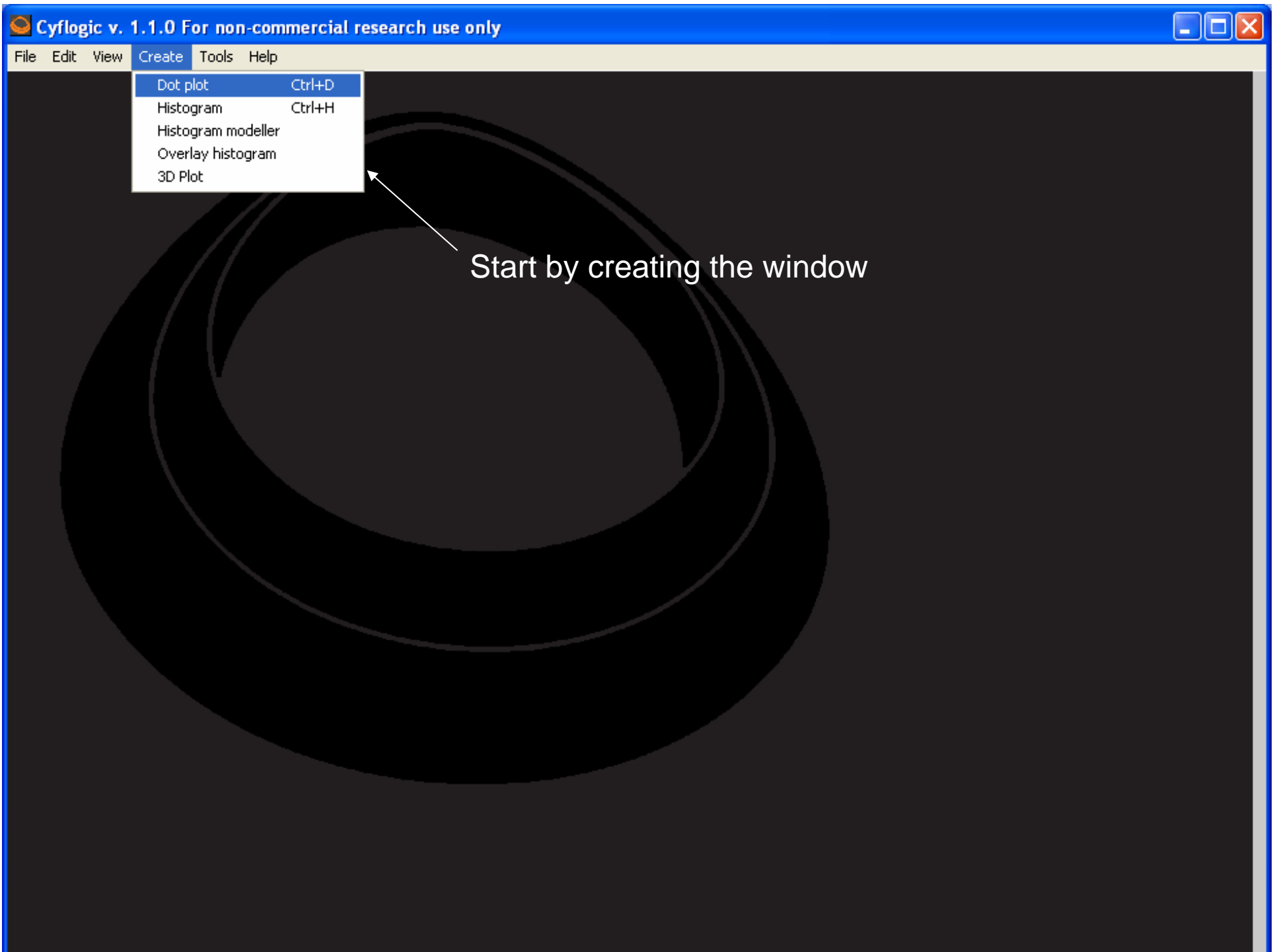


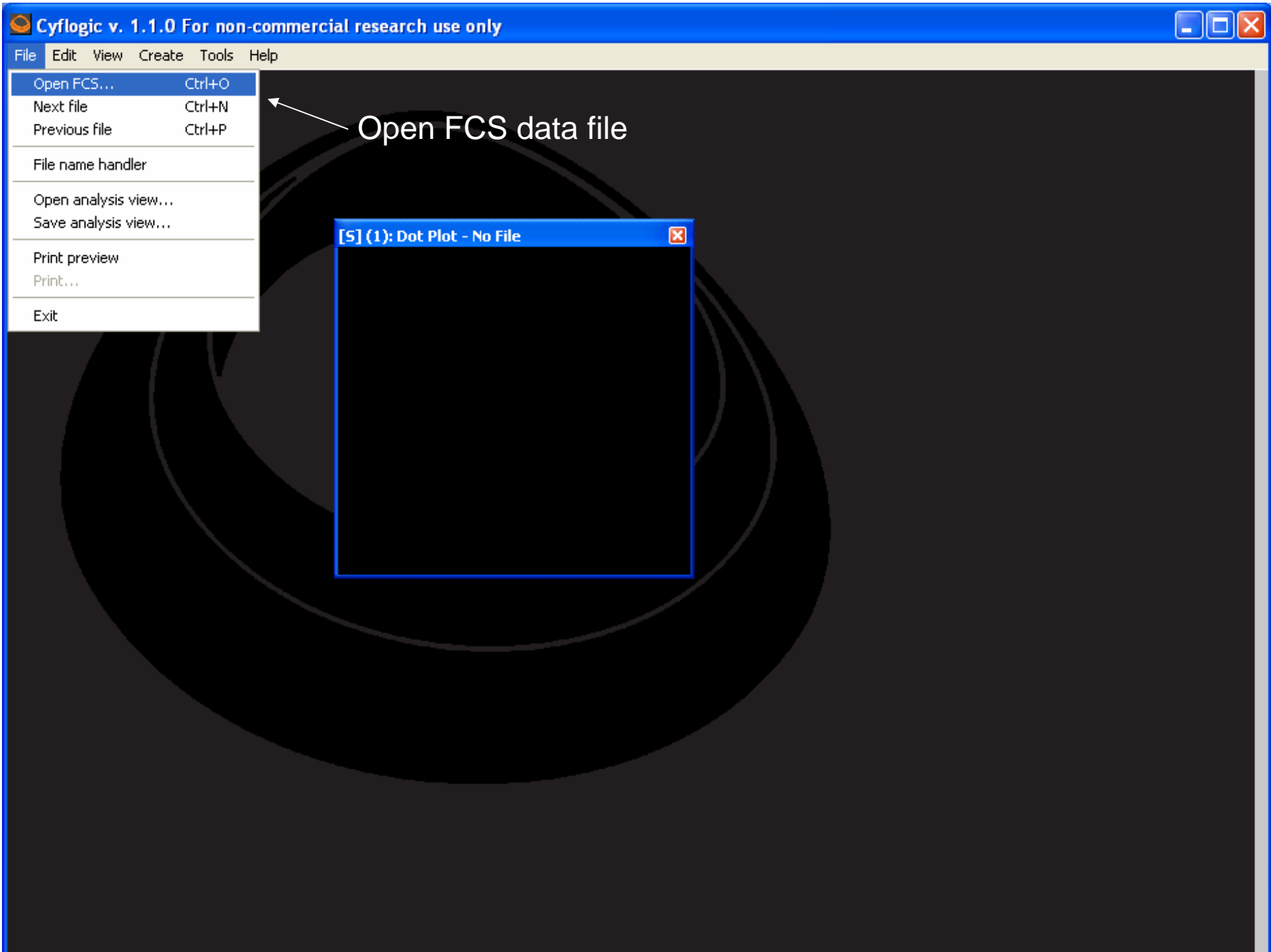


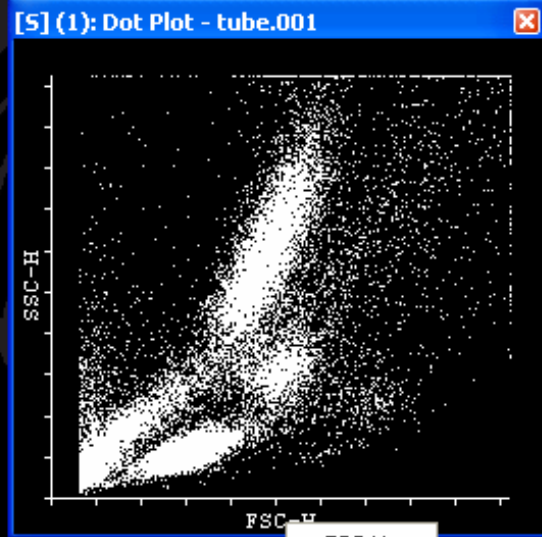
Introduction to version 1.1.0

Instruction date 16.5.2008

# Windows and Files



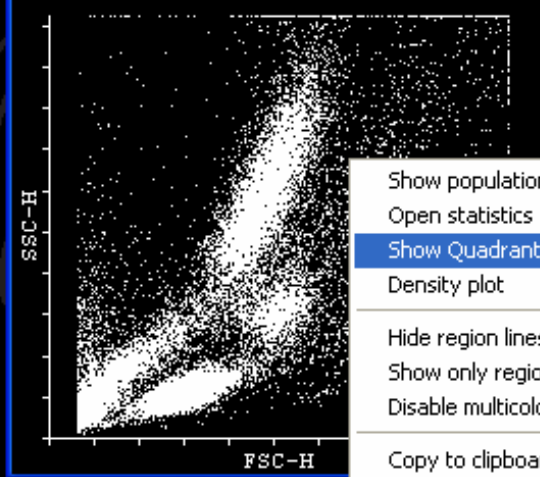




- FSC-H
- SSC-H
- FL1-H
- FL2-H
- FL3-H
- FL2-A
- FL2-W

By right-clicking the axis the list of available parameters appear.

[5] (1): Dot Plot - tube.001



- Show populations
- Open statistics
- Show Quadrant
- Density plot

---

- Hide region lines
- Show only regions created here
- Disable multicoloring

---

- Copy to clipboard (CTRL-C)
- Save as .BMP...

---

- Invert background color
- Large dots
- Fast draw mode

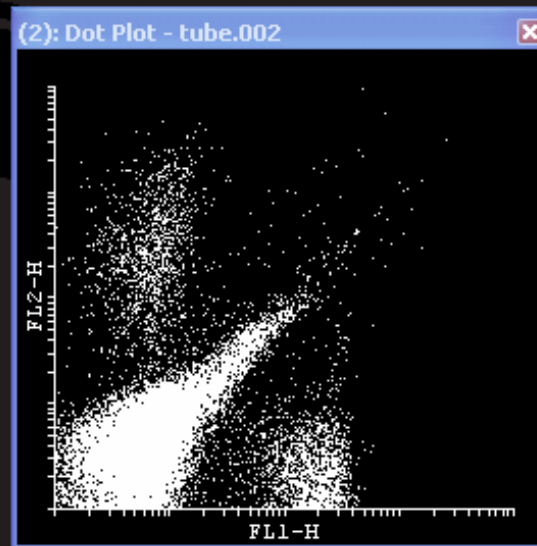
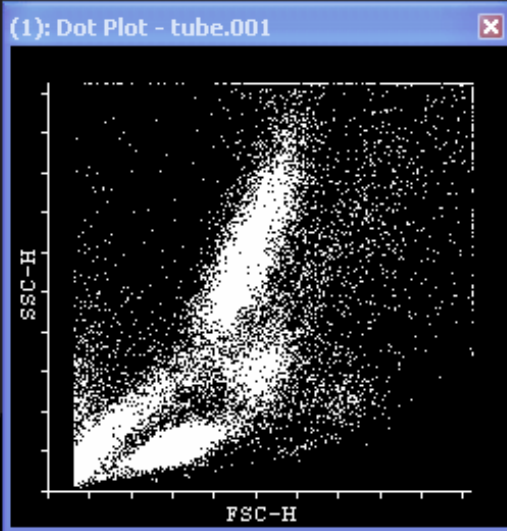
---

- Save data as .TXT...

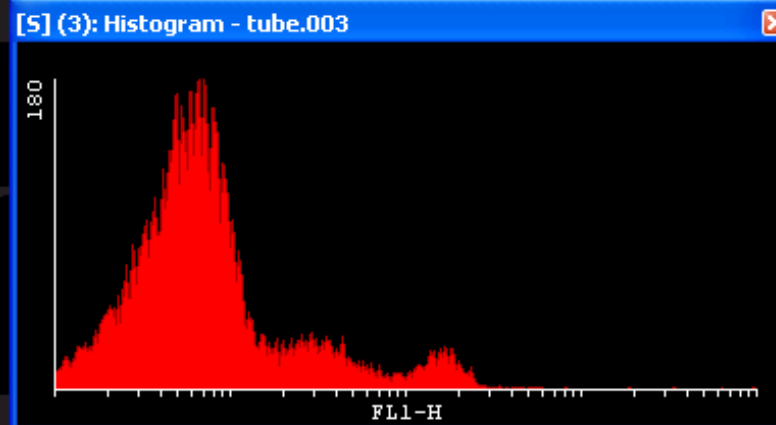
---

- Show in print preview page

Right-click somewhere else in the area opens the pop-up menu.



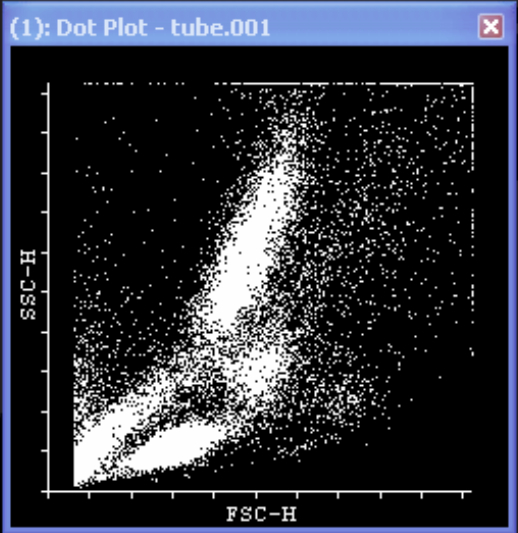
You can create several windows having different files and different parameters. They can also be resized freely.



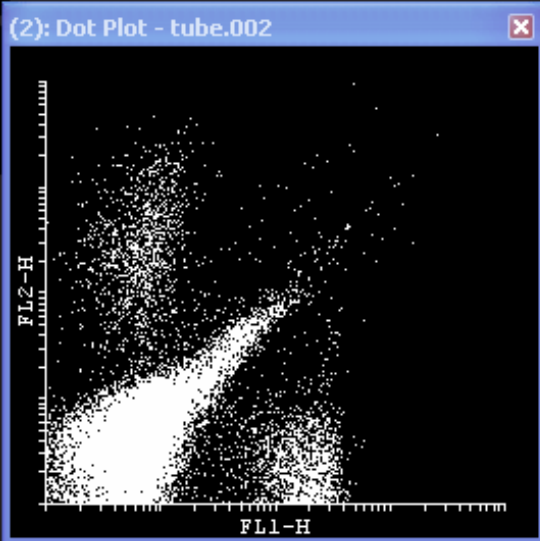
Cyflogic v. 1.1.0 For non-commercial research use only

File Edit View Create Tools Help

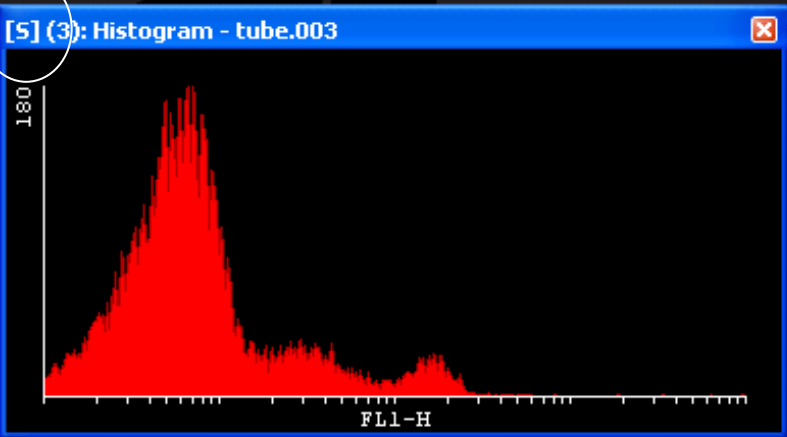
(1): Dot Plot - tube.001



(2): Dot Plot - tube.002



[5] (3): Histogram - tube.003



Microsoft Windows doesn't support multiple selection of windows. However, Cyflogic does, by marking the selected windows with [S]. Normally when you click some window, the clicked becomes selected and others become unselected.

The image displays the Cyflogic software interface. At the top, the title bar reads 'Cyflogic v. 1.1.0 For non-commercial research use only' and the menu bar includes 'File', 'Edit', 'View', 'Create', 'Tools', and 'Help'. Three windows are open: two dot plots and one histogram. The first dot plot, titled '(1): Dot Plot - tube.001', shows a scatter plot of SSC-H (y-axis) versus FSC-H (x-axis). The second dot plot, titled '(2): Dot Plot - tube.002', shows a scatter plot of FL2-H (y-axis) versus FL1-H (x-axis). The histogram, titled '[5] (3): Histogram - tube.003', shows a red-filled distribution of FL1-H values, with a peak around 180. The histogram window title is circled in red, and an arrow points from the text below to it. The text explains that Cyflogic allows for multiple window selection, which is indicated by the '[S]' in the histogram title, a feature not supported by standard Microsoft Windows.



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File Edit View Create Tools Help

(1): Dot Plot - tube.001

[5] (2): Dot Plot - tube.002

[5] (3): Histogram - tube.003

SSC-H

FSC-H

FL2-H

FL1-H

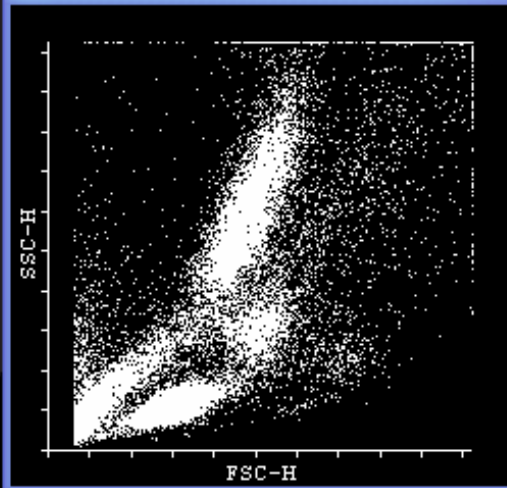
180

FL1-H

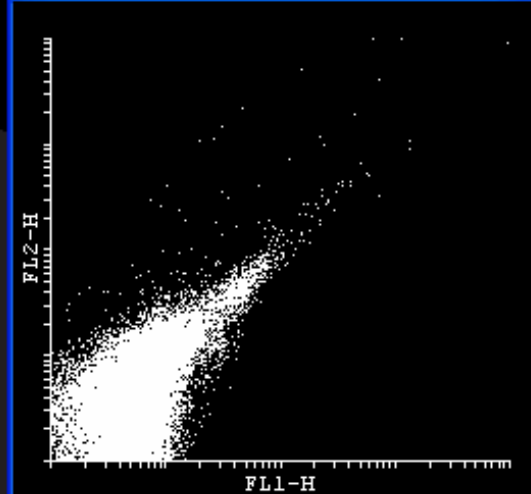
If you click windows while pressing SHIFT button, you can select several windows.

From Edit menu you can find the option "Select all". CTRL-A is the shortcut for this feature.

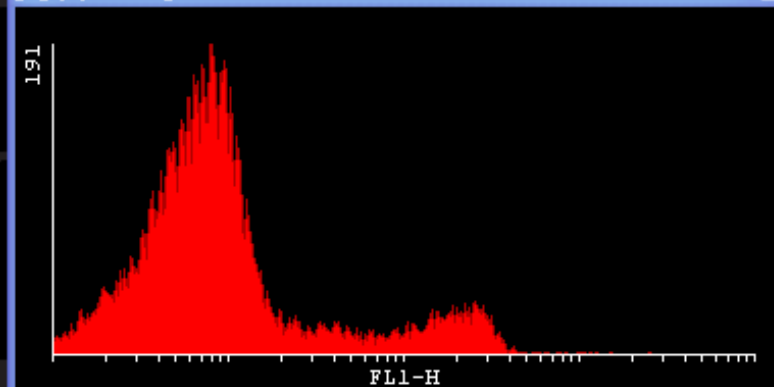
(1): Dot Plot - tube.001



[5] (2): Dot Plot - tube.001

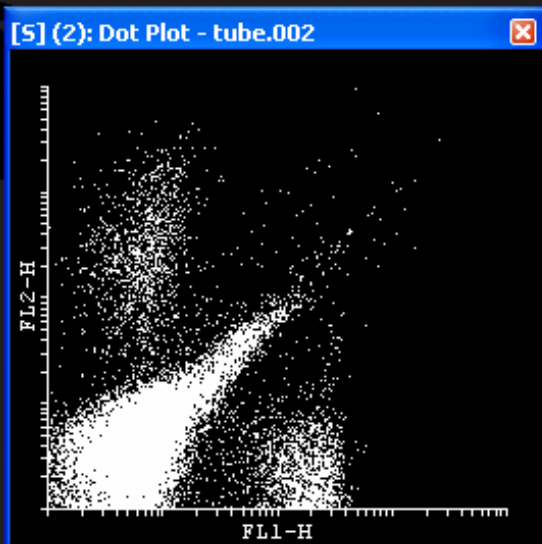
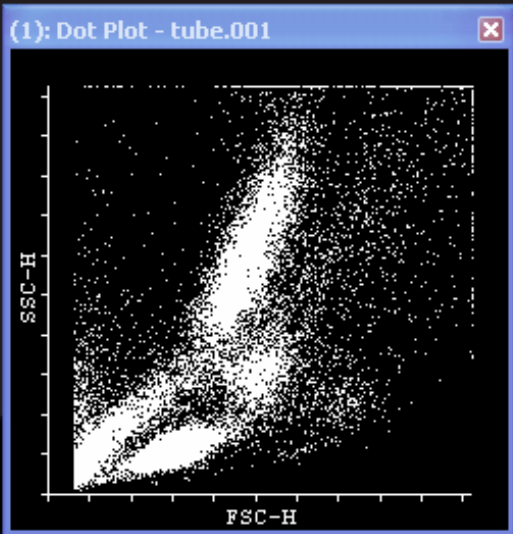


[5] (3): Histogram - tube.002



You can move forward and backward in the folder by pressing CTRL-N (next file in folder) and CTRL-P (previous file in folder). the feature affects to all selected windows.

# Populations and Definitions



Move the mouse cursor to the right side of the screen. Populations and definitions toolbar jumps up.

### Populations

Create

Rename

Delete

- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined



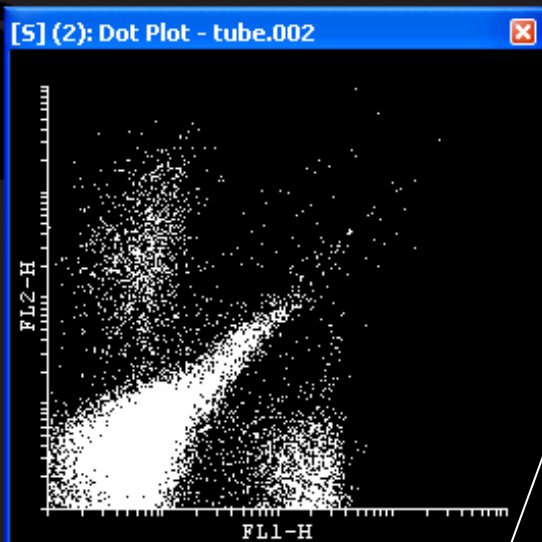
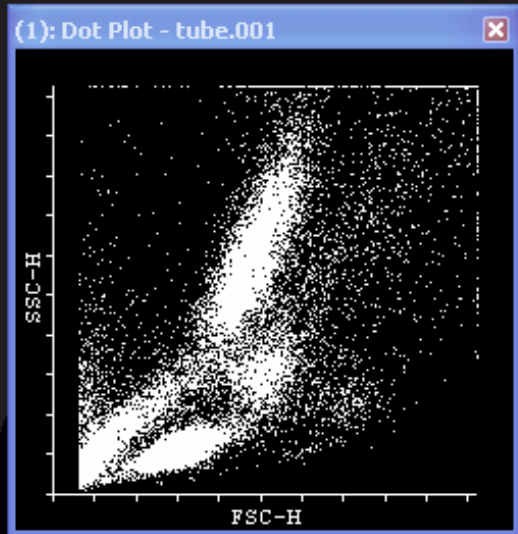
### Definitions

Define

Delete

- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined

Freeze the panel



**Populations**

Create    Rename    Delete

- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined

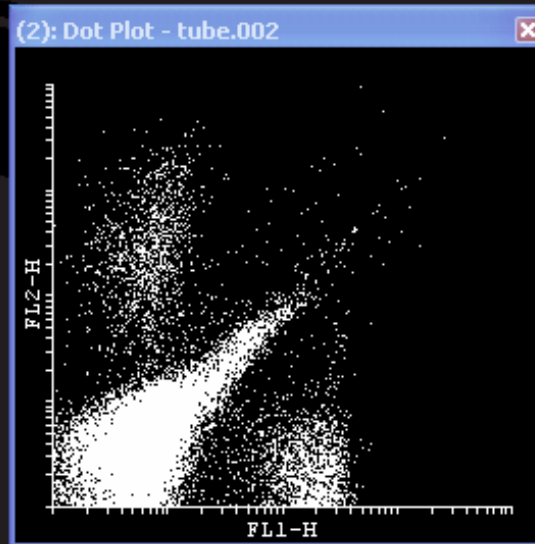
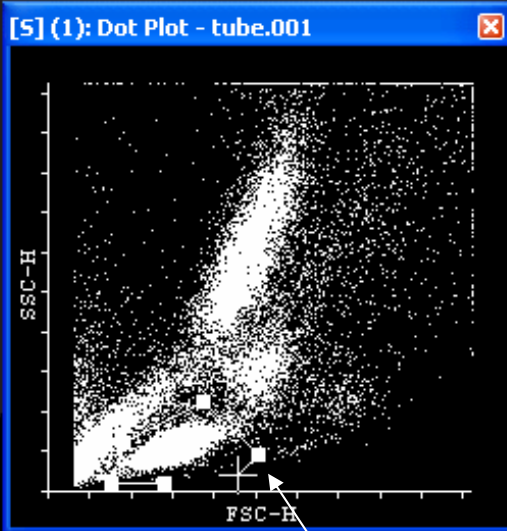
**Definitions**

Define    Delete

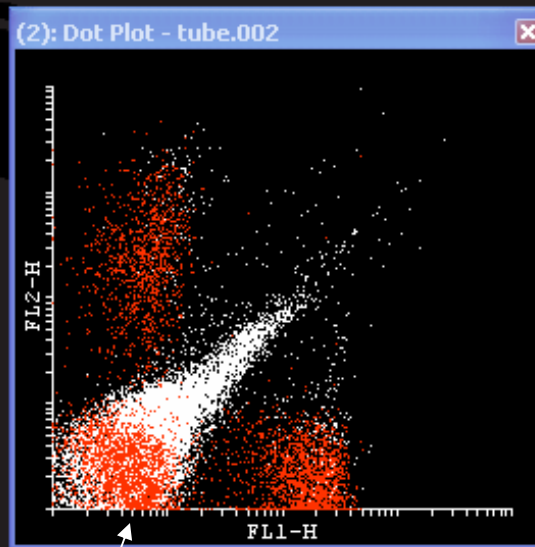
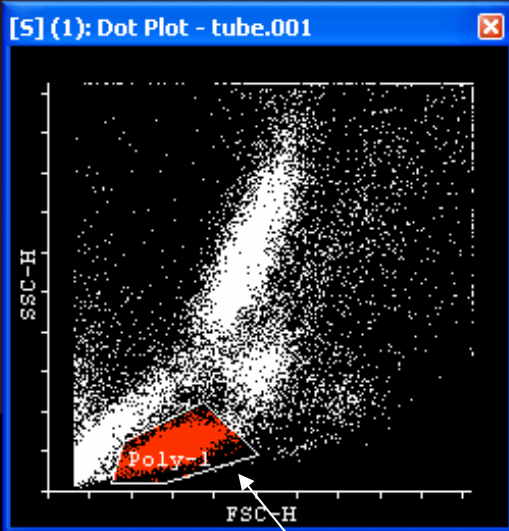
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined

Freeze the panel

Let's create a population. Select the population you want and press Create. Then start creating the population.



In dot plots, you can create polygons. Click to create corners. When you want to close the polygon, left-click the first point OR right-click anywhere. The population is defined.



The events inside the defined population are red.

### Populations

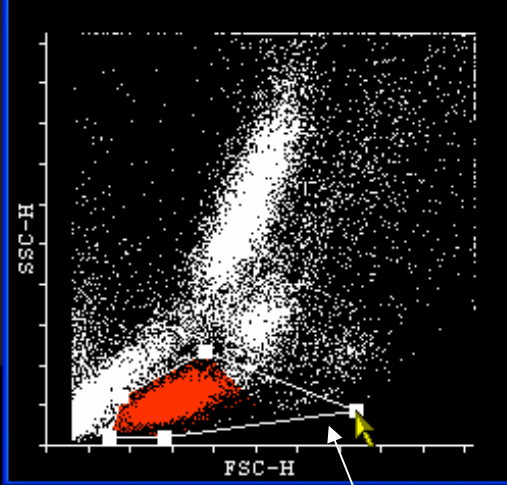
- C ■  Poly-1
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined

### Definitions

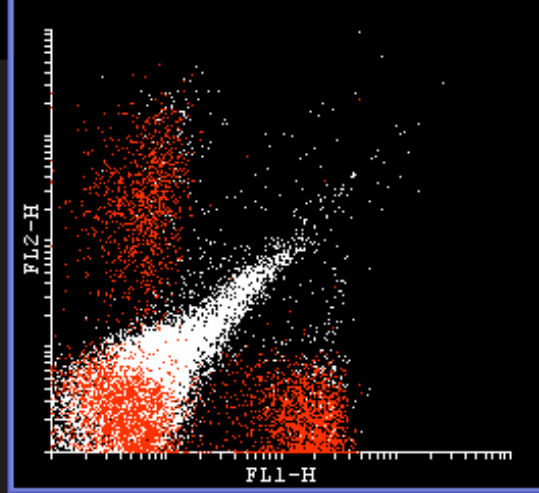
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined
- C ■  Not defined

Freeze the panel

[5] (1): Dot Plot - tube.001

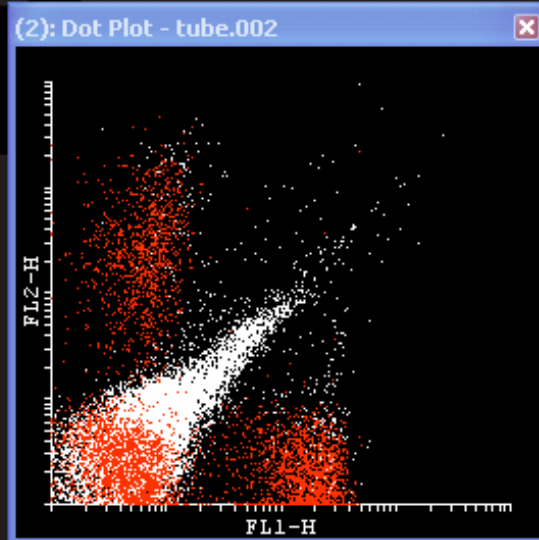
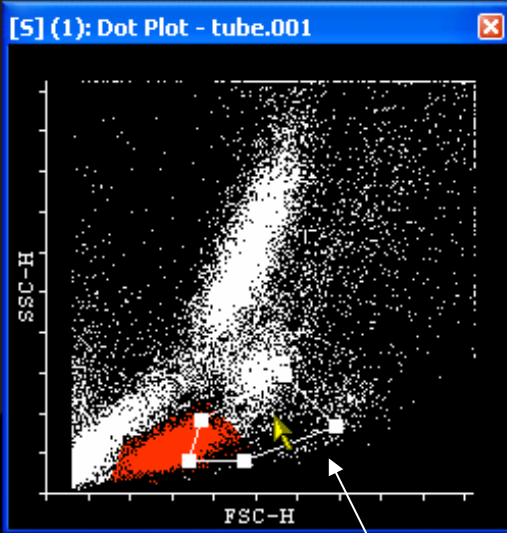


(2): Dot Plot - tube.002

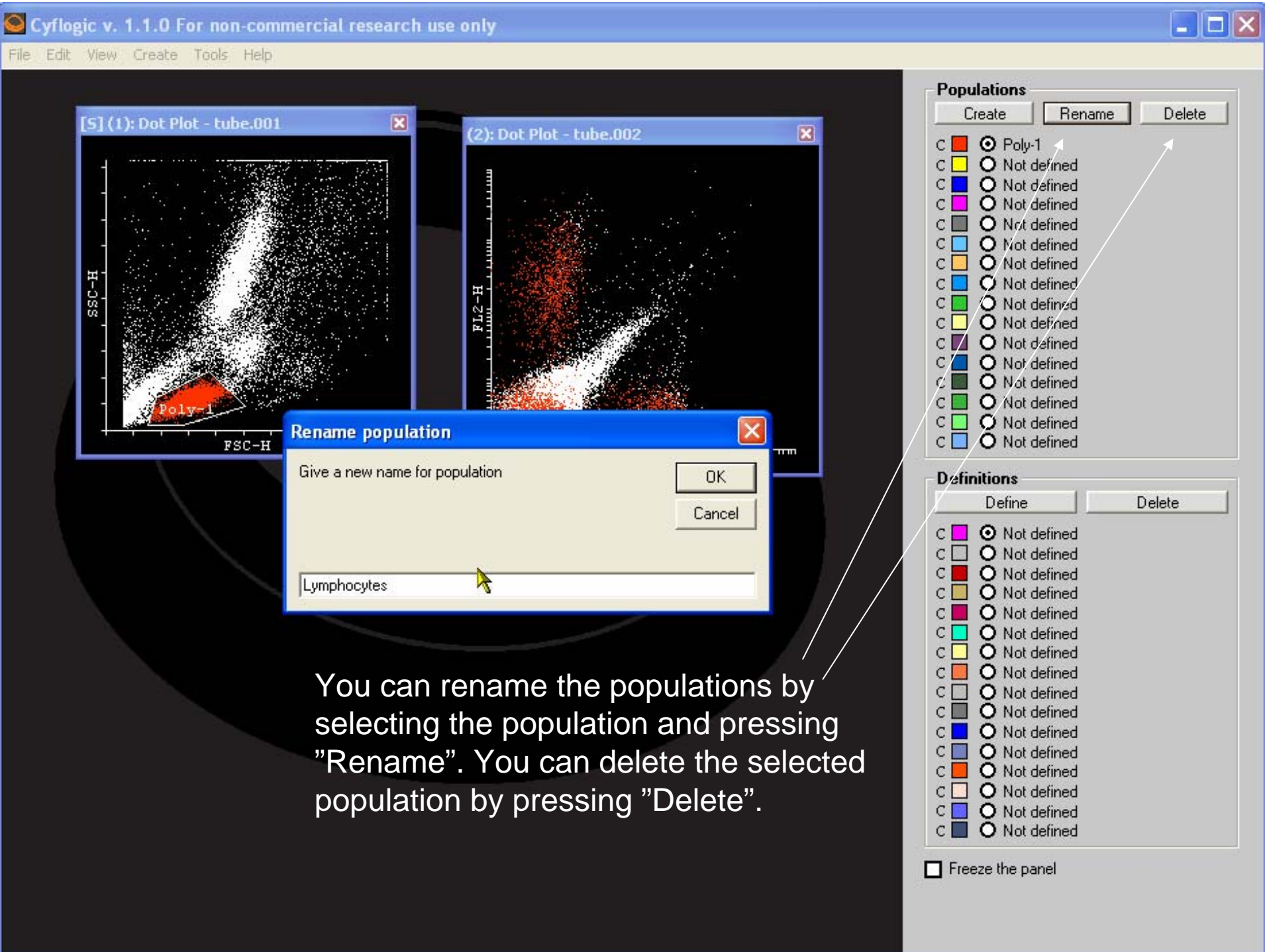


By pushing the left mouse button on the corner of the polygon the population can be resized.





Pushing the mouse button on the area of the population the whole population can be moved.



You can rename the populations by selecting the population and pressing "Rename". You can delete the selected population by pressing "Delete".

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File Edit View Create Tools Help

(1): Dot Plot - tube.001

SSC-H

FSC-H

lymphocytes

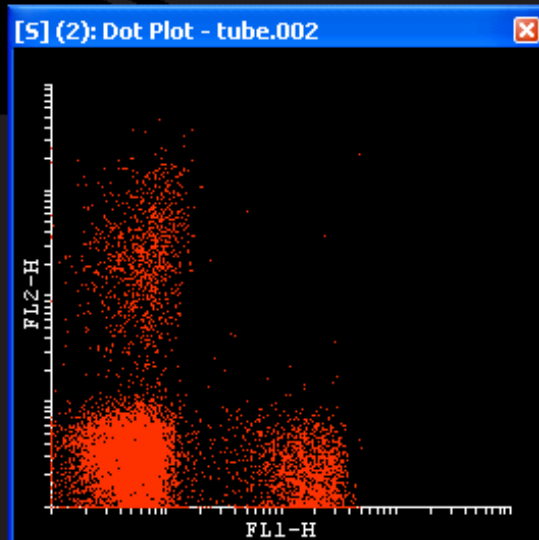
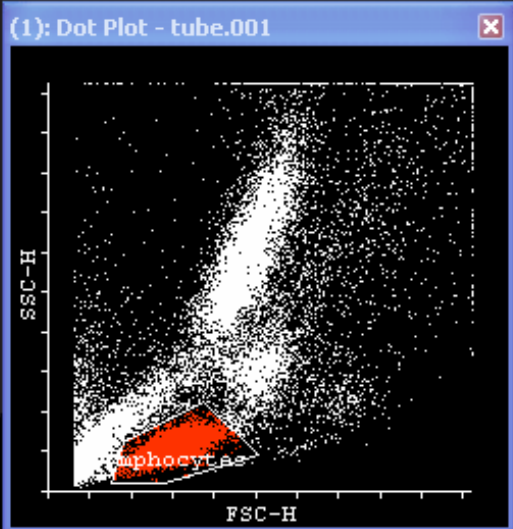
[5] (2): Dot Plot - tube.002

FL2-H

FL1-H

- Show populations
  - All events
  - Lymphocytes
- Open statistics
- Show Quadrant
- Density plot
- Hide region lines
- Show only regions created here
- Disable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page

When opening the pop-up menu (by right-clicking the window area) you can select which population can be seen in the window. All defined populations are listed.



**Populations**

Create Rename Delete

- C   Lymphocytes
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined

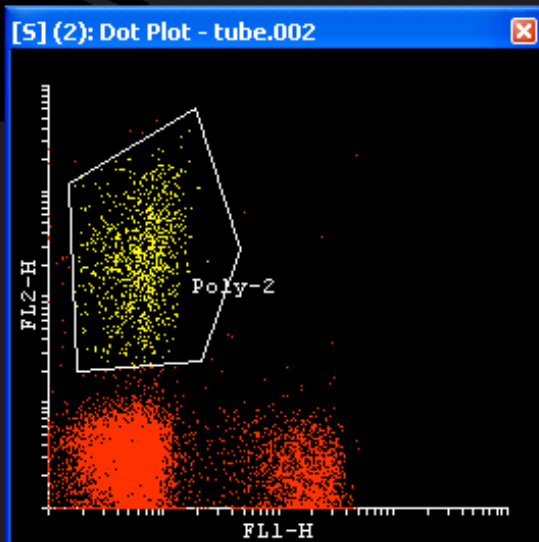
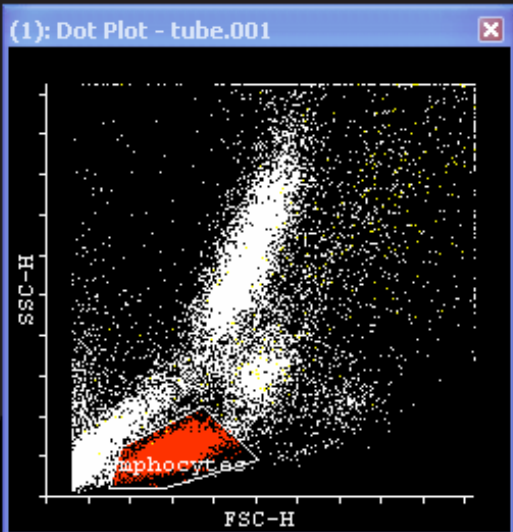
**Definitions**

Define Delete

- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined
- C   Not defined

Freeze the panel

You can change the population color by clicking  
The color box on the left side of the population.



If the event belongs into many populations, it has the color of the lowest population in the list.

**Populations**

Create Rename Delete

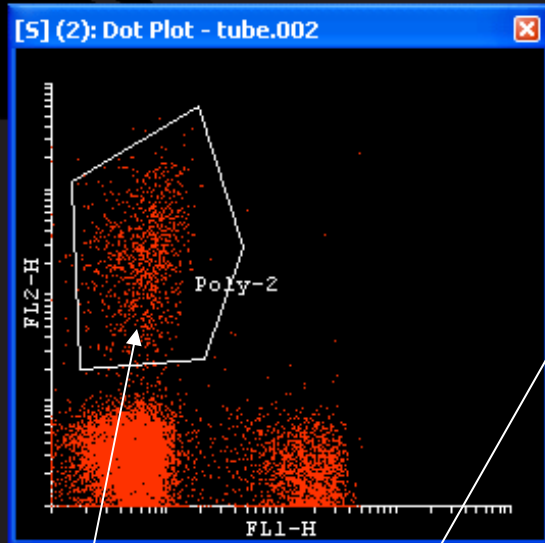
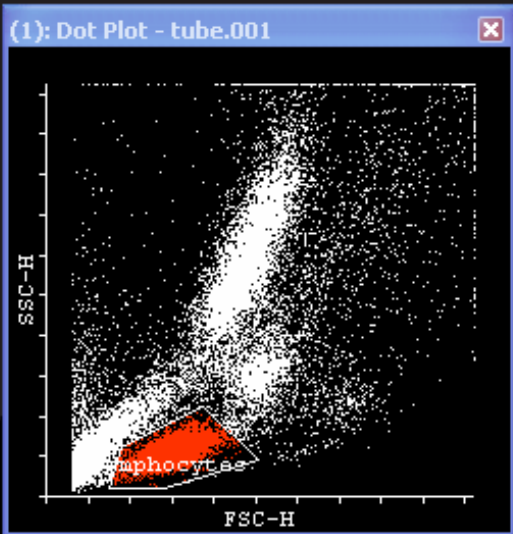
- C  Lymphocytes
- C  Poly-2
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

**Definitions**

Define Delete

- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

Freeze the panel



**Populations**

Create Rename Delete

- C  Lymphocytes
- T  Poly-2
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

**Definitions**

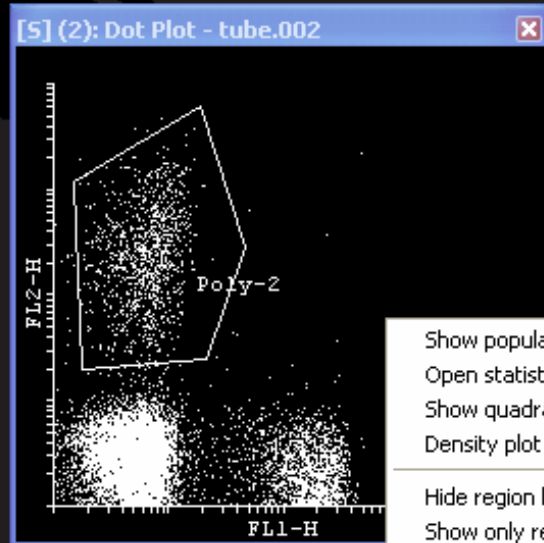
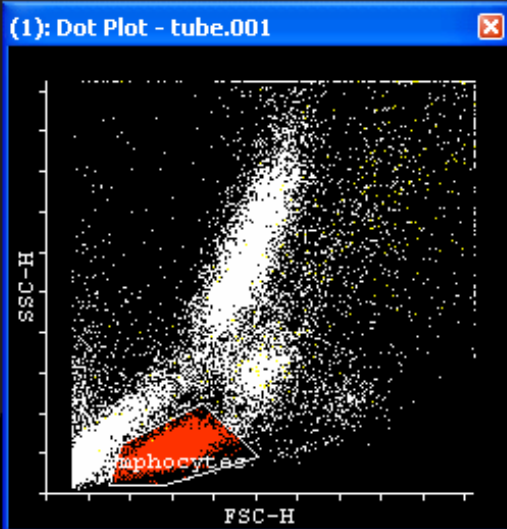
Define Delete

- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

Freeze the panel

If you want that some population doesn't colorize the events, you can turn the population into "Transparent" by double-clicking "C" (which means "Color") on the left side of the population. "C" becomes "T" and the events are not coloured with the population specific color. You can make them coloured again by double-clicking "T".

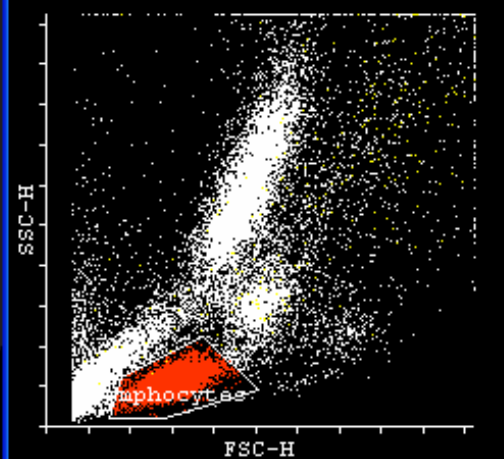




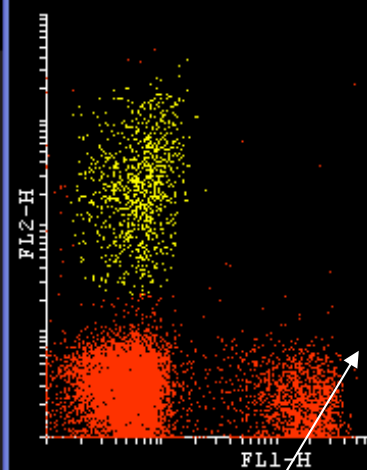
- Show populations
- Open statistics
- Show quadrant
- Density plot
- Hide region lines
- Show only regions created here
- Enable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page

You can remove the population specific colors entirely by choosing "Disable multicoloring" from the pop-up menu. you can enable it again by choosing "Enable multicoloring"

(1): Dot Plot - tube.001



[5] (2): Dot Plot - tube.002

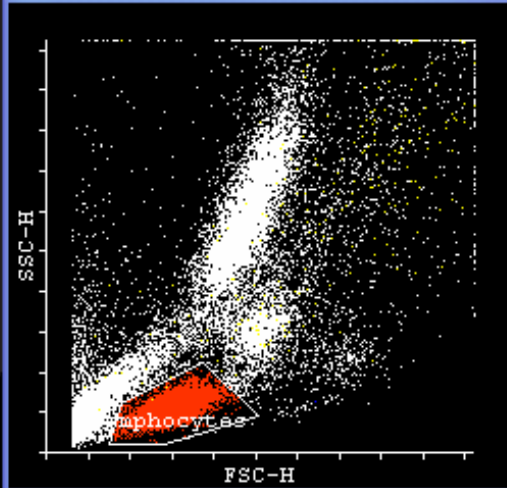


- Show populations
- Open statistics
- Show quadrant
- Density plot
- Show region lines
- Show only regions created here
- Disable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page

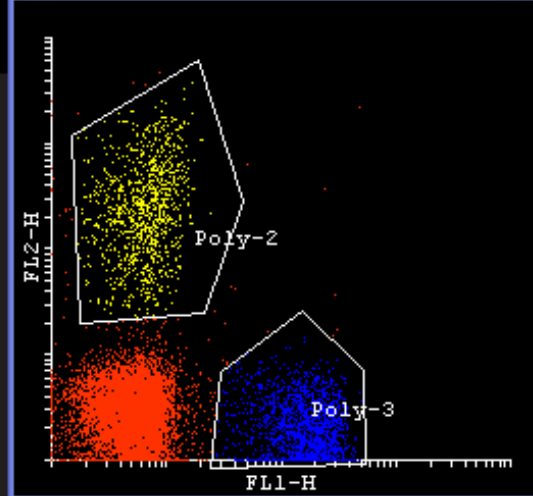
If you want to hide the lines from the window, select "Hide region lines". This doesn't affect anyhow to the definition of the population. They will appear again when you choose "Show region lines"



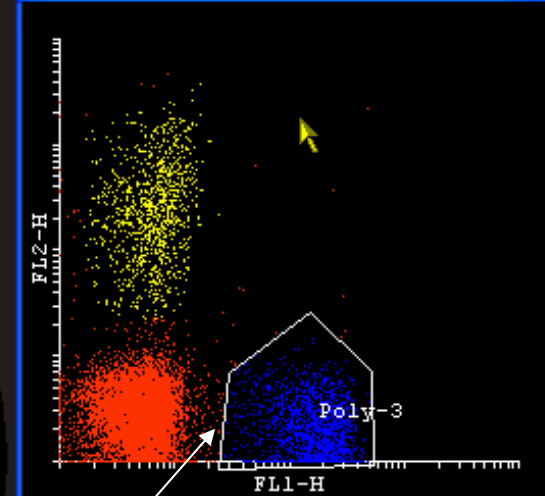
(1): Dot Plot - tube.001



(2): Dot Plot - tube.002



[5] (3): Dot Plot - tube.002



The populations are only parameter specific, in other words, they appear in every window having the same parameters (in same order). If you want to hide the unnecessary region lines, you can choose "Show only regions created here"; only the regions created in that specific window appear. In the example case, Poly-3 has been created in the rightmost window and the option has been chosen, resulting that only Poly-3 line can be seen. This affects only the visibility of the region lines.

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File Edit View Create Tools Help

[S] (1): Dot Plot - tube.001

SSC-H

FSC-H

Poly-1

Poly-2

**Populations**

Create Rename Delete

- C  Poly-1
- C  Poly-2
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

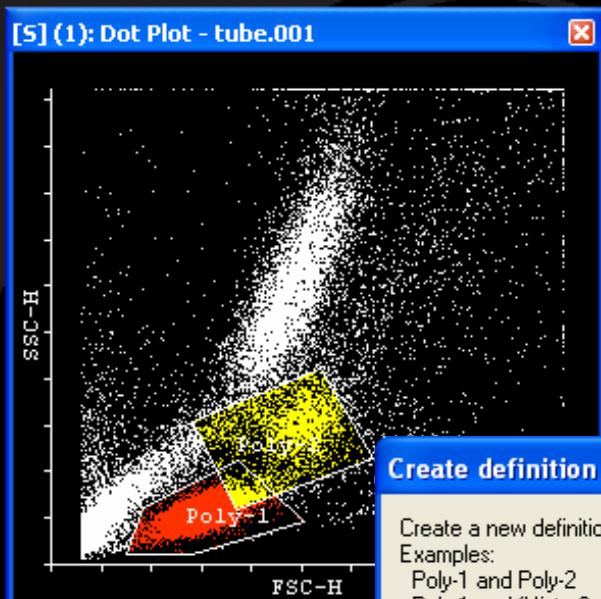
**Definitions**

Define Delete

- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined
- C  Not defined

Freeze the panel

If you need to combine two populations, you can create a definition. Select the wanted place for definition and press "Define".



**Create definition**

Create a new definition (case insensitive)  
Examples:  
Poly-1 and Poly-2  
Poly-1 and (Histo-2 or Poly-3)  
Poly-1 and Histo-2 and not Poly-3

Poly-1 and Poly-2

OK  
Cancel

Write the wanted definition. You can use "AND", "OR", "NOT" and parentheses. The definition is case insensitive.

**Populations**

Create Rename Delete

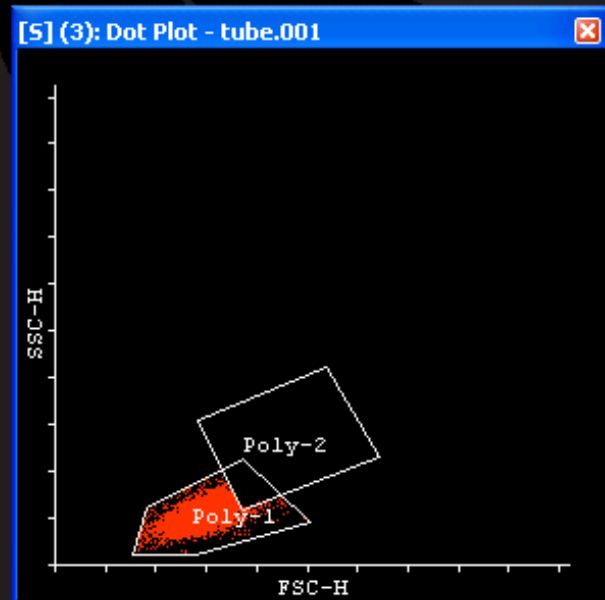
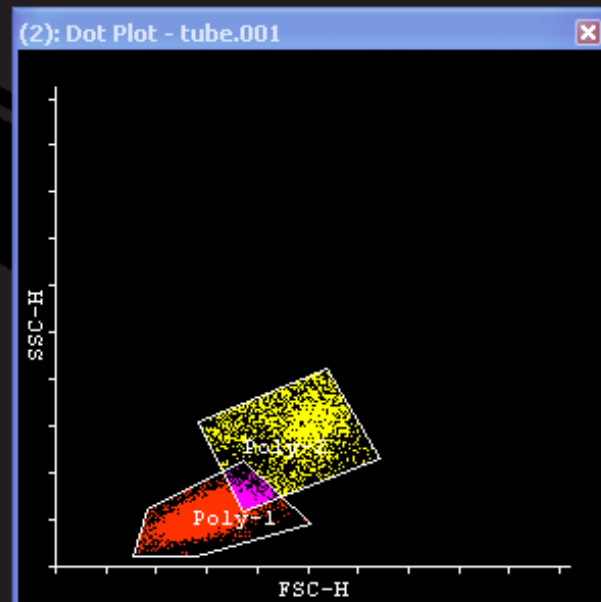
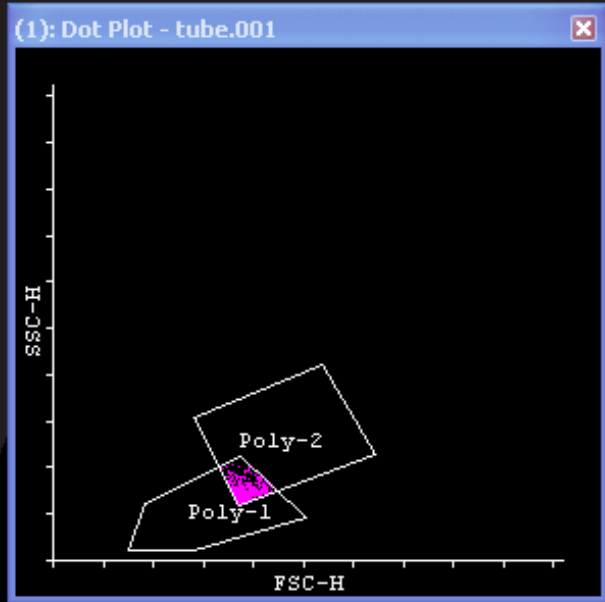
- C Poly-1
- C Poly-2
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined

**Definitions**

Define Delete

- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined
- C Not defined

Freeze the panel



Some examples:  
Poly-1 or Poly-2  
Poly-1 and Poly-2  
Poly-1 and not Poly-2

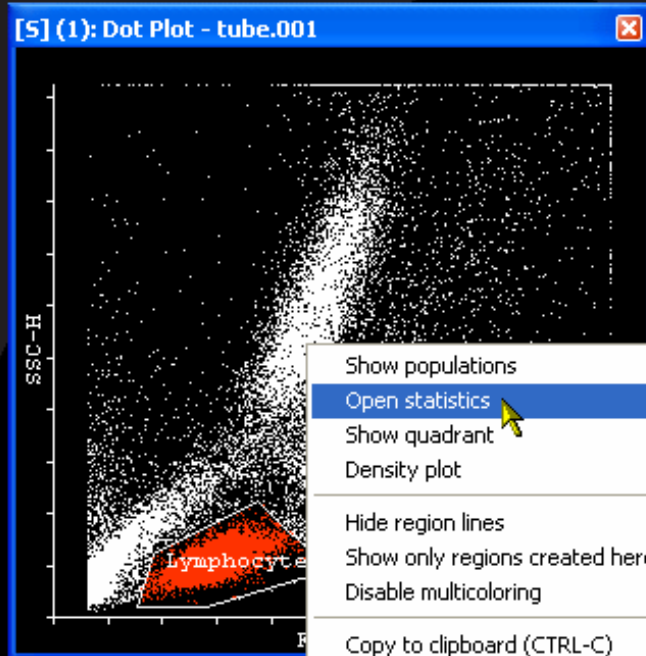
Attention! You can use only populations in the definitions. You can't use other definitions.

Other population remarks:

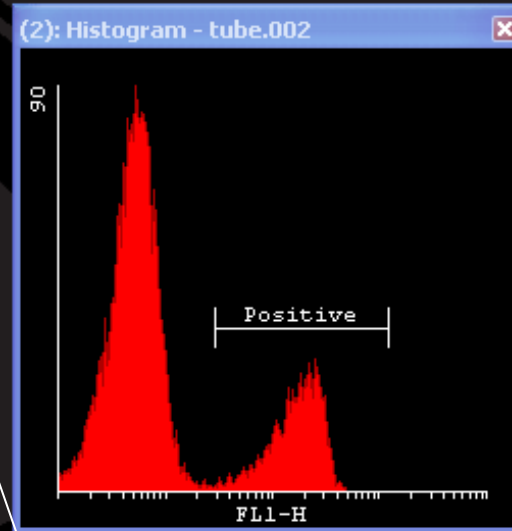
If the population is deleted, all definitions which used the deleted population will be also deleted.

You can define colors for definitions similarly as for populations.  
You can also make them transparent.

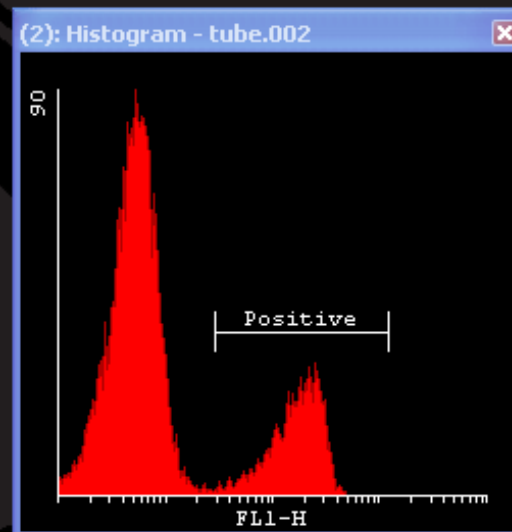
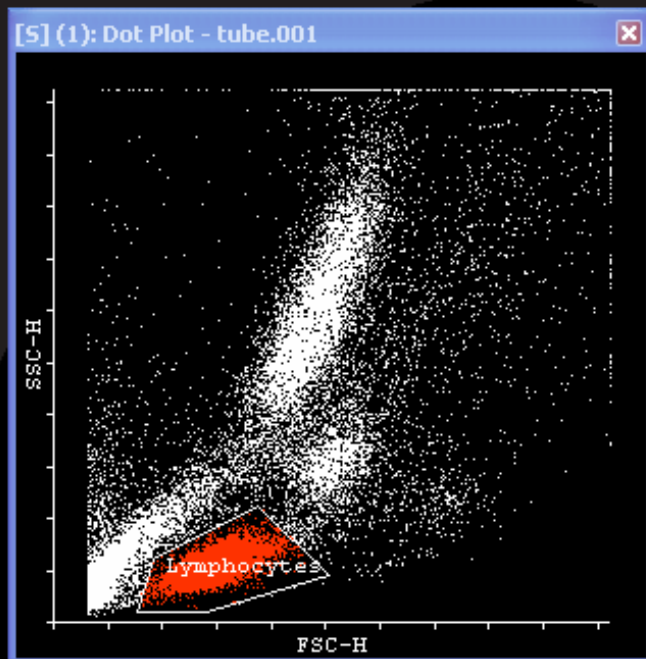
# Statistics



- Show populations
- Open statistics**
- Show quadrant
- Density plot
- Hide region lines
- Show only regions created here
- Disable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page



You can open statistics for the window by selecting "Open statistics" from the pop-up menu.

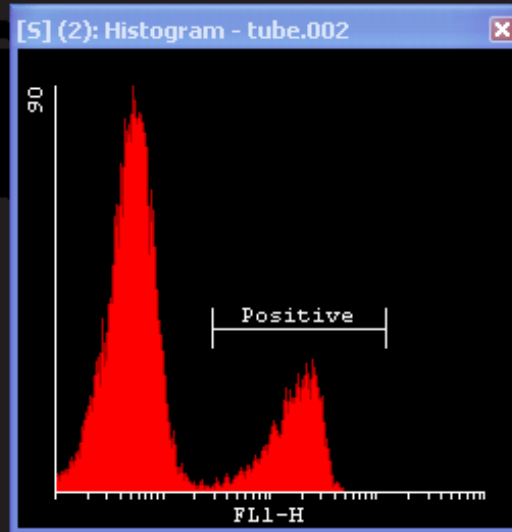
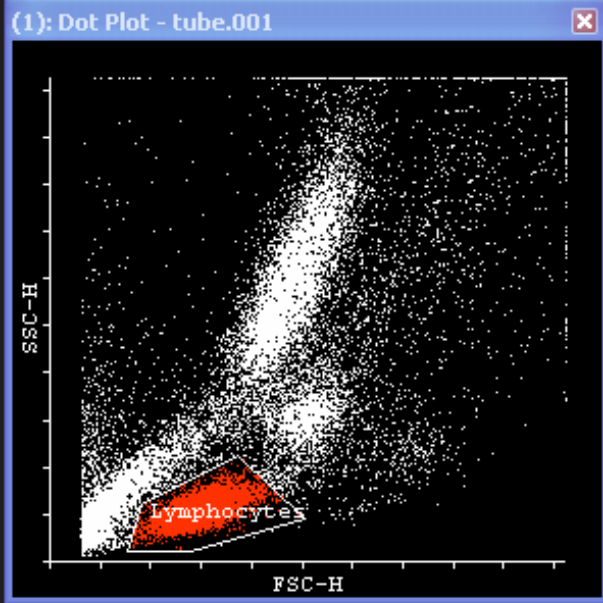


(1) Statistics: tube.001

	Number	% of vis	X mean	Y mean	X geomean	Y geomean
Visible	26418	100	364,61	294,55	316,22	205,69
Lymphocytes	11161	42,24	299,8	109,45	297,24	107,3

The statistics box shows the numbers of your data. If you add / delete the populations, change axis or change data files, statistics box is automatically updated. The number on the left corner tells from which window the statistics has been created.





(2) Statistics: tube.002

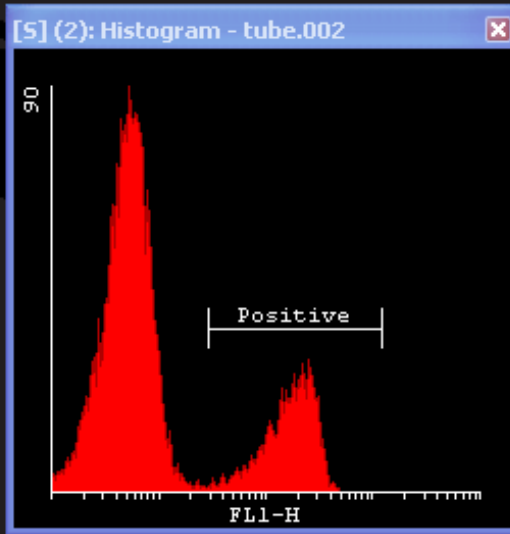
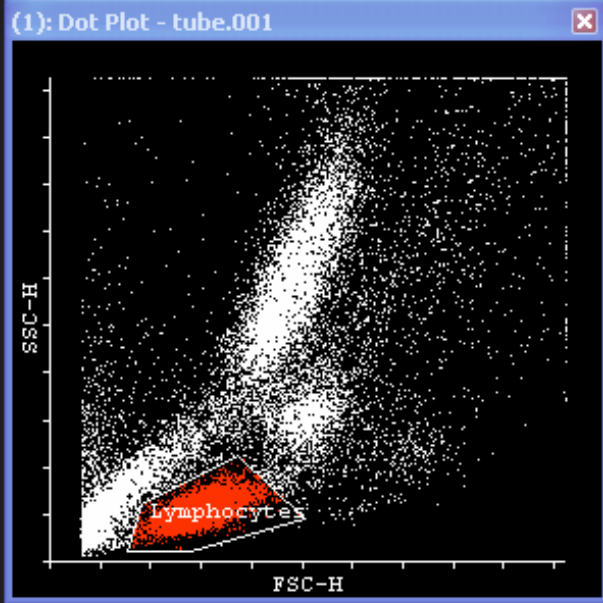
	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

(1) Statistics: tube.001

	Number	% of vis	X mean	Y mean	X geomean	Y geomean
Visible	26418	100	364,61	294,55	316,22	205,69
Lymphocytes	11161	42,24	299,8	109,45	297,24	107,3

You can create several statistics from different windows. Only the relevant populations and definitions can be seen in the box.

Attention! In this example, histogram shows only the events inside population "Lymphocytes". All the statistics have been calculated only from the events which are visible in the histogram.



(2) Statistics: tube.002

	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

(1) Statistics: tube.001

	Number	% of vis	X geomean	Y geomean
Visible	26418	100	210,22	205,00
Lymphocytes	11161	42,24		

- Copy to clipboard
- Number
- % of Vis
- XMean
- YMean
- XGeoMean
- YGeoMean

---

- File name
- Sample ID
- Comments

---

- Show in print preview page

If you don't need all provided numbers, you can hide them by right-clicking the statistics.

Other statistics remarks:

You can copy the contents of statistics window by selecting "Copy to clipboard" from pop-up menu or by pressing CTRL-C when the wanted window is active.

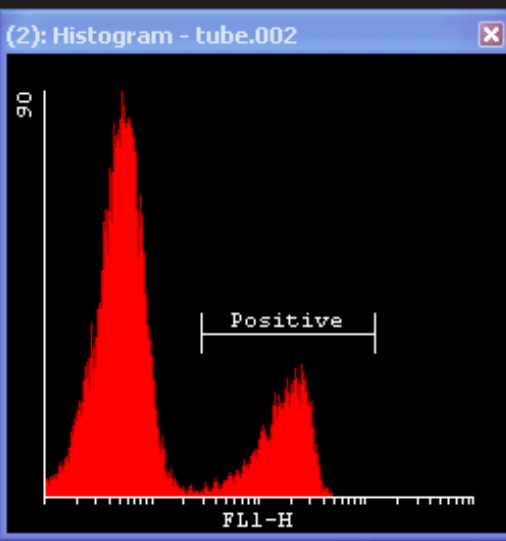
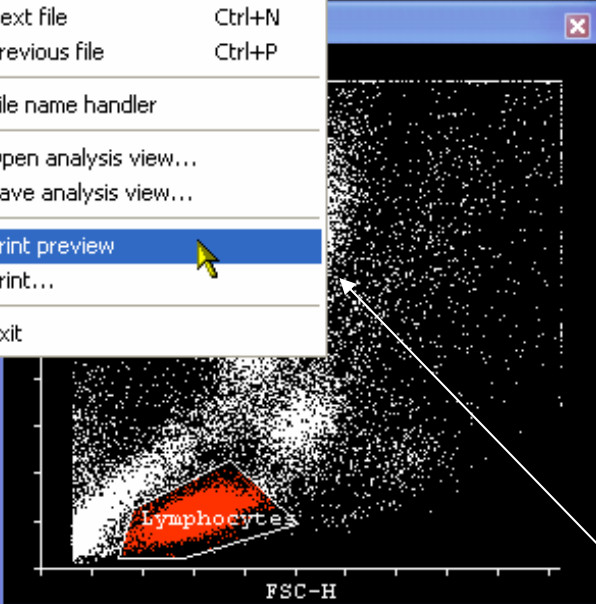
If the window which has created the statistics is closed, the statistics is also closed.

Printing

Cyfllogic v. 1.1.0 For non-commercial research use only

File Edit View Create Tools Help

- Open FCS... Ctrl+O
- Next file Ctrl+N
- Previous file Ctrl+P
- File name handler
- Open analysis view...
- Save analysis view...
- Print preview**
- Print...
- Exit



	Number	% of vis	X geomean	Y geomean
Visible	26418	100	316,22	205,69
Lymphocytes	11161	42,24	297,24	107,3

	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

Print preview window can be opened by selecting "Print preview".

Cyflogic v. 1.1.0 For non-commercial research use only

File Edit View Create Tools Help

[5] (1): Dot Plot - tube.001

(2): Histogram - tube.002

- Show populations
- Close statistics
- Show quadrant
- Density plot
- Hide region lines
- Show only regions created here
- Disable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page

(1) Statistics: tube.001

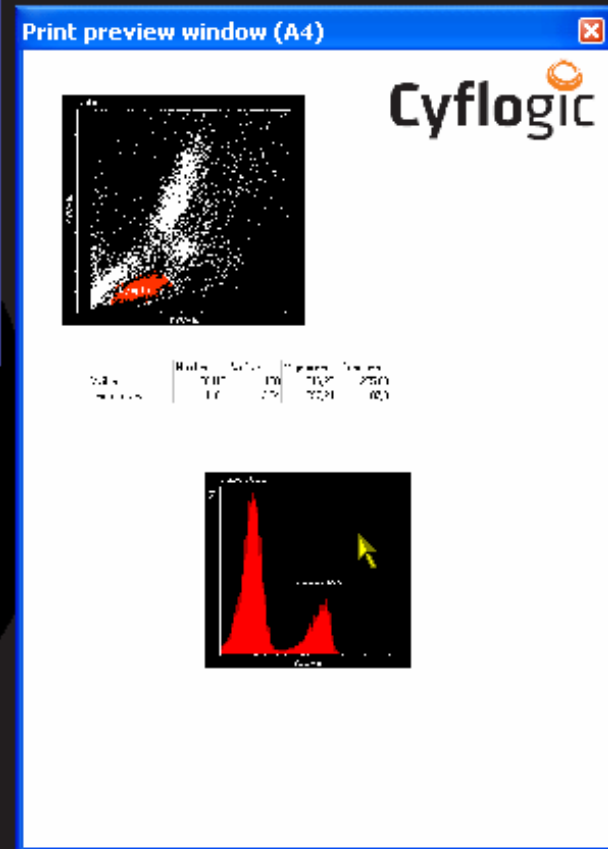
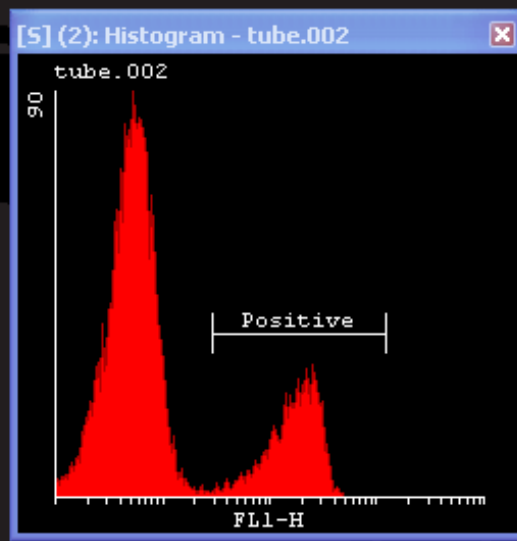
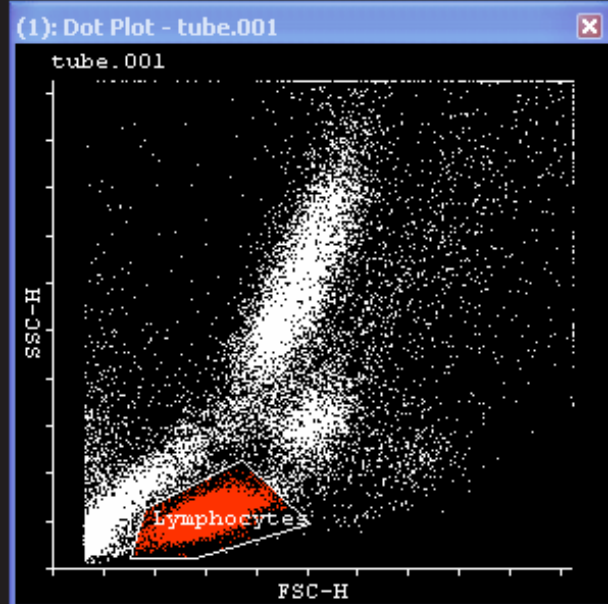
	Number	% of vis
Visible	26418	
Lymphocytes	11161	

(2) Statistics: tube.002

	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

Print preview window (A4)

Windows and statistics can be added into the preview page by selecting "Show in print preview page" from the window's pop-up menu



(1) Statistics: tube.001

	Number	% of vis	X geomean	Y geomean
Visible	26418	100	316,22	205,69
Lymphocytes	11161	42,24	297,24	107,3

(2) Statistics: tube.002

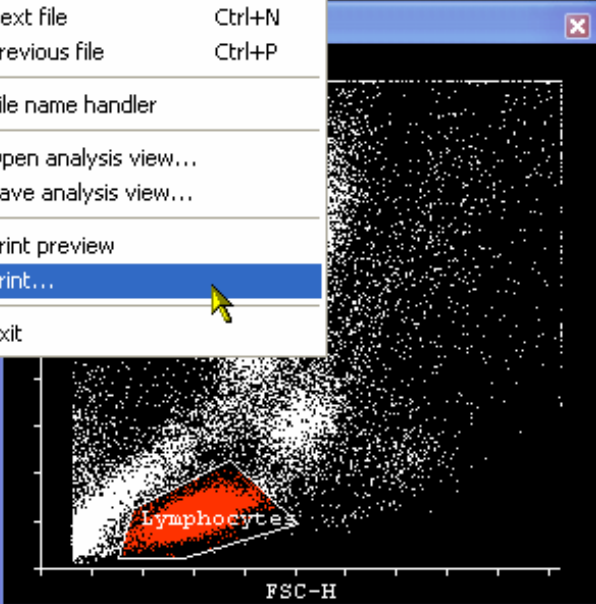
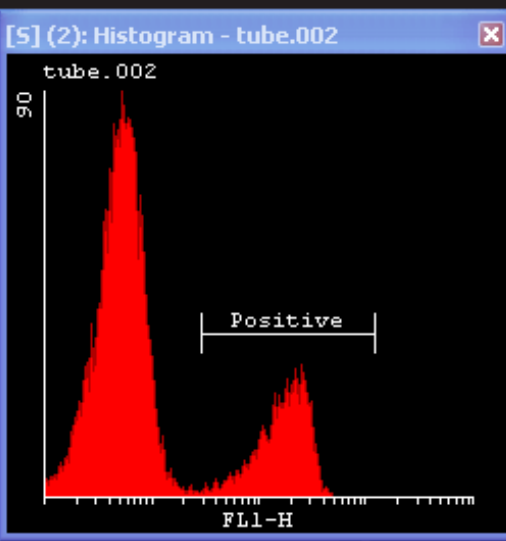
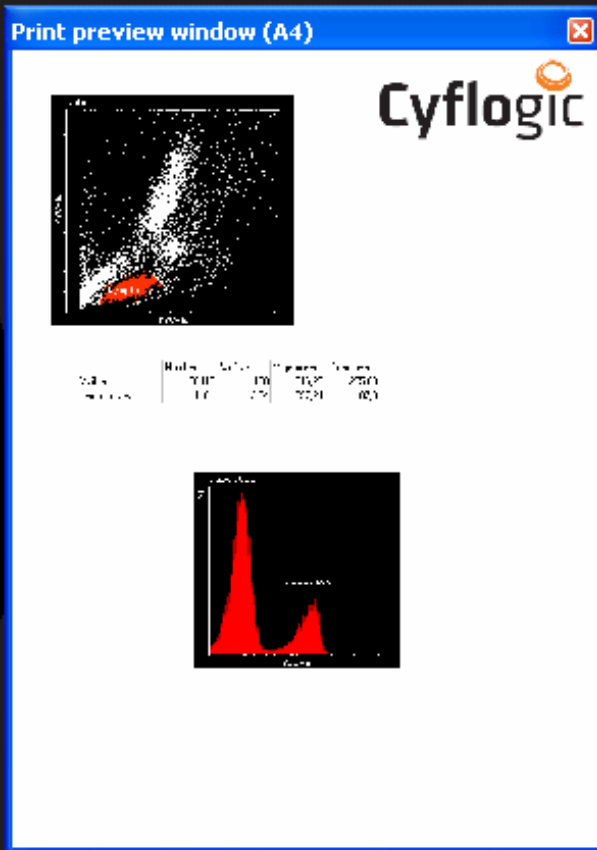
	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

The windows can be moved in the paper freely. If you change the Size of the window, it will resize also in the preview page.

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File Edit View Create Tools Help

- Open FCS... Ctrl+O
- Next file Ctrl+N
- Previous file Ctrl+P
- File name handler
- Open analysis view...
- Save analysis view...
- ✓ Print preview
- Print...**
- Exit

	Number	% of vis	X geomean	Y geomean
Visible	26418	100	316,22	205,69
Lymphocytes	11161	42,24	297,24	107,3

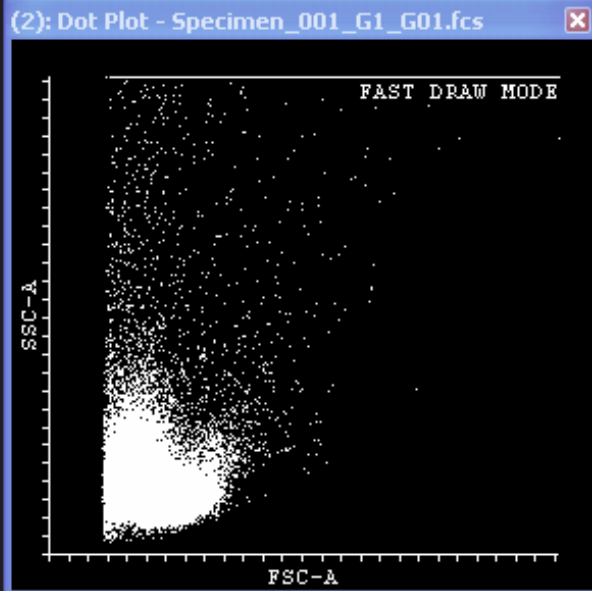
  

	Number	% of vis	X mean	X geomean	CV
Visible	11161	100	49,17	11,4	182,16
Positive	2587	23,18	193,13	170,65	45,14

When you want to print the view, select "Print..."



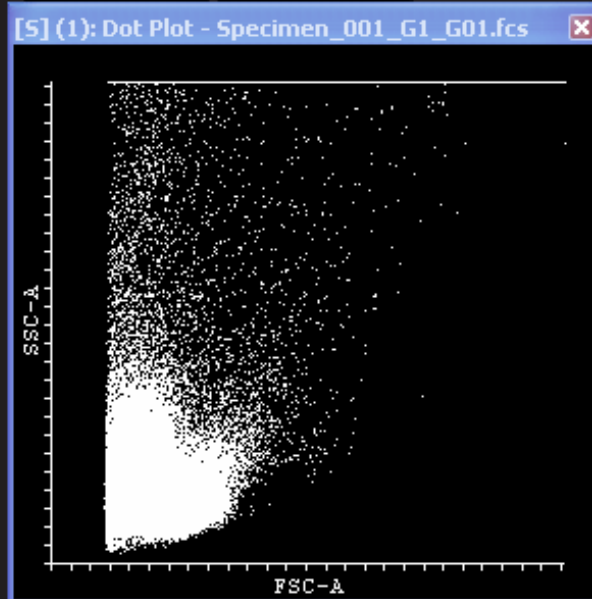
Large files



(2) Statistics: Specimen\_001\_G1\_G01.fcs

FAST DRAW	Number	% of vis	X mean	Y mean	X geomean	Y geomean
Visible	35434	100	61699,44	50189,72	56964,94	39482,82

If you load FCS file containing more than 50000 events, the window is switched into "Fast draw mode". Only every n:th event is drawn and statistics are calculated from those drawn events.



(1) Statistics: Specimen\_001\_G1\_G01.fcs

	Number	% of vis	X mean	Y mean	X geomean	Y geomean
Visible	106302	100	61856,46	50178,78	57112,58	39501,62

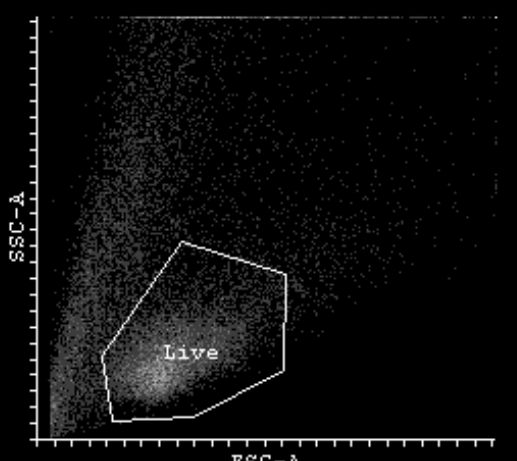
By selecting "Full draw mode" all events are drawn and the statistics are counted from every event.

# Dot plot features

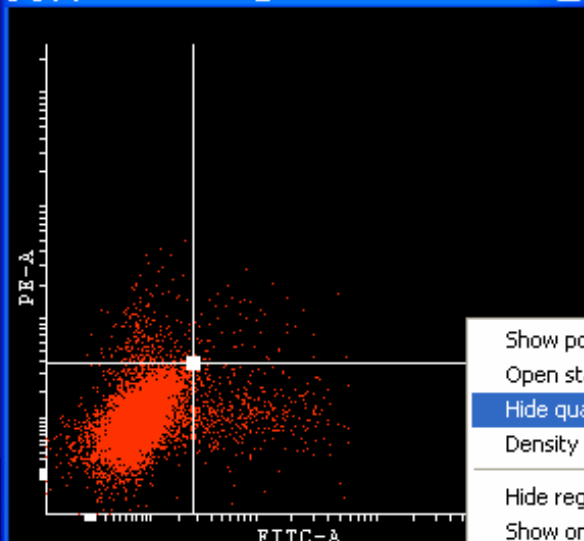
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File Edit View Create Tools Help

(1): Dot Plot - D06\_D06.fcs

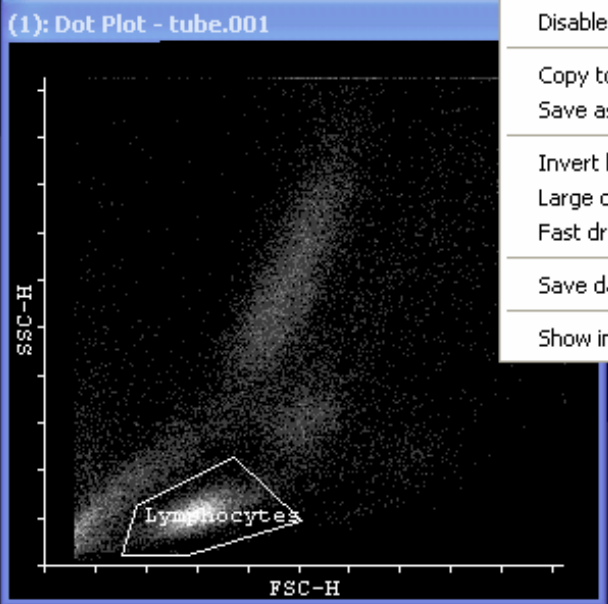
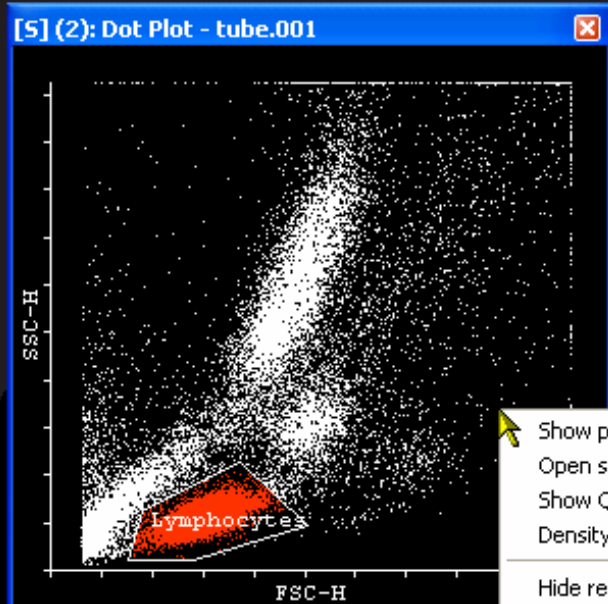


(5) (2): Dot Plot - D06\_D06.fcs



Show populations  
Open statistics  
Hide quadrant  
Density plot  
Hide region lines  
Show only regions created here  
Disable multicoloring  
Copy to clipboard (CTRL-C)  
Save as .BMP...  
Invert background color  
Large dots  
Fast draw mode  
Save data as .TXT...  
Show in print preview page

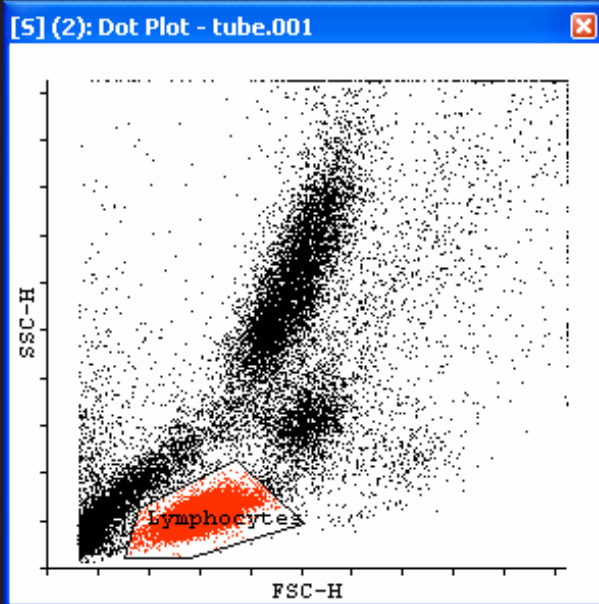
You can create the quadrant by selecting "Show quadrant" in the pop-up menu. Hide it by selecting "Hide quadrant". If you select "Show quadrant" in some other dot plot, it will appear into the same place as in the previous dot plot. By this way you can copy the quadrants from one dot plot to another.



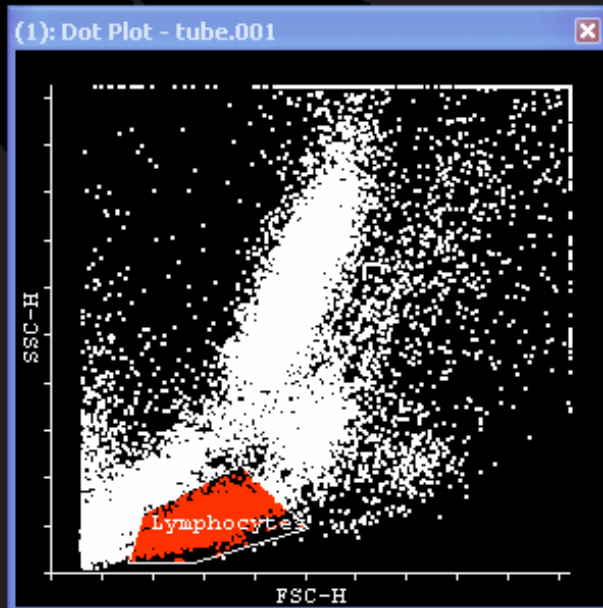
- Show populations
- Open statistics
- Show Quadrant
- Density plot
- Hide region lines
- Show only regions created here
- Disable multicoloring
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Invert background color
- Large dots
- Fast draw mode
- Save data as .TXT...
- Show in print preview page

You can change the dot plot into density plot by selecting "Density plot" from pop-up menu.

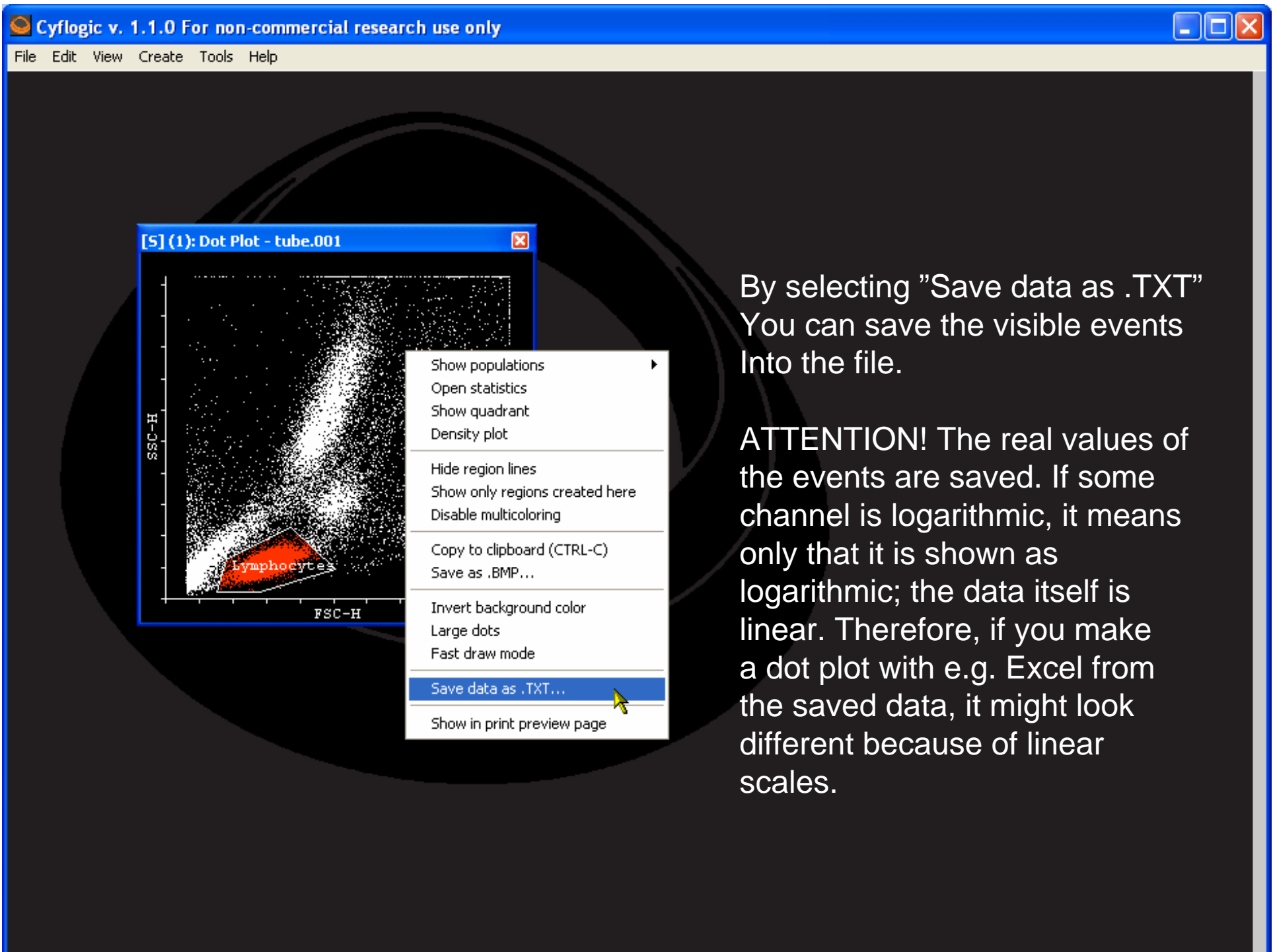
You can change it back to dot plot by selecting "Dot plot".



By selecting "Invert background color" the background color changes, from black to white or vice versa.



By selecting "Large dots" the size of the dots is doubled. You can change the size back to normal by selecting "Small dots".

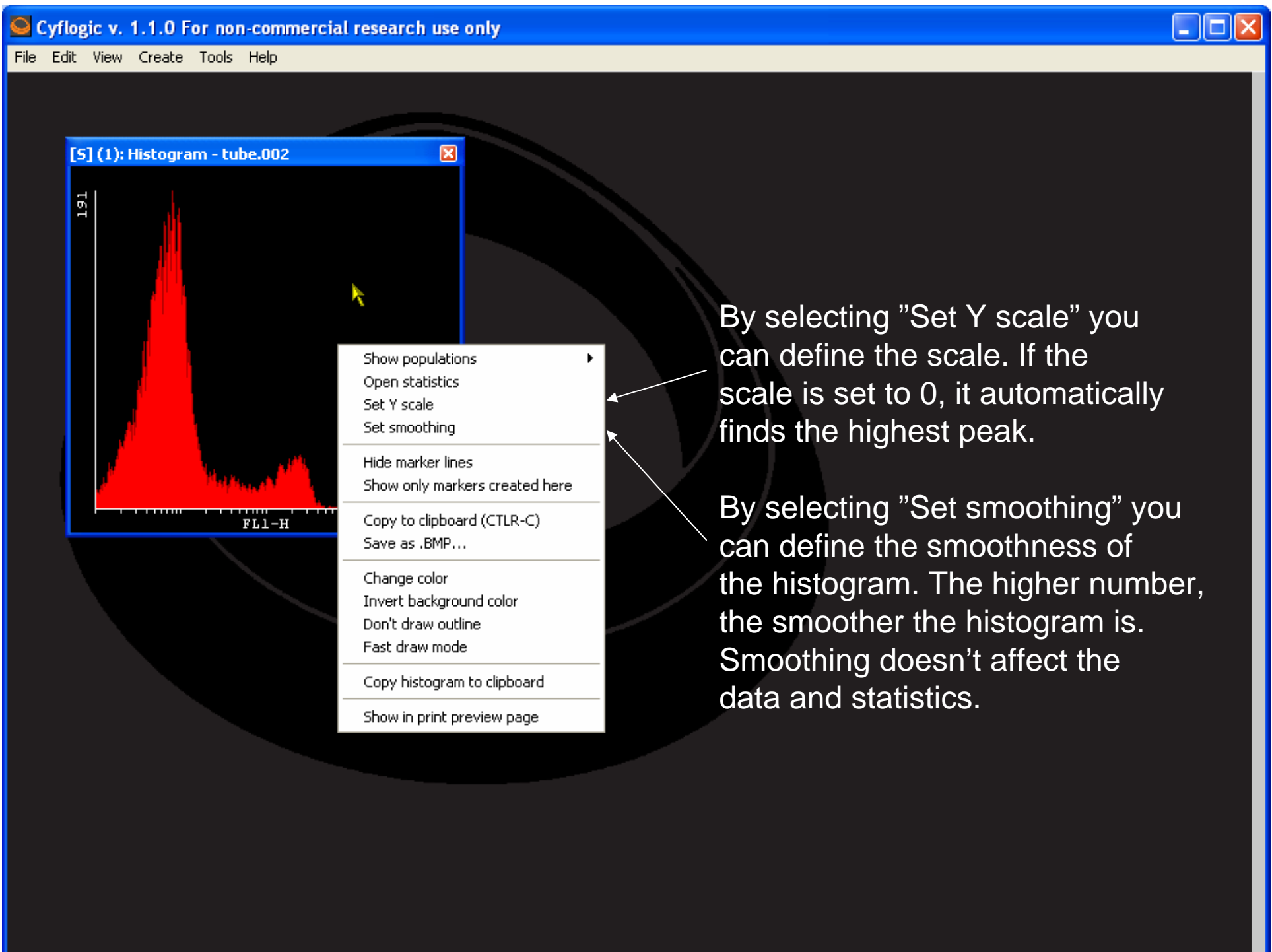


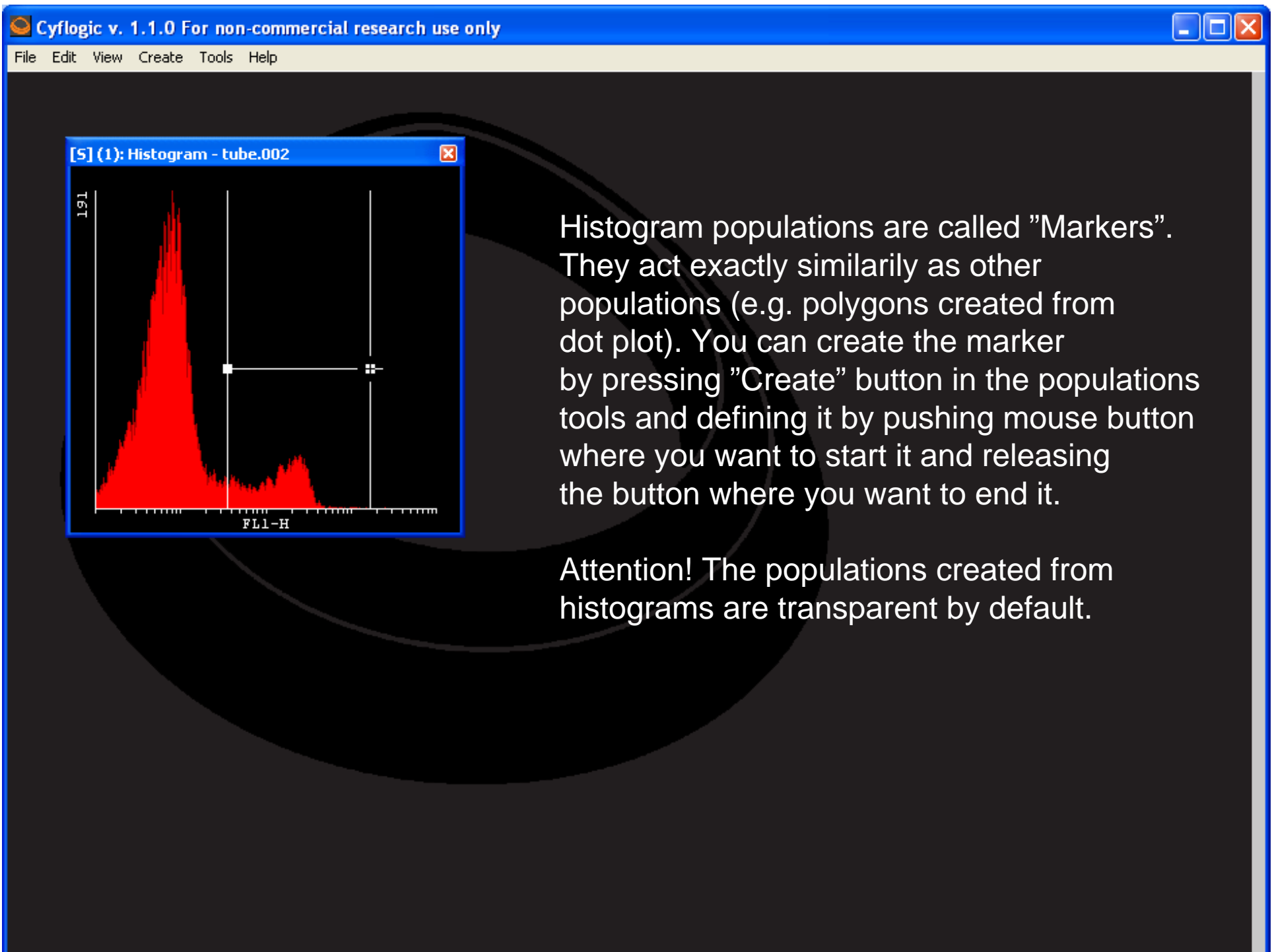
By selecting "Save data as .TXT" You can save the visible events into the file.

**ATTENTION!** The real values of the events are saved. If some channel is logarithmic, it means only that it is shown as logarithmic; the data itself is linear. Therefore, if you make a dot plot with e.g. Excel from the saved data, it might look different because of linear scales.

# Histogram features







Histogram populations are called "Markers". They act exactly similarly as other populations (e.g. polygons created from dot plot). You can create the marker by pressing "Create" button in the populations tools and defining it by pushing mouse button where you want to start it and releasing the button where you want to end it.

Attention! The populations created from histograms are transparent by default.

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File Edit View Create Tools Help

[5] (1): Histogram - tube.002

191

FL1

Show populations

Open statistics

Set Y scale

Set smoothing

Hide marker lines

Show only markers created here

Copy to clipboard (CTRL-C)

Save as .BMP...

Change color

Invert background color

Don't draw outline

Fast draw mode

Copy histogram to clipboard

Show in print preview page

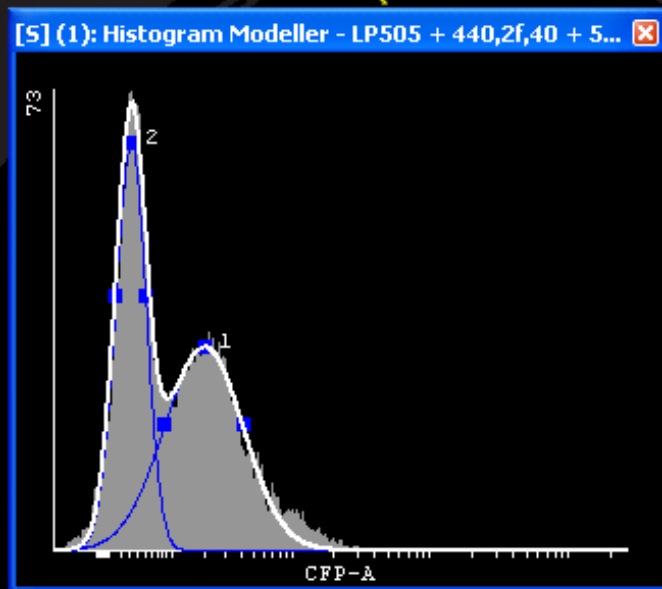
You can change the color of the histogram by choosing "Change color".

You can invert the black background to white and vice versa by selecting "Invert background color".

If you want that the darker outline is not drawn, you can select "Don't draw outline".

You can copy the histogram profile (as numbers) into the clipboard by selecting "Copy histogram to clipboard".

# Histogram modeller features



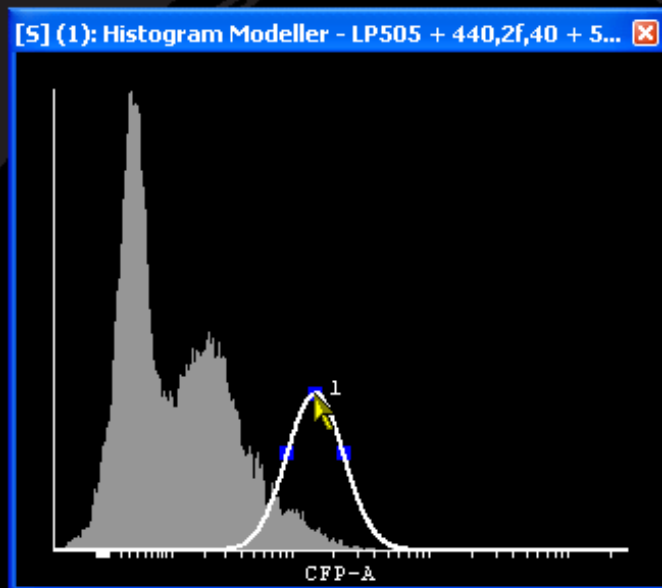
(1) Statistics: LP505 + 440,2f,40 + 585,2f,42\_CFP.fcs

	% of tot	X mean	X geomean	CV
Curve 1	56,51	253,97	181,23	81,59
Curve 2	43,48	32,81	26,16	56,09

Histogram modeller is a new tool for modelling the histogram and understanding the overlapping populations.

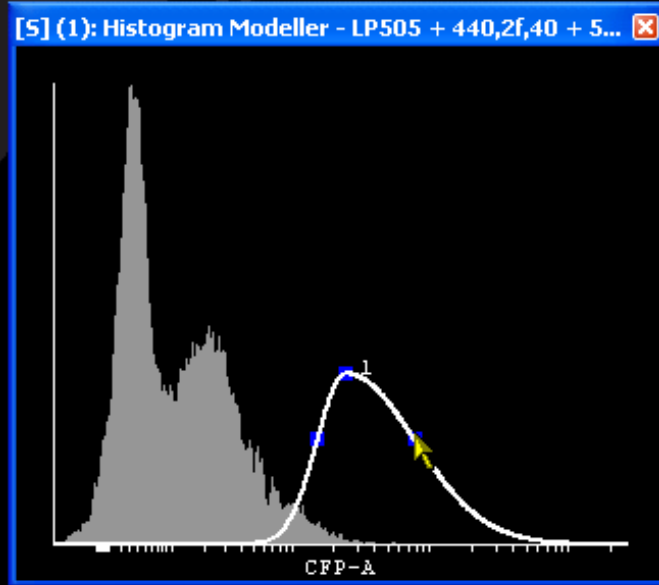
The idea is to add gaussian curves (blue lines) on the top of the histogram. White line is the sum of blue lines. The goal is to fit the white line to the histogram.

Attention! Modeller statistics doesn't tell anything about the original data; it just tells the features of the curves. This must be kept in mind when analyzing the numbers.

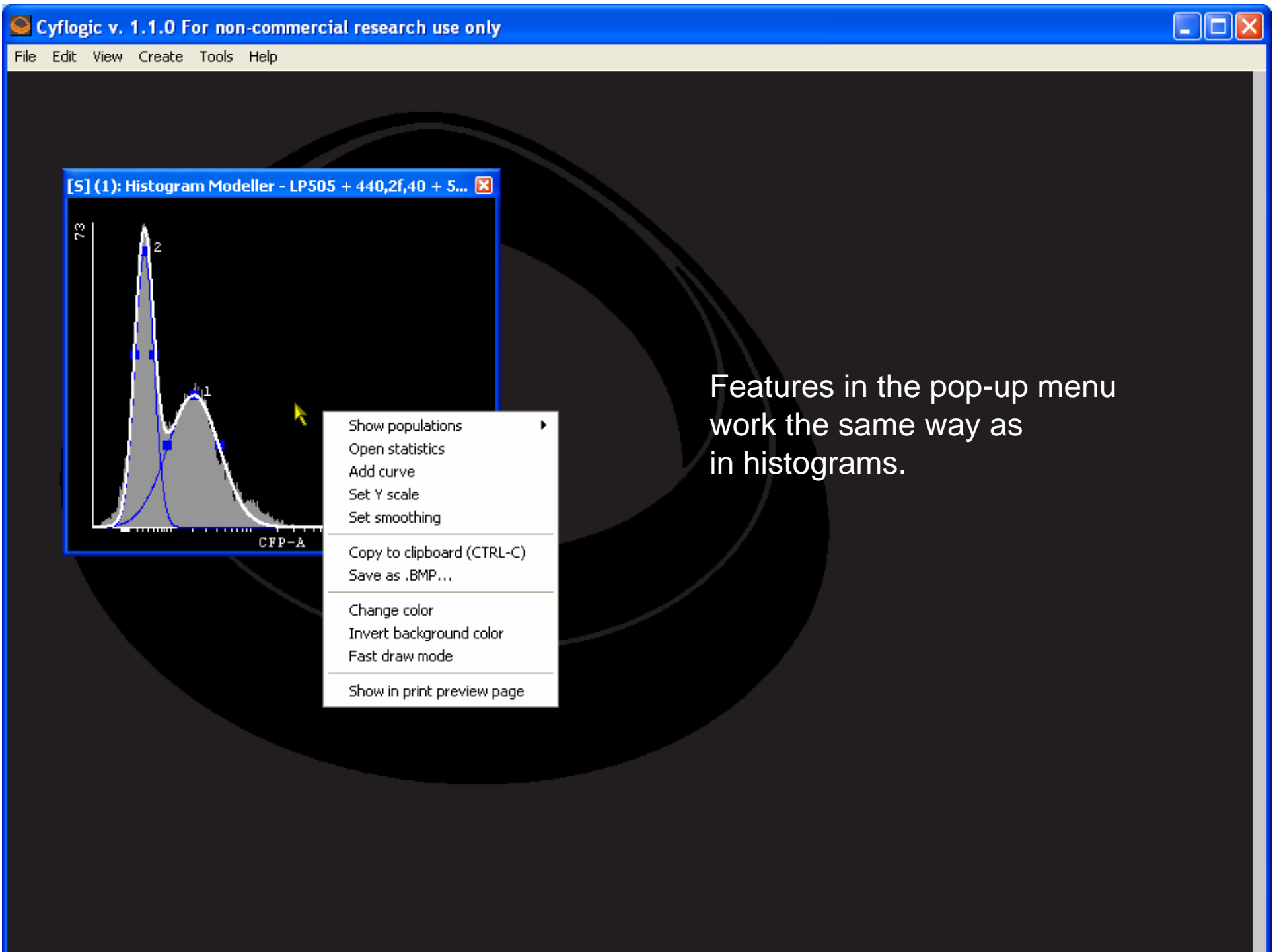


Start modelling by creating the curve. You can do it by selecting "Add curve" from pop-up menu. However, faster way is to push left mouse button down under the scale, move mouse up and put the curve into right position.

You can change the position and height of the curve by pushing the blue box on the top of the curve.



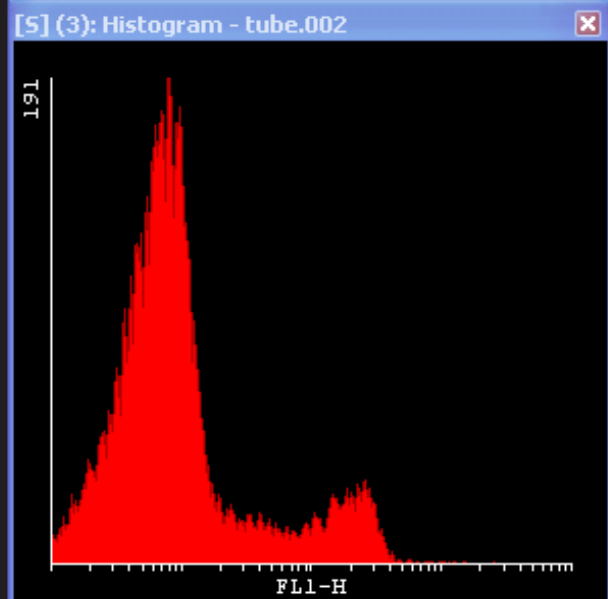
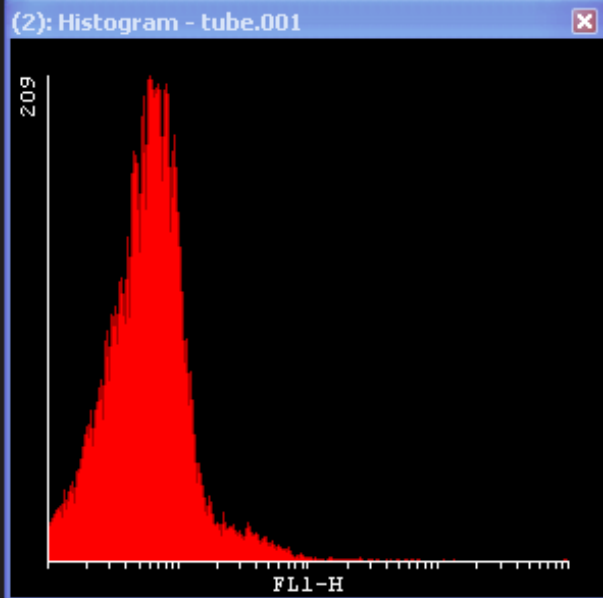
You can change the deviation of the curve from left and right blue boxes.



Features in the pop-up menu work the same way as in histograms.



Overlay histogram features



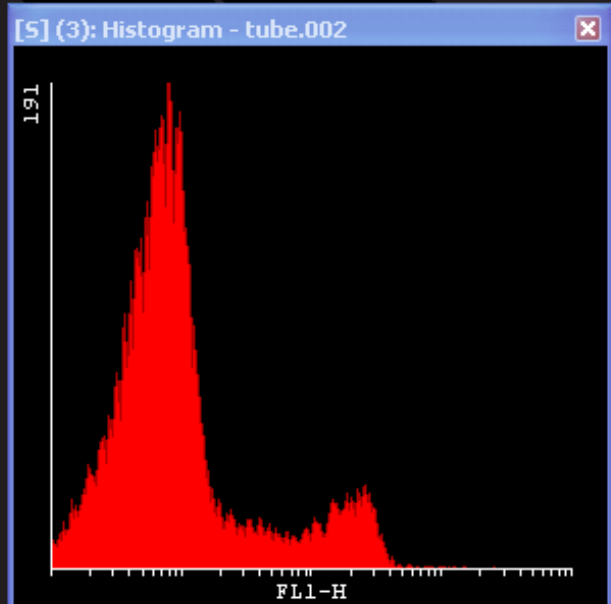
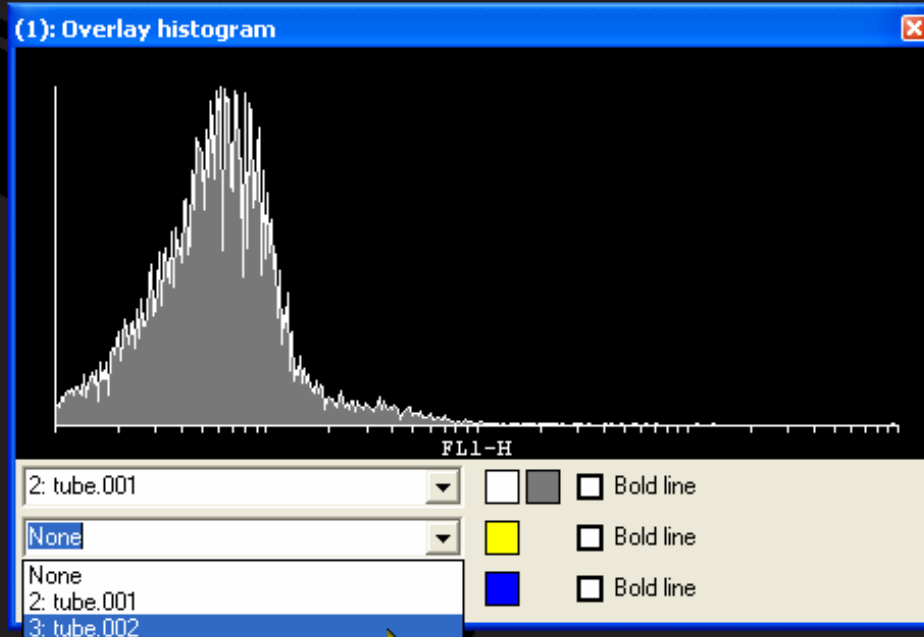
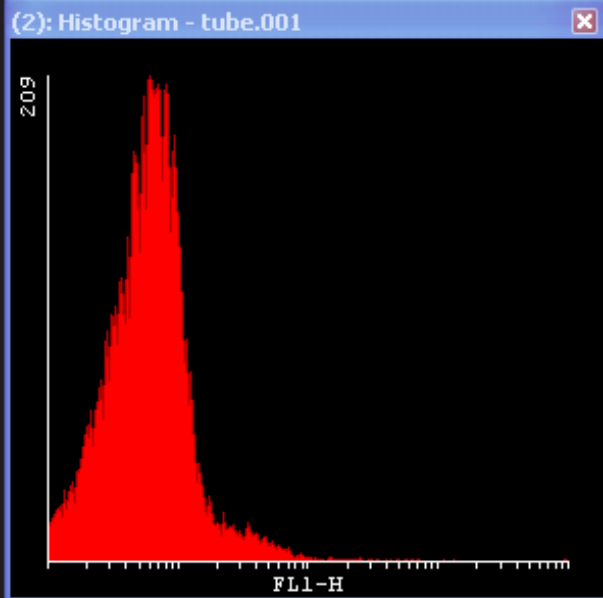
(1): Overlay histogram

None    Bold line

None   Bold line

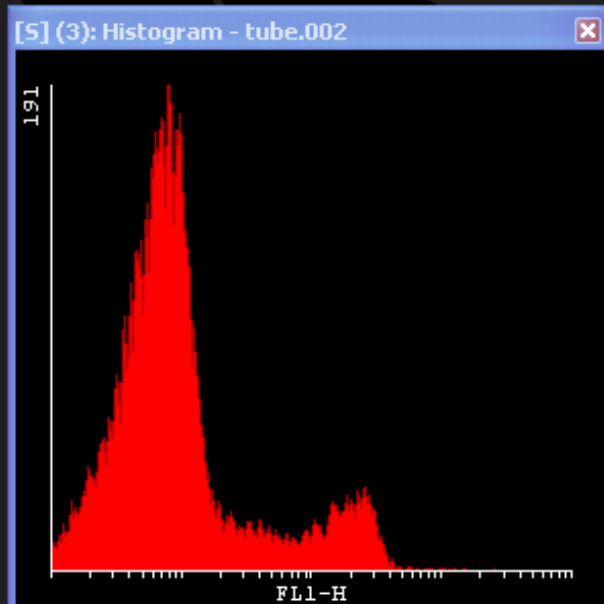
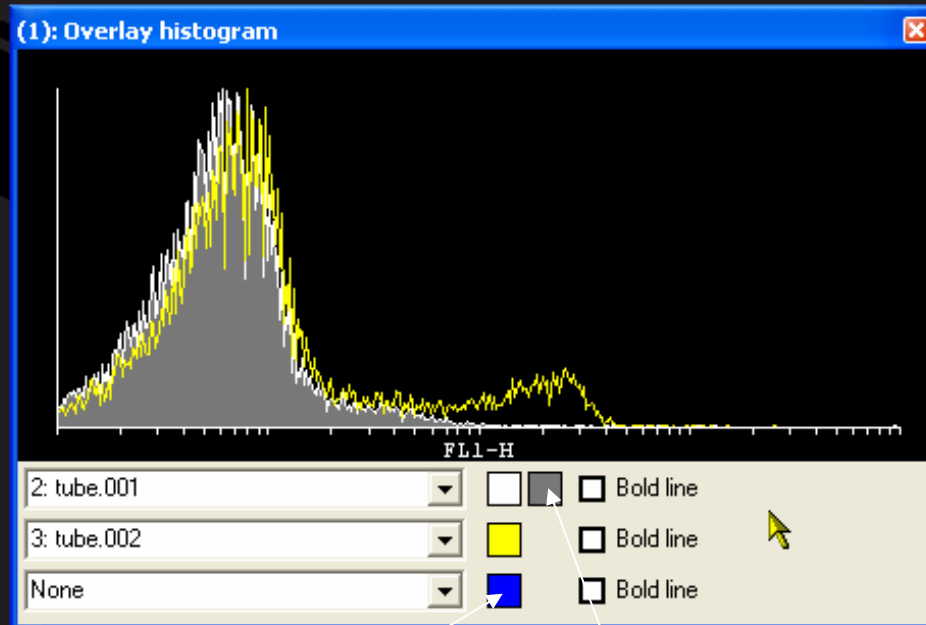
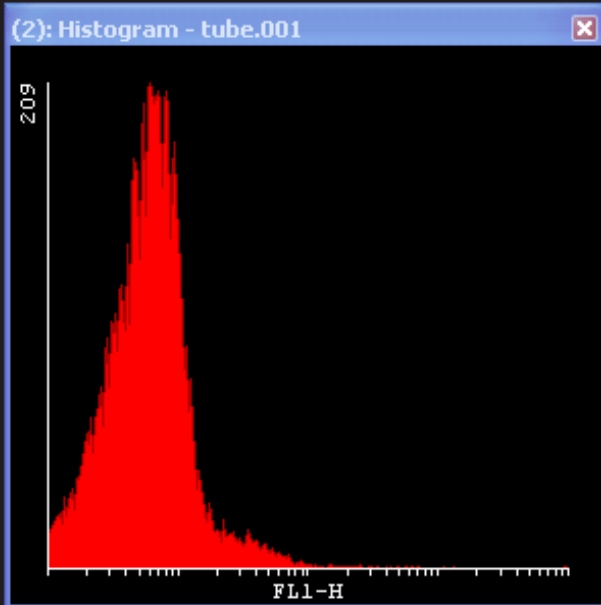
None   Bold line

Overlay histogram window doesn't read the FCS files directly. Instead, you must have normal histogram windows where you have the wanted data. Overlay histogram is the way to combine these histograms into one image.



Drop-down menus include all available histogram windows. Select the wanted histograms from the menus.

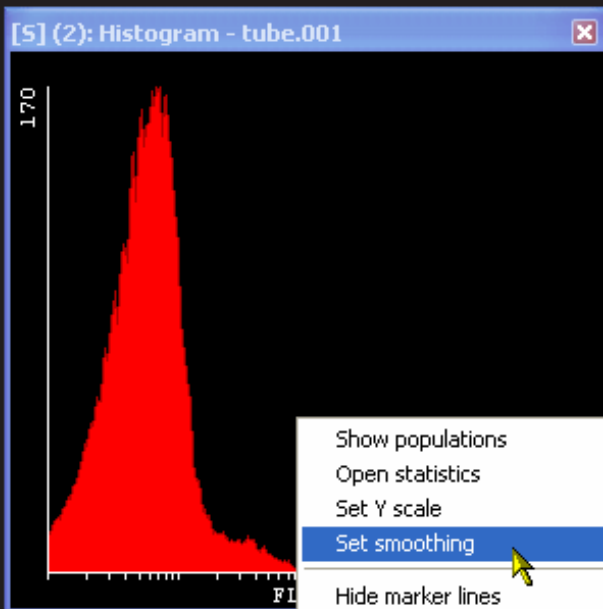
Only the first histogram can be filled.



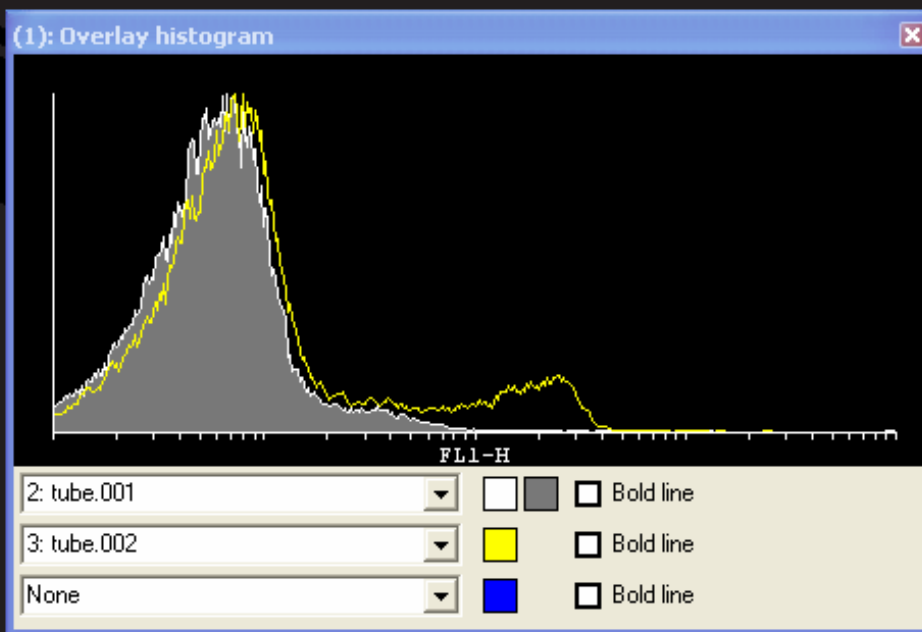
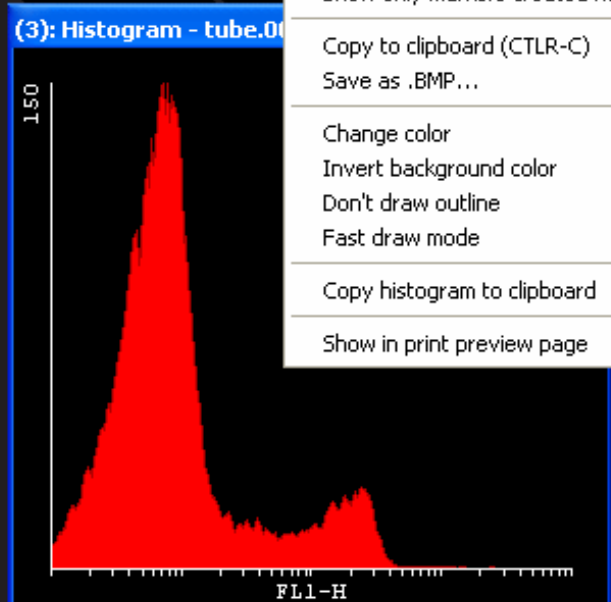
By clicking these you  
Can change the line  
Colors.

By clicking this you  
Can change the  
Fill color of the first  
histogram

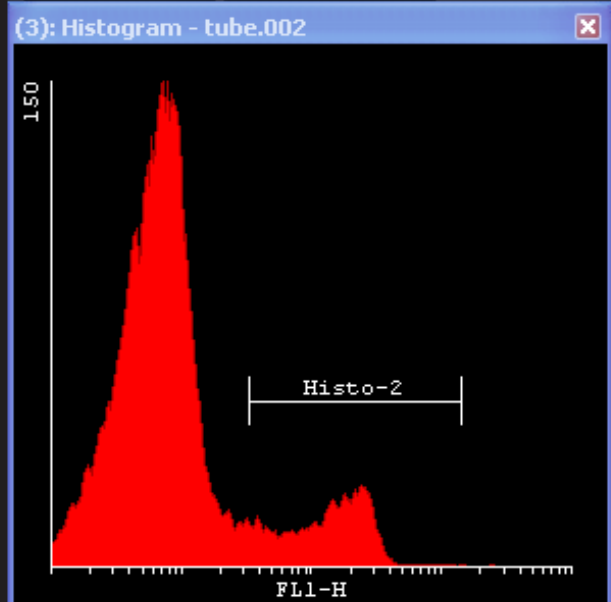
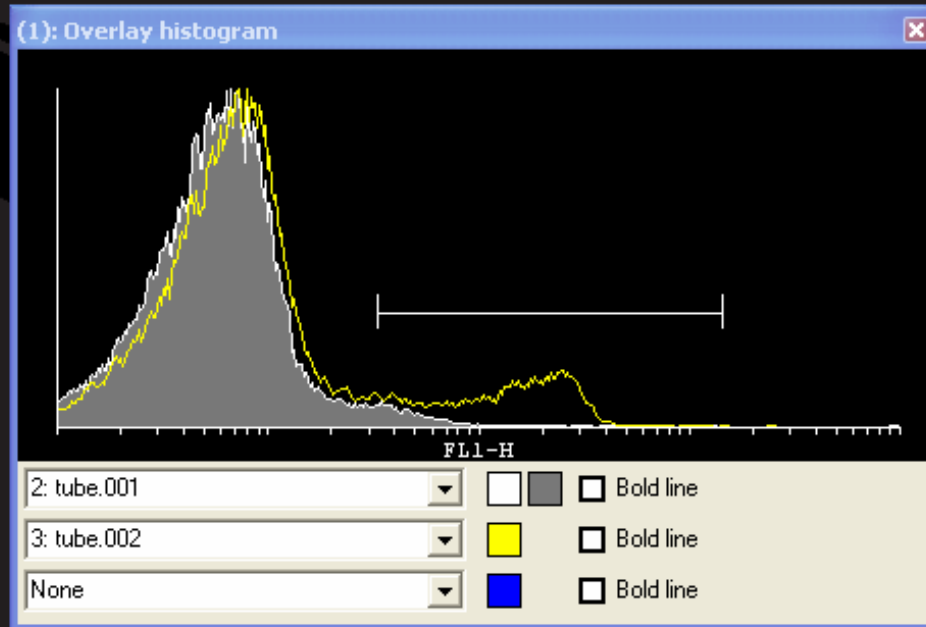
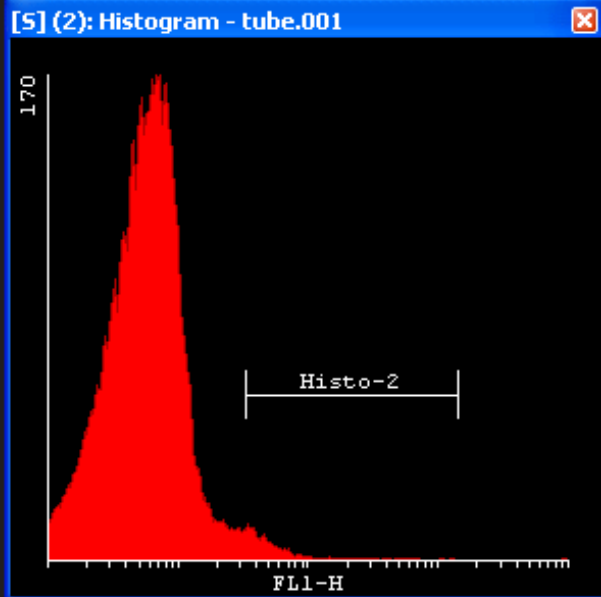
Here you can bold  
the line



- Show populations
- Open statistics
- Set Y scale
- Set smoothing**
- Hide marker lines
- Show only markers created here
- Copy to clipboard (CTRL-C)
- Save as .BMP...
- Change color
- Invert background color
- Don't draw outline
- Fast draw mode
- Copy histogram to clipboard
- Show in print preview page

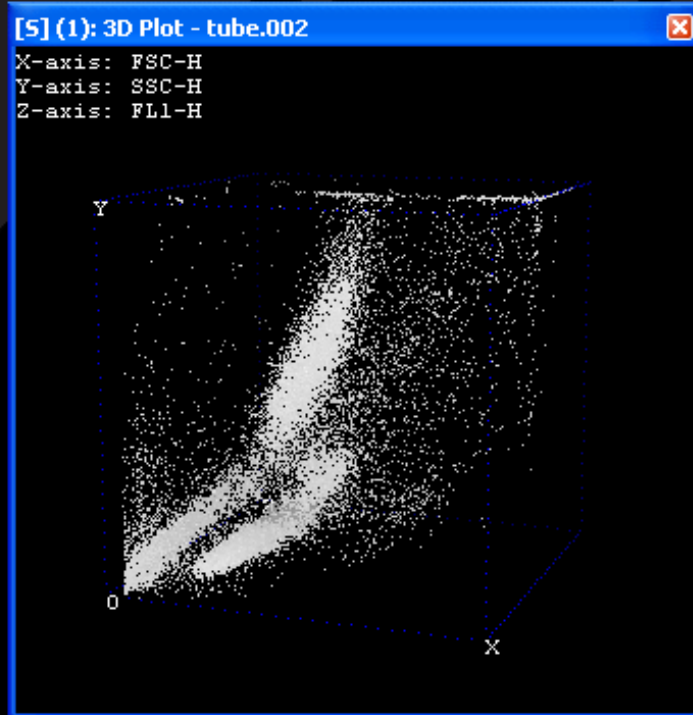


If you make changes into original histograms, they reflect immediately to the overlay histogram. You can scale, smooth and change the file.



The markers of the original histograms can be seen in the overlay histogram. If you want to hide them, select "Hide marker lines" from the pop-up menu.

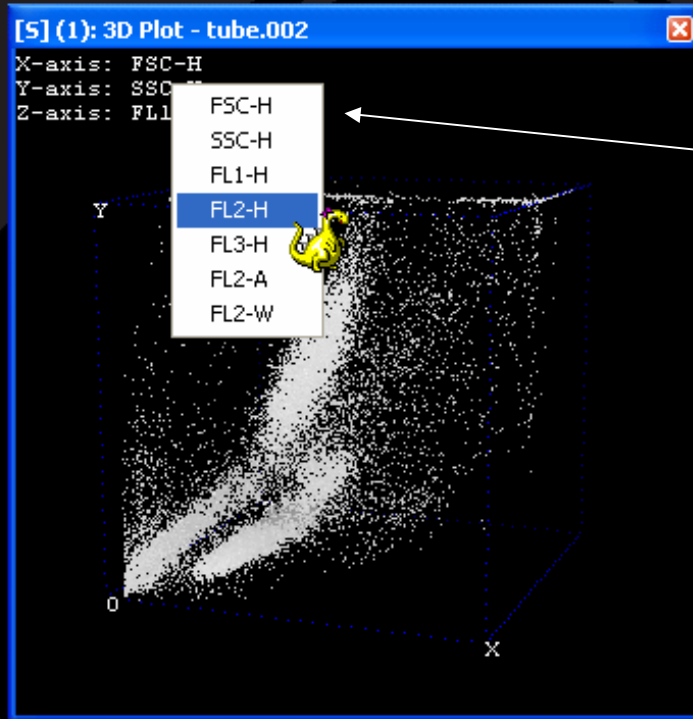
# 3D plot features



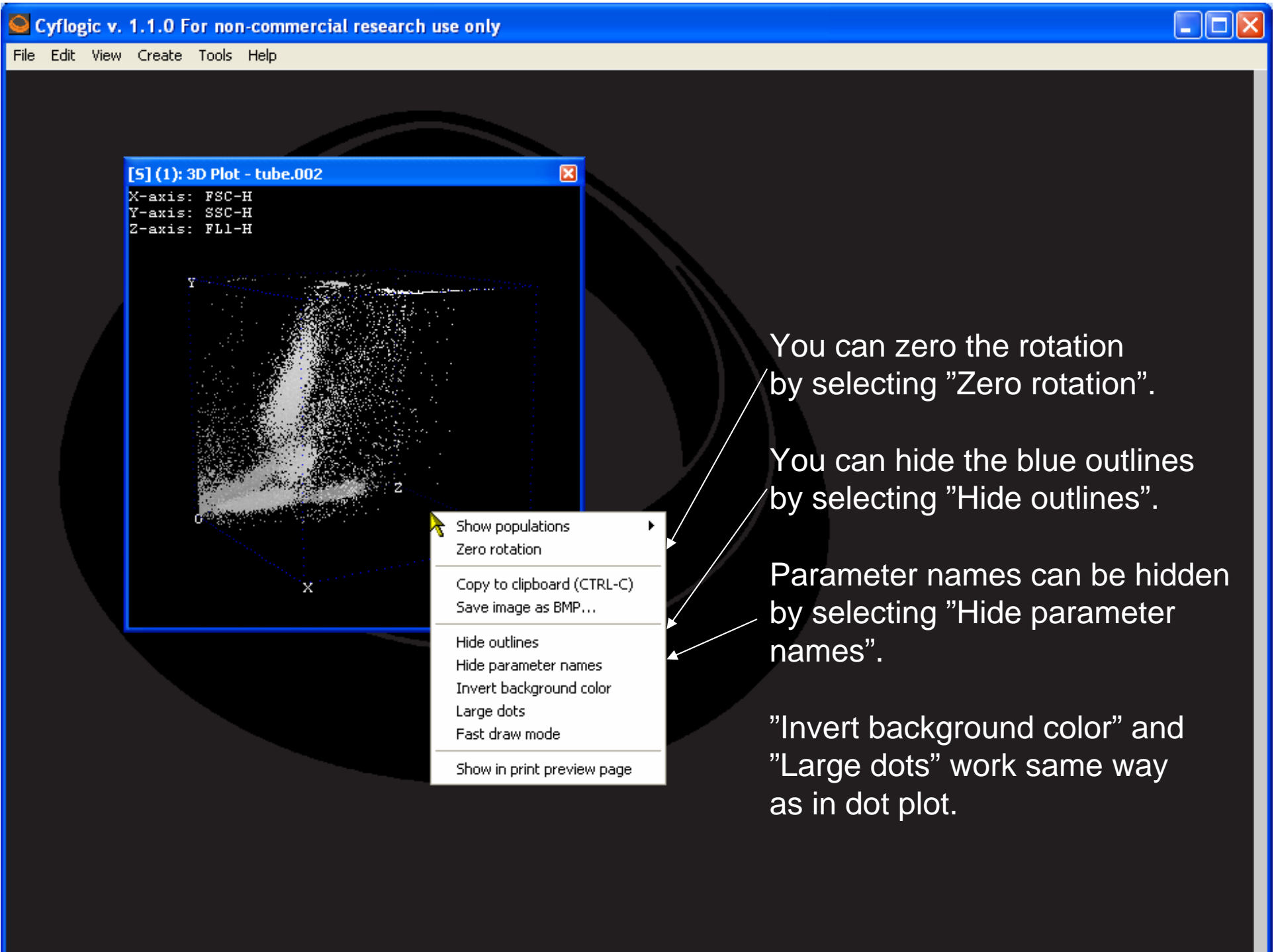
With 3D plot, you can study three Parameters simultaneously.

You can rotate the cube by Pushing the left mouse button down And moving the mouse.





You can change the parameters  
By right-clicking the parameter names.



You can zero the rotation by selecting "Zero rotation".

You can hide the blue outlines by selecting "Hide outlines".

Parameter names can be hidden by selecting "Hide parameter names".

"Invert background color" and "Large dots" work same way as in dot plot.

Tools-menu

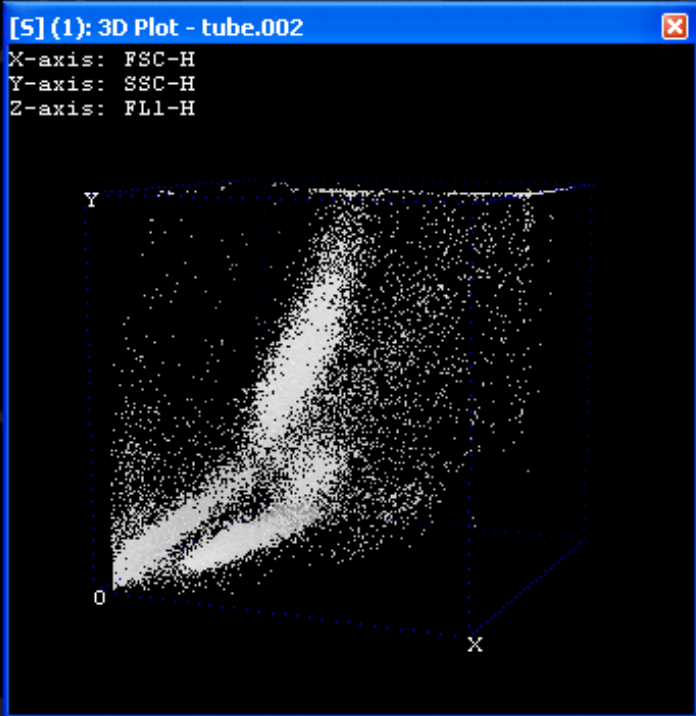
Cyflagic v. 1.1.0 For non-commercial research use only

File Edit View Create Tools Help

- 360 Degrees Rotation
- Batch Print
- Export Statistics to File

[5] (1): 3D Plot - tube.002

X-axis: FSC-H  
Y-axis: SSC-H  
Z-axis: FLL-H



360 degrees rotation:

You can create 360 degrees rotation from your 3D plot. You must have only one 3D plot open. You can have as many other windows, populations and definitions you want.

Select Tools – 360 degrees rotation.

First the tool asks how many degrees step you want. Then it asks for the output folder where the images are saved.

After that, the tool starts to rotate the dot plot. When it is finished, you have the individual images of the animation.

There are several tools how you can combine the images into animation. Check [www.cyflagic.com](http://www.cyflagic.com) for details.

Batch print:

Batch printing enables you to print several prints with one command.

Let's assume that you have 10 files in the folder, and you want that one paper has data of two files (files 1 and 2 in first paper, 3 and 4 in second paper etc).

First create the preview paper with the wanted view (e.g. with two dot plots having files 1 and 2). Then choose Tools – Batch Print.

The tool asks for file increment. That number is the number how many files the windows jumps forward before next printing. Next it asks for the printer. Select the printer.

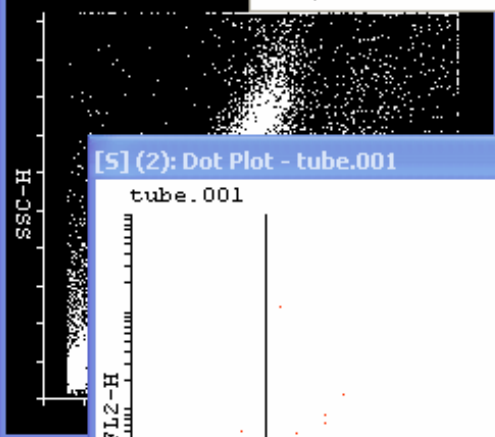
Next page shows the typical analysis layout.

360 Degrees Rotation

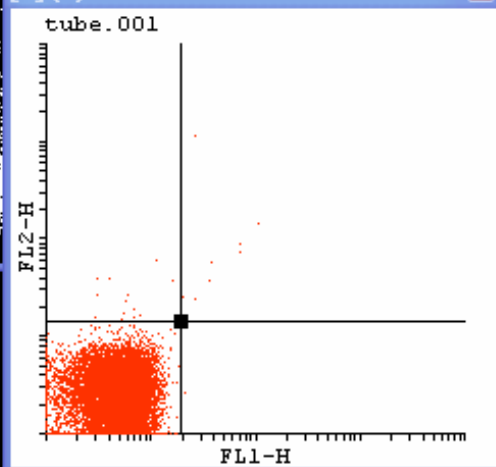
Batch Print

Export Statistics to File

(1): Dot Plot - tube.001



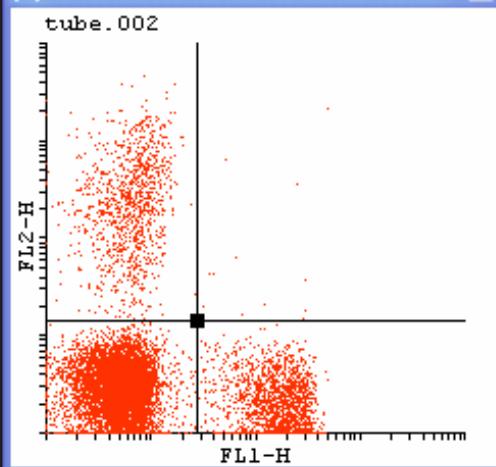
[S] (2): Dot Plot - tube.001



(2) Statistics: tube.001

File name	tube.001	
	Number	% of vis
Visible	11164	100
Upper Left	13	0,11
Upper Right	8	0,07
Lower Left	11141	99,79
Lower Right	2	0,01

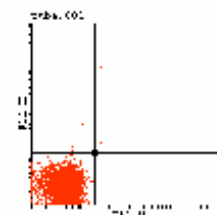
(3): Dot Plot - tube.002



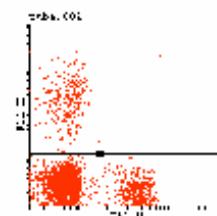
(3) Statistics: tube.002

	Number	% of vis
Visible	11178	100
Upper Left	1080	9,66
Upper Right	13	0,11
Lower Left	7503	67,12
Lower Right	2582	23,09

Print preview window (A4)



File name	Area C	% of vis
	Count	
Visible	11164	100
Upper Left	13	0,11
Upper Right	8	0,07
Lower Left	11141	99,79
Lower Right	2	0,01



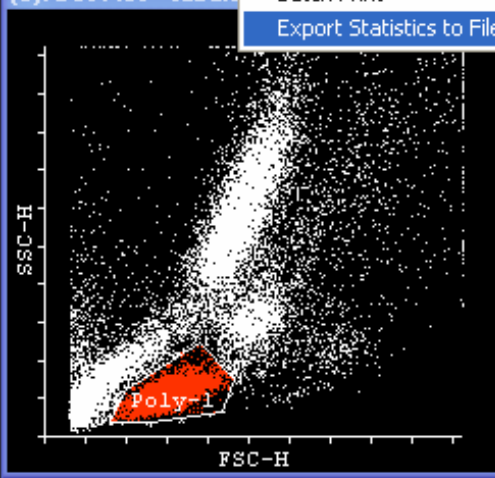
File name	Area C	% of vis
	Count	
Visible	11178	100
Upper Left	1080	9,66
Upper Right	13	0,11
Lower Left	7503	67,12
Lower Right	2582	23,09

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File Edit View Create Tools Help

Tools menu options: 360 Degrees Rotation, Batch Print, Export Statistics to File

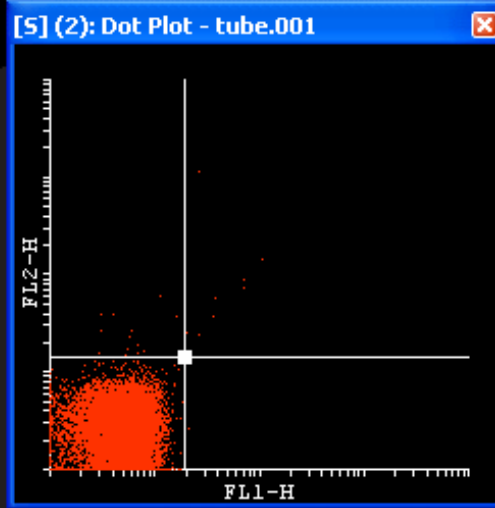
(1): Dot Plot - tube.001



(1) Statistics: tube.001

	Number	% of vis
Visible	26418	100
Poly-1	11164	42,25

[5] (2): Dot Plot - tube.001



(2) Statistics: tube.001

File name	Number	% of vis	X geomean	Y geomean
Visible	11164	100	4,73	2,5
Upper Left	13	0,11	5,78	24,37
Upper Right	8	0,07	42,88	77,91
Lower Left	11141	99,79	4,72	2,49
Lower Right	2	0,01	20,35	2,49

Export statistics to file:

This tool exports the statistics of the files in one folder to text file which can be opened with. E.g Microsoft Excel.

You must have at least one statistics window open. When selecting the tool, it asks for the file name for the export text file. Give the name, for example stats.txt.

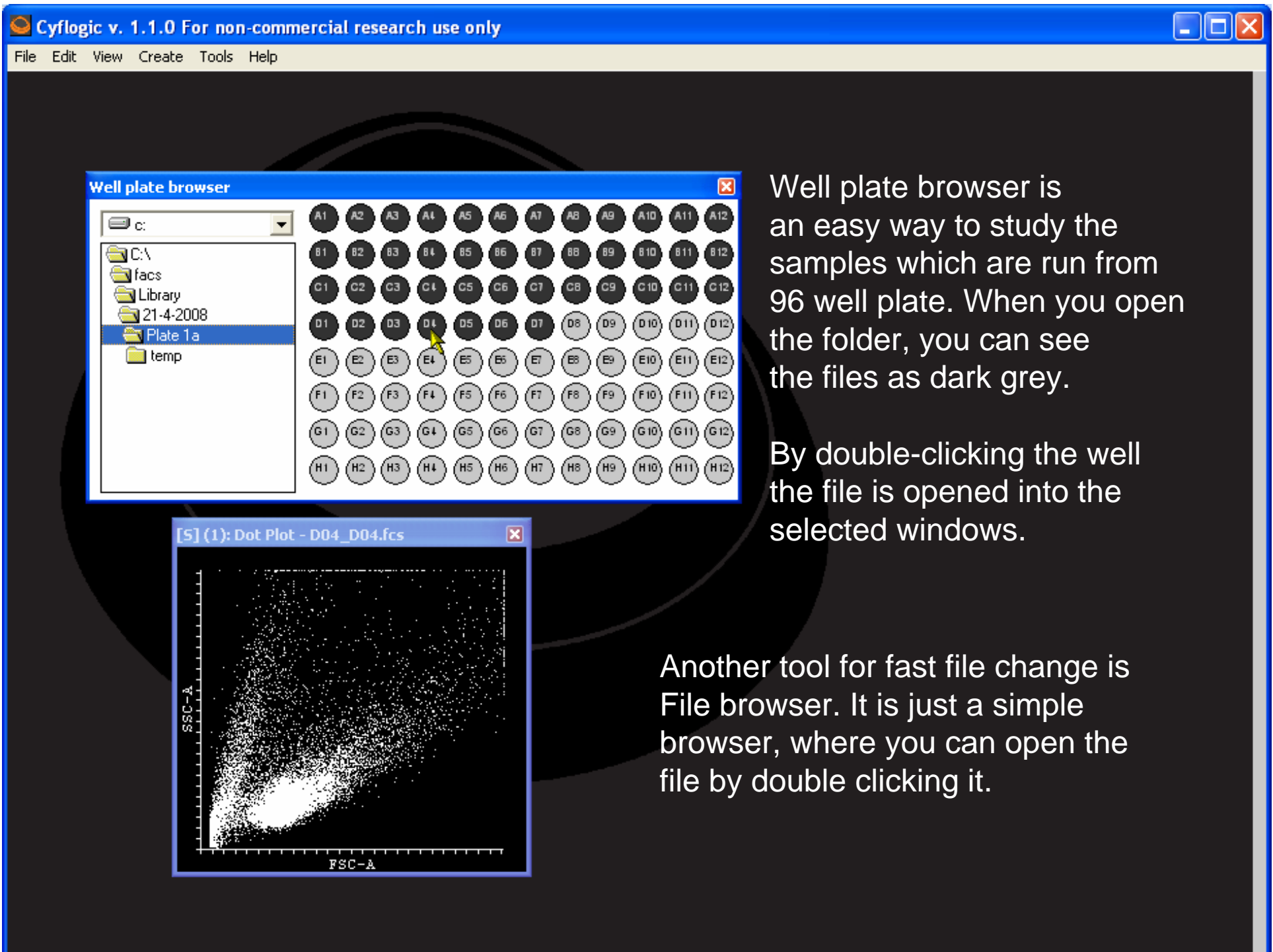
All windows are moved into the first file of the folder, The statistics are exported, and then it moves into next. This continues until all files have gone through.

## Remarks about Tools:

There will be more tools available in the address [www.cyflogic.com](http://www.cyflogic.com). Check frequently the web page.



# Miscellaneous tools

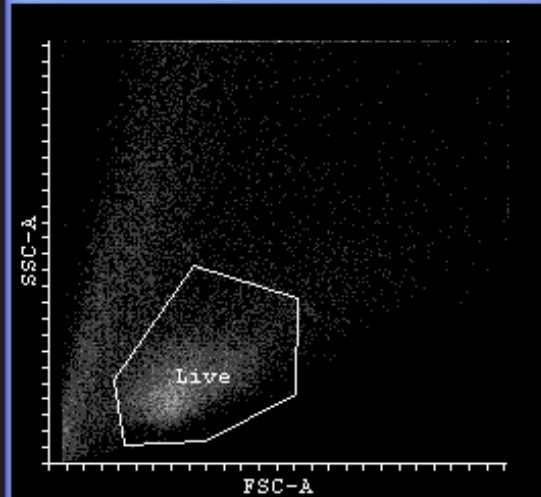


Well plate browser is an easy way to study the samples which are run from 96 well plate. When you open the folder, you can see the files as dark grey.

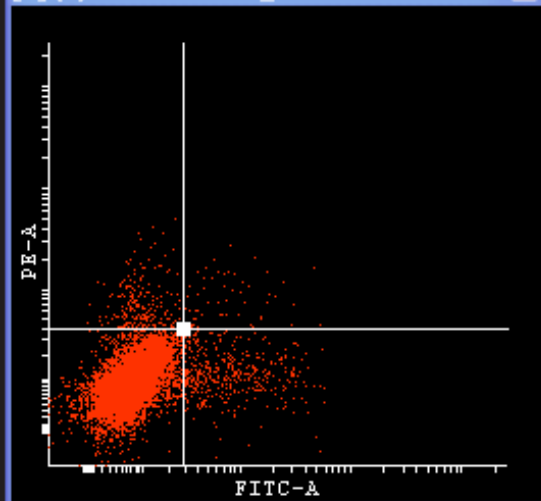
By double-clicking the well the file is opened into the selected windows.

Another tool for fast file change is File browser. It is just a simple browser, where you can open the file by double clicking it.

(1): Dot Plot - D06\_D06.fcs



[S] (2): Dot Plot - D06\_D06.fcs



File info:

**File info**

D06\_D06.fcs

- FSC-A
- SSC-A
- FITC-A
- PE-A
- PerCP-Cy5.5-A
- PE-Cy7-A
- Alexa Fluor 430-A
- Alexa Fluor 405-A
- Indo 1 (Blue)-A
- DAPI-A
- APC-Cy7-A
- APC-A
- Time

Compensate      Show first n events: 17216

	FSC-A	SSC-A	FITC-A	PE-A	PerCP	PE-Cy	Ale
FSC-A	0	0	0	0	0	0	0
SSC-A	0	0	0	0	0	0	0
FITC-A	0	0	0	14	0	0	0
PE-A	0	0	0	0	0	0	0
PerCP-Cy5.5-A	0	0	0	0	0	0	0
PE-Cy7-A	0	0	0	0	0	0	0
Alexa Fluor 430-A	0	0	0	0	0	0	0
Alexa Fluor 405-A	0	0	0	0	0	0	0
Indo 1 (Blue)-A	0	0	0	0	0	0	0
DAPI-A	0	0	0	0	0	0	0
APC-Cy7-A	0	0	0	0	0	0	0
APC-A	0	0	0	0	0	0	0
Time	0	0	0	0	0	0	0

Use these parameter values instead of file parameter values

## File info:

File info tool is a method for observing e.g. the compensation values of the file.

You can also change the values. On the left there is a list of files in memory. Click one. After this, you can change settings:

- By double-clicking the parameter name you can rename it
- By double-clicking the compensation value you can change it.

Attention! Only with FCS3.0 files the real off-line compensation is possible, with other files the results might be quite interesting.

- You can change linear parameter into log and vice versa. This works only with FCS3.0 files.
- You can select how many first events you want to see.

Attention! These changes are not permanent, if you close the file, the changes disappear.

By selecting "Use these parameters instead of file parameters" the parameter names, lin/logs and compensations of all the files are overruled. If you load new files, these parameters overrule their parameters also. Again, these changes are not permanent.

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File Edit View Create Tools Help

### File name handler

**Original files**

c:\

- C:\
- facts
- Library
- 22-4-2008
- Plate 1A

analysis.cyflagic  
Sample\_001\_Well\_001.fcs  
Sample\_002\_Well\_001.fcs  
Sample\_003\_Well\_001.fcs  
Sample\_004\_Well\_001.fcs  
Sample\_005\_Well\_001.fcs  
Sample\_006\_Well\_001.fcs  
Sample\_007\_Well\_001.fcs  
Sample\_008\_Well\_001.fcs  
Sample\_009\_Well\_001.fcs  
Sample\_010\_Well\_001.fcs  
Sample\_011\_Well\_001.fcs  
Sample\_012\_Well\_001.fcs  
Sample\_013\_Well\_001.fcs  
Sample\_014\_Well\_001.fcs

**Actions**

Change "A1" -> "A01" etc.

Replace  ->

**Preview**

Make the preview

analysis.cyflagic  
Sample\_001.fcs  
Sample\_002.fcs  
Sample\_003.fcs  
Sample\_004.fcs  
Sample\_005.fcs  
Sample\_006.fcs  
Sample\_007.fcs  
Sample\_008.fcs  
Sample\_009.fcs  
Sample\_010.fcs  
Sample\_011.fcs  
Sample\_012.fcs

Make the changes!

## File name handler

With this tool, you can change the file names of one folder.

E.g. if your file names have something extra which you want to get rid of (e.g. in this example "\_Well\_001" in every file name) you can use action "Replace". Give the original string and the string to be replaced (or leave it empty). Then press "Make the preview" to see what would be result. If you are happy, press "Make the changes".

You can also change well names so that they are alphabetized correctly.

Save analysis view

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File Edit View Create Tools Help

- Open FCS... Ctrl+O
- Next file Ctrl+N
- Previous file Ctrl+P
- File name handler
- Open analysis view...
- Save analysis view...
- Print preview
- Print...
- Exit

(3): Histogram - tube.002

[5] (2): Dot Plot - tube.002

(2) Statistics: tube.002

	Number	% of vis
Visible	11160	100
Upper Left	1054	9,44
Upper Right	10	0,08
Lower Left	7510	67,29
Lower Right	2586	23,17

(3) Statistics: tube.002

	Number	% of vis	X mean	X geomean	CV
Visible	11160	100	49,17	11,4	182,16
Positive	2561	22,94	194,76	173,51	44,22

By choosing "Save analysis View" you can save your Analysis. Windows, Populations and statistics Are saved.

When you want to restore The analysis view, choose "Open analysis view" and Open the analysis file.

# Comments, ideas, bugs?

If you find a bug, get the idea or want to comment the features of Cyflogic, go to [www.cyflogic.com](http://www.cyflogic.com) – Discussion. There you can find the idea and bug discussion pages where you can tell your ideas!