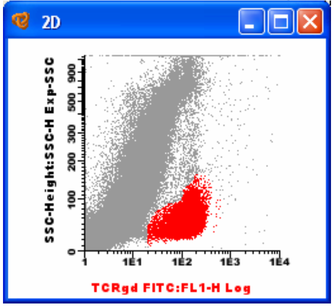
INFINICYT^{TM}, 1.1 VERSION

To improve the benefits that this software offers, Infinicyt^{TM} 1.1 has included the following:

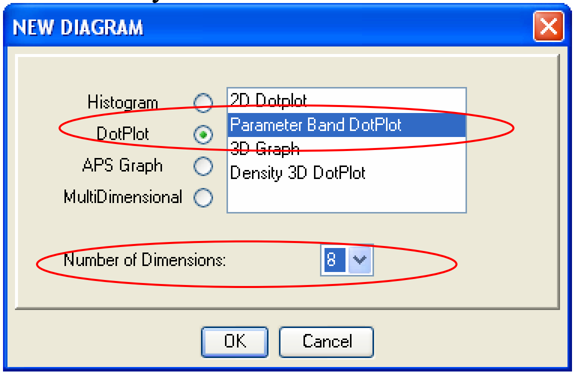
1. <u>The FL-based merger option has been deleted</u>.

In the version 1.1 there is a unique merger option since it is possible to obtain the information that in the previous version was obtained with the FL-based merger option, from the new “Parameter Band Dot Plot” diagram.

1. Select the population of interest in the file resulting from the merger.

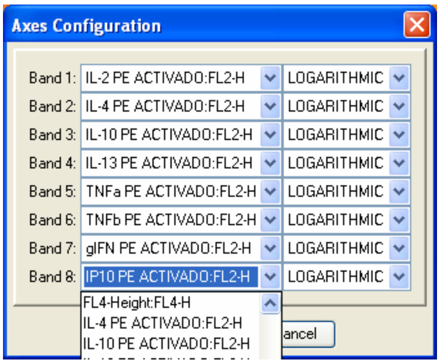


2. Open a new diagram type “Parameter Band Dot Plot” selecting as number of dimensions the number of parameters that you want to visualize from the merged file.

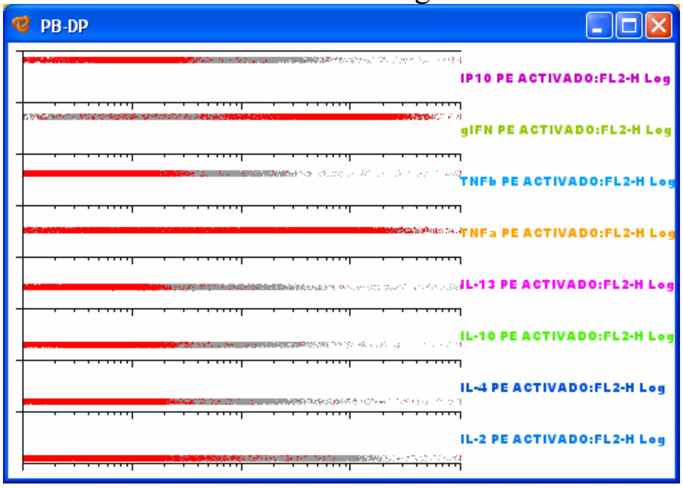


Number of Dimensions: 8

3. By right-clicking in the middle of the new Parameter Band Dot Plot, go to “Graph Configuration” menu and select “Axes Configuration”. Through this command the user may select the parameters to visualize on each band of the new diagram:



Band 1: IL-2 PE ACTIVADO:FL2-H LOGARITHMIC
Band 2: IL-4 PE ACTIVADO:FL2-H LOGARITHMIC
Band 3: IL-10 PE ACTIVADO:FL2-H LOGARITHMIC
Band 4: IL-13 PE ACTIVADO:FL2-H LOGARITHMIC
Band 5: TNFa PE ACTIVADO:FL2-H LOGARITHMIC
Band 6: TNFb PE ACTIVADO:FL2-H LOGARITHMIC
Band 7: gIFN PE ACTIVADO:FL2-H LOGARITHMIC
Band 8: IP10 PE ACTIVADO:FL2-H LOGARITHMIC

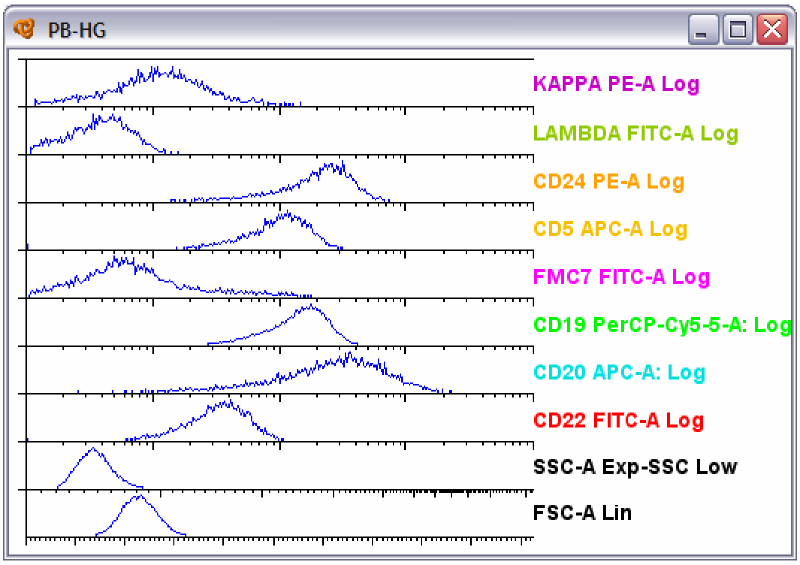


Parameter Band Dot Plot shows the legend of the analyzed parameters.
Each band represents the real expression of the parameter selected in the original file.
See detailed information about the different options related to the file merger process in chapter 4.2 of the User’s Manual.

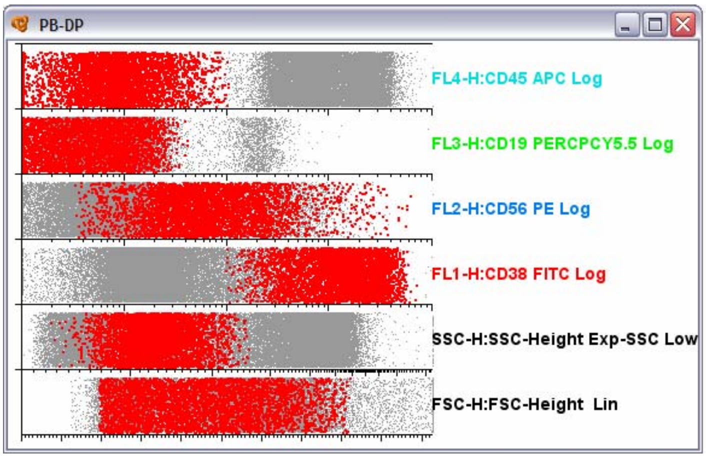
2. <u>New options and types of Graphs</u> have been created to show information.

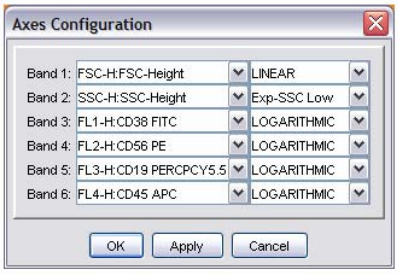
See detailed information about types of graphic representations in chapter 5.3 of the User's Manual.

In the version 1.1 of Infinicyt^{TM}, <u>Parameter Band Histogram</u> and <u>Parameter Band Dot Plot</u> have been included as new type of graphics that allow to select as many different bands as parameters desired to be represented in the same graphic. The number of bands can be as many as the number of parameters in the data file. For example in a merged file, we could obtain a Parameter Band Histogram with "n" bands, one for each parameter.



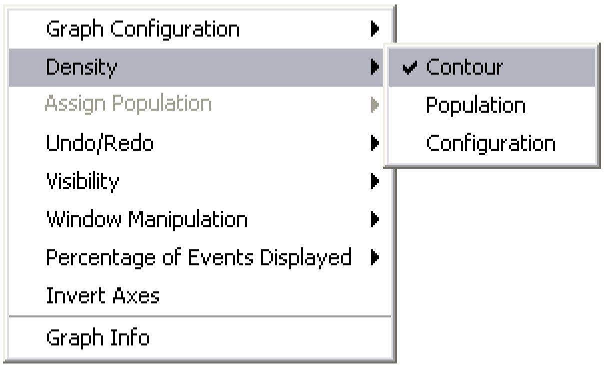
The following Parameter Band Dot Plot shows on red the phenotypic expression of the plasma cells population previously selected.



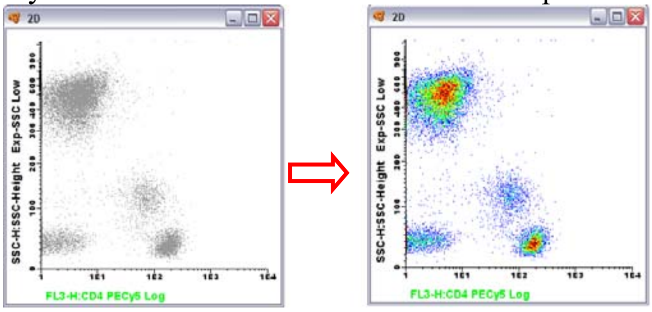


Band 1: FSC-H:FSC-Height LINEAR
Band 2: SSC-H:SSC-Height Exp-SSC Low
Band 3: FL1-H:CD38 FITC LOGARITHMIC
Band 4: FL2-H:CD56 PE LOGARITHMIC
Band 5: FL3-H:CD19 PERCPCY5.5 LOGARITHMIC
Band 6: FL4-H:CD45 APC LOGARITHMIC

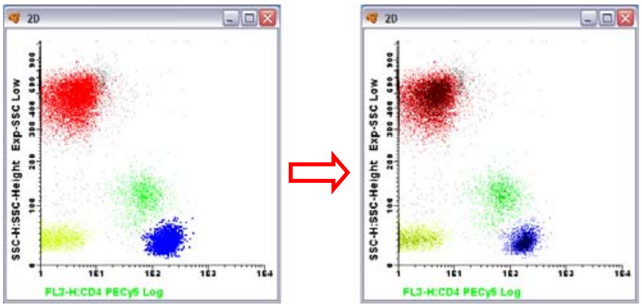
In some assays it will be very useful to visualize the data with Density Representations since they can help to better distinguish the different cellular populations. Version 1.1 of Infinicyt^{TM} allows visualizing the data as dot plots or density views in different types of graphs. To change to a density view, right-click on the diagram and select “Density”



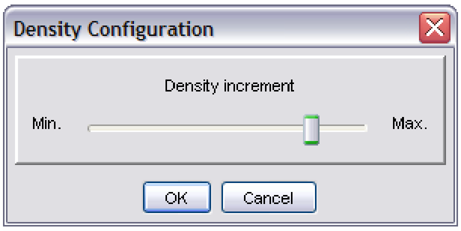
1. “Countour Density” considers all the events visualized to perform a density display.



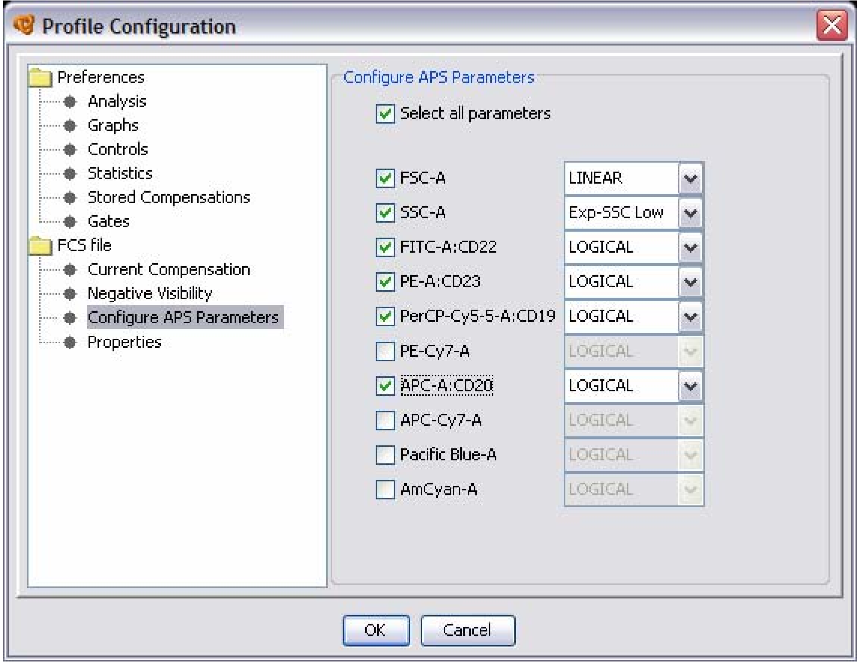
2. “Population density” considers the population already classified to maintain the colours for the density representation. The density will be represented degrading the choosen colour.



3. “Configuration” allows the user to choose the scale of density to show in the graphic.



Using the new Automatic Population Separator (APS) graph, in the 1.1 version of Infinicyt^{TM}, the user can choose the parameters to use in order to obtain the best separation of populations. The software will take into account both the parameters selected and the rest of events independently. This option is available from the "Configure APS Parameter" window of the "Profile Configuration" menu and it is especially useful in merged files where we will select only the common parameters for the APS calculation.

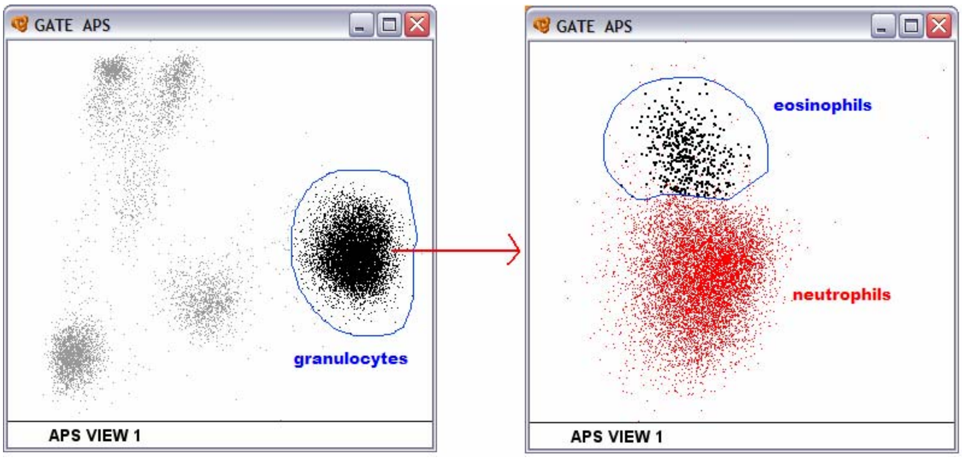


Configure APS Parameters

☑ Select all parameters

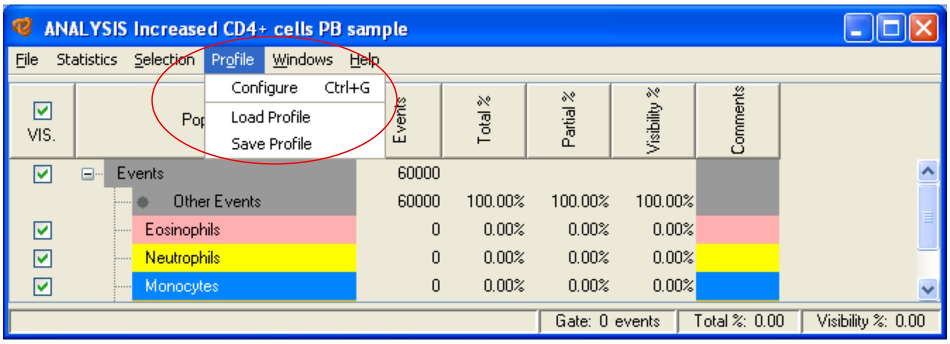
☑ FSC-A	LINEAR
☑ SSC-A	Exp-SSC Low
☑ FITC-A:CD22	LOGICAL
☑ PE-A:CD23	LOGICAL
☑ PerCP-Cy5-5-A:CD19	LOGICAL
☐ PE-Cy7-A	LOGICAL
☑ APC-A:CD20	LOGICAL
☐ APC-Cy7-A	LOGICAL
☐ Pacific Blue-A	LOGICAL
☐ AmCyan-A	LOGICAL

The aim of APS graph is for the user to be able to distinguish in these representations as many different populations as possible (especially useful when working with a high number of parameters) for a further characterization of these populations in the conventional 2D graphics.
New in this version, whenever you gate and classify a population, the APS graph will calculate again the view taking into account selected populations. This way, it is possible to distinguish even more subpopulations among already-classified events.



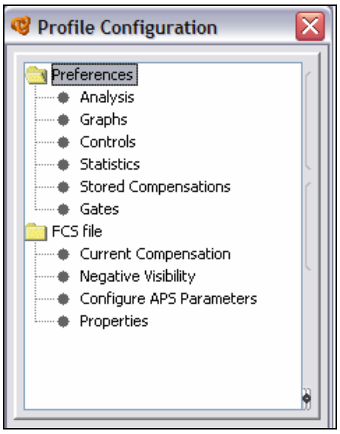
3. It is possible to create and save <u>Analysis Profiles</u> for further uses.

In order to facilitate and standardize the analysis of similar samples, version 1.1 of Infinicyt™ allows to configure, to save and to load for further uses different analysis profiles.



VIS.	Pop	Events	Total %	Partial %	Visibility %	Comments
☑	Events	60000				
	Other Events	60000	100.00%	100.00%	100.00%	
☑	Eosinophils	0	0.00%	0.00%	0.00%	
☑	Neutrophils	0	0.00%	0.00%	0.00%	
☑	Monocytes	0	0.00%	0.00%	0.00%	

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00



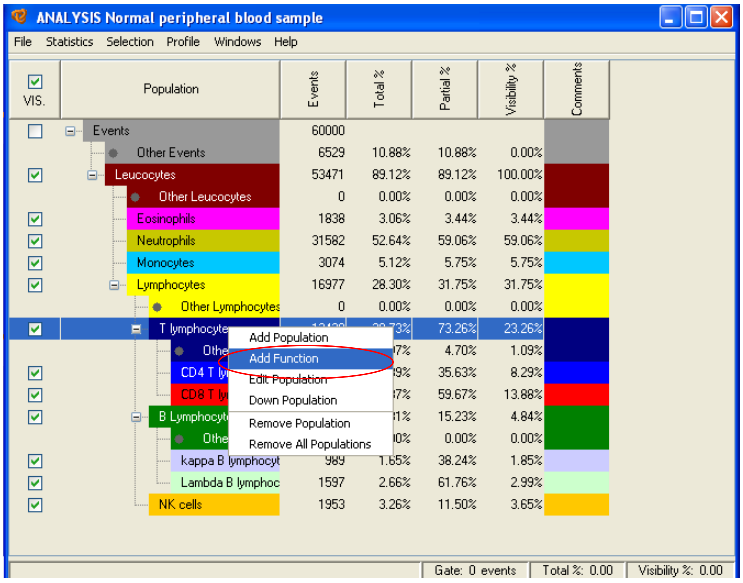
A profile is all the information about the software configuration. It is possible to create different profiles for different diseases, so the user can always work with the same graphs, population tree, controls, statistics, compensations, gates, report, etc with the same type of study.
It is recommended to see detailed information about Analysis Profiles in chapter 5.1.4 of the User's Manual.

3.1. <u>Statistics</u>

3.1.1. It is possible to use results calculation formula.

The result of the formula can be located in the populations tree, if the created formula relates different previously established populations (for example CD4/CD8 ratio), or in the columns relating to the statistics in the Analysis Window.

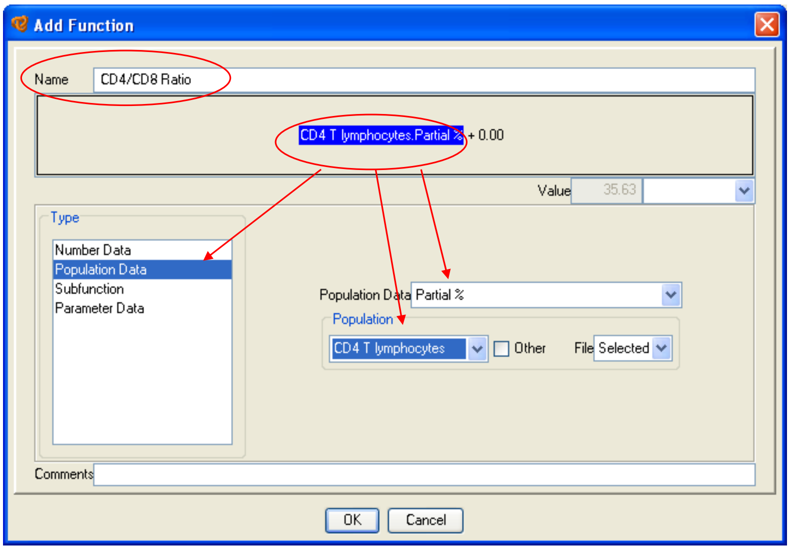
3.1.1.a) In order to place the result of the formula as a new population in the Population Tree, right-click on the first label of "Events" of the Population Tree and select "Add Function".



VIS.	Population	Events	Total %	Partial %	Visibility %	Comments
☐	Events	60000				
	Other Events	6529	10.88%	10.88%	0.00%	
☑	Leucocytes	53471	89.12%	89.12%	100.00%	
	Other Leucocytes	0	0.00%	0.00%	0.00%	
☑	Eosinophils	1838	3.06%	3.44%	3.44%	
☑	Neutrophils	31582	52.64%	59.06%	59.06%	
☑	Monocytes	3074	5.12%	5.75%	5.75%	
☑	Lymphocytes	16977	28.30%	31.75%	31.75%	
	Other Lymphocytes	0	0.00%	0.00%	0.00%	
☑	T lymphocyte	[illegible]	[illegible]	73.26%	23.26%	
	Othe		7%	4.70%	1.09%	
☑	CD4 T ly		9%	35.63%	8.29%	
☑	CD8 T ly		7%	59.67%	13.88%	
☑	B Lymphocyt		1%	15.23%	4.84%	
	Othe		0%	0.00%	0.00%	
☑	kappa B lymphocyt	989	1.65%	38.24%	1.85%	
☑	Lambda B lymphoc	1597	2.66%	61.76%	2.99%	
☑	NK cells	1953	3.26%	11.50%	3.65%	

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

A new window will open where the user can write the name of the function desired and choose the formula.



Name: CD4/CD8 Ratio

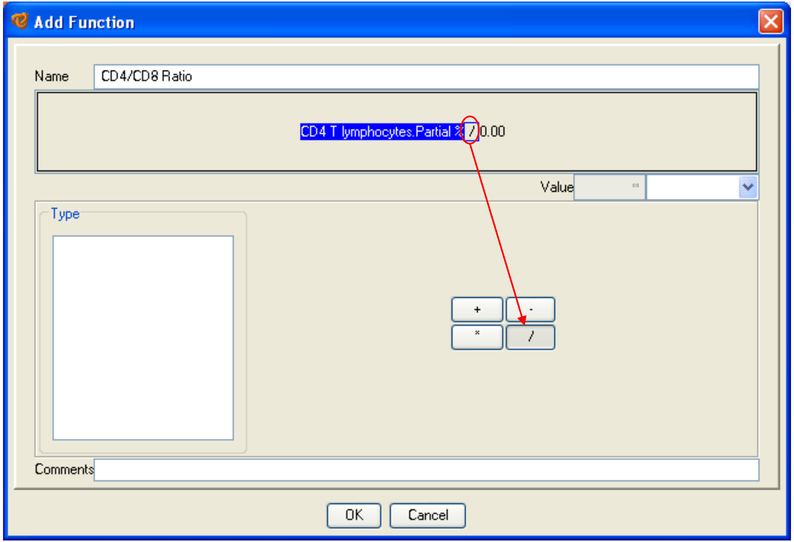
CD4 T lymphocytes.Partial % + 0.00

Value: 35.63

Population Data: Partial %

Population: CD4 T lymphocytes ☐ Other File: Selected

Comments:

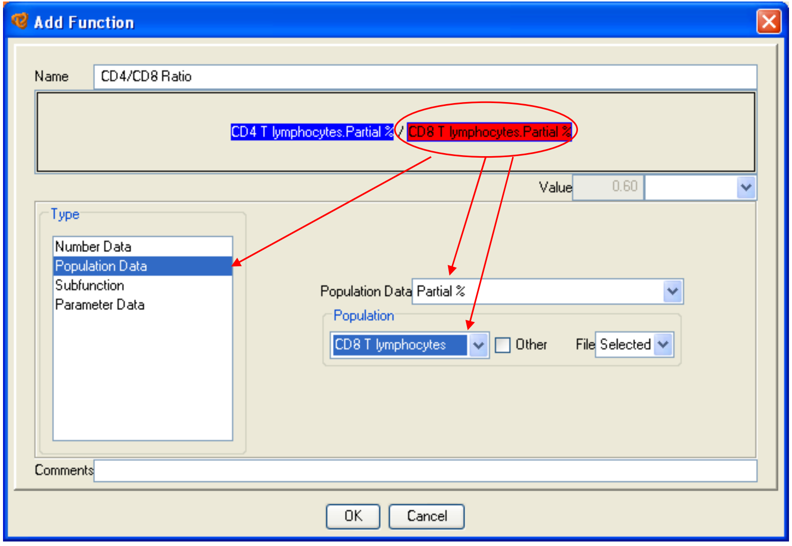


Name: CD4/CD8 Ratio

CD4 T lymphocytes.Partial % / 0.00

Value:

Comments:



Name: CD4/CD8 Ratio

CD4 T lymphocytes.Partial % / CD8 T lymphocytes.Partial %

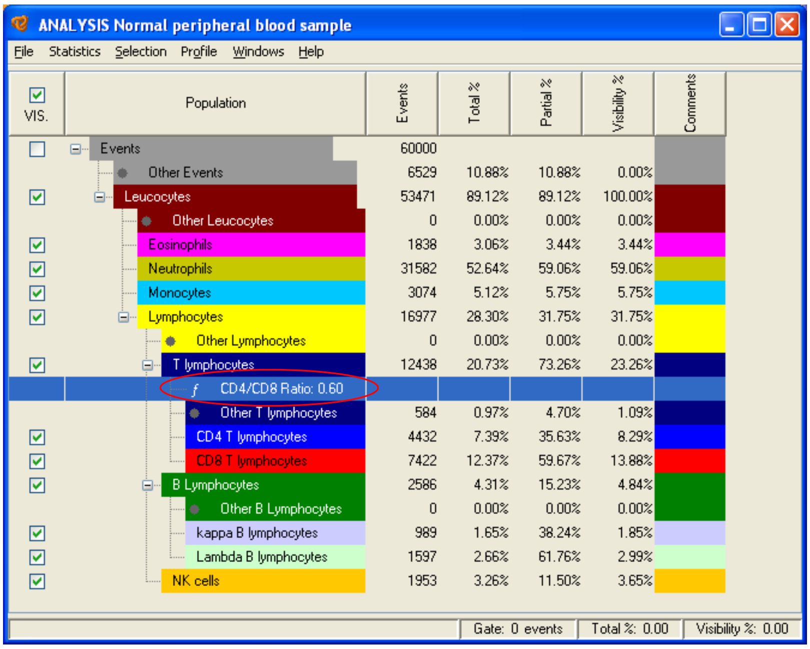
Value: 0.60

Population Data: Partial %

Population: CD8 T lymphocytes ☐ Other File: Selected

Comments:

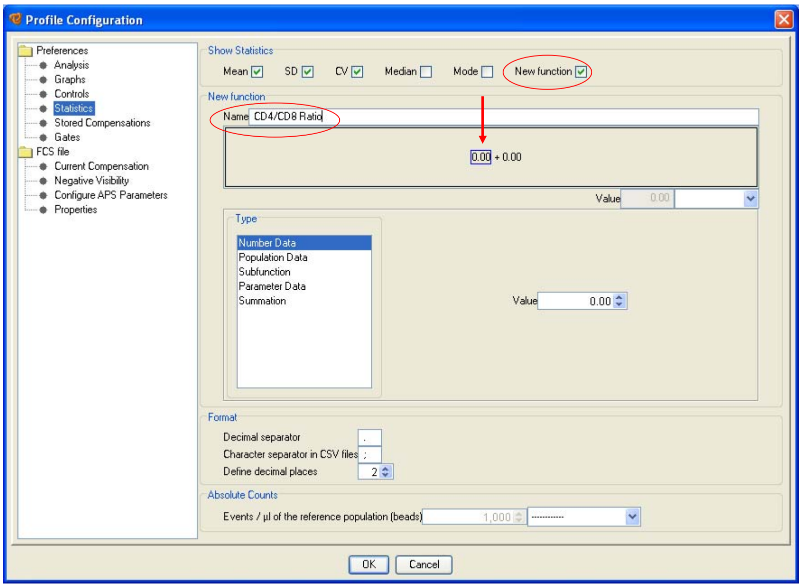
Once the formula is finished, click OK and the new function will not be a new column with values for each population, but a unique value shown as a new population of the Population Tree.



☑ VIS.	Population	Events	Total %	Partial %	Visibility %	Comments
☐	Events	60000				
	Other Events	6529	10.88%	10.88%	0.00%	
☑	Leucocytes	53471	89.12%	89.12%	100.00%	
	Other Leucocytes	0	0.00%	0.00%	0.00%	
☑	Eosinophils	1838	3.06%	3.44%	3.44%	
☑	Neutrophils	31582	52.64%	59.06%	59.06%	
☑	Monocytes	3074	5.12%	5.75%	5.75%	
☑	Lymphocytes	16977	28.30%	31.75%	31.75%	
	Other Lymphocytes	0	0.00%	0.00%	0.00%	
☑	T lymphocytes	12438	20.73%	73.26%	23.26%	
	ƒ CD4/CD8 Ratio: 0.60					
	Other T lymphocytes	584	0.97%	4.70%	1.09%	
☑	CD4 T lymphocytes	4432	7.39%	35.63%	8.29%	
☑	CD8 T lymphocytes	7422	12.37%	59.67%	13.88%	
☑	B Lymphocytes	2586	4.31%	15.23%	4.84%	
	Other B Lymphocytes	0	0.00%	0.00%	0.00%	
☑	kappa B lymphocytes	989	1.65%	38.24%	1.85%	
☑	Lambda B lymphocytes	1597	2.66%	61.76%	2.99%	
☑	NK cells	1953	3.26%	11.50%	3.65%	

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

3.1.1.b) In order to show the result of the formula in the columns relating to the statistics in the Analysis Window, go to Profile Configuration menu click Statistics and activate the “New Function” option. A new window will open, where the user can write the name of the function desired and choose the formula.



Show Statistics

Mean ☑ SD ☑ CV ☑ Median ☐ Mode ☐ New function ☑

New function

Name CD4/CD8 Ratio

0.00 + 0.00

Value 0.00

Type

- Number Data
- Population Data
- Subfunction
- Parameter Data
- Summation

Value 0.00

Format

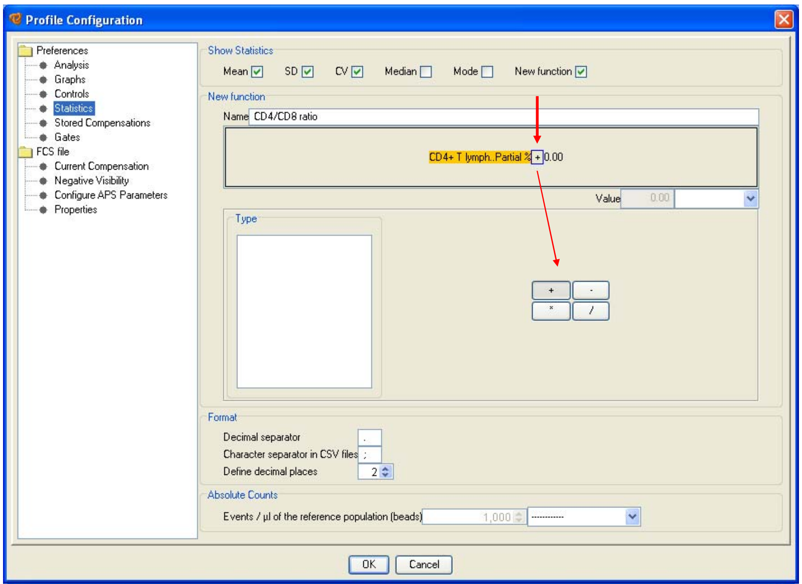
Decimal separator .

Character separator in CSV files ;

Define decimal places 2

Absolute Counts

Events / µl of the reference population (beads) 1,000 ----------



Mean ☑ SD ☑ CV ☑ Median ☐ Mode ☐ New function ☑

Name CD4/CD8 ratio

CD4+ T lymph..Partial % + 0.00

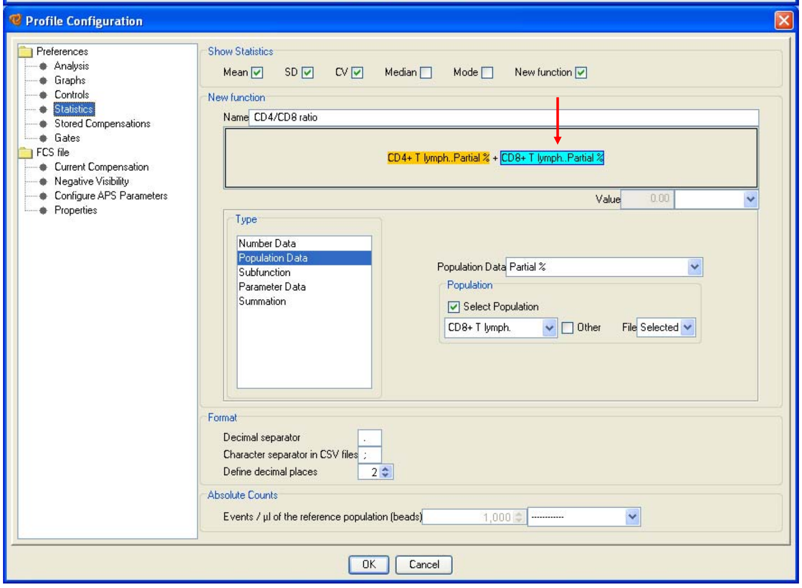
Value 0.00

Decimal separator .

Character separator in CSV files ;

Define decimal places 2

Events / µl of the reference population (beads) 1,000 ----------



Mean ☑ SD ☑ CV ☑ Median ☐ Mode ☐ New function ☑

Name CD4/CD8 ratio

CD4+ T lymph..Partial % + CD8+ T lymph..Partial %

Value 0.00

Type: Population Data

Population Data Partial %

☑ Select Population

CD8+ T lymph. ☐ Other File Selected

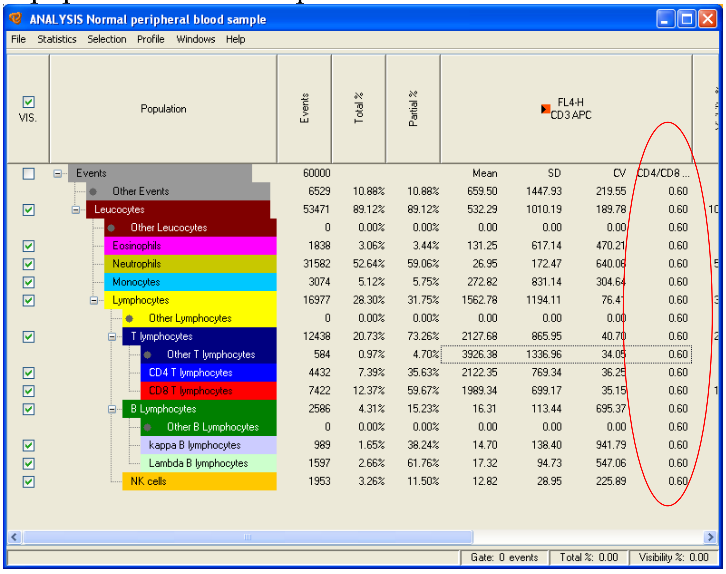
Decimal separator .

Character separator in CSV files ;

Define decimal places 2

Events / µl of the reference population (beads) 1,000 ----------

Once the formula is finished, click OK and the new function will be a new statistic column with values for each population and each parameter.

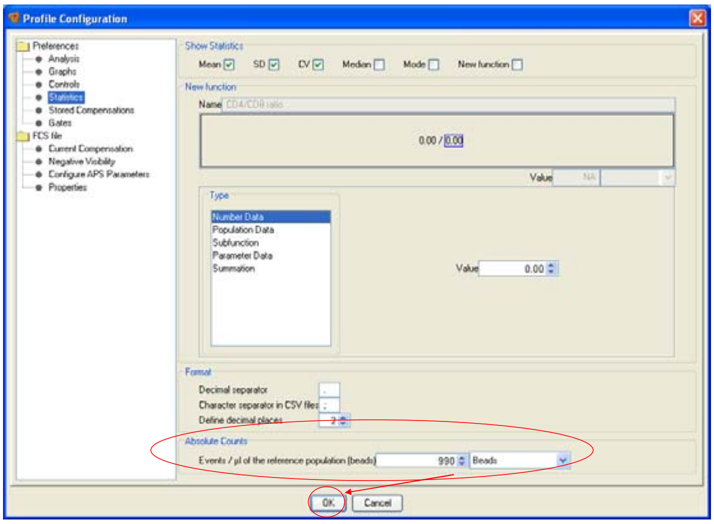


☑ VIS.	Population	Events	Total %	Partial %	FL4-H CD3 APC Mean	SD	CV	CD4/CD8 ...
☐	Events	60000						
	Other Events	6529	10.88%	10.88%	659.50	1447.93	219.55	0.60
☑	Leucocytes	53471	89.12%	89.12%	532.29	1010.19	189.78	0.60
	Other Leucocytes	0	0.00%	0.00%	0.00	0.00	0.00	0.60
☑	Eosinophils	1838	3.06%	3.44%	131.25	617.14	470.21	0.60
☑	Neutrophils	31582	52.64%	59.06%	26.95	172.47	640.06	0.60
☑	Monocytes	3074	5.12%	5.75%	272.82	831.14	304.64	0.60
☑	Lymphocytes	16977	28.30%	31.75%	1562.78	1194.11	76.41	0.60
	Other Lymphocytes	0	0.00%	0.00%	0.00	0.00	0.00	0.60
☑	T lymphocytes	12438	20.73%	73.26%	2127.68	865.95	40.70	0.60
	Other T lymphocytes	584	0.97%	4.70%	3926.38	1336.96	34.05	0.60
☑	CD4 T lymphocytes	4432	7.39%	35.63%	2122.35	769.34	36.25	0.60
☑	CD8 T lymphocytes	7422	12.37%	59.67%	1989.34	699.17	35.15	0.60
☑	B Lymphocytes	2586	4.31%	15.23%	16.31	113.44	695.37	0.60
	Other B Lymphocytes	0	0.00%	0.00%	0.00	0.00	0.00	0.60
☑	kappa B lymphocytes	989	1.65%	38.24%	14.70	138.40	941.79	0.60
☑	Lambda B lymphocytes	1597	2.66%	61.76%	17.32	94.73	547.06	0.60
☑	NK cells	1953	3.26%	11.50%	12.82	28.95	225.89	0.60

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

3.1.2. Absolute counts from the population of reference selected by the user.

To calculate absolute counts there is no need to create the formula. The user can choose which population will be taken as reference for the calculation in the “Absolute Counts” option of the Statistics Window of the “Profile Configuration” menu.



Mean ☑ SD ☑ CV ☑ Median ☐ Mode ☐ New function ☐

Name CD4/CD8 ratio

0.00 / 0.00

Value NA

Value 0.00

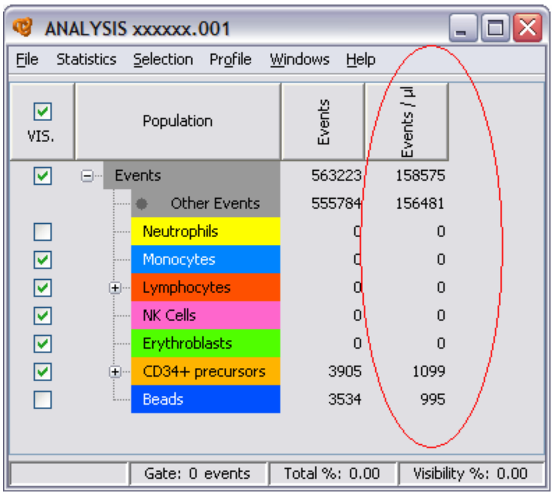
Decimal separator .

Character separator in CSV files ;

Define decimal places 2

Events / µl of the reference population (beads) 990 Beads

Once the population which will be taken as reference for the calculations and the number of event per microlitre of this population is selected, the software will automatically show a new column in the Analysis Window with the absolute counts (events /µl) of each population.

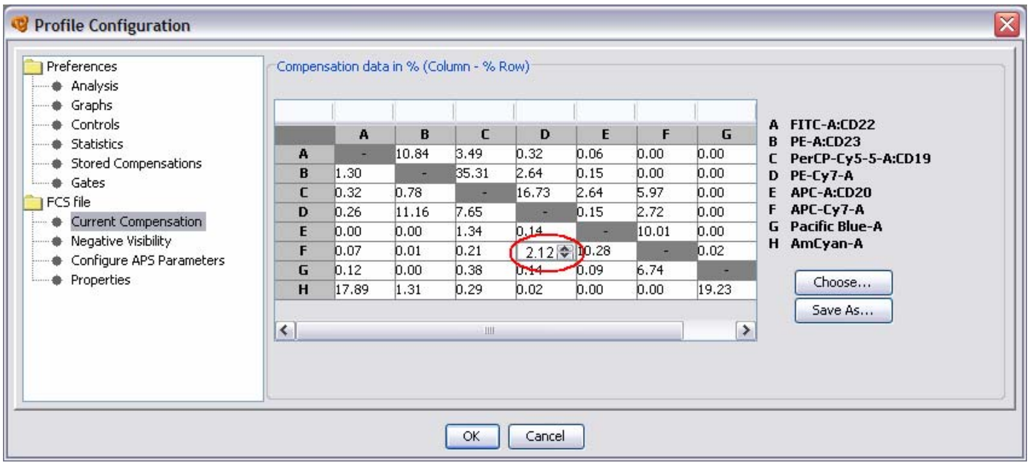


☑ VIS.	Population	Events	Events / µl
☑	Events	563223	158575
	Other Events	555784	156481
☐	Neutrophils	0	0
☑	Monocytes	0	0
☑	Lymphocytes	0	0
☑	NK Cells	0	0
☑	Erythroblasts	0	0
☑	CD34+ precursors	3905	1099
☐	Beads	3534	995

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

3.2. <u>Current compensation can be changed manually and saved</u> if the origin FCS file contains this information internally.

The new cytometric file acquisition programs can save as internal data the compensation of fluorescences used. In those cases, with the version 1.1 of Infinicyt^{TM} the user can modify the compensation values manually so that the changes will be applied to the file. In addition, the user can save the values of the new compensation and apply them to other files. These options are available in the Current Compensation Window of the Profile Configuration menu.

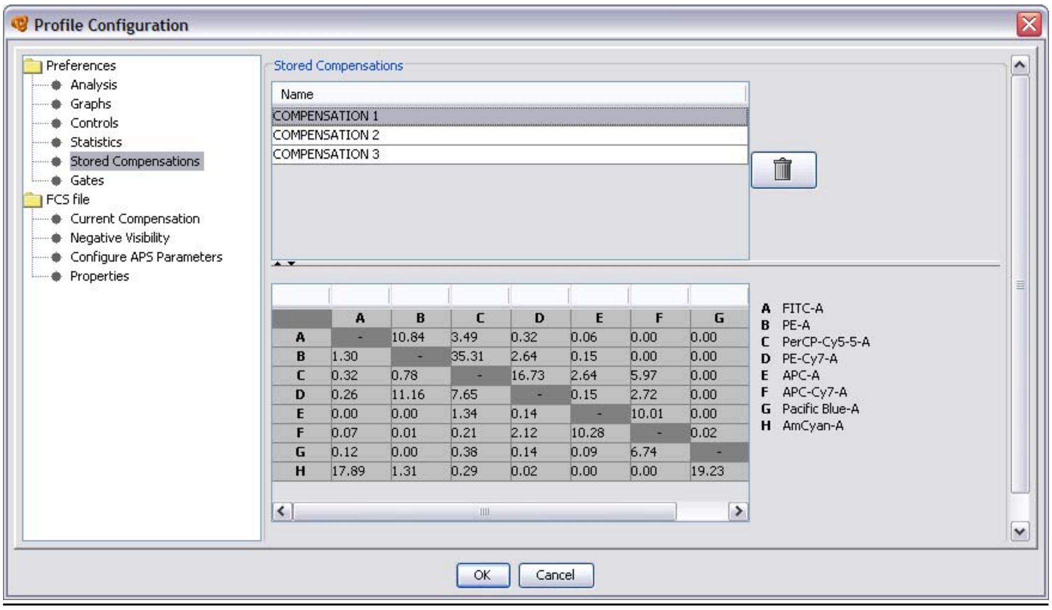


Compensation data in % (Column - % Row)

	A	B	C	D	E	F	G
A	-	10.84	3.49	0.32	0.06	0.00	0.00
B	1.30	-	35.31	2.64	0.15	0.00	0.00
C	0.32	0.78	-	16.73	2.64	5.97	0.00
D	0.26	11.16	7.65	-	0.15	2.72	0.00
E	0.00	0.00	1.34	0.14	-	10.01	0.00
F	0.07	0.01	0.21	2.12	10.28	-	0.02
G	0.12	0.00	0.38	0.14	0.09	6.74	-
H	17.89	1.31	0.29	0.02	0.00	0.00	19.23

A	**FITC-A:CD22**
B	**PE-A:CD23**
C	**PerCP-Cy5-5-A:CD19**
D	**PE-Cy7-A**
E	**APC-A:CD20**
F	**APC-Cy7-A**
G	**Pacific Blue-A**
H	**AmCyan-A**

Selecting "Stored Compensations" option of the Profile Configuration menu, the user can check, delete and change the current file compensation for one of the compensation already stored.



Stored Compensations

Name
COMPENSATION 1
COMPENSATION 2
COMPENSATION 3

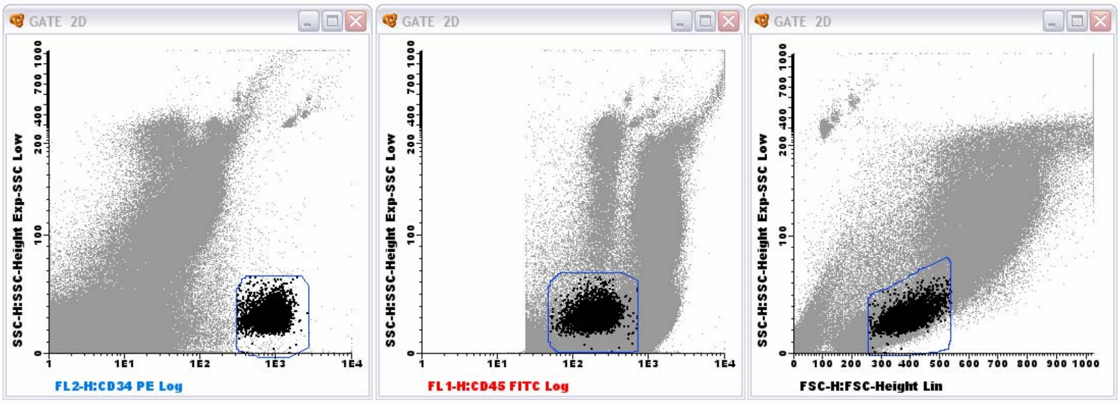
	A	B	C	D	E	F	G
A	-	10.84	3.49	0.32	0.06	0.00	0.00
B	1.30	-	35.31	2.64	0.15	0.00	0.00
C	0.32	0.78	-	16.73	2.64	5.97	0.00
D	0.26	11.16	7.65	-	0.15	2.72	0.00
E	0.00	0.00	1.34	0.14	-	10.01	0.00
F	0.07	0.01	0.21	2.12	10.28	-	0.02
G	0.12	0.00	0.38	0.14	0.09	6.74	-
H	17.89	1.31	0.29	0.02	0.00	0.00	19.23

A	FITC-A
B	PE-A
C	PerCP-Cy5-5-A
D	PE-Cy7-A
E	APC-A
F	APC-Cy7-A
G	Pacific Blue-A
H	AmCyan-A

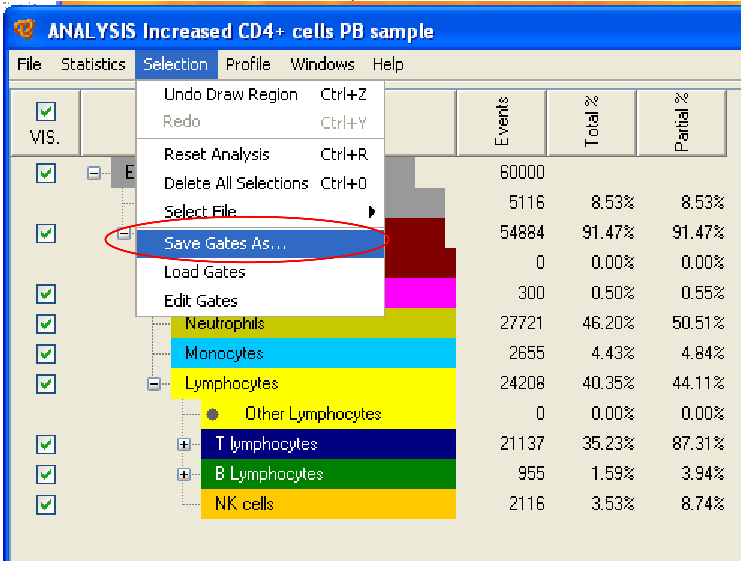
When the user selects a compensation already stored and clicks OK, then the selected compensation will be applied to the file, but whenever a new file is opened, it will be always opened with its internal compensation.

3.3. New option for <u>creating consecutive gates to identify a population and save the sequence to apply it to similar files</u>

1) Draw consecutive gates to identify a population:

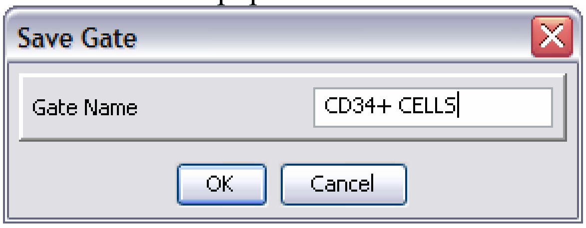


2) Go to "Selection" menu of the Analysis Window and select "Save Gate As…"



☑ VIS.		Events	Total %	Partial %
☑	E	60000		
		5116	8.53%	8.53%
☑		54884	91.47%	91.47%
		0	0.00%	0.00%
☑		300	0.50%	0.55%
☑	Neutrophils	27721	46.20%	50.51%
☑	Monocytes	2655	4.43%	4.84%
☑	Lymphocytes	24208	40.35%	44.11%
	Other Lymphocytes	0	0.00%	0.00%
☑	T lymphocytes	21137	35.23%	87.31%
☑	B Lymphocytes	955	1.59%	3.94%
☑	NK cells	2116	3.53%	8.74%

3) Write a name for the identified population.



Gate Name: CD34+ CELLS

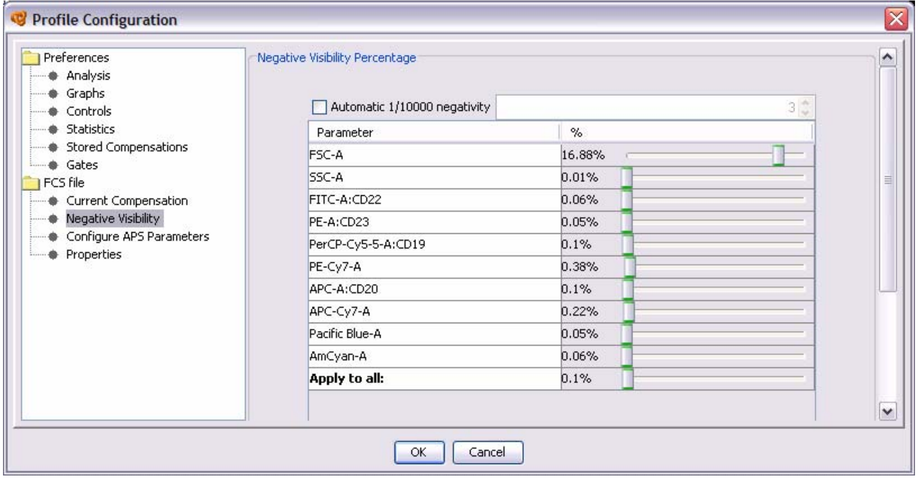
4) When you want to apply the same gating sequence to a new file, go to the "Selection" menu of the Analysis Window and click "Load Gates". Choose the appropriate gate from the list and then go to the Analysis Window to classify the population in the Population Tree.

5) The pre-defined gates are part of the current profile configuration, therefore clicking the option "Gates" of the "Profile Configuration" menu or clicking the option "Edit Gates" of the "Selection" menu, the software will open a window with options to delete them or change the color of the events and the contour.

3.4. It is allowed to configure negative visibility of the parameters with Logical scale.

Negative visibility represents the percentage of negative data range in the Logical scale which is shown in the graph. It corresponds to the part of negative axes relative to the positive axes. Thus, whenever the FCS file has been obtained from an instrument with electronic compensation, negative values that are usually close to the axes may be moved to improve the visualization of the positive events.

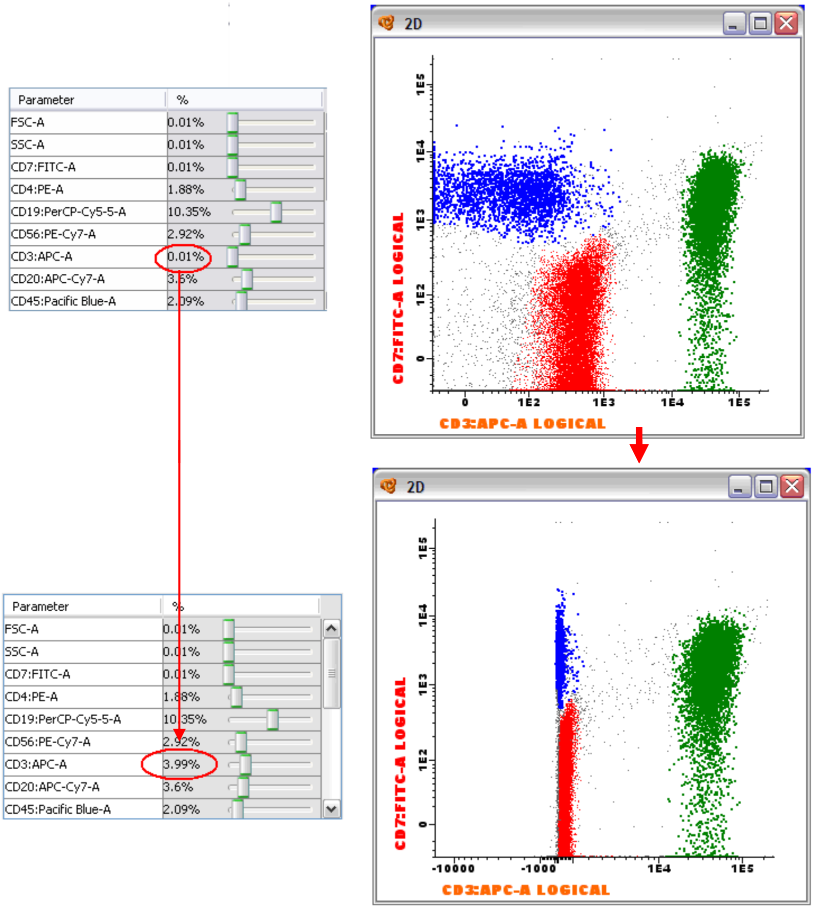
Go to “Profile Configuration” menu and click “Negative Visibility” to change the percentage shown for each parameter. Depending on this percentage, the negative part of the scale of the parameter will be more or less represented.



☐ Automatic 1/10000 negativity 3

Parameter	%
FSC-A	16.88%
SSC-A	0.01%
FITC-A:CD22	0.06%
PE-A:CD23	0.05%
PerCP-Cy5-5-A:CD19	0.1%
PE-Cy7-A	0.38%
APC-A:CD20	0.1%
APC-Cy7-A	0.22%
Pacific Blue-A	0.05%
AmCyan-A	0.06%
Apply to all:	0.1%

If we increased the negative visibility percentage applied for a certain parameter, the negative part of the scale of this parameter will be less represented although it continues being visible.



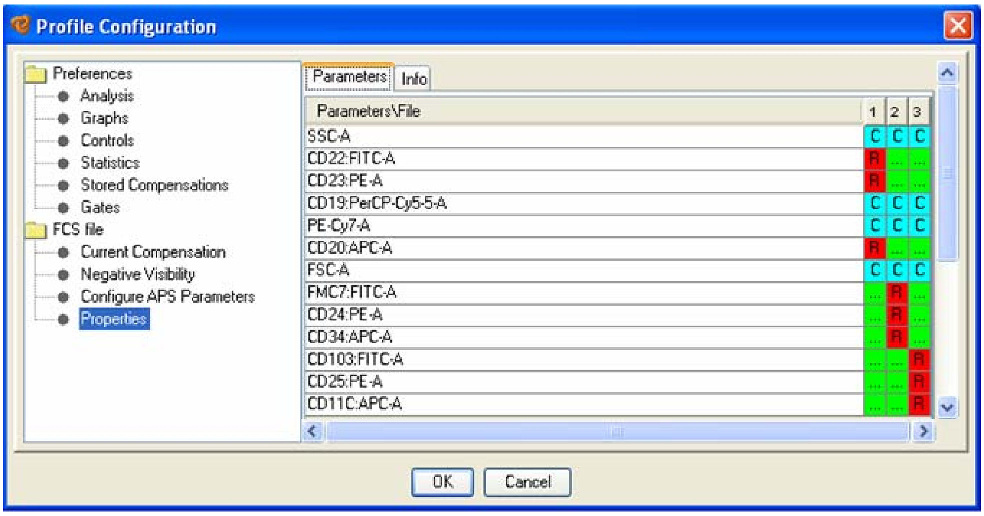
Parameter	%
FSC-A	0.01%
SSC-A	0.01%
CD7:FITC-A	0.01%
CD4:PE-A	1.88%
CD19:PerCP-Cy5-5-A	10.35%
CD56:PE-Cy7-A	2.92%
CD3:APC-A	0.01%
CD20:APC-Cy7-A	3.6%
CD45:Pacific Blue-A	2.09%

Parameter	%
FSC-A	0.01%
SSC-A	0.01%
CD7:FITC-A	0.01%
CD4:PE-A	1.88%
CD19:PerCP-Cy5-5-A	10.35%
CD56:PE-Cy7-A	2.92%
CD3:APC-A	3.99%
CD20:APC-Cy7-A	3.6%
CD45:Pacific Blue-A	2.09%

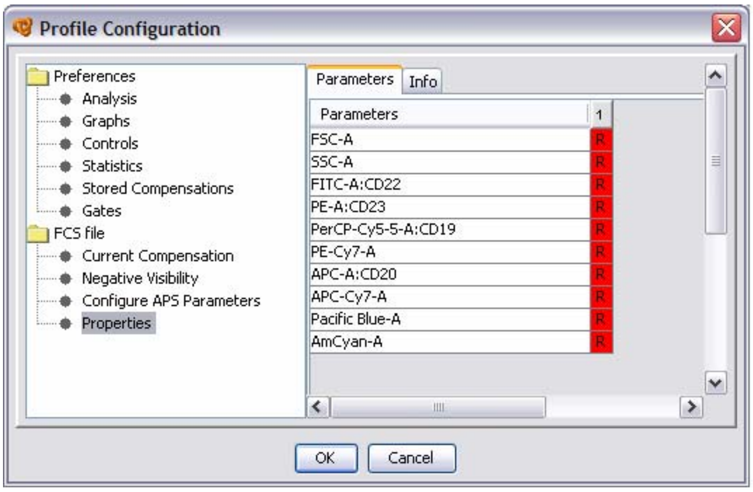
3.5. New option to check all the parameters measured of a data file (Properties)

It is possible to go to the information about the parameters measured on a given data file, by clicking "Properties" of the Analysis Window "File" menu, by clicking the "Properties" option of the "Profile Configuration" menu or with Crtl+I.

This is especially useful when working with files acquired with more than 3-4 fluorescences or with merged data files because in this window we will always have available the matrix with the information for all the parameters and tubes.



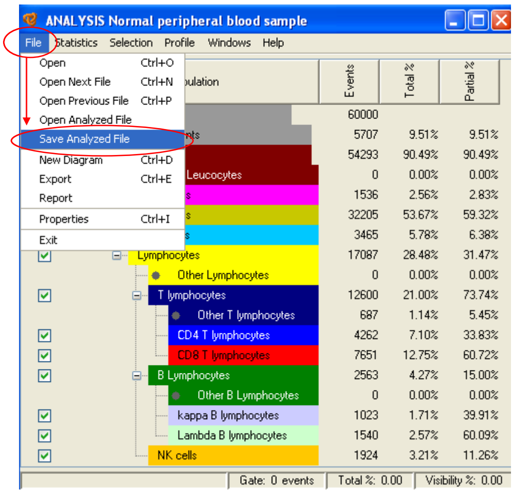
Parameters\File	1	2	3
SSC-A	C	C	C
CD22:FITC-A	R	...	...
CD23:PE-A	R	...	...
CD19:PerCP-Cy5-5-A	C	C	C
PE-Cy7-A	C	C	C
CD20:APC-A	R	...	...
FSC-A	C	C	C
FMC7:FITC-A	...	R	...
CD24:PE-A	...	R	...
CD34:APC-A	...	R	...
CD103:FITC-A	...	...	R
CD25:PE-A	...	...	R
CD11C:APC-A	...	...	R



Parameters	1
FSC-A	R
SSC-A	R
FITC-A:CD22	R
PE-A:CD23	R
PerCP-Cy5-5-A:CD19	R
PE-Cy7-A	R
APC-A:CD20	R
APC-Cy7-A	R
Pacific Blue-A	R
AmCyan-A	R

4. New options make the Manual Analysis of the data file easier

4.1. The way in which the user can save and find in his computer a document already analyzed (.cyt) has been improved



	ulation	Events	Total %	Partial %
		60000		
	nts	5707	9.51%	9.51%
		54293	90.49%	90.49%
	Leucocytes	0	0.00%	0.00%
	s	1536	2.56%	2.83%
	s	32205	53.67%	59.32%
	s	3465	5.78%	6.38%
☑	Lymphocytes	17087	28.48%	31.47%
	Other Lymphocytes	0	0.00%	0.00%
☑	T lymphocytes	12600	21.00%	73.74%
	Other T lymphocytes	687	1.14%	5.45%
☑	CD4 T lymphocytes	4262	7.10%	33.83%
☑	CD8 T lymphocytes	7651	12.75%	60.72%
☑	B Lymphocytes	2563	4.27%	15.00%
	Other B Lymphocytes	0	0.00%	0.00%
☑	kappa B lymphocytes	1023	1.71%	39.91%
☑	Lambda B lymphocytes	1540	2.57%	60.09%
☑	NK cells	1924	3.21%	11.26%

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

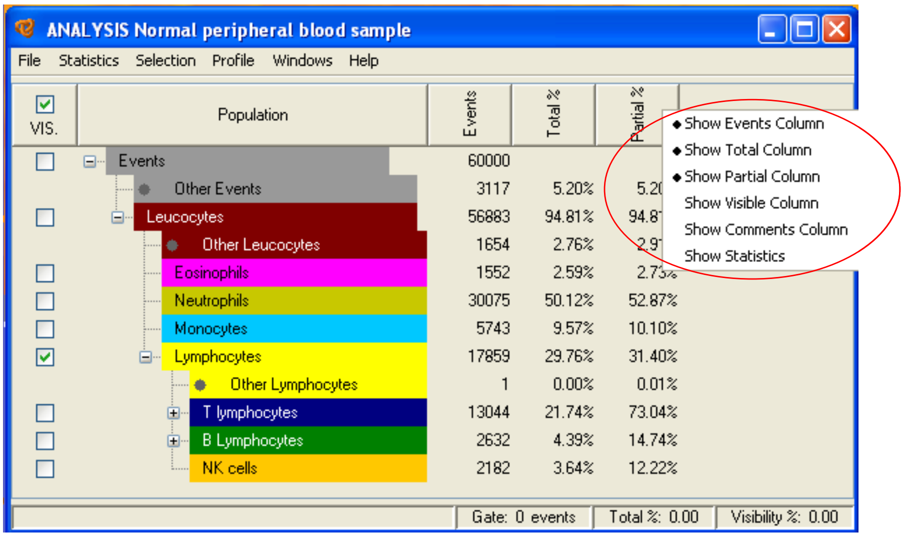
Infinicyt™ software allows saving the files analyzed, containing the information of classification, statistics, graphics, report, profile, etc. and store them in the hard disk. Select "Save Analyzed File" option of the "File" menu when you want to store the analysis of a file. The software will ask you the directory where you want to save it and the file will be saved as **.cyt**.

The new feature in version 1.1 of Infinicyt™ is that the analyzed file will be saved in the folder of the hard disk that the user selects with the extension **.cyt**. The original data file will remain the same in the corresponding folder, because the **.cyt** file does not affect the original one.

Whenever the user wants to open an already analyzed file, then the **.cyt** file must be opened from the option "Open Analyzed File" of the "File" menu.

4.2. Option to choose the columns to visualize in the Analysis Window.

By default, all columns of the Analysis Window, except the statistics column, will be visible. Nevertheless, at any time, right-clicking on upper columns, this information can be hidden or shown.

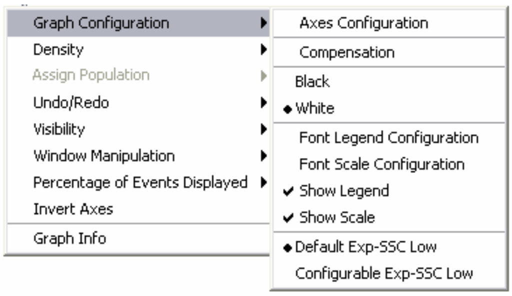


☑ VIS.	Population	Events	Total %	Partial %
☐	Events	60000		
	Other Events	3117	5.20%	5.2
☐	Leucocytes	56883	94.81%	94.8
	Other Leucocytes	1654	2.76%	2.9
☐	Eosinophils	1552	2.59%	2.7
☐	Neutrophils	30075	50.12%	52.87%
☐	Monocytes	5743	9.57%	10.10%
☑	Lymphocytes	17859	29.76%	31.40%
	Other Lymphocytes	1	0.00%	0.01%
☐	T lymphocytes	13044	21.74%	73.04%
☐	B Lymphocytes	2632	4.39%	14.74%
☐	NK cells	2182	3.64%	12.22%

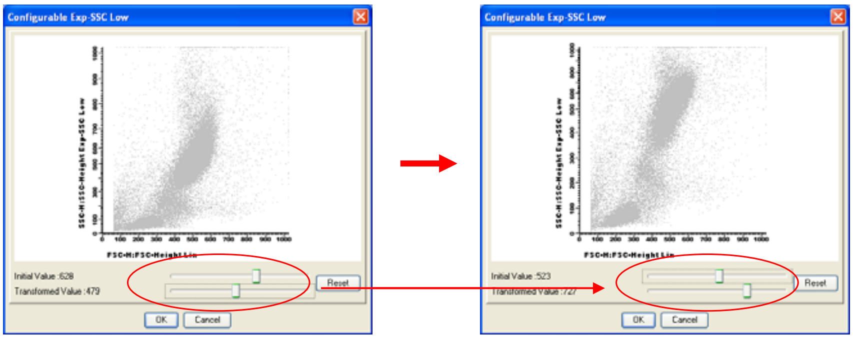
Gate: 0 events | Total %: 0.00 | Visibility %: 0.00

4.3. The user can <u>configure the mathematical transformation of the parameter SSC</u> to improve the separation of populations.

In Graph Configuration option, the possibility of selecting the values of the mathematical transformation of the SSC-parameter to apply in the data file, in order to improve the separation of populations, as been added.



The “Configurable EXP-SSC Low” option gives the possibility to expand the SSC-parameter with the values desired by the user. When clicking on it, the SSC plot will appear with two scrolls in the lower part of the graph to regulate the part of the scale to amplify.



Initial Value :628
Transfomed Value :479

Initial Value :523
Transfomed Value :727

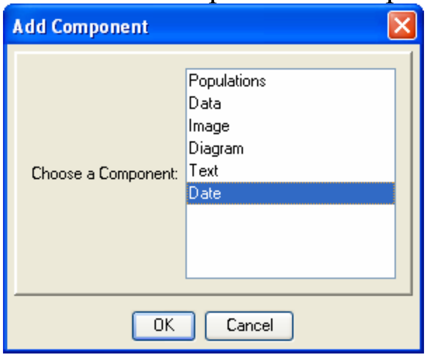
4.4. New options regarding the <u>Printed Report</u>

4.4.1. It is allowed to copy, cut and paste on Printed Report components of the software and external images or texts.

4.4.2. It is possible to choose the size of paper from the “Properties” option of the report “File” menu.

4.4.3. Undo (Cntrl+Z) / Redo (Cntrl+Y) options have been added from the Report “Edit” menu.

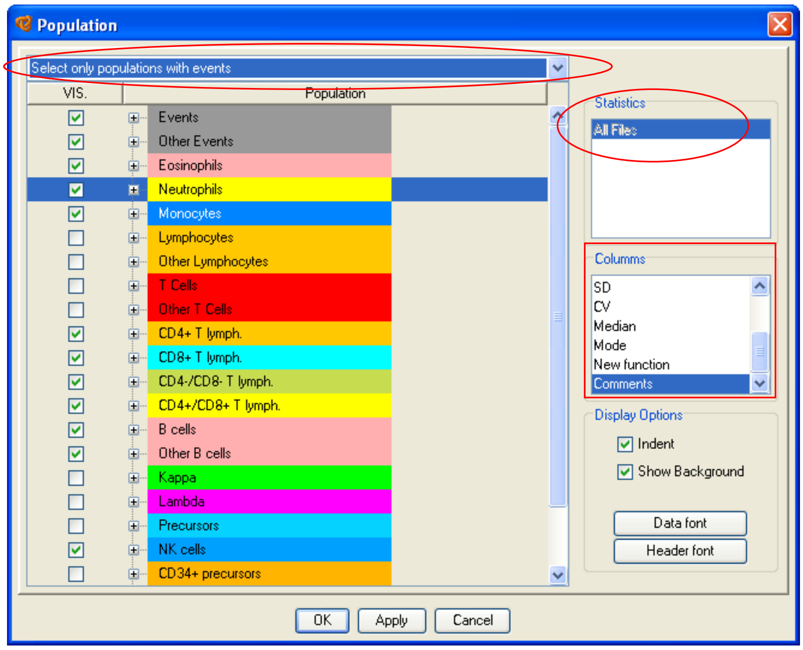
4.4.4. “Data” option has been added as component of the printed report.



Choose a Component: Date

4.4.5. Different options have been added to configure the populations, the parameters and the statistics to be shown on the population component of the report. In the version

1.1 of Infinicyt^{TM} it is possible to select more statistic data, choose different files and show comments if this option is selected in the "columns" menu.



Select only populations with events

VIS.	Population
☑	Events
☑	Other Events
☑	Eosinophils
☑	Neutrophils
☑	Monocytes
☐	Lymphocytes
☐	Other Lymphocytes
☐	T Cells
☐	Other T Cells
☑	CD4+ T lymph.
☑	CD8+ T lymph.
☑	CD4-/CD8- T lymph.
☑	CD4+/CD8+ T lymph.
☑	B cells
☑	Other B cells
☐	Kappa
☐	Lambda
☐	Precursors
☑	NK cells
☐	CD34+ precursors

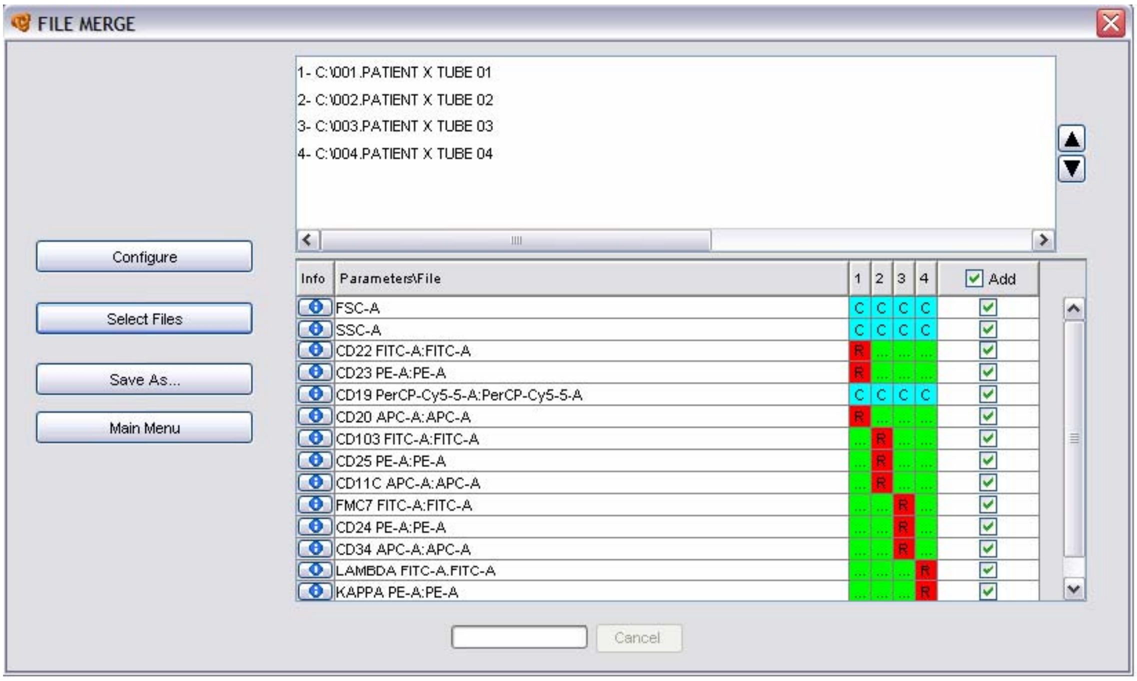
Statistics: All Files

Columns:
- SD
- CV
- Median
- Mode
- New function
- Comments

Display Options:
- ☑ Indent
- ☑ Show Background

5. New options regarding the Merger Module:

5.1 New information and commands in the Merger Screen.



1- C:\001.PATIENT X TUBE 01
2- C:\002.PATIENT X TUBE 02
3- C:\003.PATIENT X TUBE 03
4- C:\004.PATIENT X TUBE 04

Info	Parameters\File	1	2	3	4	☑ Add
ⓘ	FSC-A	C	C	C	C	☑
ⓘ	SSC-A	C	C	C	C	☑
ⓘ	CD22 FITC-A:FITC-A	R	...	...	...	☑
ⓘ	CD23 PE-A:PE-A	R	...	...	...	☑
ⓘ	CD19 PerCP-Cy5-5-A:PerCP-Cy5-5-A	C	C	C	C	☑
ⓘ	CD20 APC-A:APC-A	R	...	...	...	☑
ⓘ	CD103 FITC-A:FITC-A	...	R	...	...	☑
ⓘ	CD25 PE-A:PE-A	...	R	...	...	☑
ⓘ	CD11C APC-A:APC-A	...	R	...	...	☑
ⓘ	FMC7 FITC-A:FITC-A	...	...	R	...	☑
ⓘ	CD24 PE-A:PE-A	...	...	R	...	☑
ⓘ	CD34 APC-A:APC-A	...	...	R	...	☑
ⓘ	LAMBDA FITC-A.FITC-A	...	...	...	R	☑
ⓘ	KAPPA PE-A:PE-A	...	...	...	R	☑

Version 1.1 of Infinicyt^{TM} includes the following available commands in the Merger Screen:

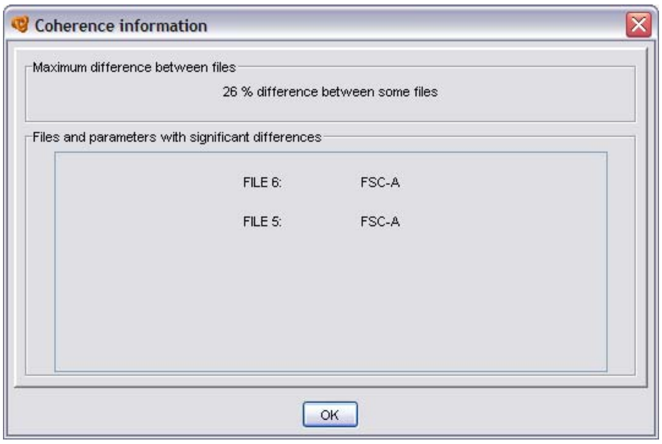
- Configure: File merge options allow manipulating the controls that will warn you in case your common parameters are not similar enough to perform a merge process.
- Select files: this command opens the directory where the user can select the files to be merged. Once the data files are selected, in the upper part of the dialogue box appears the list of the files to merge and in the lower part of the dialogue box appears the matrix (parameters/file).
    - You can select and move the files on this list with the ▲ and ▼ cursors, in order to obtain the best sequence to represent the tubes in the graphics.
    - In the matrix, the user can add or not each parameter, in order to avoid parameters that are not interesting because there is no marker on it. With this, we will optimize the memory of the new data file reducing the number of parameters to be merged.
- Save as… when the selection of the data files is done and the parameters to merge are the correct ones, the user can save this information. The software will ask you for a new name and directory for the new FCS file containing all the parameters merged.
- To go back to the main screen of the program, click the Main Menu command.

5.2 New features regarding merger process controls.

See chapter 4.2.4 of the User's Manual to obtain detailed information about Coherence Control to determine if the user is performing the merge process with the optimal files, analyzing in different aspects the similarity of expression in the common parameters.

The version 1.1 of Infinicyt^{TM} includes the following new features about the merger process controls:

a. The coherence information window about the differences relative to the global coherence control (Merge Control) has been simplified. When the program detects significant differences between the distributions of the events of the different files in the common parameters space, it shows a warning message. The warning will indicate the percentage of difference (value 0 means that there are no differences between the files and value 100 indicates that the distribution of events relative to common parameters is completely different), and will also list, by significance, the parameters and files which make the differences.



26 % difference between some files

FILE 6: FSC-A

FILE 5: FSC-A

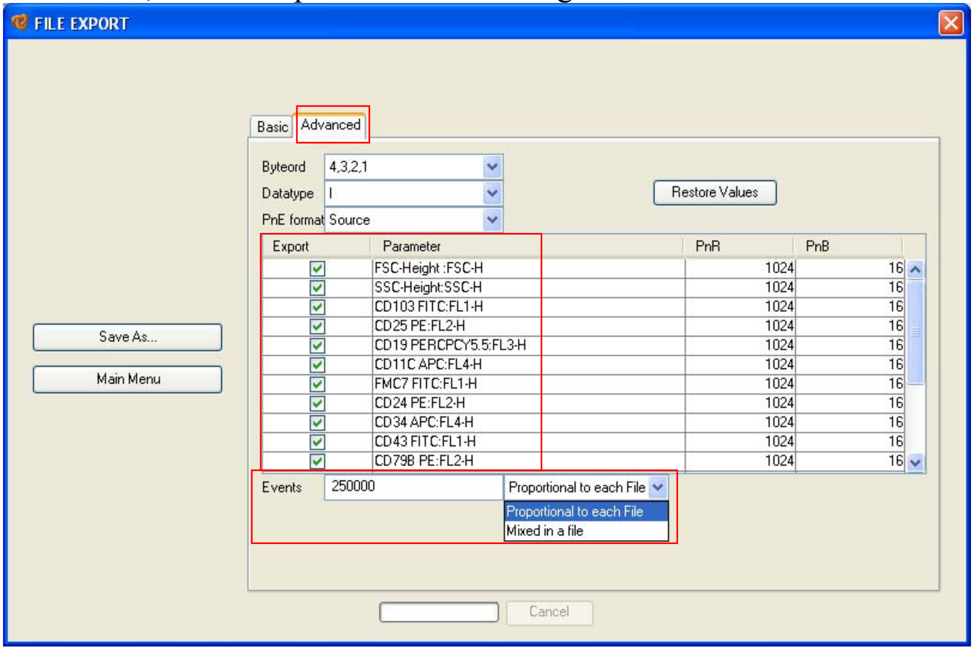
b. In order to avoid errors in analysis, the option of correcting the parameters when a difference of no coherence between populations (Population Control) is detected has been deleted

6. New options regarding <u>File Export</u>

6.1. The available export file formats have been reduced to Standard FCS or TXT. When FCS format is chosen, the data will be saved in the form of an FCS file, readable by any cytometric analysis software, but not by common worksheet or database programs. When it is chosen TXT format, the values will be saved in ASCII format as a data base, readable by worksheets or word-processing programs

6.2. Some options on the advanced functions of the File export to avoid extra memory requirements resulting from unnecessary information have been added:

- The user can export only the <u>parameters</u> which are necessary for the analysis. Initially, all the parameters are selected for export. If the user wants to eliminate some of these parameters, unclick the parameter to eliminate it from the export.
- <u>Events</u>: if the user is trying to export a merged file, Infinicyt^{TM} will give the option to either export events proportional to each file maintaining the file number separation, or mixed in a file, without separation between original files.



Byteord 4,3,2,1

Datatype I

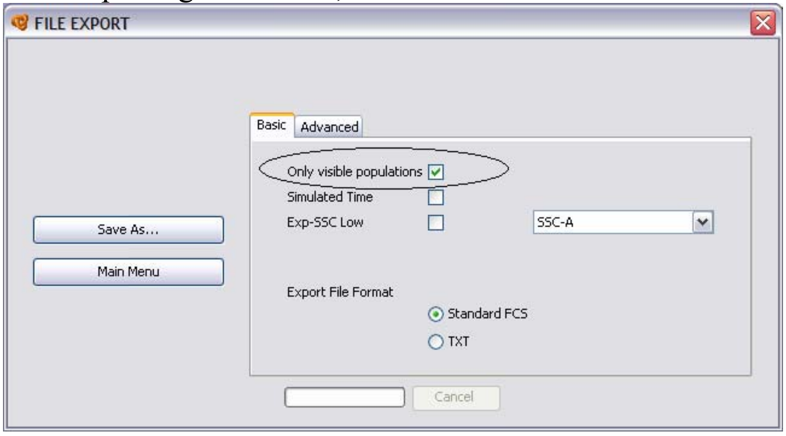
PnE format Source

Export	Parameter	PnR	PnB
☑	FSC-Height :FSC-H	1024	16
☑	SSC-Height:SSC-H	1024	16
☑	CD103 FITC:FL1-H	1024	16
☑	CD25 PE:FL2-H	1024	16
☑	CD19 PERCPCY5.5:FL3-H	1024	16
☑	CD11C APC:FL4-H	1024	16
☑	FMC7 FITC:FL1-H	1024	16
☑	CD24 PE:FL2-H	1024	16
☑	CD34 APC:FL4-H	1024	16
☑	CD43 FITC:FL1-H	1024	16
☑	CD79B PE:FL2-H	1024	16

Events 250000 Proportional to each File

Proportional to each File
Mixed in a file

6.3. From the Main Screen the File Export option will consider the export of a whole file. If the user is interested on **exporting only one or more populations**, then, the “Export” option must be opened from the “File menu” of the Analysis Window. If the box relating to “Only visible populations” is enabled, only the visible populations in the Analysis Window will be considered for exporting. If it is not, all the events contained in the file will be exported.



Only visible populations ☑

Simulated Time ☐

Exp-SSC Low ☐ SSC-A

Export File Format

● Standard FCS

○ TXT