Miltenyi Biotec



MACSQuant® Instrument short instructions

Data analysis

Before using the instrument for the first time, read the MACSQuant Instrument user manual and MACSQuantify™ Software user manual.

These short instructions are valid for the MACSQuantify Software 3.1 and later. They describe how to adapt an analysis template and create a gating strategy for analyzing data with the MACSQuantify Software.

Opening a new analysis window

1 To open a new analysis window, click the **Add** button on the toolbar.
2 Select the analysis window(s) required for analysis.

Plot properties

To change the properties of a plot, click the parameters shown in **Figure 1**. To enlarge a plot, double-click it. To return to its original view, double-click again.

- Plot title (1): Use the dropdown list to select the parent region to be shown.
- **'i'** button (2): Select the **'i'** button to open the plot properties window to change the plot type and other options of the plot.
- x-axis (3): Click the axis label to change the shown parameter.
- y-axis (4): Click the axis label to change the shown parameter.

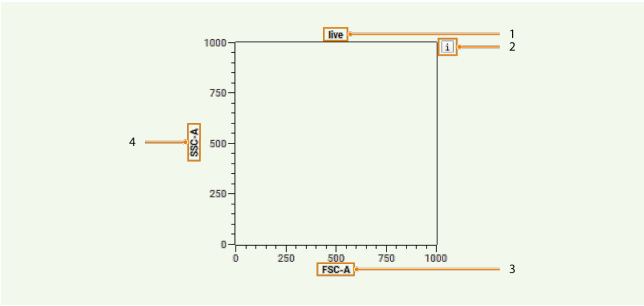
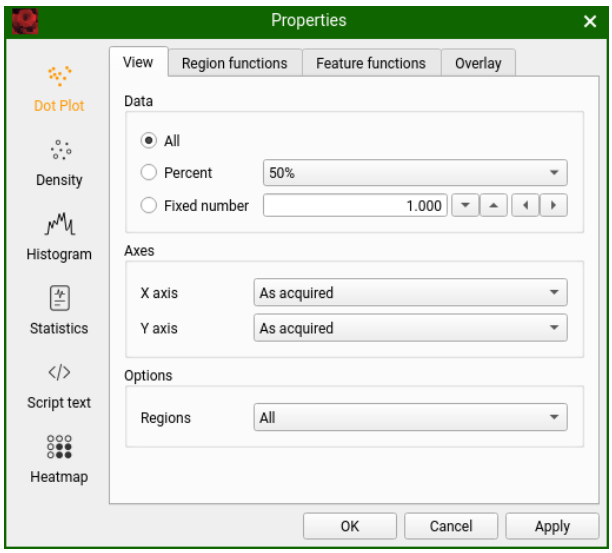


Figure 1: Plot properties

Plot types

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

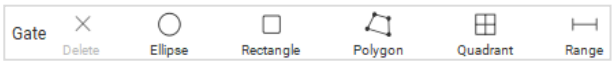
1 To open the **Properties** window, click the **'i'** button next to a plot.
2 Select a plot type from the left-hand side pane.
3 Use the tabs **View**, **Region functions**, **Feature functions**, and **Overlay** to change, for example, the shown number of events or shown statistical parameters. For details, refer to the **MACSQuantify Software user manual**.



Gating tools

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

1 Select a gating tool from the toolbar.



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

- Ellipse, rectangle, quadrant, or range: Click and hold the mouse. Drag the cursor as desired. Release the mouse to finish drawing.
- Polygon: Click once to start drawing the first point. Move the cursor and click again to draw the next point. Continue as desired. Double-click to draw the last point and finish the region. It is not possible to add additional points to a polygon once it is created.

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

- To adjust the size of the region, click and drag any edit points.
- To move the region to a new position, click and drag to the desired position.
- To change the name, the color of the region, or to set the region to a Not-gate, right-click and select **Region properties**.
- To delete the selected region, right-click and select **Delete region**.

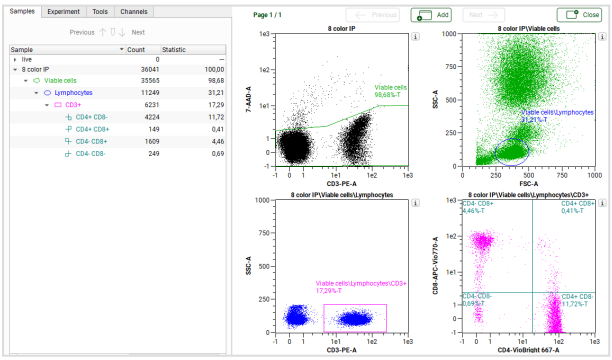
Creating an analysis template

An analysis template is a predefined layout for acquired data and can consist of plots, statistics, and tables. It usually includes gating strategies, region names, and selected statistics.

1 Optional: Open a data file.
2 To open a new analysis window, click the **Add** button on the toolbar.
3 Select the analysis window(s) required for analysis.
4 Click the **Analysis** button on the toolbar.
5 To load the samples in all plots, double-click the data file in the sample list.
6 Click the **Analysis** button.
7 Select a gating tool from the toolbar.

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

8 Optional: Change the axis parameters of plots. See **Figure 1**.
9 Draw a region around a population.
10 To show only the events of the parent region, select this region in the next plot from the plot title dropdown list. See **Figure 1**.
11 Optional: To change the plot type, click the **'i'**-button of a plot. See **Figure 1**.
12 Continue with hierarchical gating as required.
13 Optional: To expand the gating tree, click the arrow next to the sample in the sample list.
The gating tree is shown.



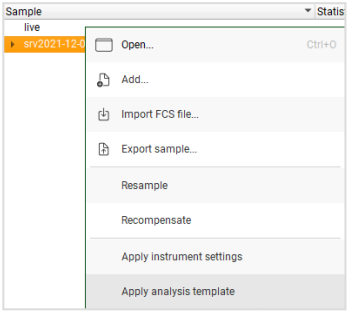
14 Optional: Save the analysis template for later use.

Applying analysis templates

Saved analysis templates can be applied to samples after acquisition.

To apply an analysis template, perform one of the following steps:

- View and analyze data using the analysis template used at acquisition:
  1 Right-click the data file in the sample list.
  2 Select **Apply analysis template**.
  The data file and corresponding analysis template is loaded. The **Analysis** button is activated.



- View or analyze data using a saved analysis template:
  1 Right-click a sample or highlight multiple samples in the sample list and right-click one of them.
  2 Select **Open** and select the desired analysis template.
  3 Click **Open**.
  The analysis template is applied to the selected data file. The analysis mode is automatically selected.
  4 Optional: To adapt an analysis template, for example move a region, the **Analysis button** must be inactivated (gray).

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