Flow Cytometry GUI for Matlab Documentation

1. Input file and toolbox requirements

1. Input file requirement

   The input file should be a .mat file containing one variable only (no matter the name) which is a structure with the following fields:

   '`.data`', a matrix containing all the cells as rows and the parameters collected for each cell as columns

   '`.colheaders`', the names of the parameters that were collected.

   In order to achieve this type of files from .fcs files the following procedure is suggested:

   1. Download `FCSExtract.exe` from:
      http://research.stowers-institute.org/efg/ScientificSoftware/Utility/FCSExtract/index.htm
      and use it in order to convert .fcs files into CSV format text files.
   2. Use the GUI in the `cvs2mat.m` in order to convert these text files into .mat file.

   This procedure could be done on all the files contained in one folder ('Batch extraction' in FCSExtract.exe).

   If you have simpler methods, feel free to use them and share.

   See `sampleDataFile.mat` as an example for an input file.

2. Toolboxes requirements

   This GUI was built with the aid of the 'GUI layout toolbox', and it is required for its functionality.
   It could be downloaded from:
   http://www.mathworks.com/matlabcentral/fileexchange/27758
   Run the '`install.m`' file there.

   Besides, the GUI requires the Matlab's image processing toolbox.

Remark:

The GUI uses 'Fast points-in-polygon test' (`private\inpoly.m`) by Darren Engwirda (See http://www.mathworks.com/matlabcentral/fileexchange/10391-fast-points-in-polygon-test), for gate computation (in manual, auto, semi-auto gates). It was found to be faster here in the .mex file version by Bruno Luong (http://www.mathworks.com/matlabcentral/fileexchange/27840-2d-polygon-interior-detection).
If you want to use it you should:

1. Download the files from Bruno Luong's submission.
2. Run the '`insidepoly_install.m`' file there, and then move the three files: '`insidepoly.m`' '`insidepoly_dblengine.mex`' '`insidepoly_sglengine.mex`' into the `private` folder.
3. Change the name of the function in '`insidepoly.m`' to `inpoly.m` and the file name accordingly.

2. GUI Overview

In startup, the GUI has the following appearance, with most of its toolbar buttons and menus disabled:
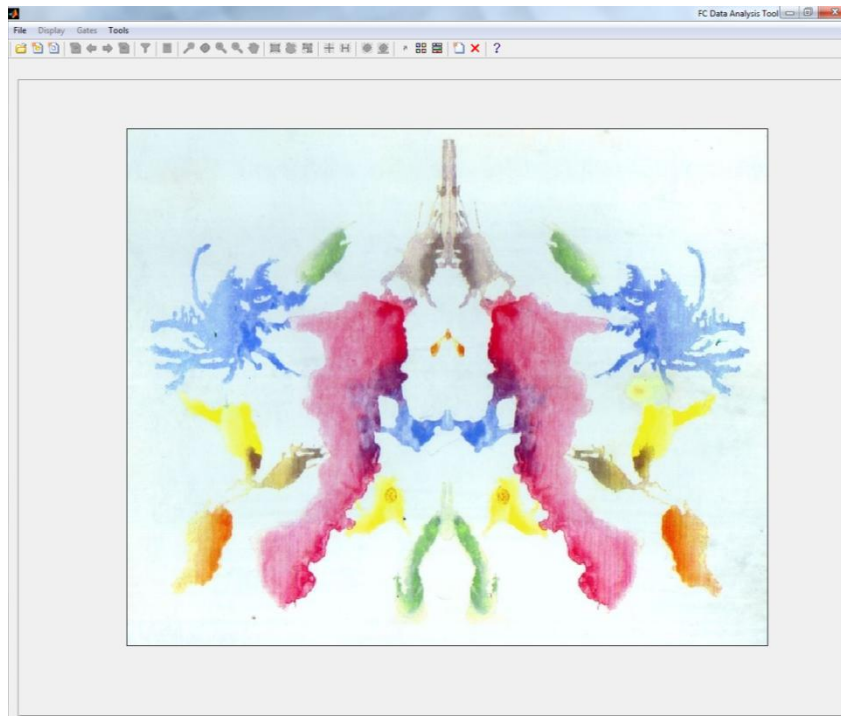
After loading a file the GUI is changed to this form:
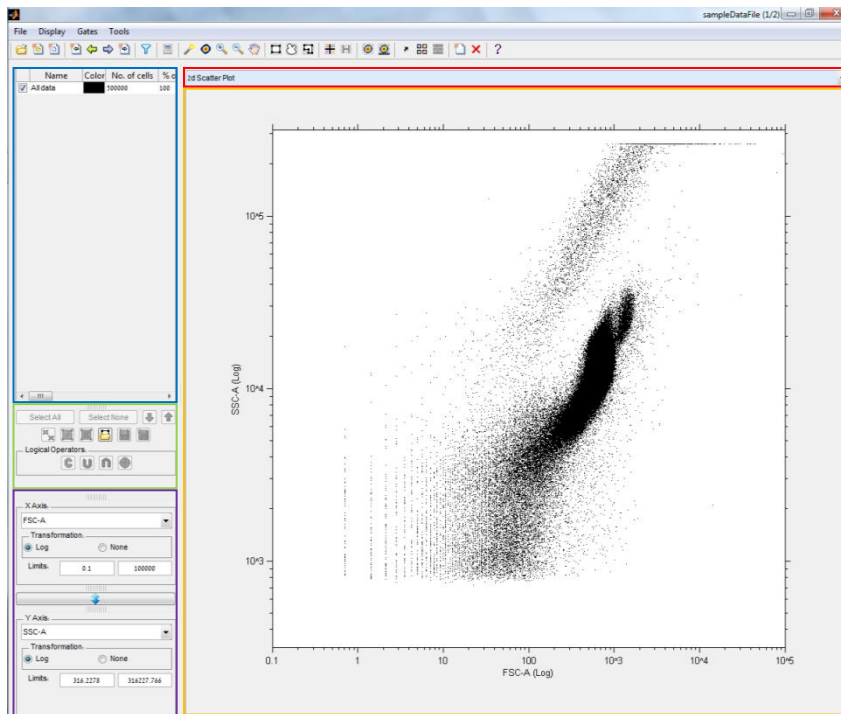
In this form most of the toolbar buttons and menus are activated, and the main window was changed. The right side (orange frame) displays a scatter plot presenting the data of

the file that was loaded. Above (red frame) there is a popup menu to choose between this display and the histogram display (see section 6).

The left side consists of three parts:

1. First (blue frame) is the gate data table, presenting information about all the gates, selecting gates for display and other purposes, and option to change gate name and color (see section 15).

2. Second (green frame) is different operations that could be made on the gates in the gate data table (see sections 13,14 and 15), the same operations could be found in the Gates menu.

3. Third (purple frame) contains different options concerning the X and Y axes (see section 6).

The toolbar contains the following buttons:

And could be divided to the following groups:

1. File and window operations (Could be found in the File menu, see section 3)

2. Display options (Display menu, see section 6)

3. Different gate creation methods (Gates menu, see sections 7, 8, 9, 10, 11,12)

4. Different tools (Tools menu, see sections 4, 5, and 16)

5. Help button ('Tools' menu, Ctrl+H shortcut, leads to this file).

3. [Loading files](#)

After startup, the only buttons* available are: 🖿 🗎 🗎 🗋 ✕ , all could be also reached through the **File** menu:
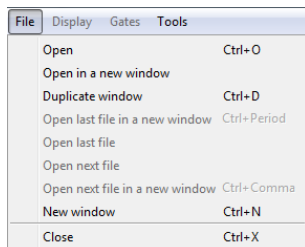
1. Open: 🖿 (keyboard shortcut `Ctrl+O`)

   Open a new .mat file containing the FCS data (see [section 1](#) for input files requirements) in the current GUI window, preserving the same display settings ([section 6](#)) and filters (see [next section](#)) as the previous file opened in this GUI window (if it is the first file opened, the settings are the default settings). In case you open a new file in the same window (either by simply open, or by ⬅ and ➡ as described below), the new file must have the same colheaders (i.e. the same parameters collected) as the current file. If it doesn't have them use 🗋 in order to avoid error.

2. Open in a new window: 🗎

   Open a new .mat file in a new GUI window, preserving the same display settings as the previous file opened in this GUI window (if it is the first file opened, the settings are the default settings, and it is the same as pressing New window).

3. Duplicate window: 🗎 (keyboard shortcut `Ctrl+D`)

   Duplicate the GUI window (if no file was opened, it is the same as pressing New window).

4. New window: 🗋 (keyboard shortcut `Ctrl+N`)

   Open a new GUI window without preserving the display settings and filters.

5. Close: ✕ (keyboard shortcut `Ctrl+X`)

   Close the current GUI window.

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* (For these 🔳 🔳 tools, see [section 16](#))

After loading a file the following buttons becomes available (ONLY IF there is another .mat file in the folder): as well as in the File menu:
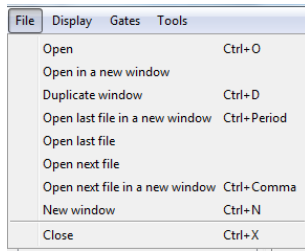
1. Open previous file: (keyboard shortcut Ctrl+,)
    Open the previous file in the folder in a new GUI window preserving the same display settings and filters.

2. Open previous file in a new window:
    Open the previous file in the folder in the current GUI window preserving the same display settings and filters.

3. Open next file:
    Open the next file in the folder in the current GUI window preserving the same display settings and filters.

4. Open next file in a new window: (keyboard shortcut Ctrl+.)
    Open the next file in the folder in a new GUI window preserving the same display settings and filters.

4. Filtering

The filtering tool could be reached through this button ⧩ or through the `Tools` menu. This tool could be used in order to create a filter according to time of read (the first # cells) or according to extreme values of the collected parameters (percentage of cells with low or high value of a selected parameter). More sophisticated methods of filtering are described in sections [7](#), [8](#), [9](#), [10](#), [11](#), [12](#)).

The filter is defined in the following sub-GUI window that will be opened:
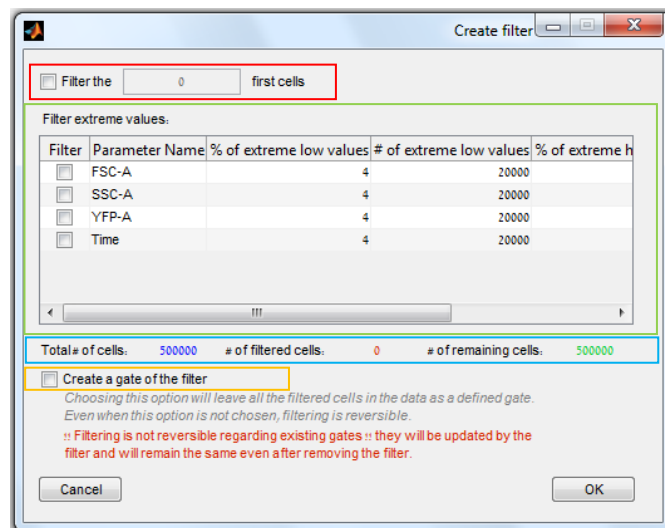
Red frame - Choosing the checkbox in the red frame will make the text box white and editable. You can define the number of first cells you want to filter.

Green frame – In the first column of the table you could choose which parameters the filter will be based on. Columns 3-7 are editable, and the values will determine the percentage or number of cells that will be filtered.

Blue frame – the total number of cells is displayed in blue. The number of filtered cells (red) and the remaining cells (green) is updated according to the chosen filters and entered values. The value may not be equal to the sum of the numbers of cells defined in the filters as the same cells might match different filters.

Orange frame – there are two ways to save the filter:

1. Checkbox marked – create a gate of the filtered cells. This option will add a gate containing all the filtered cells to the gate data table and logical operation could be performed in order to exclude them.

2. Checkbox unmarked – the filtered cells will be excluded from analysis – all the new gates will be based on the filtered data, and files loaded in the same window will be filtered the same way. The filtered cells will be excluded also from existing gates.

The toolbar button (⧩) will remain down, as an indication that the data is filtered, and a next push on this button will open the existing filters to change them. The filters will be passed to a file opened using open, open in new window, open last\next, open last\next in new window, described in the previous section. In order to cancel the filter you could push a new button that will appear in the lower part of the sub-GUI window: `Cancel all filters` and the filtered cells will be returned to analysis. However the gates that were updated by the filter will remain the same. Therefore, it is better to make this type of filtering prior to gating.

5. Parameters calculations

This tool could be reached through this button ▦ or through the `Tools` menu, and is used in order to make mathematical operations on the collected parameters.

The operations are defined in the following sub-GUI window that will be opened:
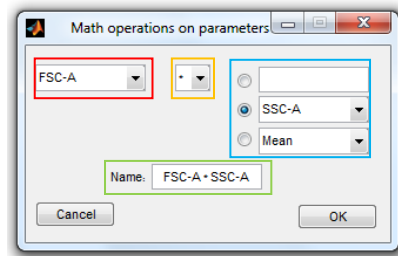
Red frame – in this popup-menu you can choose one of the collected parameters in order to make the math operation on it.

Orange frame – in this popup-menu you can choose mathematical operation that could be either: "+" plus,"-" minus, "x" multiplication,":" division, or "^" power.

Blue frame – the second element in the operation could be:
1. A number – as entered in the upper text box.
2. One of the collected parameters – as selected in the middle popup menu.
3. A statistical measure – as selected in the bottom popup menu.

If power is selected in the popup of the orange frame, only a number could be used as the second element in the operation.

Green frame – the name of the new 'parameter' is updated according to the selections, you can also change it. It could not be empty, or the same as one of the existing parameters.

Push ⬚OK⬚ to finish and the new 'parameter' will be added to the X-axis and Y-axis popup-menus (see next section).

6. Display options
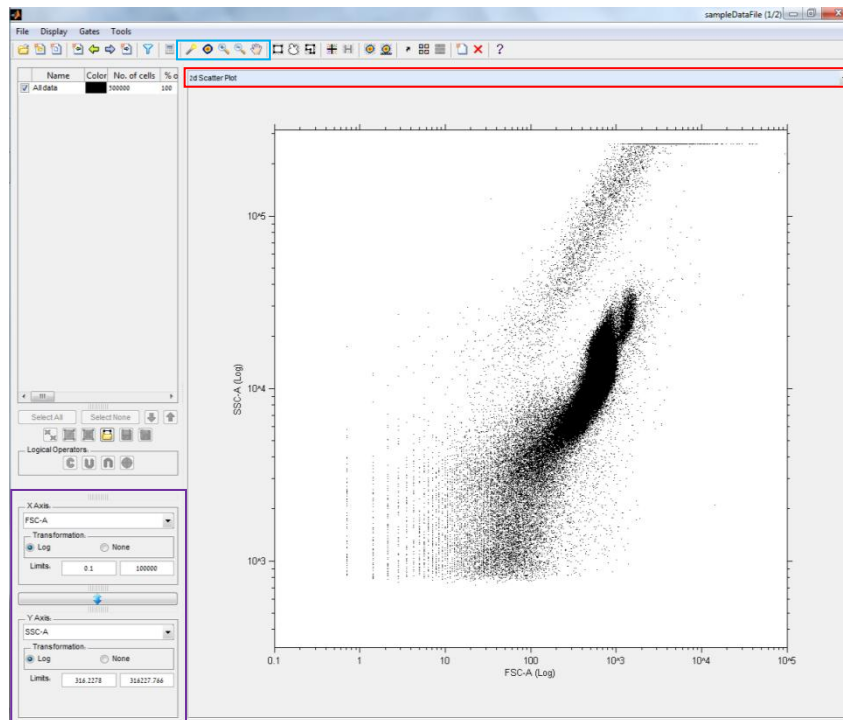
1. Red frame – Change plot mode – 2d scatter plot or 1d histogram
2. Purple frame – X axis and Y axis properties (or histogram options in histogram plot mode)
3. Blue frame - Different display tools could be also reached through the Display menu, as shown below:

1. [Plot mode – 2d scatter plot or 1d histogram](#)

Changing plot mode could be done using the popup menu in the red frame seen above, or through the `Display` menu, or by pressing `ctrl+M`.

If in the loaded file there is only one collected parameter (one column), plot mode will be 1d histogram without the ability to change to 2d scatter plot.

After changing to histogram plot mode the window will be transformed into this:
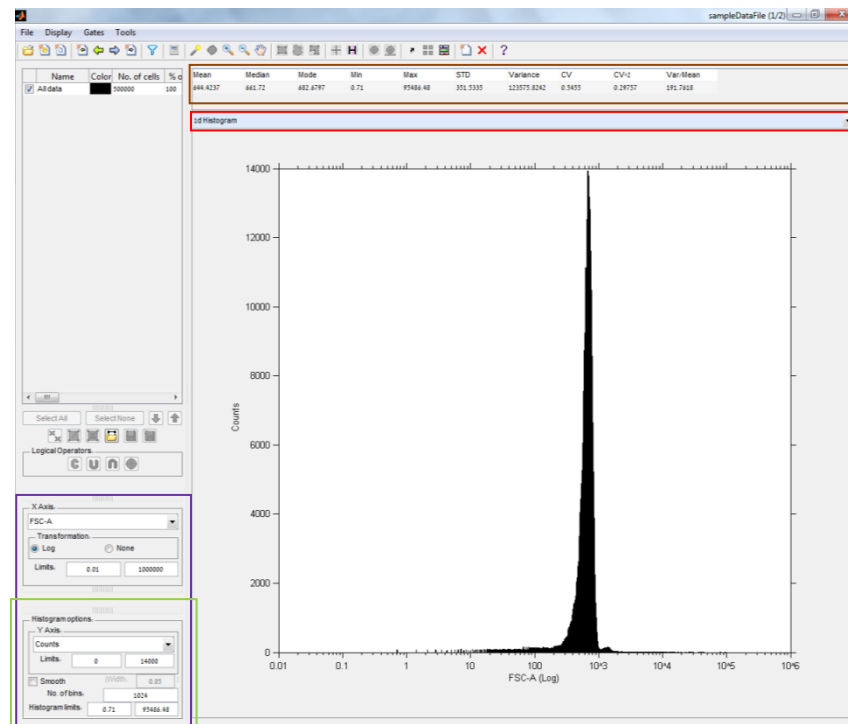
The upper table (in the brown frame) presents statistical measures of the data, and in the green frame, instead of the Y axis options, there are histogram options that will be described below.

Some of the buttons of the toolbar and menus were disabled, as the histogram plot mode does not allow these tools.

2. <u>X axis, Y axis and histogram properties</u>

Changing X axis, Y axis and histogram properties (in histogram plot mode) could be changed in the following panel on the left bottom corner of the GUI window (the purple frame in the overview in the beginning of section).
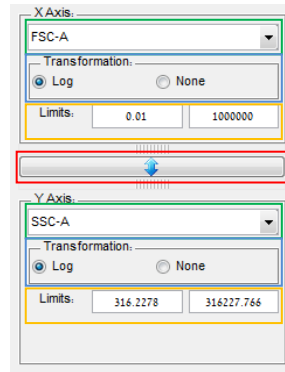
Green frames – change the parameter used as X or Y axis through the popup menu.

Blue frames – change the transformation of display of X or Y axis.

Orange frames – change the limits of display (in Logarithmic scale must be positive). (Could also be done using the display tools mentioned below)

Red frame – Switch the X axis into Y axis and the opposite

In the histogram plot mode the panel looks like this:
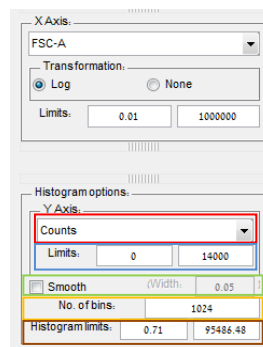
The X axis part remains the same as above, the switch button is hidden as there is no switching option in histogram plot mode, and the Y axis panel turned into the histogram options panel:

Red frame – choose between Y axis that is based on 'Counts' or 'Percentage'

Blue frame – define Y Axis limits

Green frame – choose this option in order to smooth the histogram plot using the Matlab function `ksdensity` instead of `hist`. Marking the checkbox will activate the text box to define smooth width parameter.

Yellow frame – define number of bins of the histogram, default is 1,024.

Brown frame – define histogram limits, default is the minimum and maximum values of the X axis parameter.

3. [Different display tools](#)

- Reset view – could be reached through the Display menu or by pressing `ctrl+R`. Reset the view to the original default view.

- Density color map – could be reached through the Display menu or by pressing `ctrl+Q`. Show isolines with color according to the density of cells, while isoline is an area in which there is a similar cell density. The isolines are defined by the Matlab function `contourc` (see `doc contour`) based on a 2d histogram (or density map), defining the cell density inside each 2d bin (see the .m file `private\histmap.m`). The default bin number and the number of isolines are defined as follows:

If total # of cells > 70,000, there will be 100 bins on each axis, and 100 isolines.

If total # of cells > 10,000 and < 70,000, there will be 50 bins on each axis, and 50 isolines.

If total # of cells < 10,000, there will be 10 bins on each axis, and 10 isolines.

- Zoom in - could be reached through the Display menu or by pressing `ctrl+=`.

- Zoom out - could be reached through the Display menu or by pressing `ctrl+-`.

- Pan - could be reached through the Display menu or by pressing `ctrl+P`.

See [section 13](#) for information on using the gate data table in order to change the display of gates.

7. Gating overview

There are six ways to create gates (+ the filtering tool discussed in section 4).

All are available through the toolbar icons mentioned below (except of the logical gates which their buttons appear below the gate data table), and could be also reached through the Gates menu.

1. Manually – define a gate by drawing shapes on the scatter plot area.
2. Automatically – take # cells from a center.
3. Semi-automatically – take # cell from a center, get a closer feeling of cell density and the desired size of gate (opens a new sub-GUI window).
4. Quadrant gates – divide the scatter plot area to four sections with 5 points (each section is a gate).
5. Histogram based gate – define boundaries on a histogram plot.
6. Logical gates – make logical operations on gates to create new gates.

Some of the tools are available only under specific conditions: the manual, automatic, semi-automatic, and quadrant gates are available only in 2d scatter plot mode, while the histogram gate is available only in 1d histogram plot mode. The logical gates are available if sufficient number of gates is selected in the gate data table (see sections 13 and 14).

All of the gates will be saved and displayed in the gate data table, as well as in the plot, having specific colors. Different operations could be made on existing gates, using the gate data table, the buttons below the table ( ) or the Gates menu (see sections 13 and 15).

Using the gate data table, you could deselect the 'All data' row, and by this a gate that will be created will be a child of the currently selected gates, namely, its data will include only cells that are also included in the parent gates. This could be also achieved using the logical gates (see section 14 for both methods).

Manual, quadrant, and histogram gates are editable, i.e. after finishing the gating process you could access them and edit their coordinates, using the edit coordinates button below the gate data table (see section 15). If they have children, editing will affect their children (unless they are of auto and semi-auto type), but not their grandchildren if exist.

Auto, semi-auto and logical gates are not editable, i.e. after finishing the gating process the gate data could not be changed, only gate color and name might be changed using the gate data table. As mentioned auto and semi-auto types will not be affected by changes in parent gates (if exist).

8. Creating gates manually

There are three possible methods to create gates manually, i.e. by drawing shapes on the scatter plot area:

1. Rectangular gate ⬚ (using Matlab function `imrect`)
2. Free shape gate ⬚ (using Matlab function `imfreehand`)
3. Polygonal gate ⬚ (using Matlab function `impoly`)

All of them could be also reached through the `Gates` menu, in 2d scatter plot display mode.

After choosing one of these methods the GUI window will become like this:
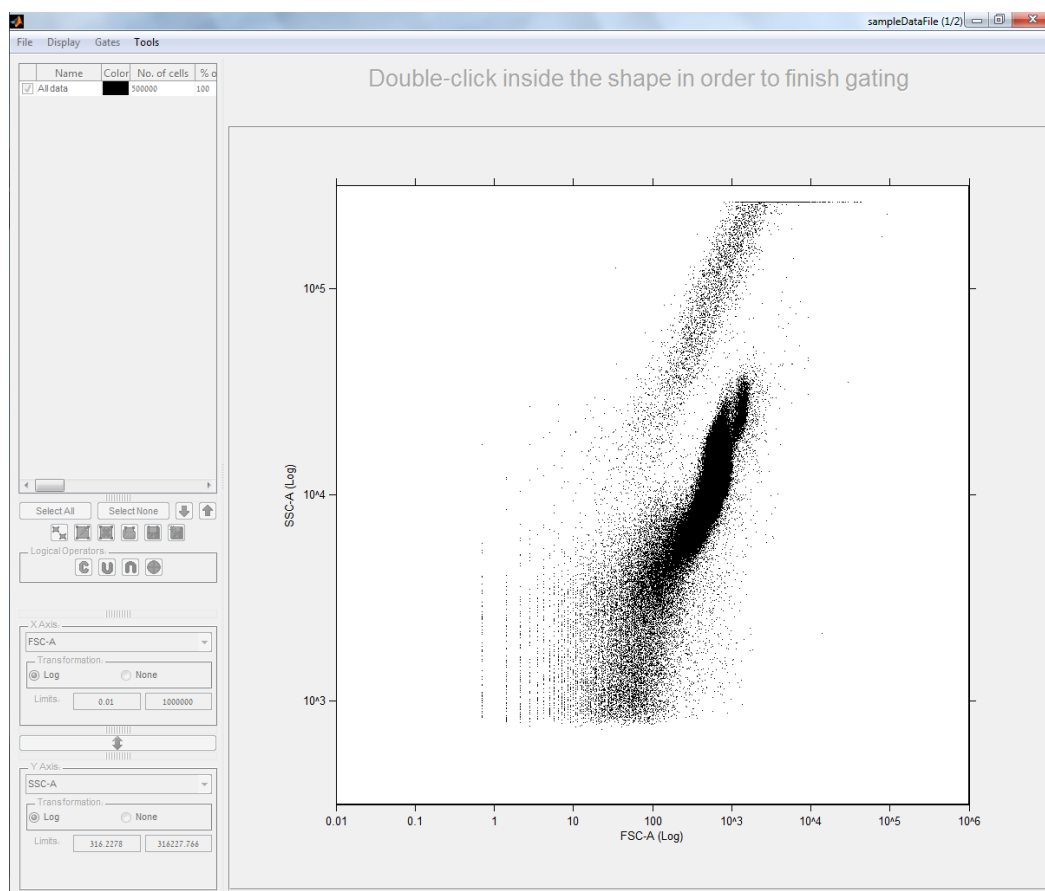
Only after double click inside the finished shape, there will be a possibility to return to the regular GUI window (this was done in order to prevent errors using the `imroi` tools).

In order to draw the gate shape:

1. In rectangular gate - Click inside the scatter plot area and drag the mouse to create a rectangle in a desired size. In order to change the size of the rectangle click on any of the edges or vertices and drag the mouse. In order to move the rectangle without changing the size – click inside it and drag the mouse. Double click inside the rectangle in order to finish this step (`doc imrect` for more options).

2. In free shape gate – Click inside the scatter plot area and drag the mouse to define the desired shape, leave the mouse button in order to finish drawing the shape (and close it if it is not closed). In order to move the shape, click inside it and drag the mouse. Double click inside the shape in order to finish this step (`doc imfreehand` for more options).

3. In polygonal gate – Click inside the scatter plot to place the first vertex. Each additional click places another vertex. In order to close the polygon, move the mouse over the first vertex and click, or double click in any location. After closing the polygon click on any or the vertex and drag to change its location, or click on an edge or inside the shape and drag, in order to move the entire polygon. Press 'A' and click on an edge in order to add a new vertex. Double click inside the polygon in order to finish this step (`doc impoly` for more options).

After double clicking inside the shape, the shape will be still available for editing in the same way mentioned above, and the window will become like this (presented is a polygonal gate):
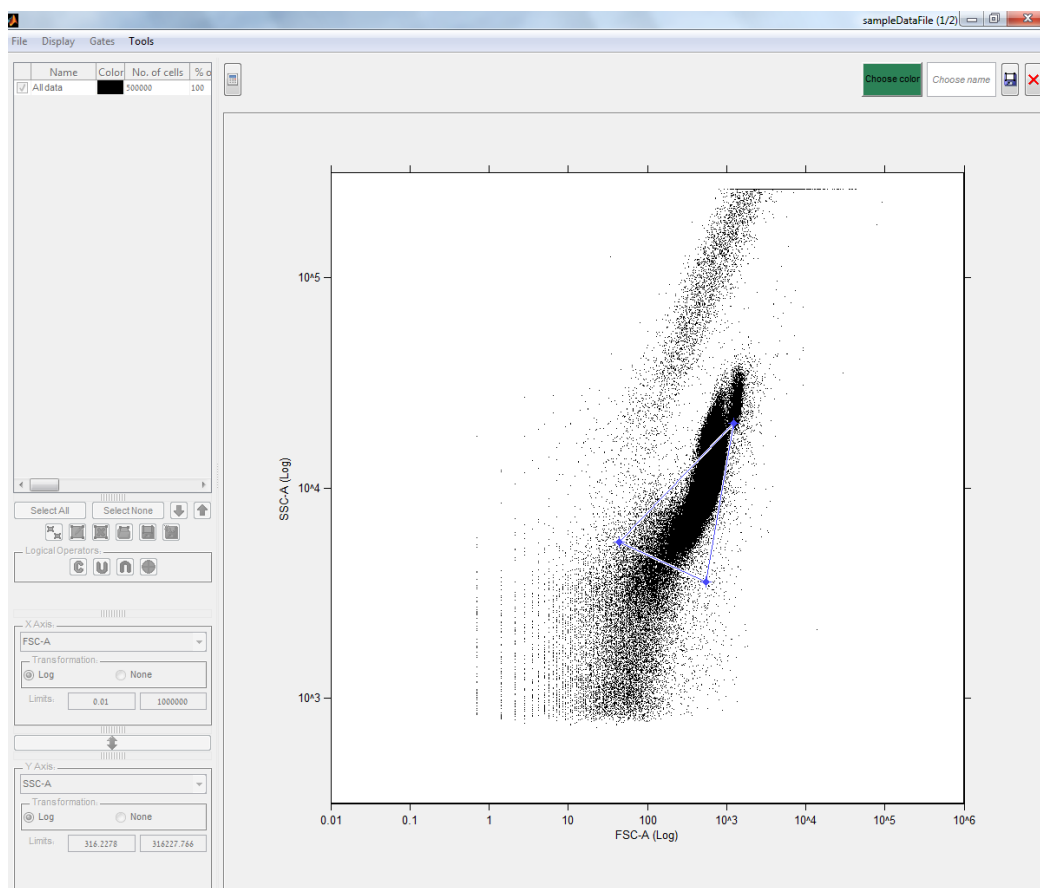
The upper panel contains the following buttons with the described functions:

Click on the calculator button in order to show how many cells are inside the shape, and the percentage of total.

 Click on the choose color button in order to change the color of the gate, the background color of the button is the current color.

 Define the name of the gate, if not define default is gate# (# is the current number of gates + 1).

 Finish gate creation and save the gate to the [gate data table](#).

 Finish gate creation without saving the gate.

After saving the gate, the GUI window will come back to its regular state and the new gate will be presented in the [gate data table](#) and the scatter plot as can be seen below (the lines of gate boundaries will be visible only when the transformation and the X and Y axis are the same as in the gate creation, otherwise only the cells in the gate will be colored in the color of gate):
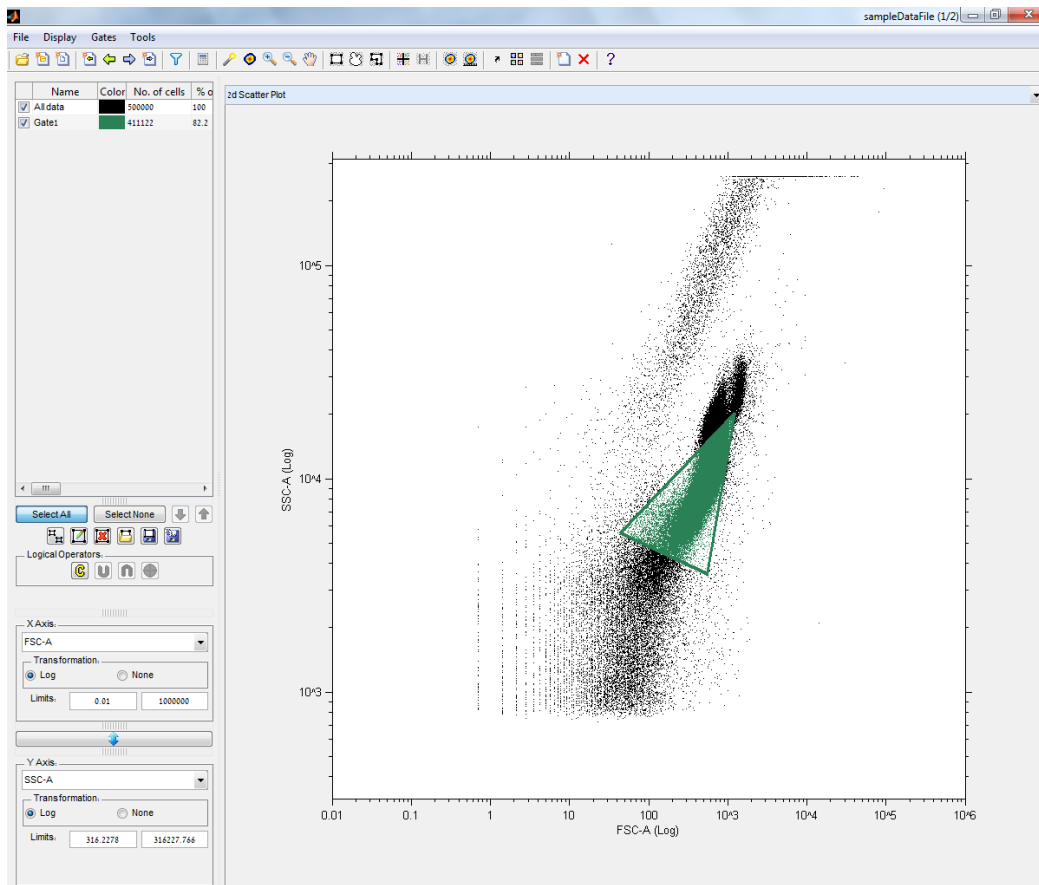
See [Section 13](#) for information about using the gate data table and operations that could be done on the existing gates.

9. Creating gates automatically

In order to create a gate automatically, push this button ⊙ or select it in the Gates menu, in 2d scatter plot display mode.

In this method, the gate is created taking # cells from a center. The method of picking those cells could be:

1. Radial – the cells are sorted by their distance from the center, and the first closest cells are taken.
2. Isolines – the cells are divided to groups, each group resides in one isoline, while isoline is an area in which there is a similar cell density. The isolines are defined by the Matlab function `contourc` (see `doc contour`) based on a 2d histogram (or density map), defining the cell density inside each 2d bin (see the .m file `private\histmap.m`). The default bin number and the number of isolines are defined as follows:
   If total # of cells > 70,000, there will be 100 bins on each axis, and 100 isolines.
   If total # of cells > 10,000 and < 70,000, there will be 50 bins on each axis, and 50 isolines.
   If total # of cells < 10,000, there will be 10 bins on each axis, and 10 isolines.
   The calculation is made as follows: The program finds the last isoline that contains number of cells that is smaller than the desired number of cells in the gate. Then it adds cells from the next isoline to reach the desired number of cells, sorted by their distanced from the edges of the last isoline.

The isolines could be visualized by activating the density color map, by pushing the toolbar button (⊙), or through the Display menu, or by pressing `ctrl+Q`. They are also visualized during the automatic gate creation.

This is how the GUI window looks when activating automatic gating:
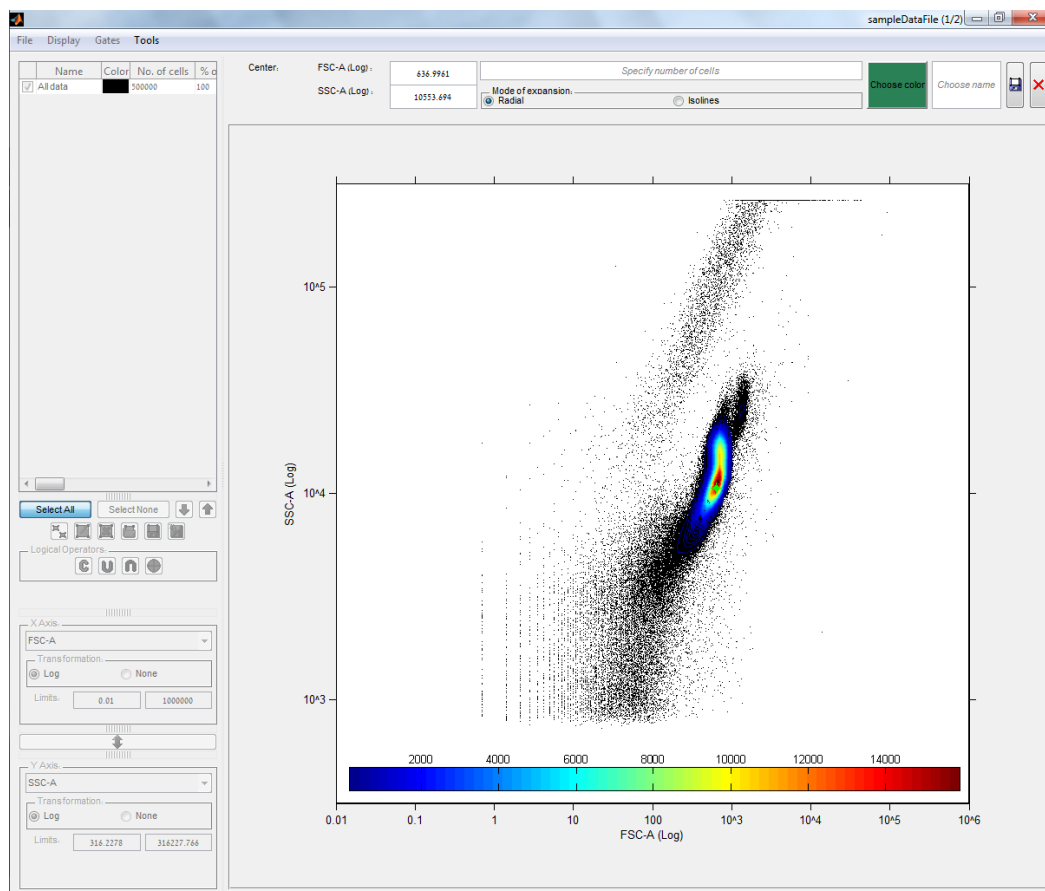
On the scatter plot a green dot is visible, this is the center as calculated automatically, the point in which the cell density is the highest.

The upper panel looks like this:

Orange frame – Controlling the center position. Could be done also by moving the green dot thorough the plot. The gate will be calculated from this center, using the expansion mode mentioned above (radial vs. isolines).

Red frame – Specifying the number of cells to be include in the gate (must be specified in order to create the gate).

Blue frame – Change mode of expansion as mentioned above.

Click on the choose color button in order to change the color of the gate, the background color of the button is the current color.

Define the name of the gate, if not define default is gate# (# is the current number of gates + 1).

 Finish gate creation and save the gate to the [gate data table](#).

 Finish gate creation without saving the gate.

After saving the gate, the GUI window will come back to its regular state and the new gate will be presented in the [gate data table](#) and in the scatter plot (if it is not visible, deactivate the density color map, by pushing the toolbar button ( ), or through the menu, or by pressing `ctrl+Q`).

10.Creating gates semi-automatically

In order to create a gate semi-automatically, push this button 🔘 or select it in the `Gates` menu, in 2d scatter plot display mode.

In this method, the gate is created in a similar way to the automatic method (See section 9), i.e. taking # cells from a center; however it gives more control on deciding which number of cells to take, based on cell density.

Activating the semi-automatic gating will open a new window (it might take a while as the number of cells in each isoline is calculated... be patient!), and deactivate most of the main GUI window, as in the other gating methods. Only after closing the semi-automatic gating window, the main GUI window will return to its regular form.

This is how the semi-automatic gating window looks like:
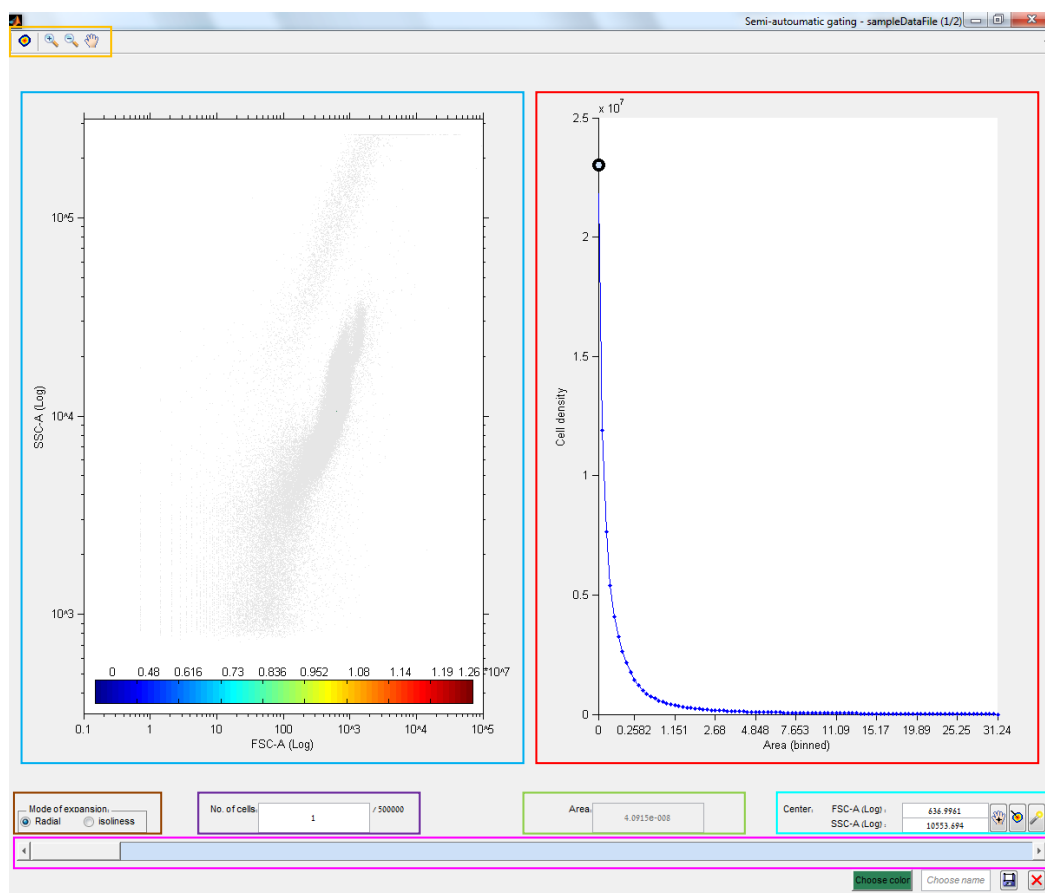
Brown frame – The same as in automatic gating, change mode of expansion between:
1. Radial – the cells are sorted by their distance from the center, and the first closest cells are taken.
2. Isolines – the cells are divided to groups, each group resides in one isoline, while isoline is an area in which there is a similar cell density. The isolines are defined by the Matlab function `contourc` (see `doc contour`) based on a 2d histogram (or density map), defining the cell density inside each 2d bin (see the .m file `private\histmap.m`). The default bin number and the number of isolines are defined as follows:

If total # of cells > 70,000, there will be 100 bins on each axis, and 100 isolines.

If total # of cells > 10,000 and < 70,000, there will be 50 bins on each axis, and 50 isolines.

If total # of cells < 10,000, there will be 10 bins on each axis, and 10 isolines.

When changing to isolines mode the window looks like this:
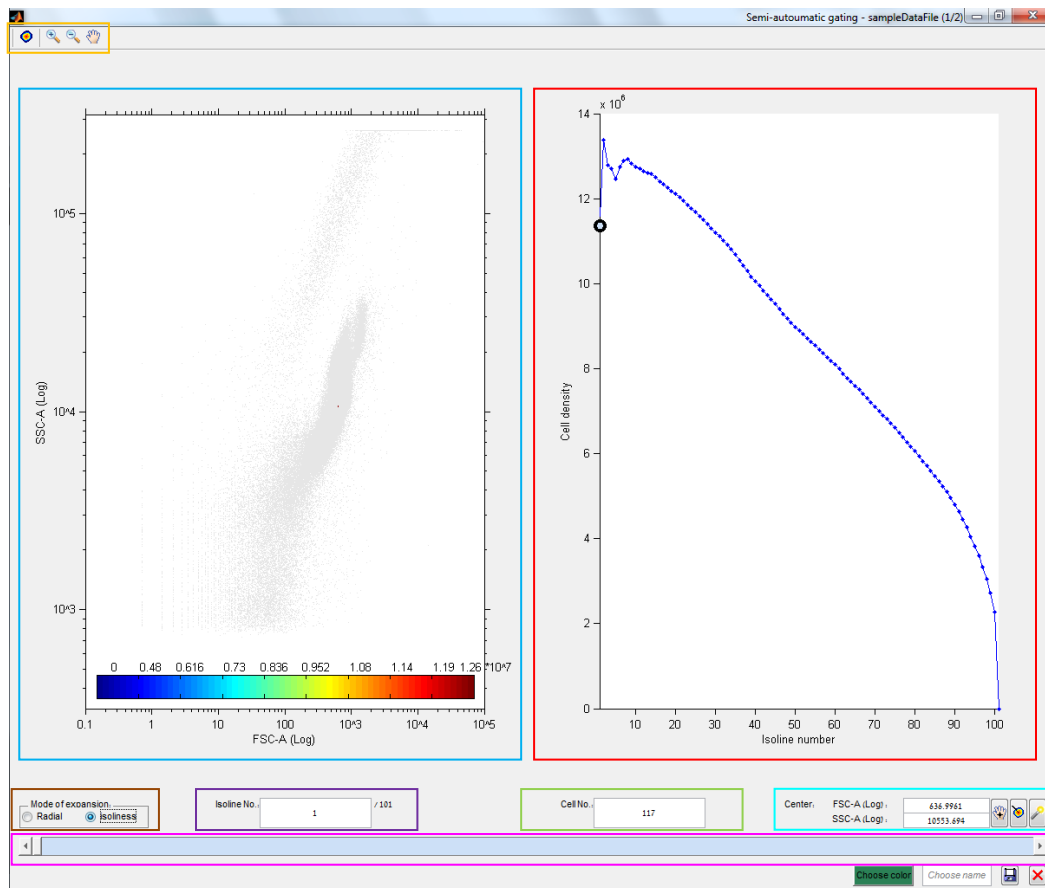
The differences are in the purple, the green, and the red frames and will be discussed below.

Orange frame – Toolbar, same as in the main GUI:

    ⬤ - Show isolines based on the cell density map on the left axis.

    🔍🔍✋ - Zoom in, zoom out and pan, works on both axes.

Blue frame – Presents the data, the same as in the main GUI, just that all the presented cells are in grey to help visualization.

Purple frame – In the radial expansion mode it shows the desired number of cells in the gate.

In the isolines expansion mode it shows the number of the selected isoline. The value in this edit box could be changed either directly, or by using the slider in the pink frame.

Green frame – In the radial expansion mode it is inactive, and shows the area that the desired number of cells occupy (it is π x (the distance of the farthest cell)^2).

In the isolines expansion mode it is active, and shows the desired number of cells in the gate. The value is updated automatically by changing the selected isoline. If you want to

specify a different number of cells it is possible. The program finds the last isoline that contains number of cells that is smaller than the desired number of cells in the gate. Then it adds cells from the next isoline to reach the desired number of cells, sorted by their distanced from the edges of the last isoline.

Red frame – In the radial expansion mode the axes displays the cell density at the area specified in the X axis (it was calculated as a histogram – bins are based on the distances of all the cells, the number of cells that fall in each bin was calculated, and the cumulative sum of this in each bin was divided by the area (π x (the distance of the bin)^2).
In the isolines expansion mode it displays the cell density as a function of isoline number.
On each of the modes the axes display also a pointer that shows the location on which the specified gate (by number of cells or isoline number) in this plot.

Cyan frame – Change the center and the isolines settings.
There are two ways to change the center that automatically was set to the place with the highest cell density:

1. Center: FSC-A (Log): 636.9961 SSC-A (Log): 10553.694 - changing the values in the edit boxes to change the X or Y values of the center.

2. [button icon] - changing the values manually. Pushing the button will change the window to this:
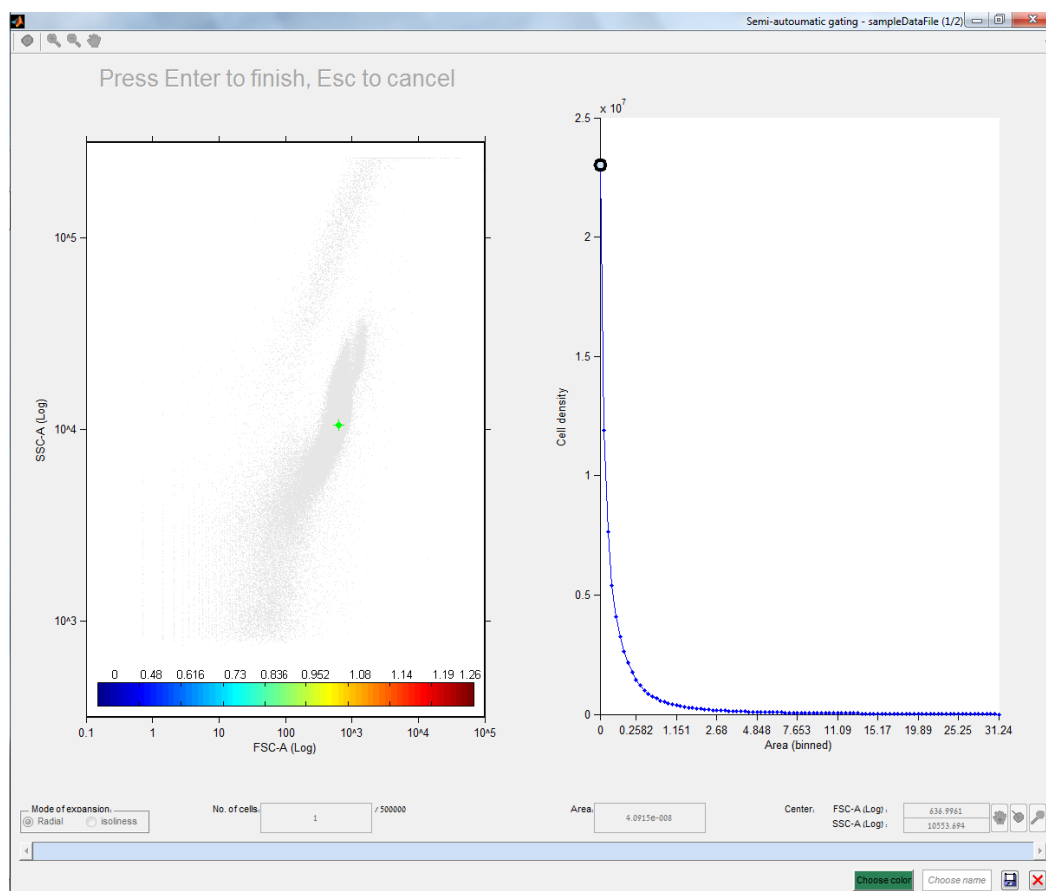
The green dot on the left plot is the center and could be moved by clicking on it and drag the mouse. To finish changing the center press Enter, to cancel press Esc.

Calculating the new center might take a while as the number of cells in each isoline is calculated.

 - In order to change the parameters on which the isolines are based (the bin number of the 2d histogram, and the total number of isolines), use this tool. It will open a sub-GUI to change these parameters. Again, the calculation might take time.

- Reset all parameters to the default values (bin number and total number of isolines as described under the brown frame and the center in the place of highest density). Again, the calculation might take time.

And as in the rest of the gates:

 Click on the choose color button in order to change the color of the gate, the background color of the button is the current color.

 Define the name of the gate, if not define default is gate# (# is the current number of gates + 1).

 Finish gate creation and save the gate to the gate data table.

 Finish gate creation without saving the gate.

After saving the gate, the window is closed and the main GUI window will return to its regular state. The new gate will be presented in the gate data table and in the scatter plot.

11. Quadrant gates

In order to create a quadrant gate, push this button [icon] or select it in the Gates menu, in 2d scatter plot display mode.

In this method the user defines 5 points that split the plot to four quadrants, each forms a separate gate.

The first step after activating the quadrant gate creation is to define the first point that will reside in the middle. The window will become like this:
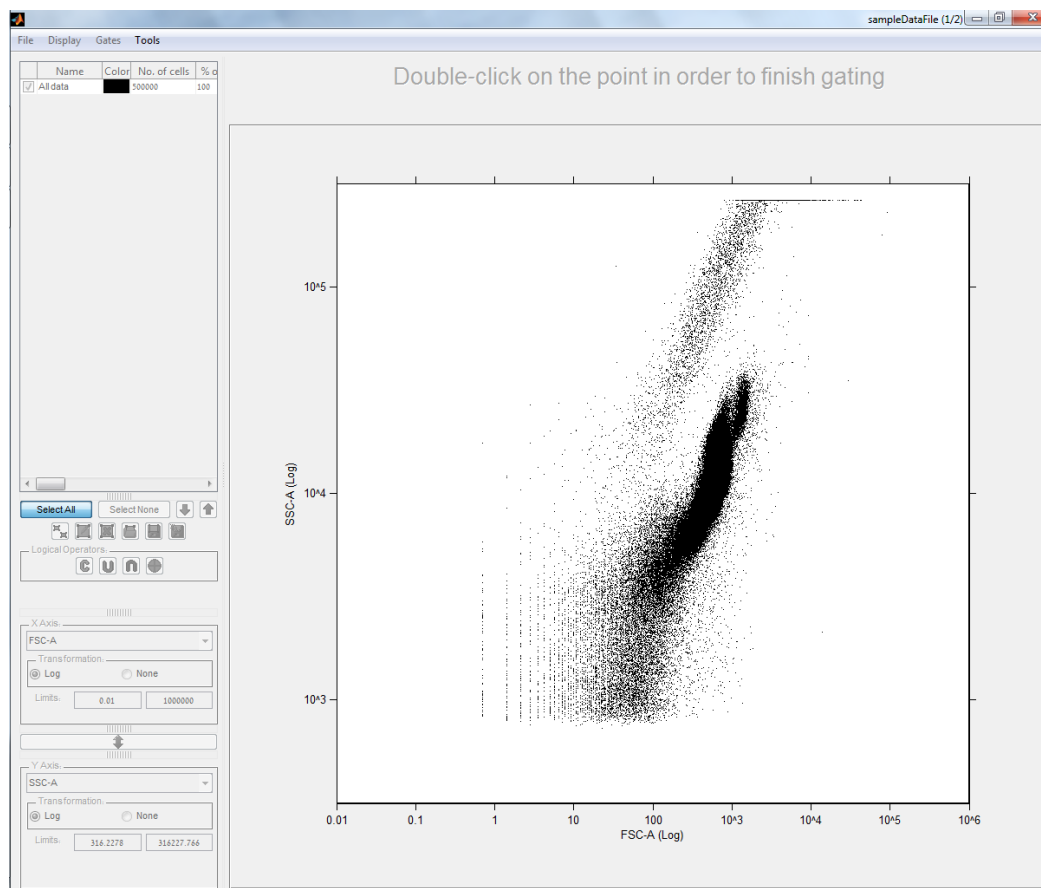
And you should click on the plot in the place you want the first point. After clicking a blue dot appears, which can be moved by a click and drag of the mouse. When finishing placing the first dot, you should click twice on it in order to proceed. The four lines of quadrants limits now appear:
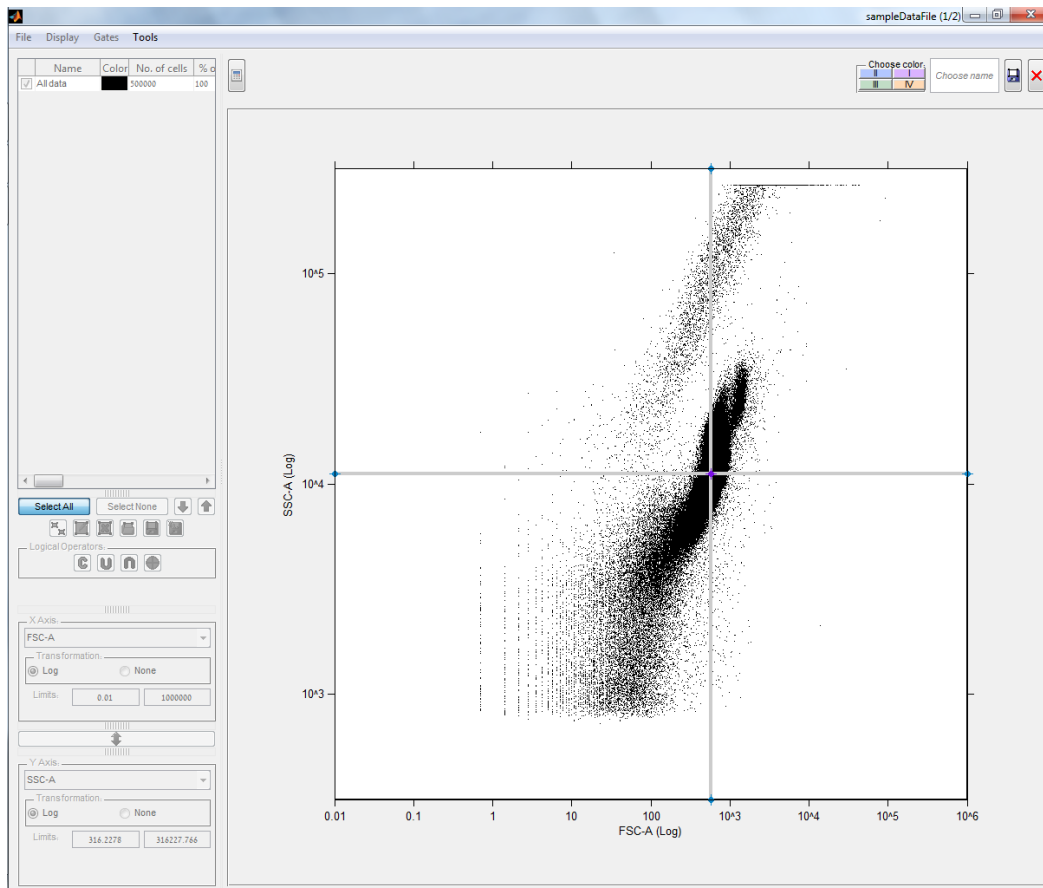
Each of the 5 dots is draggable; the four that appears on the axes are limited to the axes line. So you could define any angle you want the limits of the quadrant to take. Moving the center point will reset all the angles to 90°.

Click on the calculator button in order to show how many cells are inside each quadrant, and the percentage of total. In this case, for instance, clicking the calculator will lead to this:

II. 15787 / 500000 3.16%	I. 270938 / 500000 54.2%
III. 120458 / 500000 24.1%	IV. 89377 / 500000 17.9%
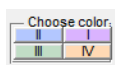
Click on each of the buttons to change the color of the quadrant. The current color is the background color of this quadrant.

Define the name of the gate, if not define default is gate# (# is the current number of gates + 1). Four gates will be saved, each for each quadrant. Each gate will have the suffix _q#, while # is the number of quadrant.

Finish gate creation and save the gate to the gate data table.

Finish gate creation without saving the gate.

After saving the gate, the GUI window will come back to its regular state and the four new gates will be presented in the [gate data table](gate-data-table) and the scatter plot as can be seen below (the lines of the quadrant boundaries will be visible only when the transformation and the X and Y axis are the same as in the gate creation, otherwise only the cells in each quadrant will be colored in the color of gate):
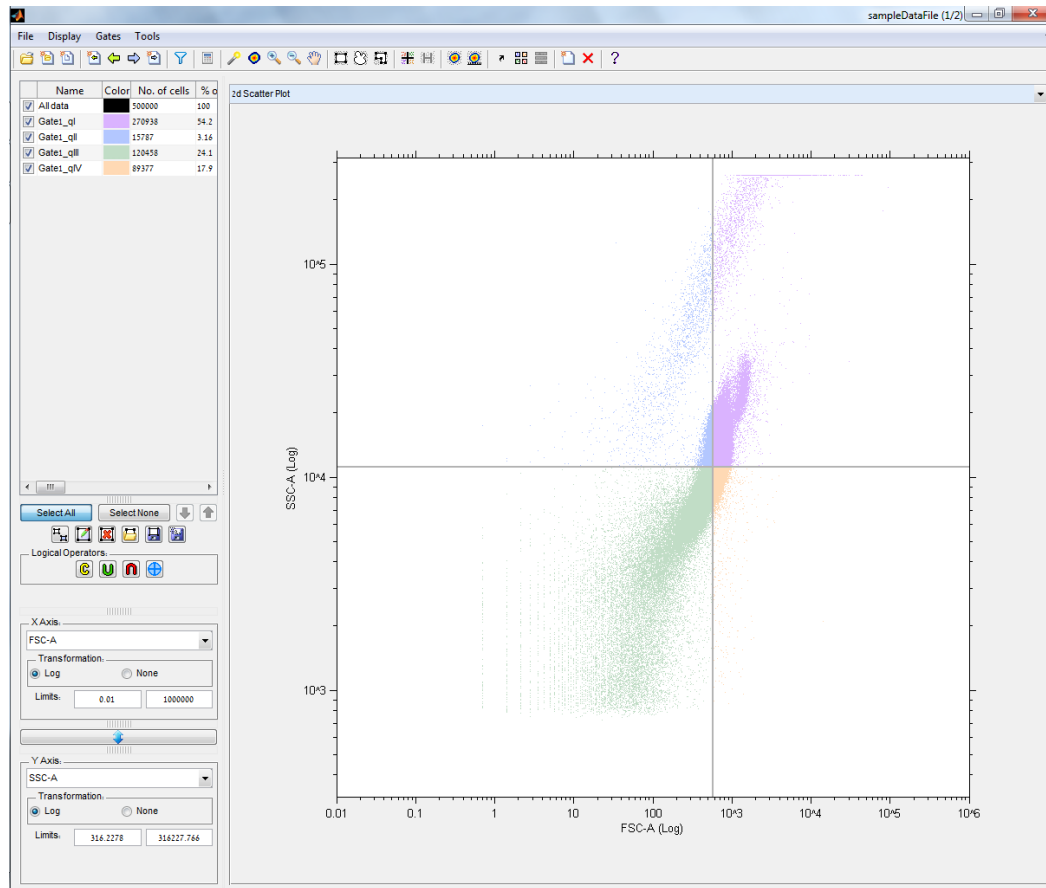
12. Histogram based gates

In order to create a gate based on a histogram, push this button [H], or select it in the `Gates` menu, in 1d histogram plot display mode.

This is how the GUI window looks when activating histogram based gating:
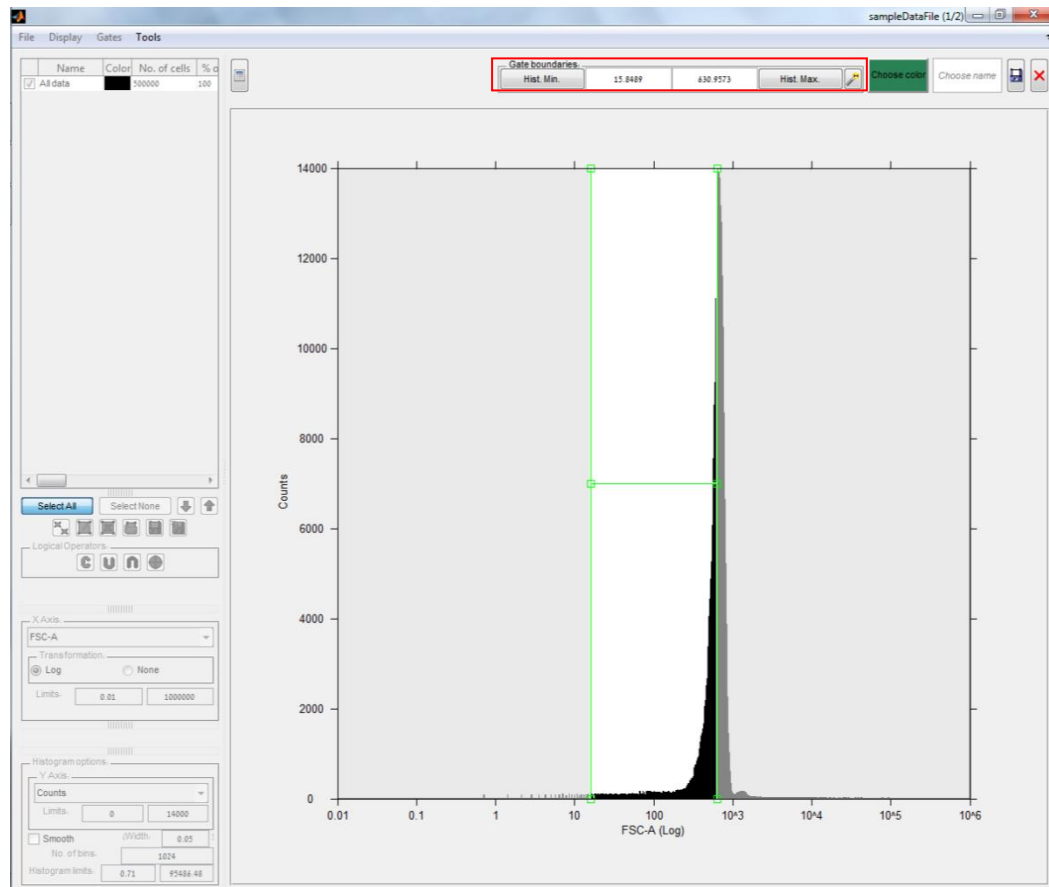
[calculator button] Click on the calculator button in order to show how many cells are inside the gate, and the percentage of total.

Red frame – A panel to control gate boundaries:
The left edit box contain the low boundary, the right edit box the high boundary.
Clicking 'Hist. Min.' button will set the low boundary value to the lowest value of histogram.
Clicking 'Hist. Max.' button will set the high boundary value to the highest value of histogram.
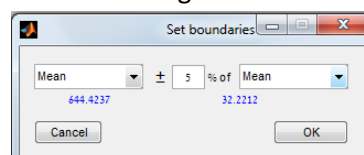
[button] - This button will open a sub-GUI to define gate boundaries based on statistical measures:

The value of the selected measure is presented in blue, and the value of the defined percentage of the second measure.

Another way to control boundaries is by dragging the green lines on the histogram plot. The middle line is also draggable and moves the two boundaries together.

Click on the choose color button in order to change the color of the gate, the background color of the button is the current color.

Define the name of the gate, if not define default is gate# (# is the current number of gates + 1).

Finish gate creation and save the gate to the gate data table.

Finish gate creation without saving the gate.

After saving the gate, the GUI window will come back to its regular state and the new gate will be presented in the gate data table and in the histogram plot.

13. Using gate data table

The gate data table is used for:

1. **Information** – Displaying all the information on each of the gates.
2. **Selection** – Selection of gates for: display, change the stack order of the display, delete gate, duplicate gate, save gate to file, and create gate hierarchy and logical operations on gates.
3. **Editing** – Change the name or color of a gate.

1. Information

Here is an overview of a gate data table with 7 gates, one of each type, as can be seen in the 'type' column:

	Name	Color	No. of cells	% of total	% of parent(s)	Parent(s)	Type	X,Y	Transformation X,Y	Coordinates
☑	All data		500000	100						
☑	FSC-A, 4% Low, 4% High; SSC-A, 4% Low, 4% High;		56123	11.2			filter			
☑	Gate1		434839	87			manual	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[1.51e+003 2.36...
☑	Gate2		100000	20			auto	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[637 1.06e+004]
☑	Gate3		50000	10			semiauto	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[637 1.06e+004]
☑	Gate4_qI		330635	66.1			quadrant	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[536 1.03e+004;...
☑	Gate4_qII		11558	2.31			quadrant	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[536 1.03e+004;...
☑	Gate4_qIII		88741	17.7			quadrant	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[536 1.03e+004;...
☑	Gate4_qIV		65626	13.1			quadrant	‹FSC-A›,‹SSC-A›	‹Log›,‹Log›	[536 1.03e+004;...
☑	Gate5		95999	19.2			hist	‹FSC-A›	‹Log›	[612 677]
☑	UNION(Gate4_qI,Gate4_qII)		342193	68.4	100	‹Gate4_qI›,‹Gate4_qII›	logical (OR)			

All the information about each of the gates is presented in the table (use the scroll if not visible, or widen the table using the draggable separator between the axes and the table).

The gate name, and color (could be modified, see below), number of cells in each gate and the percentage of total, percentage of parents and parents identity (see next section about parenting), the type of the gate, the X and Y axis when the gate was created, the transformation of each of the axes when the gate was created, and the coordinates, if exist, that define the gate (varies between gate types: for a manual gate, simply x and y coordinates that define the shape, for an auto and semi-auto gates, the x and y coordinates of the center, for quadrant gate, the x and y coordinates of the 5 points defining the gates, for hist gate, the low and high boundaries on the x axis).

2. Selection

Gates could be selected by clicking on the checkbox on the left-most column, or by clicking the ⟨Select All⟩ or ⟨Select None⟩ buttons placed under the table.

First of all, selection affects the display on the plot (in both plot modes) – only the selected gates are displayed.

Second – when selecting only one gate there is an option to change the stack order of the display, i.e. which gate is displayed on top of which. The stack order is the order of the gates in the gate data table; the last will be displayed on top of all the others, the first on bottom. The 'All data' row cannot be moved and will be always below all of the gates. When only one gate is selected ('All data' row doesn't count), the two arrow buttons below the table (⬇⬆)

become activated (if the gate already the first or the last, only one of the buttons will be active). Click on them will move the gate up or down in the gate data table and will change the stack order of the display accordingly.

Third, selection affects different operations that could be made on gates such as: [delete gate, duplicate gate, save gate to file](#), and [create gate hierarchy and logical operations on gates](#), as will be described in the following sections.

3. Editing

Through the gate data table it is possible to change gate name or color ('All data' row cannot be edited).

To change gate name, click on the gate name cell and simply change the text, press 'Enter' or click on another place in order to finish editing the name.

To change gate color, click on the colored cell in the gate data table, and press OK after selecting the desired color.

14.Gate hierarchy and logical operations on gates

1. Gates hierarchy:

Selection could create hierarchy between gates:
If the 'All data' row (the first row in the gate data table) is unselected (possible only if another gate is selected), the plot displays only the cells of the selected gate(s) and they will be the parents of the gates that will be created in this display, meaning, the new gate will include only cells that are included in the parent gate. The percentage of the created gate will be displayed in the '% of parent' column in the gate data table.
The children gates will be affected by editing of the parent gate (unless they are of auto or semi-auto type, see next section).

Quadrant gates cannot be children, and the 'All data' row will be selected automatically.

2. Logical operation on gates:

Using the gate data table it is possible to select specific gates (see previous section), and by this making the logical gates activated or deactivated.

The description and requirements for the logical operation are as follows:

Symbol and name	Logical operation	Description	Requirements
C Complement	NOT	The cells that are not included in the chosen gate(s)	At least one gate chosen
U Union	OR	The cells that are included in any of the chosen gates	At least two gates chosen
n Intersection	AND	Only the cells that are included in all of the chosen gates	At least two gates chosen
⊕ XOR	XOR	The cells that are included in any of the chosen gates, but not in all of them	

All the operations are available also through the Gates menu.
Choosing one of the operations will open a small sub-GUI to define the gate name and color:
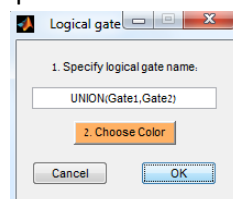
After pressing OK, a new gate based on the chosen gates and logical operation will be presented in the gate data table and in the plot. The new gate is a child of the chosen gates.

15. Other operations on gates and managing gate files

Using the buttons below the gate data table, or through the Gates menu, the following operations could be performed:

1. Duplicate selected gate(s) - This option is available only when at least one gate is selected. Copy the selected gate(s) to create new gates, having the same data and properties.

2. Edit gate coordinates - This option is available only when a gate of type manual, quadrant or hist exists. Open a dialog box to choose one among the gates available for editing (manual, quadrant or hist). After choosing the file the display is turned into the display suiting the gate type, axes, transformations, and coordinates. Although this is done automatically, it is usually better to choose the correct transformation and axes before clicking this button, and manage the display to best suit the gate. Editing will affect the gate's children that are not of auto or semi-auto types. It will not affect grandchildren.

3. Delete selected gate(s) - This option is available only when at least one gate is selected. Delete the selected gates.

Gates could be saved to files and loaded into the GUI.

There are two ways to save gates:

1. Save selected gate(s) reads – This option is available only when at least one gate is selected. The data of the cells that are included in the gates will be saved into .mat files, one for each selected gate, containing a structure named: `gateData`, containing two fields: '`.data`', a matrix containing all the cells as rows and the parameters collected for each cell as columns; and '`.colheaders`', the names of the parameters that were collected. The file could be loaded into the GUI as a regular input file (not as a gate!). The .mat files will be saved in the selected folder, in names according to the gate names; they will overwrite any .mat files in this folder that has the same names.

2. Save selected gate(s) coordinates – This option is available only when at least one gate is selected. If gates that are not of type manual, quadrant or hist, are selected, the operation could not be done. The gates will be saved into .mat files, one for each selected gate (quadrant gates will be saved to a single file) containing a structure named: `gateData` containing the following fields: `name`, `type`, `axes`, `trans`, `coordinates`, `color` and `colheaders`. The data of the cells included in the gate will not be saved (!). The file could be loaded using the load gate coordinates option mentioned right below. The .mat files will be saved in the selected folder, in names according to the gate names; they will overwrite any .mat files in this folder that has the same names.

3. Load gate coordinates - This option is always available. Load gate coordinate file that were saved as mentioned right above (a .mat file containing a structure named: `gateData` containing the following fields: `name`, `type`, `axes`, `trans`, `coordinates`, `color` and `colheaders`). The gate data will be updated according to the currently loaded file data. This enables passing gates between different FC data files.

16. Exporting plot to a Matlab figure and creating multiple plot figures

Access these tools through the following toolbar buttons or the Tools menu.

1. Exporting plot to a Matlab figure ⬈ - This option is available only when a FC data file is loaded. It will export the current plot to a separate Matlab figure file.

2. Creating multiple plot figures of histograms or scatter plots ⊞ ☰ - This tool is available also before loading a FC data file, and through the .m file: `private\subplotsFigureTool.m`, without any connection to the main GUI window. After loading a FC data file, only one of the buttons will be activated each time, according to the current plot mode (scatter plot or histogram). In any case, in the tool's GUI there will be options to change any of the plot parameters, including the plot mode, so the two buttons are practically equivalent. This tool will load all the .mat files in the current folder that should all have one variable with the two fields: '`.data`', and '`.colheaders`', and the same colheaders (collected parameters). If the current folder include a .mat file that does not fulfill these requirements the folder will not be loaded and the user will have to choose another folder.

This is how the tool's GUI window looks like after loading a folder with 15 files, in histogram plot mode:
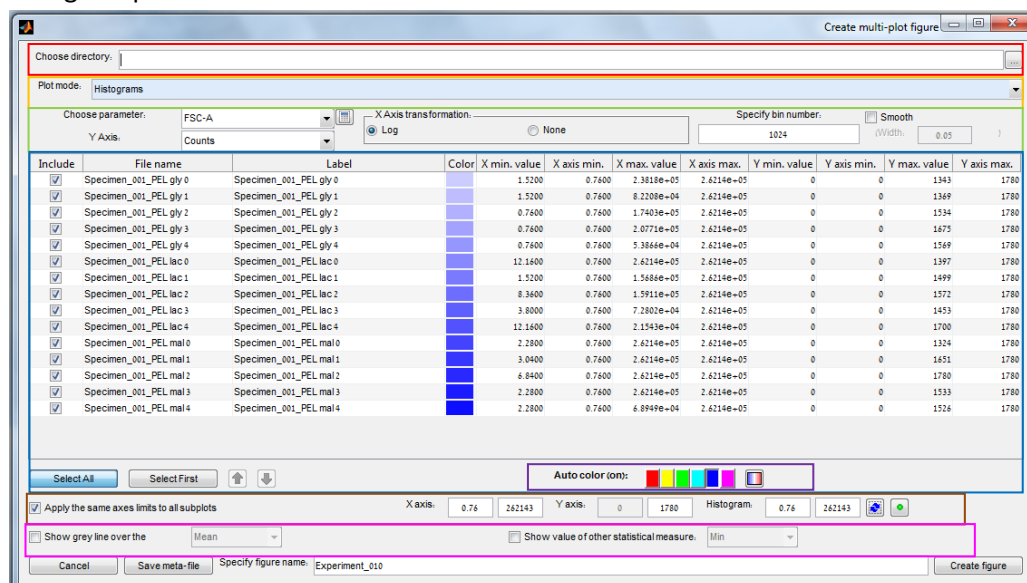
And in scatter plot mode:
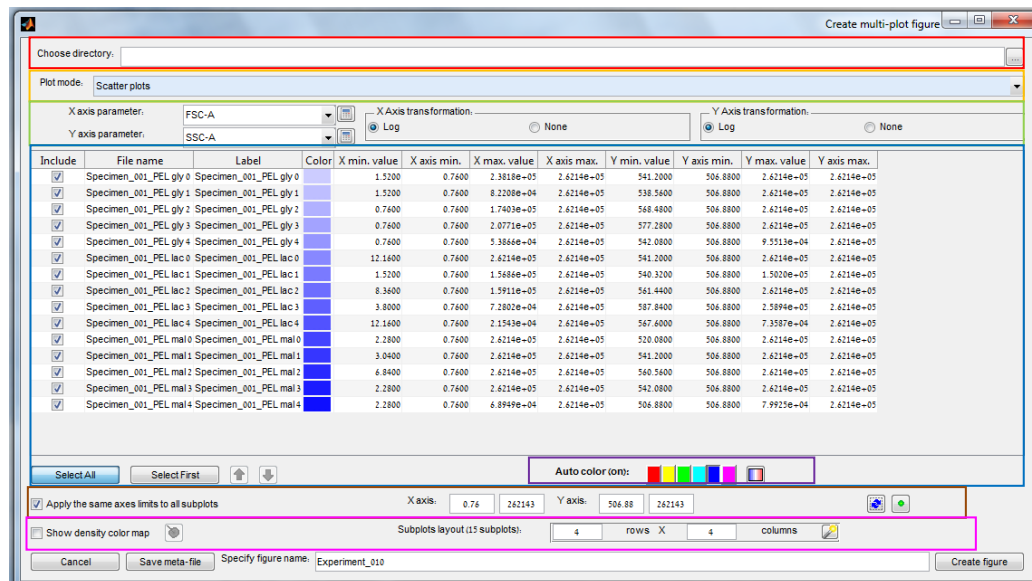
Red frame – Select the folder (the folder should contain only .mat files that contain one variable which is a structure with the fields '.data', and '.colheaders', and the same colheaders (collected parameters)). You could either type the path or click on the ⋯ button). If the folder contains other .mat files that not fulfill these requirements it will not be loaded.
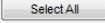
Orange frame – Change plot mode between histogram or scatter plots.

Green frame – Control axes properties, similar as in the main GUI window (see section 6). The differences: clicking on the ▦ button will lead to a dialog to make calculations on parameters as in section 5, after finishing it will make the calculation for all the files and turn the popup-menu of selecting the parameters inactive. To return to original parameters, click again on the ▦ button. The limits could be defined either using the brown frame, in order to specify the same limits to all the subplots, or through the table in the blue frame (see information below).

Blue frame – The table summarizes all the files. The checkbox in the left column is for selecting the files that will be used in the multiplot figure, files could be also selected using the Select All and Select First buttons. When only one file is selected, the two arrow buttons below the table (⬇⬆) become activated (if the gate already the first or the last, only one of the buttons will be active). Click on them will move the file up or down in the data table and it will change the order in which the files will be presented in the multiple-plot figure.

The 'Label' column is editable; you can change the value to any label you want to appear on the subplot. The color could be changed by clicking the colored rectangle inside the 'Color' column, or through the purple frame below. The 'X min. value', 'X max. value', 'Y min. value', and 'Y max. value', could not be changed, and present the maximal and minimal values of the parameter in each of the files. The 'X axis min.', 'X axis max.', 'Y axis min.' and 'Y axis max', could be changed when the checkbox 'Apply the same axes limits to all subplots' in the brown frame is unselected (in the histogram plot mode the 'Y axis min.' could not be

changed from 0). Selection does not change the values of the limits in the brown frame below. Selection will change the subplots layout in scatter plot mode (see pink frame below).

Purple frame – Choosing each of the colored buttons will activate 'auto color', which means that the colored of the files will be changed to a gradient of brighter to darker tone of the selected color. Pushing the 'set color sequence' button (🔲) will open a sub-GUI, and you could choose the files to which you want to apply the color sequence (i.e. the gradient of brighter to darker tone of the selected color in the sub-GUI), you can have more than one color sequence in this method.

Brown frame – If you want to apply the same limits to all the subplots, this is the way to do it (to apply different limits, you can specify them in the table in the blue frame when the checkbox is unchecked). The principle is the same as in the main GUI figure (see section 6). There are also options to define the limits automatically:
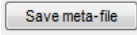
1. Update limits based on selection 🔄 - change the limits to include the minimal value of 'X min. value' and 'Y min. value', and the maximal value of 'X max. value' and 'Y max. value' among the selected files.

2. Reset limits based on all 🟢 - change the limits to include the minimal value of 'X min. value' and 'Y min. value', and the maximal value of 'X max. value' and 'Y max. value' among all the files.

Pink frame – Additional properties:
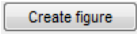
In histogram plot mode: Show grey line over the mean, median or mode of histogram and display their value, and display the value of other statistical measures.

In scatter plot mode:

1. 'Show density color map' – the same as in the main GUI figure (see section 6). Use the 'Set density color parameters' button (🔵) in order to change the parameters on which the isolines are based (the bin number of the 2d histogram, and the total number of isolines). It will open a sub-GUI to change these parameters.
2. Subplots layout – the layout in which the subplots will be arranged. If the total number of subplots in the layout is smaller than the number of selected files, the text boxes will become red, and figure could not be created. Use the 'Reset based on selection' button (🖊) in order to reset layout to match the number of files.

Save meta-file - Push this button to save a meta-file, a 3d matrix in which, the first dimension is the cells, the second is the collected parameters (could be 1 or two columns, depends on plot mode), and the third dimension is each of the selected files.

Specify figure name: [   ] - Change the name of the figure, default is folder name.

Create figure - Push this button to create the multiplot figure.

17. File list and description

`cvs2mat.m`

This file contains a script for a GUI for parsing comma-separate-vector (CSV) format file into .mat file containing one workspace variable `'fcData'`, a structure containing two fields, `'.data'`, a matrix containing all the cells as rows and the parameters collected for each cell as columns, and `'.colheaders'`, the names of the parameters collected. It is unrelated to `FCGUI_main.m`. Its input files are the output files of `FCSExtract.exe` (see [section 1](#)).

`FCGUI_help.pdf`

This file.

`FCGUI_main.m`

This file contains the script of the Flow Cytometry GUI.

`sampleDataFile.mat`

An example for an input .mat file.

`private` (Folder)

This folder contains all the files necessary for `FCGUI_main.m` to run (except of the 'GUI layout toolbox' that should be downloaded from http://www.mathworks.com/matlabcentral/fileexchange/27758.

`private\calCellsInIsolines.m`

A function to calculate the number of cells inside in each isoline, used by `FCGUI_main.m` (`saveGate_callback` in auto gate), and by `semiAutoGate.m` (see section [9](#) and [10](#)).

`private\color2htmlStr.m`

A function to convert RGB color array (e.g. [1 1 1]) into html string for use in uitable object in `FCGUI_main.m`. See: http://www.mathworks.com/support/solutions/en/data/1-D782JW/index.html?product=ML&solution=1-D782JW

Used by `FCGUI_main.m` and `subplotsFigureTool.m`.

`private\contourMat2contour3DMat.m`

A function to create a 3d matrix from a contour matrix (see contour plot function help). Columns are each of the vertices, rows are the vertices coordinates in X (3rd dim 1) or Y (3rd dim 2). First row in X contain the number of vertices, and in Y the height.

Used by: `FCGUI_main.m` (`saveGate_callback` in auto gate), and `semiAutoGate.m`.

`private\createFilter_dlg.m`

A GUI to create a filter (see [section 4](#)).

`private\FCGUI_build.m`

A function that builds the GUI figure of `FCGUI_main.m`. Returns the handles of all the objects that were created.

`private\histGate_boundSet_dlg.m`
A sub-GUI to define the boundaries of a histogram based gate based on statistical measures (see [section 12](#)).

`private\histmap.m`
A function to create a 2d histogram, or density plot. Based on: https://gist.github.com/883933
Used by: `FCGUI_main.m` (`helperfcn_plot`), `semiAutoGate.m`, and `subplotsFigureTool.m`.

`private\htmlstr2rgb.m`
The reverse function of: `color2htmlStr.m`, used by: `subplotsFigureTool.m`

`private\icons.mat`
This .mat file contains a workspace variable `'icons'`, which is a structure with different fields, each field contains the 'CData' of the icons of all the buttons of the different GUIs.
Used by: `FCGUI_build.m`, `semiAutoGate.m`, and `subplotsFigureTool.m`.

`private\inpoly.m`
A function to determine if a point is inside or outside a polygon. Taken from: http://www.mathworks.com/matlabcentral/fileexchange/10391-fast-points-in-polygon-test
Used by: `FCGUI_main.m` (`saveGate_callback` in manual and quadrant gates, and `quadrantGate_cal_callback` and `manualGate_cal_callback`), and `calCellsInIsolines.m`.

`private\isolineParam_dlg.m`
A sub-GUI to determine the parameters of 2d histogram (density map, contour plot…), number of isolines, number of X axis bins and Y axis bins. Used by: `semiAutoGate.m` and `subplotsFigureTool.m`.

`private\logicalGate_dlg.m`
A sub-GUI to make logical operations on gates (see [section 14](#)).

`private\mathParam_dlg.m`
A sub-GUI for making mathematical operations on parameters (see [section 5](#)).

`private\p_poly_dist.m`
A function to calculate the distance of a point of a polygon. Taken from: http://www.mathworks.com/matlabcentral/fileexchange/19398-distance-from-a-point-to-polygon
Used by: `FCGUI_main.m` (`saveGate_callback` in auto gate), and `semiAutoGate.m`.

`private\parseArgs.m`
See `private\subaxis.m`

`private\rorschach.mat`
This .mat file contains a workspace variable `'rorschach'`, which is a Rorschach figure used as the opening plot of `FCGUI_main.m`.

`private\semiAutoGate.m`
A sub-GUI to define a gate semi-automatically (see [section 10](#)).

```
private\subaxis.m
private\parseArgs.m
```
A function to create customable subplots.

Taken from:

```
private\subplotsFigureTool.m
private\subplotsFigureTool_semiAutoColor_dlg.m
```
A GUI to create multi-plot figure of histograms or scatter plots. Can be used independently of `FCGUI_main.m` (see).

```
private\trans_Log.m
```
A function to make logarithmic transformation of data (input and output a matrix), turns negative values and Infs into NaNs.