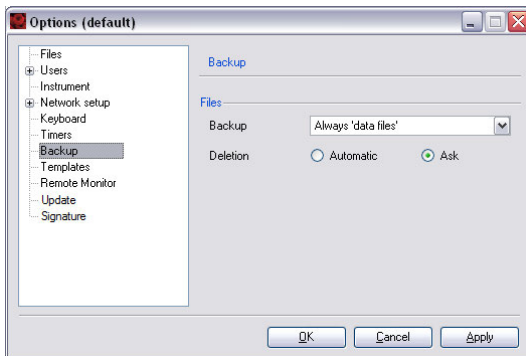
2 Choose the desired default options for file backup from the Backup drop-down list:

- Always ask will prompt the user to specify all file types for backup,
- Always 'all files' will back up all files without prompting,
- Always 'data files' will back up all data files without prompting.

3 Click **Automatic** to automatically overwrite or delete files during backup, or click **Ask** if deletion or overwriting of files during backup needs to be confirmed by the user.

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

8.4.9 Templates

New analysis window templates or changes to the default appearance of the analysis window can be defined by clicking **Edit > Options (default) > Templates**.
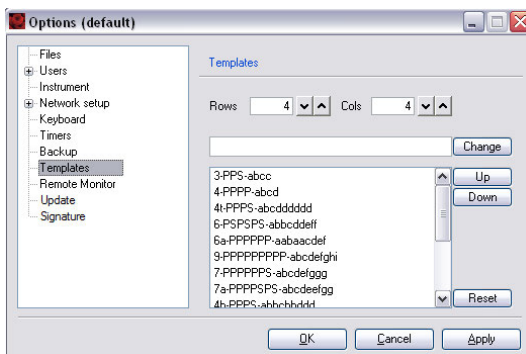
1 To change the default appearance of the **New analysis window** dialog box, define the number of Rows and columns (Cols). In this example, four rows and four columns were selected. Each of the 16 analysis windows shows a different appearance regarding number and size of displayed dot plots and statistics. E.g., Plot7 consists of seven elements, six dot plots and one statistic. It is defined as 7-PPPPPPS-abcdefggg.

2 Add or change window templates: Define the format of each template to be displayed in an analysis window:

- Double-click on a predefined template, change the format as desired.
- Specify the layout of each analysis window template according to this syntax: Tag-Format-Layout. Tag, format, and layout need to be separated by hyphens, not dashes. A tag is a short text displayed in the New analysis window dialog. In the example below the tag 3 was used.

3 Format specifies the data format for each element, defined by the following abbreviations:

- **P**: Dot plot
- **H**: Histogram
- **D**: Density plot
- **S**: Statistics
- **T**: Text
- **N**: None (blank)

4 Layout specifies the positions of the elements: the position and layout are assigned by the letters a, b, c, ..., e.g., a is the position at the top left corner of the template. Letters are used in replicates in order to assign a data format over two or more positions.

5 Click **Change** to save the changes of the template.

6 Continue to change formats as desired.

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

> **Note:** The original settings can be restored by clicking Reset.

8.4.10 Express Mode updates
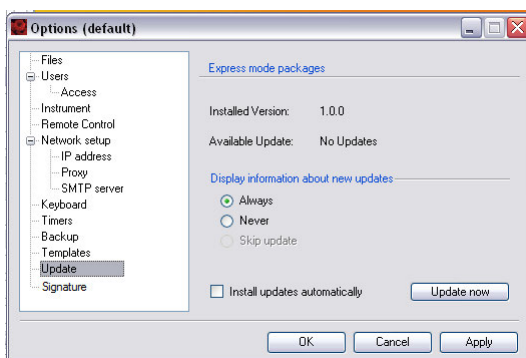
1 Express Modes Packages can be updated from a USB stick. To install a new express modes package on the MACSQuant Instrument, insert the USB stick containing the new express modes package into the USB port of the MACSQuant Instrument. During login, the software will search for new updates. **Under Display information about new updates**, select either:

- **Always** to search for the availability of new express mode packages every time the login dialog is shown. If a new update is found, a respective message will be displayed in the login window.
- **Never** to disable search for new express modes package.
- **Skip** update to skip update of the currently available update version.

2 Automatic installation can be selected by checking **Install updates automatically**.

3 Click **Update now** to initiate a manual search for updates and the subsequent installation. Click **Restart** to restart the MACSQuantify Software.

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

8.4.11 Manage signatures

Delete existing signatures or create new for signing Analysis reports.

1 Go to **Options (default) > Signature**.

2 To delete, chose the respective signature and click **Delete**.

3 To create a new signature, enter a name and click **Add**.

4 Confirm with **Apply** and click **OK** to close the window.
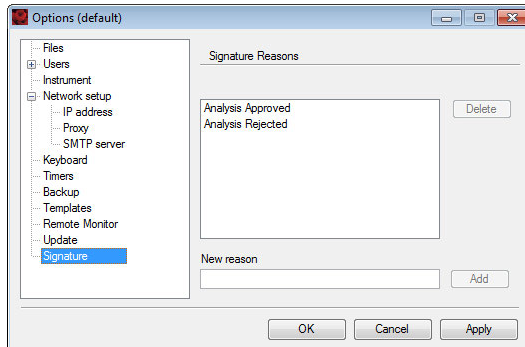
9

The Custom mode user

9.1 Modify the default file name

The default file name structure of the MACSQuant Instrument is composed of the initials of the logged-in user, followed by the acquisition date, and an index that counts up beginning with 001. This file name structure can be changed within the following restrictions. Avoid the Windows characters **? / \ < > : * | "** plus any character that can be typed using the ctrl key. The characters **. - , µl ( ) &** can be included in the file name. The folder structure under **Path** can also be specified using the listed in the table above. Take the utmost care to keep the forward slash / properly placed, i.e., **%Project%/%Date%**. Data file names can be changed using the Experiment tab. Uncheck the checkbox to the right of the file name and overwrite the name. Please note that the counter will revert back to 001 as soon as you have changed the file name.

1 Click **Edit > Options > File**.
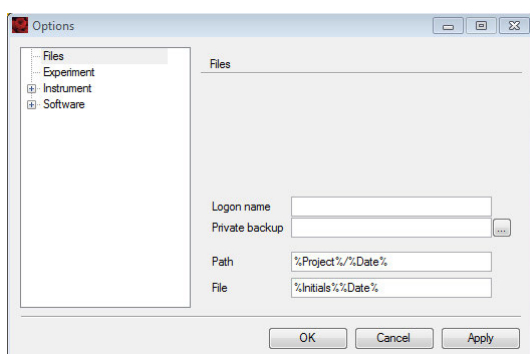
2 Specify the automatic file name in the Files section, adhering to the identifiers listed below.

3 Confirm your changes by clicking **OK** or **Apply**.

The **Files section** can handle free text and the following case-sensitive commands:

Command	Description
%Initials%	Initials of currently logged-in user
%User%	Name of the currently logged-in user
%Date%	Acquisition date in the format YYYY-MM-DD
%Description%	User description
%Project%	Project name

Command	Description
%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, e.g. A1

Table 9.1: Patterns for automatic file naming.

9.3 Assign default experiment settings

1 Click **Edit > Options > Experiment** to access and assign default values for experiment settings. The settings will be displayed in the Experiment tab. For more information, see section .
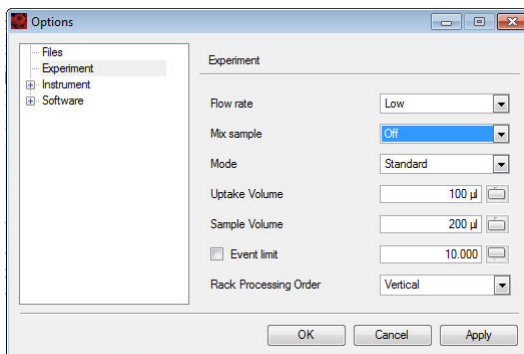
2 Modify the default values for **Flow rate**, **Mode**, **Uptake**, **Volume**, **Event limit**, **Rack processing order** and mixing as desired. Checking the box **Event limit** limits data acquisition to the specified number of events, which might preclude volumetric cell counting.

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

9.4 Instrument profile

1 Go to **Edit > Options > Instrument** to access and assign default values for the hardware profile and annotations of the optical channels.
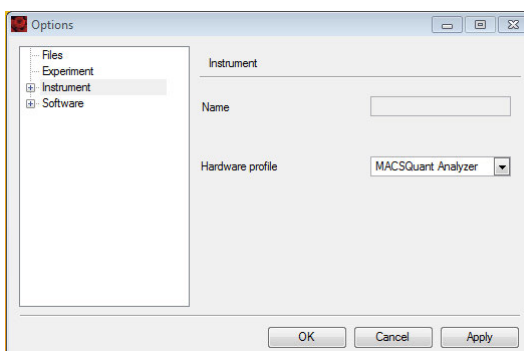
Note: The hardware profile can only be changed if MACSQuantify Software is run on a PC. It is necessary to choose the appropriate instrument in order to handle files generated with or prepare experiments for different MACSQuant Instruments.

2   Select the appropriate instrument from the Hardware profile drop-down list.

9.4.1   Optical channel annotations

Annotations provide descriptions of the nine or ten optical analysis channels that are available on the MACSQuant Analyzer, the MACSQuant Analyzer 10, and the MACSQuant VYB, respectively. The default values for the annotations can be changed, which might be desired if other than the displayed fluorochromes are used.

1   Go to **Edit > Options > Instrument > Annotations** .
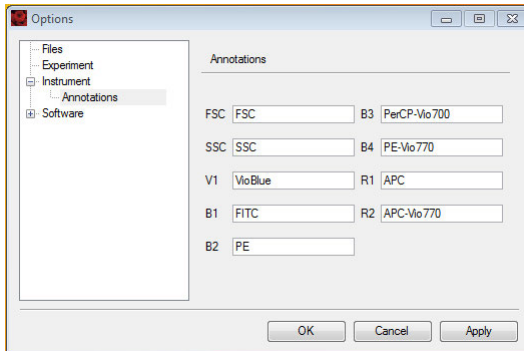
1   Highlight the default name and enter a new name.

2   Click **Apply** to implement changes and **OK** to close the window. The saved values will be displayed in the **Experiment tab**.

9.5   Adjust Software settings

9.5.1   Adjust rack colors for red-green colorblind users

Under **Software**, you can switch the sample color scheme in order to support users with red-green color discrimination deficiency.

1   Go to **Edit > Options > Software**.
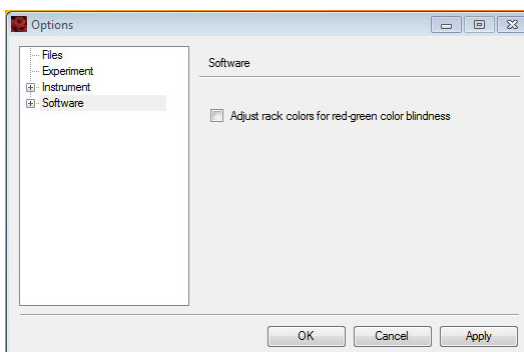
2   Check the box **Adjust rack colors for red-green color blind users**.

9.5.2   Acquisition

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

1   under **Edit > Options > Software > Acquire**.
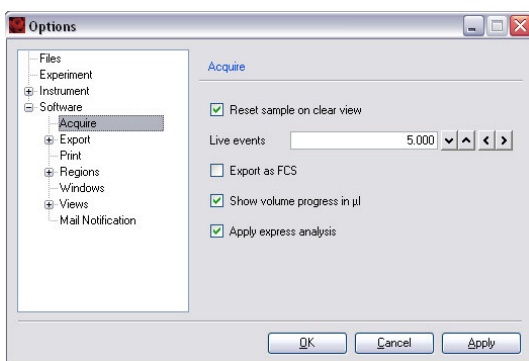
2   Check the according boxes to activate a feature that will be available during data acquisition (live mode).

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

Reset sample on clear view

- Unchecked: When **Clear button** in the instrument status bar is pressed during acquisition, only the view will be cleared but the events in the file will not be discarded.

- Checked: When **Clear button** in the instrument status bar is pressed during acquisition, the view will be cleared and all events in the file will be discarded.

Export as FCS

- Unchecked: During acquisition, only an MQD file will be created.

- Checked: Following acquisition, the MQD file will also be exported as an FCS file.

Show volume progress in µL

- Unchecked: Volume progress is shown in percent.

- Checked: Volume progress is show in µL.

> **Note:** During sample uptake and processing the Instrument status bar continually displays the remaining sample uptake volume.

Apply express analysis

- Unchecked: After the acquisition with an express mode, the file will not be analyzed with the express mode.

- Checked: after the acquisition with an express mode, the file will be analyzed with the express mode.

9.5.3   Export

Statistic

To change the default export settings for sample statistics go to **Edit > Options > Software > Export > Statistic**. Click **Apply** to implement all changes and **OK** to close the window.
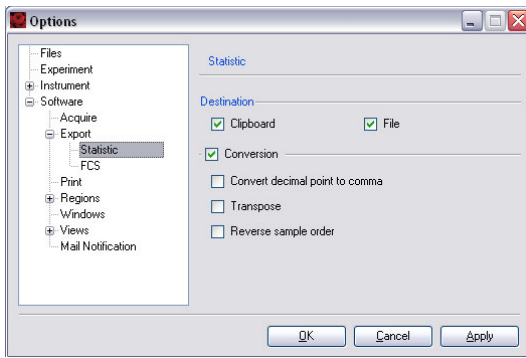
Options for data export can be selected by checking one or both boxes under **Destination**.

- **Clipboard**: copy data to the clipboard. Data can be pasted into a spread sheet.

- **File**: export data to an Excel file.

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

- Convert decimal point to comma for compliance with your regional language settings.

- Transpose if you wish to invert rows and columns.

- Reverse sample if you wish to sort samples in ascending instead of descending order.

FCS

The MACSQuant Instrument will store all acquisition data in the .mqd file format. However, by checking the box Export as FCS under **Edit > Options > Software > Acquire**, FCS files will be automatically generated after data acquisition. Click **Apply** to implement all changes and **OK** to close the window.
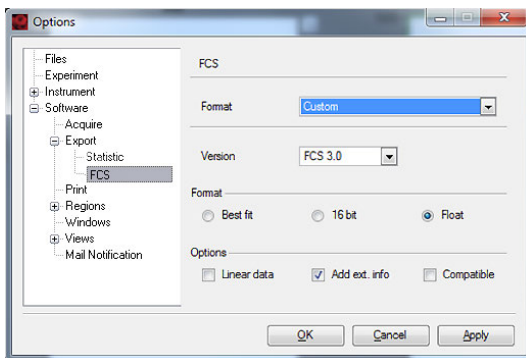
Format options can be selected from the drop-down list. Choose FCS2, FCS3, FCS 3.1, Compatible, or Custom. If Custom is selected, individual parameters can be selected from the drop-down list for Version (FCS 2.0, FCS 3.0, FCS 3.1), the Format can be specified as Best fit, 16 bit, or Float. 16 bit is compatible with most data handling software.

Checking the according **Options** boxes lets you:

- Save all data in **Linear data format** without any logarithmic manipulations.

- Save extended data information (Add ext. info), such as information on the file format, time and data, 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.

- Save all data in **Compatible format** for use with other flow cytometry analysis software.

Different data analysis software, such as FlowJo® or FCS Express® have different requirements for FCS file export. To export FCS files for use with FlowJo and FCS Express software:

1   Select **Custom** from the **Format drop-down list**.

2   Select **FCS3.0** from the **Version drop-down list**.

3   Check **Compatible** from **Options**.

4   Confirm with **OK**.

9.5.4   Print

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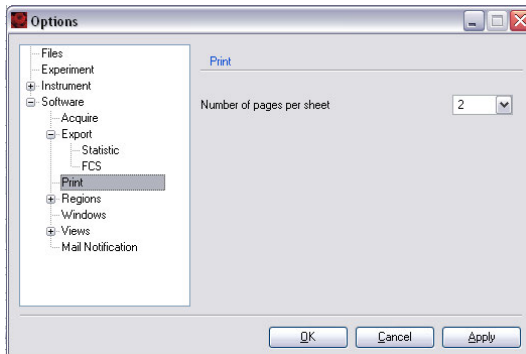
9.5.5   Regions

Default settings for the Drag & drop command for moving regions can be changed under **Edit > Options > Software > Regions**.
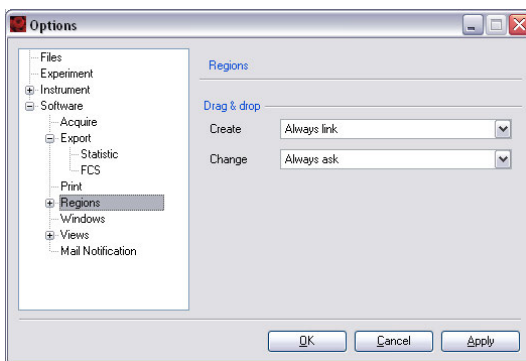
Create a region

You can activate/deactivate a feature from the according drop-down list for creating a new region (Create). Select the desired option from the drop-down list:

- Always copy: A copy of the region is created
- Always link: A link to the region is created
- Always ask: A dialog box will be displayed to choose one of the options: **Link**, **Copy**, or **Cancel**.

In general, regions are unlinked, and a region is related to a particular population of a sample. In this example, the change of region R1 would apply to Population 1 of the Sample:

- Sample\P1 – R1
- Sample\P2 – R2

If regions are linked, they are related to more than one population. In this example, 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

Change a region

You can activate/deactivate a feature from the according drop-down list for changing a region (Change). Select the desired option from the drop-down list:

- **Current**: Any link is removed and only the current population is changed.
- **All**: All linked populations are adjusted.
- **Always ask**: Whenever a linked region is changed, a dialog box is opened allowing the user to choose between **Current**, **All** and **Cancel**.

Colors

Default settings for region colors can be modified under **Edit > Options > Software > Regions > Colors**. Clicking on the color panel button next to the according region number opens a dialog box from which colors can be defined by the user. Please note that region numbers are assigned in ascending order from 1 to 10. Click **Apply** to implement all changes and **OK** to close the window.
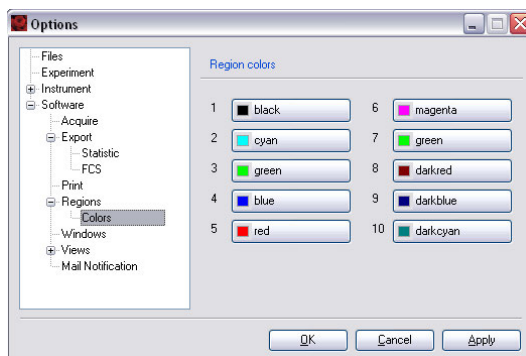
9.5.6 Windows

The default setting for the closure of windows can be modified under **Edit > Options > Software > Windows**.
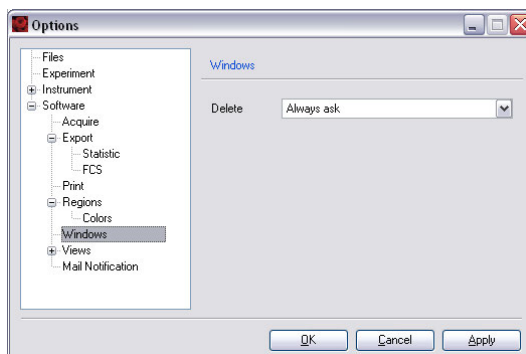
1 Select the desired option from the drop-down list:

- **Always ask**: You will be prompted to confirm closing an analysis window when selecting **Windows > Close** from the menu bar or clicking the Close window button from the toolbar. This is the default setting of MACSQuantify Software.

- **Never ask**: Analysis windows will be closed without displaying a dialog box.

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

9.5.7 Views

To change default display settings for plots, histograms, and tables, go to **Edit > Options > Software > Views**. Change the font color (text), the workspace color (background), and the plot background color as desired. Check or uncheck the Multilayer mode box to enable or disable multilayer mode.
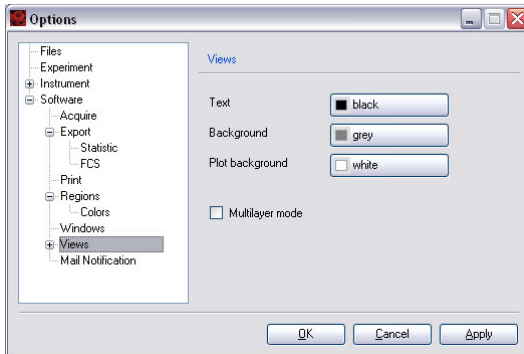
Statistic

Under **Statistic** you can decide whether to show a header for the statistic table or not. Check or uncheck the box **Show header** accordingly. For details, see section **Statistics tables**.
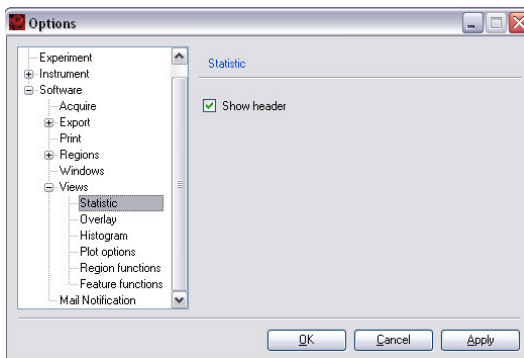
Overlay

Under **Overlay** you can define the colors of histogram and dot plot overlays. Clicking on the color panel button next to the according overlay number opens a dialog box from which colors can be defined by the user.
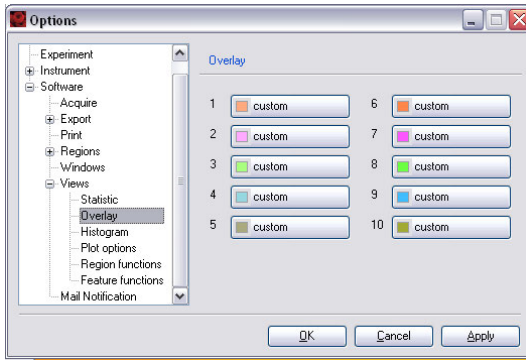
Histogram

Under **Histogram**, use the drop-down lists to select default values for displaying histograms (Normalization, Smoothing, or Mode). For details, see also section **Histograms**.
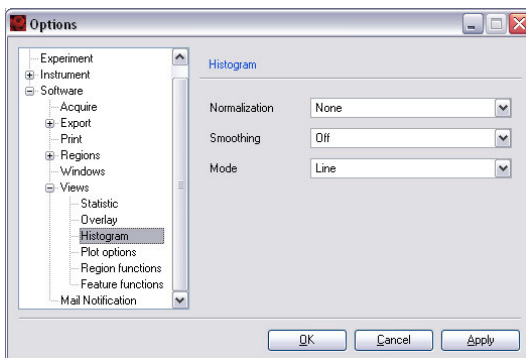
Plot options
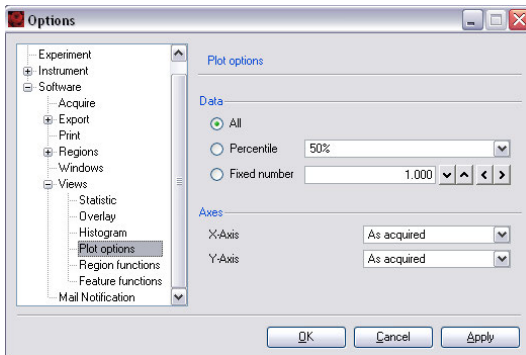
Under Data, you can define the default stetting for the display of events:

- **All**: all acquired events

- **Percentile**: select percentage of acquired events to be displayed from the drop-down list, i.e. 1%, 2%, 5%, 10%, 25%, 50%

- **Fixed number**: use arrows to enter a fixed number of acquired events to be displayed

Under Axes you can define the default scaling for the X-Axis and Y-Axis. The drop-down lists offer the options As acquired, lin, log2, log3, log4, log5, and hlog. It is recommended to use the default setting As acquired and modify the axis scaling when performing data analysis.

Region functions

Under **Views**, you can select and deselect functions by moving them from **Unused** to **In use** and vice versa. The order of in use can be modified using the **Up and Down buttons**. Click **Apply** to implement changes and **OK** to close the window.
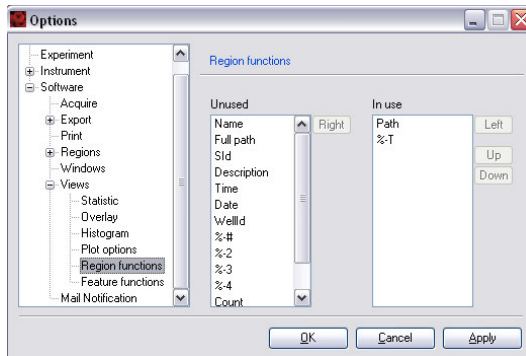
Feature functions

Under Feature functions you can select and deselect functions by moving them from **Unused** to **In use** or vice versa. The order of Feature functions in use can be modified using the Up and Down buttons. Click **Apply** to implement changes and **OK** to close the window.
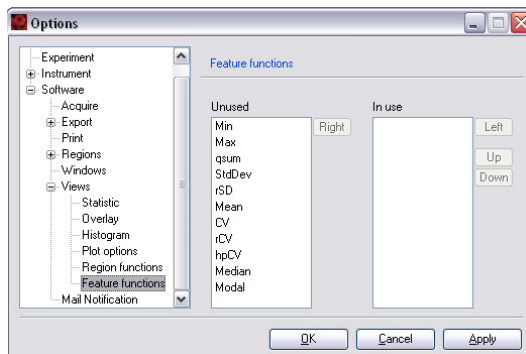
9.5.8   Mail Notification

Enter your email address (optional). An email is sent to the user when a process is complete or if an error occurred, e.g. a fluid bottle is almost empty.
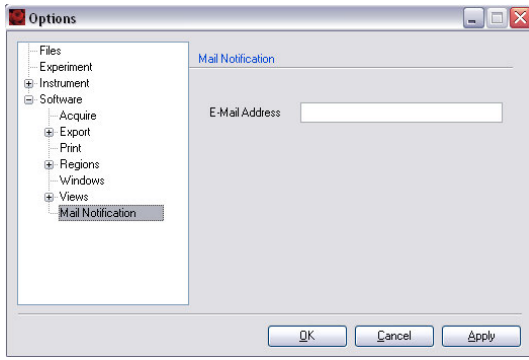
10

The Express mode user

⚠ WARNING Read the chapter **Important Safety Information** in the MACSQuant™ user manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

The Express mode is designed to simplify the setup, running, and analysis of experiments. With only a few actions, users with minimal flow cytometry experience can perform complex flow cytometry experiments. At the time of the creation of the user profile, the administrator determines each user's settings. Express mode users are allowed to perform only minimal alterations to settings. They do not have permission to perform calibration or compensation. To modify Express mode settings and/or gain access to more advanced options, users must have administrator rights. Modifications of calibration and compensation can be performed by users with administrator rights after switching to custom mode and selection of **Edit > Calibration** or **Edit > Instrument settings** from the menu bar, or by selecting **setup** from the **Mode** drop-down list as described below.

10.1 Login to Express mode

1 Select **user** name from the drop-down list and enter the password, if required.
2 Click **login** to proceed. If the user registers as an Express mode user, MACSQuantify Software will automatically log into the Express mode. If the user registers as a Custom mode user, MACSQuantify Software will automatically log into the Custom mode. Initials of the logged-in user will be displayed in the top left corner, in this example, EU.

10.2 Switch from Custom mode to Express mode

1 If logged-in to Custom mode click the **Express Mode** button in the toolbar. The MACSQuantify Software window will change to the Express mode. If windows are active in the Custom mode (e.g., analysis window), the user will be prompted to confirm closure of all views in Custom mode.
2 Click **Yes** to confirm or **No** to abort the process.

Note: Any active work will not be transferred to the Express mode. All data or settings must be saved before switching to Express mode.

10.3   Logout from Express mode

Logout

1 Click the **Logout button**.

2 If prompted to continue, click **OK**. The software will return to the login menu.

10.4   Change passwords

Logout

1 Click on the **user name** at the left upper had of the screen.

2 If prompted to continue, click **OK**.

10.5   The Express mode user interface

10.5.1   The toolbar

	Open	To open Instrument Settings, Experiments, or Data files
	Save	To save experiments
	Print	To print experiments
	Backup	Backs up data to DVD or initiate data transfer to USB stick or network location.
	2D code reader	Activate the 2D code reader.
	Touchscreen keyboard	Activate/deactivate the touchscreen keyboard of the MACSQuant Instrument.
Help	**Help**	Opens a help file.
Custom Mode	**Custom mode**	Switch to Custom mode. Only available for administrators or custom mode users.
	Lock	Lock the screen. To unlock, enter your password.
Logout	**Logout**	Logout of current user without shutting down MACSQuantify Software.

(power icon) **Main instrument control**	Switch between Acquisition mode and Data analysis mode. Also used to switch off the MACSQuant Instrument or shut down MACSQuantify Software.

10.5.2 The side panel

Definition	Assign rack and sample positions, enter sample details such as name, description, and define the Mode.
Acquisition	Display live data that is being acquired.
Analysis	Analyze acquired data, for example, using an analysis template.
Rack	Several racks are available for the MACSQuant Instrument. Choose the appropriate rack for your experiment from the drop-down list.
Filename	The filename is automatically shown in this field (not changeable).
Sample ID	Optional. Can be entered during definition of an experiment
Description	Sample description can be entered during definition of an experiment.
Mode	Drop-down list to select **Analysis, Analysis template** or **Setup**Selecting **Setup** from the Mode drop-down list reveals three options for instrument setup: **Compensation** and **CompensationMultiColors**

10.6 Define an experiment

Express mode users may apply defined experiment and analysis templates to newly acquired data. However, analysis templates can only be defined by an administrator or a Custom mode user. In order to perform an experiment the following criteria must be defined:

- select and configure rack
- enter sample ID and description
- select mode

10.6.1 Select a rack

Choose an appropriate rack type for the experiment, depending on the sample number and volume. The Chill 5 Rack, Chill 15 Rack, Chill 50 Rack, and Chill 96 Rack must be used with the MACS MiniSampler. For details about racks and how to choose the correct rack format refer to section **Chose a rack**.

1   Click on **Definition** to define the experimental setup.

2   Choose the rack format using the **Rack** drop-down list. In this example, **Chill 5 rack** was chosen for the measurement.

10.6.2 Configuring the sample rack

Click on a sample to select and activate the sample position. Use a right-click to clear sample positions. For some Express modes, grouped samples are required. After selecting the desired sample position, click the **Group** button to designate this. Please note that colors might change if the red-green color blindness option is active (see section **Adjust rack colors for red-green colorblind users**).

The entire rack may also be selected/deselected or even cleared using the **multiple sample menu** button located at the top left-hand corner of the dialog box. Rows or columns can also be selected or deselected by clicking on the letter or number, respectively.
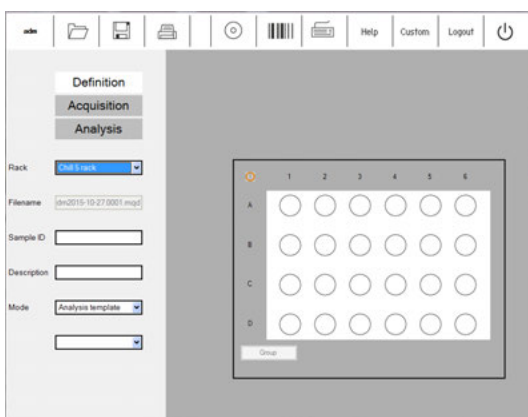
For more information about selecting samples, see section **Sample rack configuration**.

○	**Clear**	Default open circle indicates no operation.
●	**Closed green circle**	Sample selected for measurement. Double-click on circle to select.
●	**Closed green circle with orange rim**	Sample selected and activated for editing. Single click on circle to activate. The orange circle indicates that the sample is activated for editing.
●	**Closed yellow circle**	Processing of sample has commenced, e.g., sample has been labeled and incubation is underway.
●	**Closed blue circle**	Measurement in progress.
●	**Closed gray circle**	Measurement finished.

10.6.3   Sample ID and Description

The **Sample ID** and **Description** boxes are text fields in which alphanumeric characters may be entered in order to name the sample (Sample ID) and provide a more extensive description (Description) about the sample. Both fields are optional.

1   Click on the **Sample ID** or **Description** text box.
2   Enter the appropriate alphanumeric text using the keyboard. The data is automatically stored in the field.
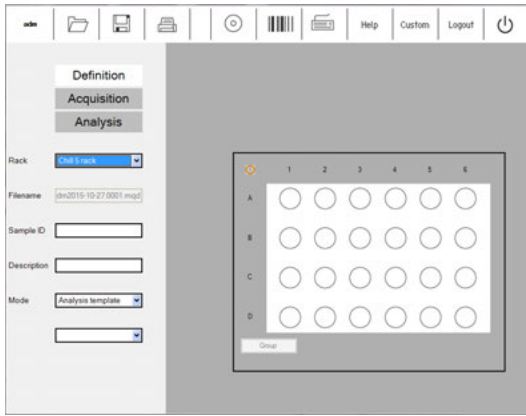
10.6.4 Select a Mode

The **Mode** drop-down list allows for choosing Analysis templates and Analysis options. Please note that Analysis templates or Analysis options cannot be created or modified by Express mode users; templates can only be created and managed by administrators or Custom mode users.

- **Analysis template**: Analysis templates simplify data analysis so that even inexperienced flow cytometry users can perform complex data analysis. Analysis templates (e.g., gating strategies) created by administrators or custom users and saved to public locations can be applied by Express mode users.

- **Analysis**: The **Analysis** option from the **Mode** drop-down list reveals a list of options available to perform flow cytometry cell analysis. Numerous Express mode applications are available for selection from the lower drop-down list.

- **Setup**: reveals two options for instrument settings, **Compensation** and **CompensationMulticolor**. If logged in as an administrator, a third option **Calibration** is available.
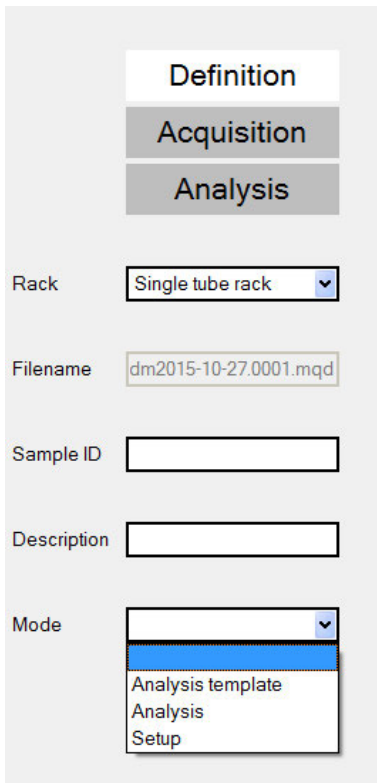
Selecting an analysis template

1. Select **Analysis template** from the **Mode** drop-down list.
2. From the lower drop-down box select the desired template.

3 Click **Start Measurement** using the instrument status bar.

Selecting an Express mode program

1 Select **Analysis** from the **Mode** drop-down list.

2 From the lower drop-down box select the desired analysis criterion.

3 Click **Start Measurement** using the instrument status bar.

10.7   File management

This section describes how data files can be opened, saved, and backed-up in Express mode. Data files may be stored to and therefore opened from a Public, Private, or External file location.

- Public files are located on the local hard drive of the MACSQuant Instrument (or personal computer) and are accessible by all users.
- Private files are located on the local hard drive of the MACSQuant Instrument (or personal computer) and are only accessible by the logged-in user account.
- External files are located on an independent file storage device, which is connected to the MACSQuant Instrument (or personal computer) via the USB stick.

Access to different files is defined for each user individually by an administrator. For information on access rights, see section **Create new user accounts**.

10.7.1   Open files

1   Click the **Open button**. Highlight **Instrument settings**, **Experiment**, or **Data file**.
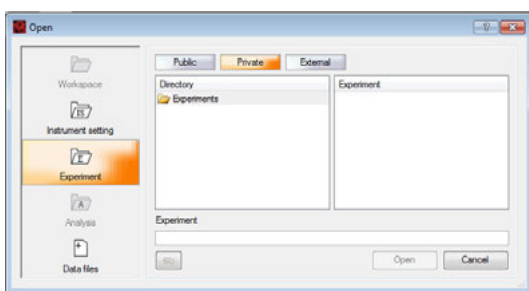
2 Highlight the file location: **Private**, **Public**, or **External**.

3 Select file and click **Open**.

10.7.2 Save files

1 Click the **Save button** to open the **Save window** and highlight **Experiment**.

2 Highlight the desired file location **Private**, **Public**, or **External**. By default the experiment definition will be saved to the user's private folder. To save to an external drive, highlight **External**.

3 Enter the filename in the Experiment field and click **Save**.

10.7.3 Print

MACSQuantify Software uses installed windows printer drivers to print active workspaces. Please contact your MACSQuant Instrument administrator or Miltenyi Biotec Technical Support for more information.

1 Open the desired analysis window.

2 Click the **Print button**.

3 Select the desired printer. Click **Print**. The printer can be networked to or directly connected to the MACSQuant Instrument or to the PC running MACSQuantify Software.

4 The current analysis page is printed.

10.7.4 Data Backup

Data can be transferred from the public location of the MACSQuant Instrument to a remote storage location (network folder, USB stick, external hard drive, DVD, etc.).

1 Click on the **Backup button**.

2 Select all files or all data files when prompted.

3 When backup is completed, a dialog window appears.

4 When the next backup is performed, all previously backed up .mqd files will be deleted, if selected by choosing delete cloned files.

11
Pre-enrichment

The MACS Technology permits the magnetic enrichment of cells prior to fluorescence analysis to reduced numbers of total analyzed cells. This is particularly useful for the analysis of cells present in low abundance, such as stem cells, dendritic cell subsets, or natural killer cell subsets. Miltenyi Biotec offers many kits and reagents optimized for the efficient identification and enumeration of a number of cell types and their subsets, e.g.:

- dendritic cells,
- endothelial progenitor cells,
- cancer stem cells,
- cytokine-secreting cells,
- antigen-specific T cells,
- circulating tumor cells.

11.1 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

11.2 Example

Here, the use of the EPC Enrichment and Enumeration Kit, human (# 130-093-477) in combination with the MACSQuant Instrument is described. The EPC Enrichment and Enumeration Kit was developed for the enumeration of circulating endothelial progenitor cells (cEPCs) from human peripheral blood, cord blood, or leukapheresis products as well as EPCs from bone marrow, following the pre-enrichment of EPCs.

Materials required

- EPC Enrichment and Enumeration Kit, human
- autoMACS Running Buffer
- 12×75 mm (5 mL) tubes
- Micro-centrifuge tubes 1.5 mL
- 50 mL tubes
- Fresh human whole blood

- MACSQuant Instrument with installed MACSQuant Column
- Pre-chilled Chill 5 Rack

Protocol

1   While preparing the samples for analysis, turn on the MACSQuant Instrument, perform a PMT calibration if necessary, and open or generate appropriate instrument settings.

2   Prepare three samples 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

3   Resuspend each sample to 500 µL and transfer to a 12×75 mm tube.

4   Place the three samples in the following positions in the Chill 5 Rack:

- CD133 control sample: A1
- CD309 control sample: B1
- EPC sample: C1

5   Go to the Experiment tab and select Chill 5 rack.

6   Highlight A1, B1, C1.

7   With all three positions highlighted, enter 500 µL in the sample volume.

8   (Optional) Enter a project.

9   Under the Autolabel tab, click on any **<add...>** button to open the Reagent manager.

10   In position R1, select Universal as category and Propidium Iodide Solution as reagent.

11   Ensure a 0 incubation time and 1:99 titer.

12   Click **Apply** and close the reagent manager.

13   Check the button next to PI in the Autolabel tab.

14   With only position A1 highlighted:

- Enter CD133 Control in Sample ID.
- Select Low fluidics.
- Select the mode Standard.
- Enter 250 µL in uptake volume.
- In Annotations tab enter: B1 = CD34-FITC, B2 = mIgG2b-PE, B3 = PI/CD14-PE-Cy5.

15   With only position B1 highlighted:

- Enter CD309 Control in Sample ID.
- Select High fluidics.
- Select the mode EnrichS.Measure Pos from the drop-down list under Pickup and measure.
- Enter 450 µL in uptake volume.
- In Annotations tab enter: B1 = CD34-FITC, B2 = CD133-PE, B3 = PI/CD14-PE-Cy5, R1 = mIgG1-APC.

16 With only position C1 highlighted:

- Enter EPC Sample in Sample ID.

- Select High fluidics.

- Select the mode EnrichS.Measure Pos from the drop-down list under Pickup and measure.

- Enter 450 µL in uptake volume.

- In Annotations tab enter: B1 = CD34-FITC, B2 = CD133-PE, B3 = PI/CD14-PE-Cy5, R1 = CD309-APC.

17 Create the following display template:
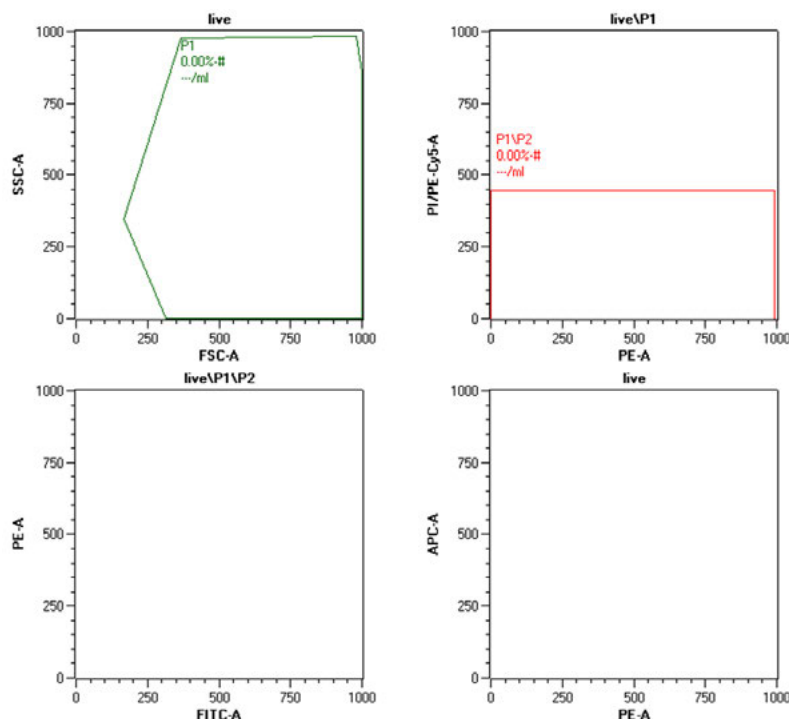
18 Place the **Chill 5 Rack** on the MACS MiniSampler with the Reagent Rack.

19 Place an open vial of PI in **Reagent Rack position 1**.

20 Check MACSQuant Running Buffer and waste bottle fluid level.

21 Go to **View > Experiment table...** to check all settings.

22 Start acquisition.

23 Follow product data sheet for proper gating guidelines for EPC enumeration.

11.3   Pre-enrichment programs

	Enrich.Measure Pos	EnrichS.Measure Pos	EnrichS2.Measure Pos
Process description	Designated sample volume is loaded onto MACSQuant Column. Negative fraction is disposed of. Positive fraction is eluted and directed to 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

	Enrich.Measure Pos	EnrichS.Measure Pos	EnrichS2.Measure Pos
Elution rate	37.5 mL/min	25 mL/min	37.5 mL/min
Elution volume	450 µL	450 µL	450 µL

Table 11.1: Pre-Enrichment programs available on the MACSQuant Instrument.

12

Instrument monitoring

⚠️ **WARNING** Read the chapter **Important Safety Information** in the MACSQuant™ user manual before operating the instrument. When processing infectious, radioactive, poisonous, or any other hazardous liquids, always abide by the necessary safety precautions.

12.1 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. Here you find also the serial number of the MACSQuant Instrument and informations on the photomultiplyer tubes (PMT).

12.1.1 Fluidics

The fluidics tab in the hardware monitor displays the pumps and valves in the live status, as well as the status of the fluid bottles.

Component	Further information
Fluid bottle monitor	Displays buffer/solution levels in real time
Pump monitor	Displays the status of the waste (W), air (A), and fill (F) pumps
Separation unit monitor	Displays the status of the MACSQuant Column and MACS Enrichment Unit
Valves 1–3 monitor	Displays the position of the valves for the MACSQuant Column: C = closed, O = open, green = in use
Valves 4–6 monitor	Displays the position of the general fluidics system valves
Syringe drive monitor	Displays the position of the dilutor
Sensor monitor	Displays the general system pressure and fluid reservoir levels.

Table 12.1: Panels of the **Fluidics tab**.

The waste (W), air (A), and fill pump symbols (F) are illuminated in green when active. Active separator valves (Valves 1-3) are indicated in green, defective rotary valves in red. Valve status is indicated by o for open and c for closed. The fluid bottles in the hardware monitor as well as on the instrument will be illuminated in red when empty; the waste bottle will be illuminated in red when full.
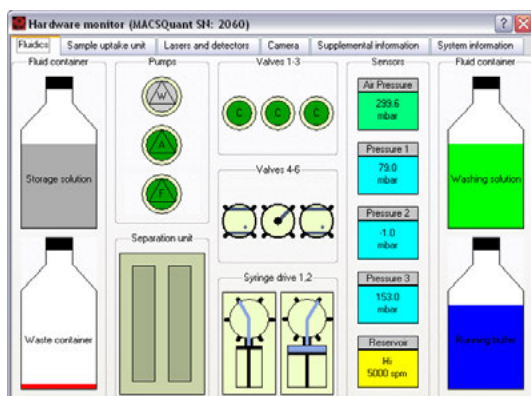
Figure 12.1: Real-time Hardware monitor of the fluidic components.

12.1.2 Sample uptake unit

The sample uptake tab indicates the status of the robotic needle arm as well as whether the MACS MiniSampler is connected or not. The relative position of the robotic needle arm is listed in the lower left box as well as whether the Single tube rack is connected or not.
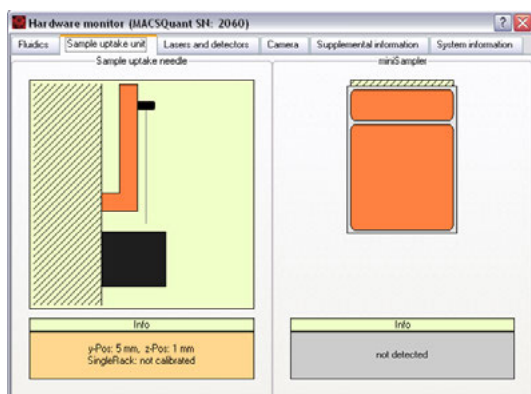
Figure 12.2: Real-time hardware monitor of the sample uptake arm.

12.1.3 Lasers and detectors

The lasers and detectors tab displays the status of the optical bench. It is possible to monitor the status of each laser fluorescence channel. The temperature, fan speed, PMT voltage, and annotated path of each laser is shown. A status overview of the optical bench is schematically represented. The bench temperature is kept between 33 °C and 37 °C. Therefore the fan speed is regulated automatically depending on the ambient room temperature and internal temperature of the MACSQuant® Instrument. Please note that the temperature for the lasers should be between 10 °C and 45 °C. In case of errors, please contact Miltenyi Biotec Technical Support.

Figure 12.3: Real-time Hardware monitor of the optical bench.

12.2 Instrument status LEDs

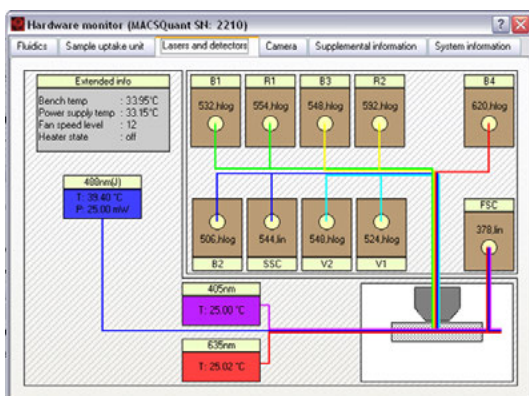The MACSQuant Instrument is equipped with LEDs, illuminating each bottle to indicate the status of the instrument in Acquisition mode. If the fluid bottles are not illuminated, the MACSQuant Instrument is in data analysis mode and the lasers are off.

Bottle illumination	Description
Green	The MACSQuant Instrument is ready to measure, liquid levels are sufficient, and the instrument is primed. Please note that the lasers can take up to 30 minutes to warm-up after performing the initial instrument priming.
Yellow	The MACSQuant Instrument reports sensor error. Please ensure that the sensor is correctly attached to the bottle.
Red	Liquid level error or general instrument error. The liquid levels are too low in a particular bottle or that the waste bottle is full. The blinking red light will indicate which bottle needs to be tended to. Additionally, a message on the Instrument status bar will specify the instrument error. All bottles can be replaced during a measurement. Blue The MACSQuant Instrument is measuring, liquid levels are sufficient.
Blue	Indicates normal instrument function during sample processing, or that the instrument is busy.

Table 12.2: Color code of the fluid bottle illumination on the MACSQuant Instruments.

12.3 Remote monitoring

The Instruments' status can be monitored from a different computer or mobile device. As a prerequisite, the MACSQuant Instrument needs to be connected to a Network. To set up remote monitoring, administrator rights are necessary.

Note: If logged out on the instrument, the user is automatically logged out on the Remote Monitoring website. Only one user can log onto the website at a time.

1 Go to **Options (default) > Remote Monitor**.

2 Check box next to **Enable Remote Monitoring**.

3 To access remote monitoring, log in to the Remote Monitoring website: Go to the web address provided under **Options (default) > Remote Monitor** (usually http: //MQ serial number: 9000/).

4 Log in with your user name and password (same as for the instrument). Only the user currently logged in on the instrument can log in to remote monitoring.

12.3.1 Email notification

The Instruments' status can be monitored from a different computer or mobile device. As a prerequisite, the MACSQuant Instrument needs to be connected to a Network. To set up remote monitoring, administrator rights are necessary (see section **Network**).

1 Go to **Options > Software > Mail Notification**.

2 Enter your email address.

3 An email is sent to the user when a process is complete or if an error occurred, e.g. if a fluid bottle is almost empty.

MQ2160 Experiment: finished

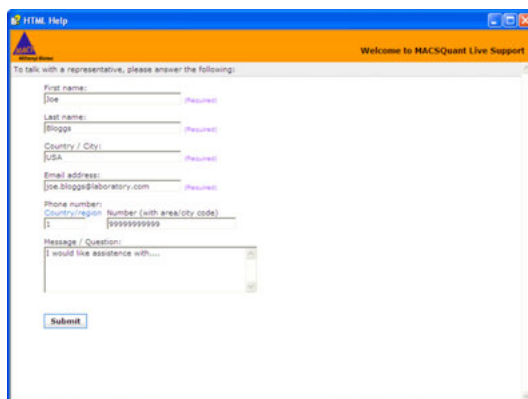| Sample	Count	Sample ID	Description	Time	Well ID
srv2014-09-01.0002	3430		qca	17:23	B1
srv2014-09-01.0003	15093	Blank		17:33	A3
srv2014-09-01.0004	5511		compmix	17:36	D4

13
MACSQuant® Live Support

MACSQuant® Live Support is a real-time diagnostic service provided by Miltenyi Biotec Technical Support. Highly trained MACSQuant Specialists can be reached in real-time to assist with any queries you may have. A webcam is provided for communication with the MACSQuant Live Support, which is attached via USB connection to the MACSQuant Instrument. The webcam is installed to a free USB port at the back of the instrument and detected automatically by the software. During normal operation, the webcam should not be installed on the instrument. Please note that the MACSQuant Instrument must have network access to the internet in order to use the MACSQuant Live Support.

1   Select **MACSQuant Live Support** from the **Tools tab** in the **Sidebar** of the MACSQuantify Software to access remote assistance.
2   Complete all fields and detail any queries you may have using the **Message/Question box**.
3   Click **Submit** to commence MACSQuant Live Support.

14

21 CFR Part 11 compliance

For 21 CFR Part 11 compliant use of the software, connect a 21 CFR Part 11 compliant MACSQuantify™ software to the user management system of your institute (e.g. a Microsoft Active Directory or LDAP system). The MACSQuantify Software will pass requests to validate a user's name and password to the user management system. During the setup of your instrument, this connection to your institute's user management system needs to be configured.

Users need to login on the MACSQuantify Software with the user name and password as defined in the institute's user management system.

14.1   LDAP configuration

1   Go to the **Tools** tab and select **LDAP Configuration**.

2   Expand the drop-down menu **LDAP server** and click on **Connection**.
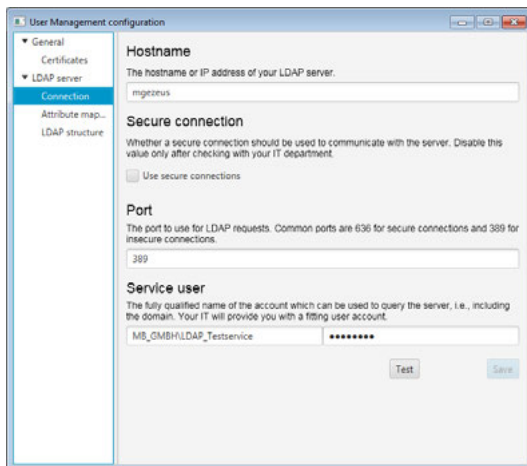
3   Enter the **hostname** or **IP address** of your LDAP server.

4   Under **Secure connection**, check box to use a secure connection via a LDAPS protocol (recommended). Uncheck to use a LDAP protocol.

5   Enter the port number (usually 636 for secure connections or 389 for insecure connections).

6   Enter a user name and password that can be used to send queries to the server. The user name should include the correct domain (e.g. Domain_Name\LDAP_TestUser)

7   Click on **Test** to check the connection.

8   Click **Save**.

Note: The **Save button** becomes available after the connection was tested and confirmed.

9 If using a **secure** connection, you will need the server's certificate. Expand the **General** drop-down menu and click on **Certifications**.
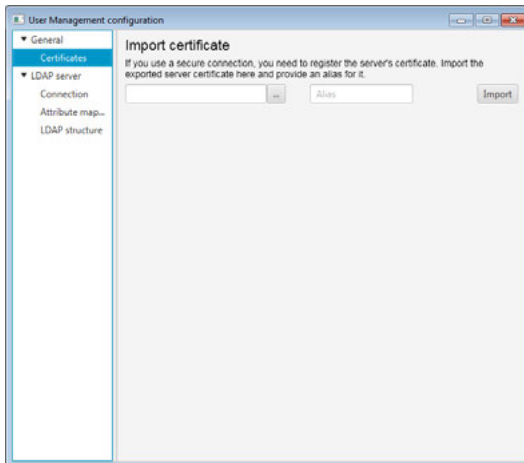
**10** Click on the **browse button** to search for your server's certificate.

11 Enter an alias and click on **Import**.

12 Define which attributes of your LDAP server setup correspond to the information needed by the MACSQuant Instrument. Go to **LDAP Server > Attributes**.
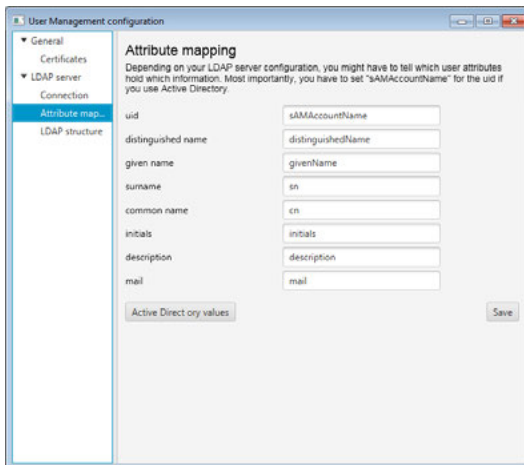
13 Enter the following Information:

- user id (for an Microsoft Active Directory setup, this has to be "sAMAccountName")
- given name
- surname
- common name
- initials
- description
- mail address

14 For Microsoft Active Directory, click the button "Active Directory values" to fill in the correct user id mapping.

15 Click **Save**.

16 Go to **LDAP Server > Structure**.
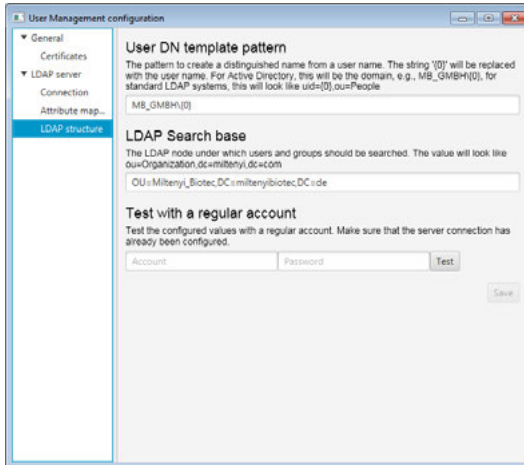
17 Enter the pattern to construct a distinguished name from a user name (i.e. user id) in order to send the correct credentials as request for authentication to the LDAP system. The sequence '{0}' within the pattern will be replaced with the user id for authentication requests.

- For Microsoft Active Directory this will be something like 'Domain_Name\{0}'.
- For standard LDAP systems this will be something like 'uid={0}, ou=People'.

18 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. You will need to 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 the LDAP structure of your server.

19 Test the configuration using a regular account: Enter the user id and passwort and click **Test**.
20 Click **Save**.

> **Note:** The **Save button** becomes available after the connection was tested and confirmed.

14.2 User accounts

14.2.1 Rights

Within the MACSQuantify™ Software there are 3 categories of rights:

- Rights that control the access to actions within the User Management (**User Management**)
- Rights that control the access to actions in the MACSQuantify™ Software (**Interface / Access to Functions**)
- Rights that control the read and write access to files that are created by user interactions (**MQ Private Files, MQ Public Files**)

File Access

Files	Preconfigured roles					
	MQ Administrator		**MQ Custom**		**MQ Express**	
	Private location	**Public location**	**Private location**	**Public location**	**Private location**	**Public location**
Analysis template	Write	Write	Write	Read	Write	Read
Data files	Write	Read	Write	Read	Write	Read
Experiment files	Write	Write	Write	Read	Write	Read
Instrument settings	Write	Write	Write	Read	Write	Read

Table 14.1: File access rights for the predefined roles **MQ Administrator**, **MQ Custom** and **MQ Express**.

View Access

Right	Description	Predefined roles		
		MQ Administrator	**MQ Custom**	**MQ Express**
Administrator access	Access to administrative actions, such as changing instrument wide default settings (e.g. manage signature reasons), calibrate uptake unit, change system time etc.	Yes	No	No
Custom access	Access to custom user interface. Allows custom actions, such as save instrument and experiment settings.	Yes	Yes	No
Express access	Access to express user interface, where the user does not have the possibility to define experiments or analyses.	Yes	Yes	Yes

Table 14.2: View access rights for the predefined roles **MQ Administrator**, **MQ Custom** and **MQ Express**.

User management

Right	Description	Predefined roles	
		UM Administrator	**UM User**
Read details of other users' accounts	Allows the user to retrieve account details such as user name, full name for an account other than the own, in order to be able to administer the account.	Yes	No
Read rights of other users' accounts	Allows the user to retrieve rights assigned to an account other than the own, in order to administer the account.	Yes	No
Read details of own account	Allows the user to retrieve account details such as user name, full name for own account, e.g. in order to be able to authenticate credentials of own account.	Yes	Yes
Read rights of own account	Allows the user to retrieve rights assigned to own account, in order to correctly grant access to authorized actions only in the user interface.	Yes	Yes

| Right	Description	Predefined roles	
		UM Administrator	UM User
Create role	Allows the user to create new roles, that can be assigned to accounts.	Yes	No
Read role	Allows the user to read details of existing roles.	Yes	No
Update role	Allows the user to modify rights assignment to existing roles	Yes	No
Assign role	Allows the user to assign roles to accounts.	Yes	No
Export	Allows the user to export role definitions and assignments.	Yes	No
Import	Allows the user to import role definitions and assignments.	Yes	No

Table 14.3: User management rights for the predefined roles **UM Administrator** and **UM User**.

14.2.2   Assign user roles

The user management tool can be accessed by a user with administrator rights i.e. who has the **UM Administrator** and **MQ Administrator** role assigned (see).
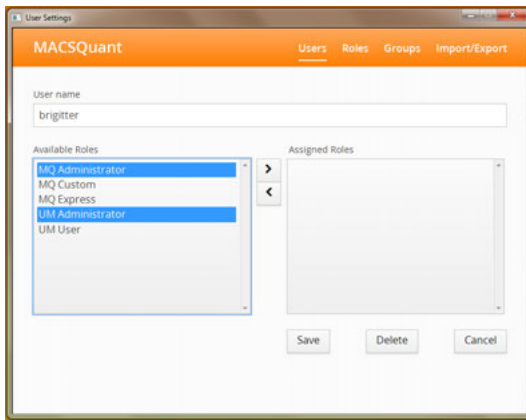
Assign roles to an individual user

1 Make sure you know the user name as defined in your user management system.

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

3 Go to the **Users tab**. A list of all currently assigned users is displayed.
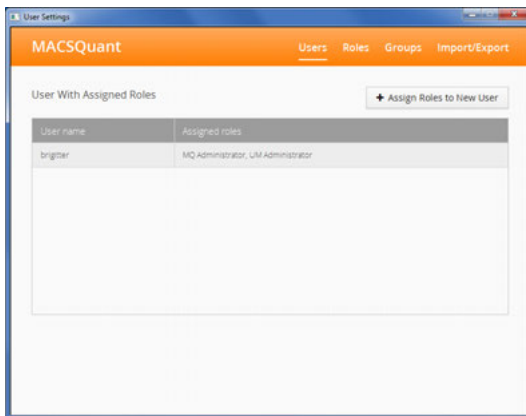
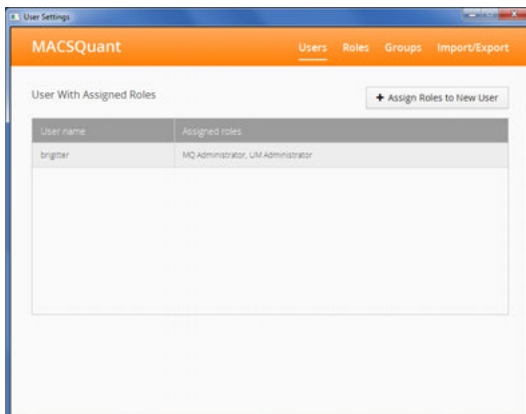> **Note:**   Users that have roles assigned only via a group assignment will not be displayed here

4 To add a new user, click on **Assign Roles to New User**. Enter the user name and choose a role. If the user name is not found in the connected user management system, the input field will be highlighted in red.

5 Within the left panel **Available Roles**, select the desired role.

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

7 Click **Save**.

Modify role assignment for an individual user

1 Go to **Edit > User settings** to open the user settings dialog.

**2** Go to the **Users tab**. A list of all assigned users is displayed.

> **Note:** Users that have roles assigned only via a group assignment will not be displayed here

3 Within the displayed list of users, click on the user whose role you want to modify.

4 Within the left panel **Available Roles**, select the desired role. Selected roles are highlighted in blue.

5 Click on the **right arrow button** to move the selected role(s) to the panel **Assigned Roles** .
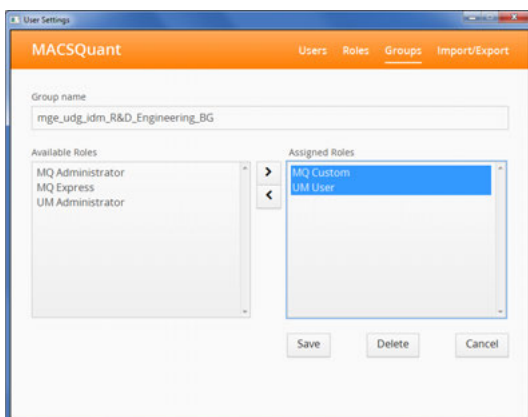
6 Click **Save**.

Assign roles to a group

1 Find out the correct name of the group from your institutes user management system. (Contact your system administrator to find out the correct group name.)

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

3 Go to the **Groups tab**.
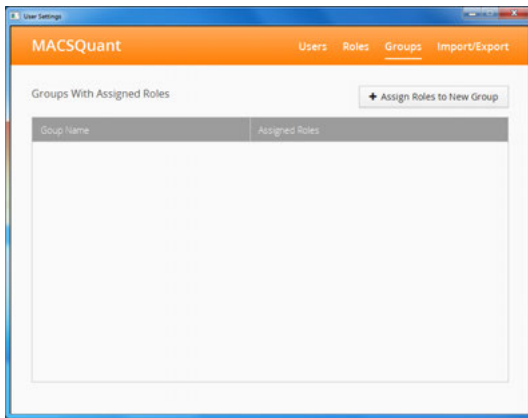
4 The page displays the list of groups, to which roles are currently assigned.

5 Click on **Assign Roles to New Group**. The display will change to provide an input field for the group name and the possibility to choose from the available roles.
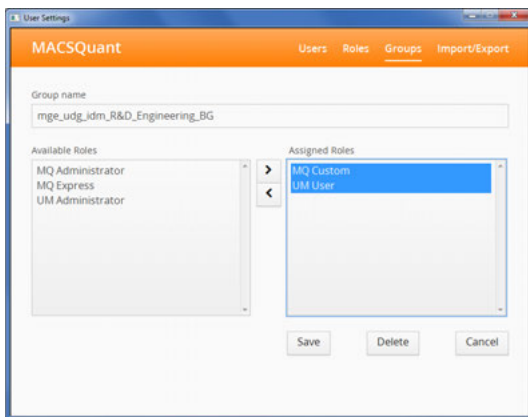
6 Enter a group name and click on one of the available roles. If the group name is not found in the LDAP system, the input field will be highlighted in red.

7 Within the left panel "Available Roles", select the roles you want to assign. Selected roles are highlighted in blue.

8 To assign the selected role(s) to the group, move them to the panel "Assigned Roles" by clicking the **right arrow button**.

9 Click **Save**.

Modify role assignment for a group

1 Go to **Edit > User settings** to open the user settings dialog.

2 Go to the **Groups tab**.

3 The page displays the list of groups to which roles are currently assigned.

4 Click on the group whose role you want to modify.

5 Select a role in the left panel **Available Roles** and click the **right arrow button** to move it to the right panel **Assigned Roles**.

6 To remove an assigned role, select it within the right panel **Assigned Roles** and click the **left arrow button** to move the selected role to the left panel **Available Roles**.

7 Click **Save**.

14.2.3 Log in

Log in to the MACSQuantify™ Software with the user name and password as defined in your institute's user management system.

> **Note:** Only a user with assigned roles will be able to login to the MACSQuantify™ Software.

14.2.4 Delete an individual user account

1 Go to **Edit > User settings** to open the user settings dialog.

2 Go to the **Users tab**. All users with assigned roles are listed. Users that have roles assigned only via a group assignment will not be displayed here. Those users cannot be deleted individually.
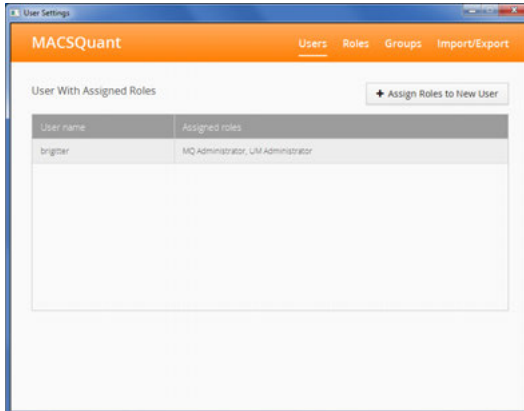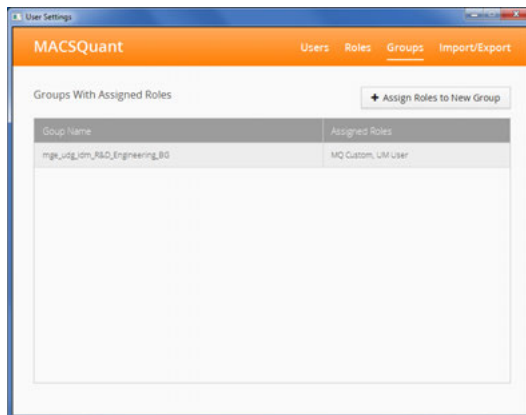

3 Click on the user that you want to remove.
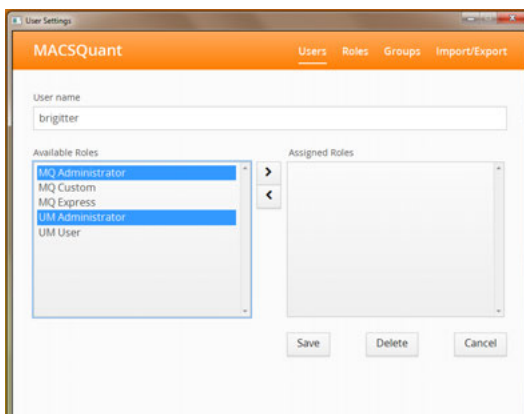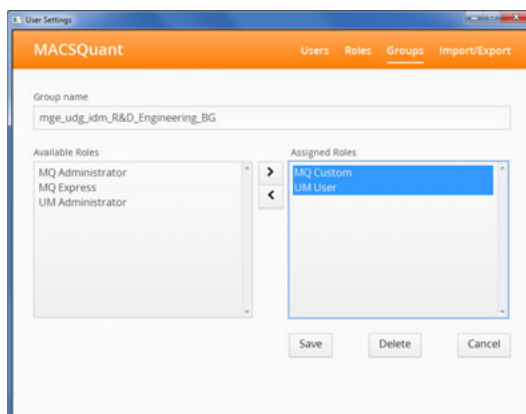
4 Click **Delete** and confirm with **OK**.

14.2.5 Delete a group account

1 Go to **Edit > User settings** to open the user settings dialog.

2 Go to the **Groups tab**. All groups with assigned roles are listed.

3 Click on the group that you want to remove.

4 Click **Delete** and confirm with **OK**.

14.2.6 Export roles and role assignments

1 Login as user with administrator rights.

2 Connect a USB stick called "MQSOFTSETUP" to the instrument.

3 Go to **Edit > User settings** to open the user settings dialog.

4 Click on the **Import/Export tab**.

5 Click **Export Roles and Role Assignments** to export this system's user configuration to the connected USB stick.

6   Once the blue progress bar at the top of the window has reached the right side and disappears, the export is finished.

7   Eject the USB stick on the instrument: Open the copy dialog, select the USB drive and click **Eject**.

14.2.7   Import roles and role assignments

1   Login as user with administrator rights.

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

3   Go to **Edit > User settings** to open the user settings dialog.

4   Click on the **Import/Export tab**.

5   Click **Upload File** to import the user configuration from the connected USB stick.

> **Note:**   If no USB stick is attached to the system, an error message will appear.

6   Select **RoleAssignmentExport.xml** and confirm the dialog with **OK**.

7   When the user settings are successfully imported, a dialog box is displayed.

8   Eject the USB stick on the instrument: Open the copy dialog, select the USB drive and click **Eject**.

9   The next time, the login screen is displayed, all imported users can login and have the assigned rights.

14.3   Analysis reports

14.3.1   Create and sign reports

1   From the **File menu**, choose **Analysis report**.

2   Enter name and password to electronically sign the report.

3   A report in PDF-A format is generated. The report includes:

- a front Page including the electronic signature

- the current Analysis

- a section with meta information about all samples used in Analysis)

14.3.2   Transfer the record

1   To access the generated report, go to **File > Copy**.

2   Choose file type **Analysis reports**.

3   Choose a destination.

4   Click **Copy**.

14.4   Standard operating procedure (SOP)

14.4.1   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 SOP's)

- The customer must have relevant SOP's / Work instructions, 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 SOP's-Corporate Compliance Policy). This is to prevent forgery of entries and signatures. Which means the customer should have a company policy, which describes this in detail.

14.4.2 Training of employees

- 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.

14.4.3 Miscellaneous

- The customer must comply with the legal archiving periods.

- The customer must ensure that the times on the machines as well as the times on the corresponding PCs can only be changed by an Administrator. Regular checks of the time as well as regular matching with the customer´s system 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.

14.5 SOP template forms

For your convenience, Miltenyi Biotec GmbHprovides SOP template forms. Please refer to the appendix of this user guide.

15
Technical Support

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

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macstec@miltenyibiotec.de

Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

16
Limited warranty

Except as stated in a specific warranty statement, which may accompany this product, or unless otherwise agreed in writing by an authorized representative of Miltenyi Biotec, Miltenyi Biotec's warranty, if any, with respect to this product is subject to the terms and conditions of sale of the company within the Miltenyi Biotec group, which supplied the product. 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's warranty for this product only covers product issues caused by defects in material or workmanship during ordinary use; it does not cover product issues caused by any other reason, including but not limited to product issues due to use in a manner other than specifically described in this manual, for example: inappropriate or improper use; incorrect assembly or installation by an operatoror a third party; reasonable wear-and-tear; negligent or incorrect handling, servicing, or maintenance; non-adherence to the operating instructions; unauthorized modification of or to any part of this product; or use of inappropriate accessories or work materials.

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 immediately, if a claim is made under such warranty. If a material or manufacturing defect occurs within the warranty period, Miltenyi Biotec will take the appropriate steps to restore the full usability of your product.

Limitation on damages: Miltenyi Biotec shall not be liable for any incidental or consequential damages for breach of any express or implied warranty or condition on this product.

Some states or jurisdictions do not allow the exclusion or limitation of incidentalor consequential damages, so the above limitations or exclusions may not apply to you. This warranty statement gives you specific legal rights and you may have other rights, which may vary from country to country or jurisdiction to jurisdiction.

17
Appendix

The following documents can be used as templates and customized if necessary:

- Letter to FDA
- Corporate compliance policy
- SOP Access to (company) database
- SOP Repeated account lockings
- SOP Data transfer

(Company Name)   Date: March 9, 2016
(Company Address)
(Company Address)
(Company Address)

U. S. Food and Drug Administration
5600 Fishers Lane,
Rockville MD 20857-0001
United States

Re.: 21 CFR Part 11 – Use of Electronic Signatures

Ladies and Gentlemen,

Pursuant to Section 11.100 of Title 21 of the Code of Federal Regulations, this is to certify that our organization intends that all electronic signatures executed by our employees, agents, or representatives, located anywhere in the world, are the legally binding equivalent of traditional handwritten signatures.

Sincerely

Yours

(Name)
(Title)

For internal use only
Information security

(Company) Corporate Compliance Policy

The (Company) Corporate Compliance Policy defines the principles and requirements in regard to the responsibility of (Company) for humans and the environment.

Version:
Released on

For internal use only.

The (Company) Corporate Compliance Policy defines the principles and requirements in regard to the responsibility of (Company) for humans and the environment.

- ❖ **Law compliance**
  - ♦ to comply with the laws of the applicable legal system(s).

- ❖ **Prohibition of corruption and bribery**
  - ♦ not to tolerate or get involved in any type of corruption or bribery, including any type of illegal payment offers or similar contributions to government officials in order to manipulate their decision making.

- ❖ **Respect for the employees' fundamental rights**
  - ♦ to promote equality for each employee regardless of their race, skin color, nationality, social background, possible disability, sexual orientation, political or religious beliefs as well as gender and age;
  - ♦ to respect each individuals personal dignity, privacy and personal rights;
  - ♦ not to employ anyone or to force them to work against their will;
  - ♦ not to tolerate unacceptable treatment of employees such as psychological harshness, sexual or personal harassment or discrimination.
  - ♦ not to tolerate any type of behavior (including gestures, language and physical contact) of sexual, enforcing, threatening, abusive or exploiting nature.
  - ♦ to provide reasonable pay and to ensure the national minimum wage.
  - ♦ to observe the maximum working hours set by the respective state.
  - ♦ to acknowledge the employees' freedom of association within the scope of applicable law, and to neither favor nor discriminate against the members of workers' organizations or labor unions.

- ❖ **Prohibition of child labor**
  - ♦ not to hire any workers under the age of 15. Countries which fall under the exception for developing nations in the ILO Convention 138, may reduce the minimum age to 14.

- ❖ **Health and safety of employees**
  - ♦ to take responsibility for the health and safety of the employees;
  - ♦ to reduce risks and to ensure best possible precaution against accidents and occupational diseases;
  - ♦ to offer trainings and to ensure that all employees are familiar with work safety.

- ❖ **Separation of company and personal interests – no interest conflicts**
  - ♦ All employees must separate between their personal and the interests of (Company) (e.g. personnel decisions, business relations with others etc.).
  - ♦ In case of a conflict of interest employees shall inform the management or their point of contact.

- ❖ **Environmental protection**
  - ♦ to observe the environmental protection with regards to legal norms and international standards;
  - ♦ to minimize environmental damage and to continuously improve environmental protection.

- ❖ **Supply chain**
  - ♦ to promote the observance of the content of the Corporate Compliance Policy to the suppliers in the best possible way;
  - ♦ to comply with the principles of non-discrimination within the selection of suppliers and in dealing with the suppliers.

(Company)	Standard Operating Procedure	Effective Date:
	Access to (Company) Database	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Title: Access to (Company) Database

Contents

- Purpose
- Signatures / Approval
- Scope
- Procedure
- Roles and Responsibilities
- Document History

Purpose

To assure that modifications or access to the (Company) database with administrative privileges by the Database-Administrator is under supervised control.

Signatures / Approval			
Author	Name:		
	Function:	Date	Signature
Reviewed by	Name:		
	Function:	Date	Signature
Approved by	Name:		
	Function:	Date	Signature

This document is confidential and property of (Company)

(Company)	Standard Operating Procedure	Effective Date:
	Access to (Company) Database	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Scope

Access to the (Company) database with administrative privileges by the Database-Administrator.

Procedure

1) The Database-Administrator needs to access the database.

2) A second qualified person must monitor all actions of the Database-Administrator when accessing the (Company) database.

3) The Database-Administrator accesses the (Company) database in the presence of the qualified person.

Roles and Responsibilities

The *Database-Administrator* accesses the (Company) database only with a monitoring qualified person.

Document History

Version-No.	Date of Revision	Reason for Change / Description of Changes	Revision done by
1.0		Initial Release	

(Company)	Standard Operating Procedure	Effective Date:
	Repeated Account Lockings	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Title: Repeated Account Lockings

Contents
- Purpose
- Signatures / Approval
- Scope
- Procedure
- Roles and Responsibilities
- References
- Document History

Purpose
Before unlocking an account the number of lockings during a defined time period need to be investigated and the reason for repeated lockings need to be clarified with the user.

Signatures / Approval			
Author	Name: Function:	 Date	 Signature
Reviewed by	Name: Function:	 Date	 Signature
Approved by	Name: Function:	 Date	 Signature

	Standard Operating Procedure	Effective Date:
(Company)	**Repeated Account Lockings**	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Scope

Repeated account lockings of (Company) users.

Procedure

1) After a locking of an account, the corresponding Administrator (Project-Administrator for Experts and Image Senders; Partner-Administrator for Project Administrators) needs to contact the Super-Administrator.

2) The Super-Administrator retrieves the number of lockings during the last 4 weeks from the Audit Trail.

3) If the Account was locked more than 5 times during the last 4 weeks the corresponding Administrator needs to contact the account holder and investigates the reason for the repeated lockings.

   a. If a plausible explanation was found the account will be unlocked.

   b. If no plausible explanation was found the management at (Company) needs to be informed, the account will not be unlocked until the reason for the repeated lockings is found.

Roles and Responsibilities

The *Project-, Partner-,* and *Super-Administrators* have to make sure that a locked account will only be unlocked if it was not repeatedly locked during a defined period of time.

Project- and *Partner-Administrator* inquire the Super-Administrator about repeated account lockings and further investigate the reason for any repeated lockings.

The *Super-Administrator* investigates the number of repeated account lockings in a given time interval.

References

Related SOPs in the effective version
   - (Company) SOP: Password confidentiality
   - (Company) SOP: Periodic Change of Passwords

(Company)	Standard Operating Procedure	Effective Date:
	Repeated Account Lockings	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Document History

Version-No.	Date of Revision	Reason for Change / Description of Changes	Revision done by
1.0		Initial Release	

(Company)	Standard Operating Procedure	Effective Date:
	Secure data exchange between IT-Systems	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Title: Secure data exchange between IT-Systems

Contents
- Purpose
- Signatures / Approval
- Scope
- Procedure
- Roles and Responsibilities
- References
- Document History

Purpose
The exchange of data between IT Systems within a regulated environment must be secure.

Signatures / Approval			
Author	Name: Function:	Date	Signature
Reviewed by	Name: Function:	Date	Signature
Approved by	Name: Function:	Date	Signature

This document is confidential and property of (Company)

(Company)	**Standard Operating Procedure**	Effective Date:
	Secure data exchange between IT-Systems	Revision Date:
	SOP No. XXX Version-No. 1.0	Replaces Version NA

Scope

Regulatory secure data exchange between IT-Systems.

Procedure

If data in a secure environment (e.g. within a company network) is exchanged between 2 IT-Systems, the route between the source system and the target system must be secured.
Data transfer can either take place:
   a) directly from the source system to the target system (without the possibility of external access e.g. by users) respectively by clipboard in a secure and protected folder.
   b) or by clipboard in an unsecured or partly secured folder.

In case a) no action is required.
In case b) opening the transfer folder and manipulating the data must be prohibited. For this purpose all users with access to the transfer folder must be informed that accessing the folder is prohibited. This must be ensured through training this SOP.

Roles and Responsibilities

All users with access to the data transfer folder.

References

   - n.a,

Document History

Version-No.	Date of Revision	Reason for Change / Description of Changes	Revision done by
1.0		Initial Release	

18

Index

Index

18

140-003-409.05

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.