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HOW TO EDIT A POPULATION TREE

1.7 VERSION — V2.0 *de*

*see updates by
Gosie*

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INTRODUCTION

Infinicyt™ is the most powerful software for data integration and multidimensional analysis of flow cytometry data.

It brings you a wide range of possibilities on a potentially infinite number of dimensions that will increase your laboratory efficiency.

This tutorial will introduce you to **Infinicyt™** performing a simple analysis. **Infinicyt™** will allow you to:

- **Analyse** individual flow cytometry files.
- **Merge** different data files from one or more samples, what enables to perform a multiple-tube analysis in a single step.
- **Represent** integrated information in an easy way with the most powerful tools for **N-Colour analysis**, statistics and reports.

In the following tutorials you will notice the capacity of this analysis tool in an easy way. These tutorials will allow you to learn about Manual Analysis module through a series of steps: configure diagrams and population tree, configure statistics, create a report, analyse a file, etc.

The aim of this first tutorial is to explain how to edit the Population Tree in the Manual Analysis in order to analyse our files in the simplest way with Manual Analysis.

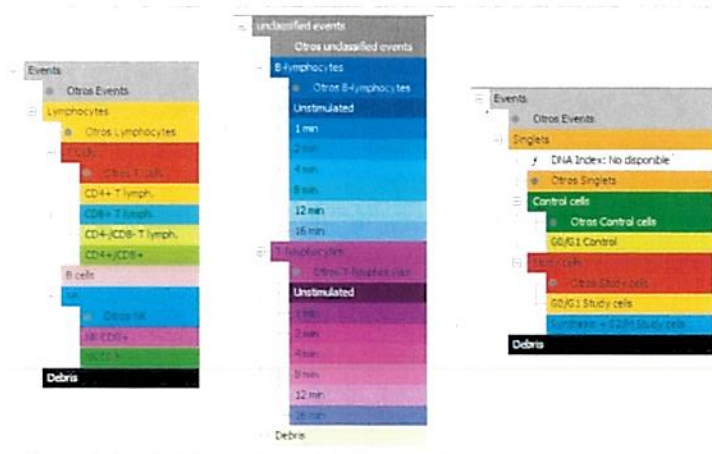


Figure 1. Different examples of Population Trees

WARNING:

Please in order to learn properly how to use this module follow the six tutorials about Manual Analysis in the suggested order.

OBTAIN SAMPLE DATA FILES

Visit www.infinicyt.com and click on **Support** → **Tutorials and Videotutorials** → **Tutorials** or use the quick access to Tutorials at the bottom menu from the Homepage.

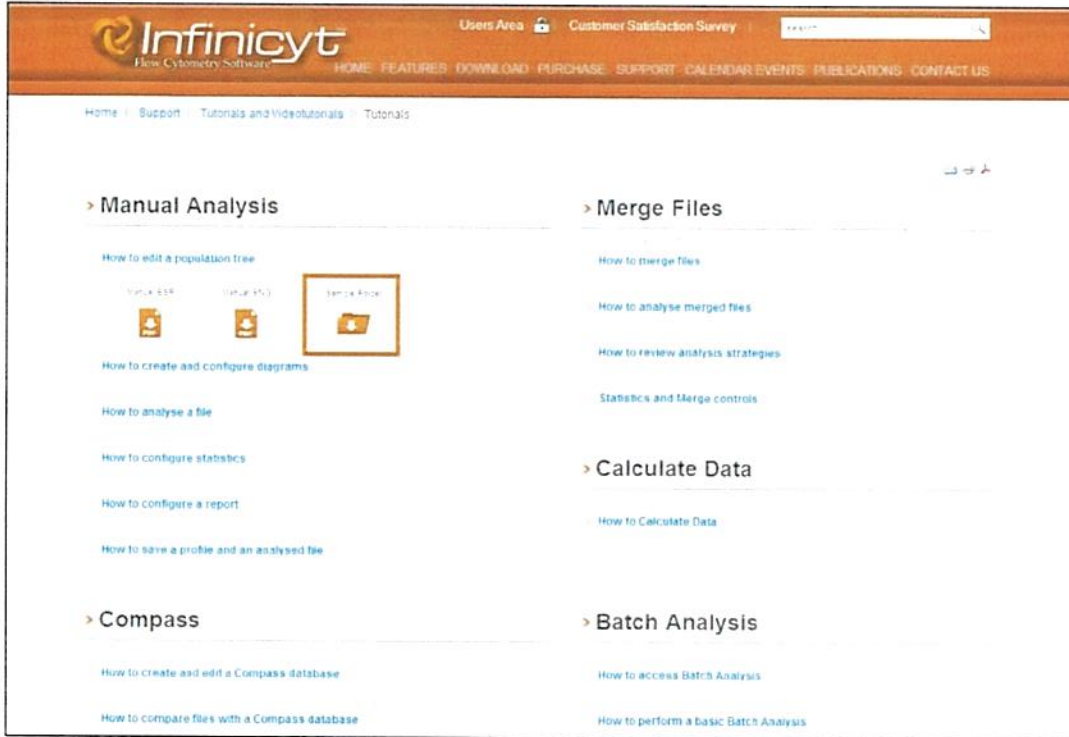


Figure 2. Download Sample Directory

Download the **Sample Folder**, which contains the file **Tutorial.fcs**. *Done by Goni* ← are located in folder:

This FCS file belongs to a normal peripheral blood sample stained with a **Lymphoid Screening Tube (LST-11)** from **Cytognos SL**. LST-11 kit is an antibody cocktail developed for evaluation of the major lymphocyte subpopulation in several suspected clinical conditions (lymphocytosis, lymph node enlargement, splenomegaly, etc.) with a single test.


*Peripheral Review 2
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FCS FILES TUTORIAL*

2 FCS files are in this folder →

- ① LST_Manual
- ② LST-B-CLPD

LST-11 kit has been designed following the EuroFlow™ recommended combination:

PacB	OC515	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CD20 + CD4	CD45	CD8 + Smlgλ	CD56 + SmlgK	CD5	CD19 + TCRγδ*	SmCD3	CD38

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Note: *Antibody not included in the mixture. OC515 is equivalent to Pacific Orange™. PE- Cyanine7 is equivalent to PE-Cy™7. PerCP-Cyanine5.5 is equivalent to PerCP-Cy™5.5. APC-C750 is equivalent to APC-H7.

Those markers will allow identifying different populations such as total Leucocytes (CD45+), T-Cells (CD3+\CD45+), B-Cells (CD19+\CD45+) and NK cells (CD56+\CD45+), besides some subpopulations we will see in the course of these tutorials.

RUN THE MANUAL ANALYSIS

1. Open Infinicyt™

Launch **Infinicyt™** by double-clicking on the icon that appears on the desktop, once it is installed. ✓

2. Go to Manual Analysis Option

When you click on ~~Manual~~ **Analysis**, a file selection window will be shown.

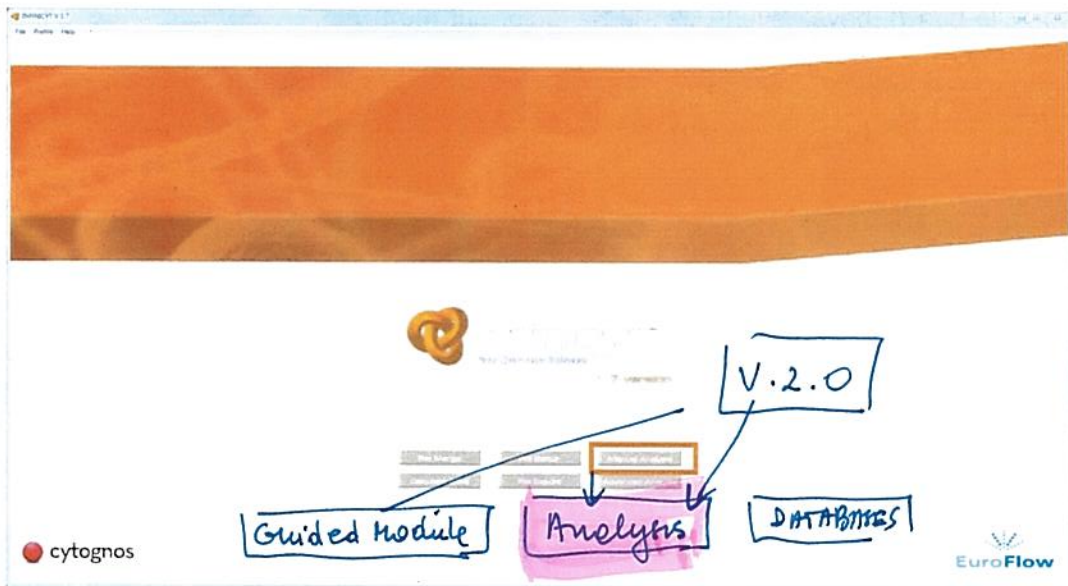


Figure 3. Infinicyt™ main screen (Infinicyt v1.7)

Choose the file **Tutorial.fcs** (from the folder where you have saved it) and click on **Select**.

“ FCS Files Tutorial ”
(see page 3)

Go to Folder: Patient

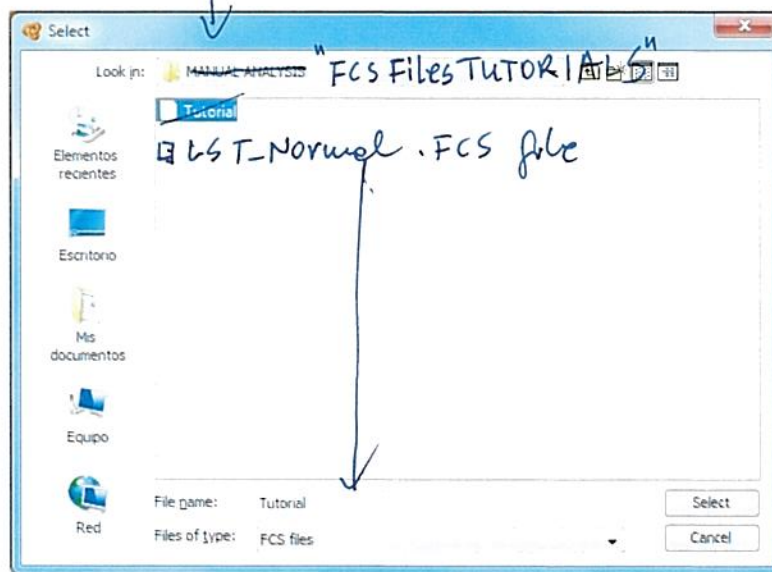


Figure 4. File Selection window

Downloaded files can also be opened by dragging and dropping them from the folder where it is saved into **Infinicyt™** main screen or on **Infinicyt™** desktop icon.

3. Manual Analysis Window

The first time we launch the Manual Analysis module, the default profile will be loaded with five DotPlots (A) and the Manual Analysis Window with the Population Tree (B). Events will appear in grey colour in the DotPlots.

The Manual Analysis Window (Figure 5) contains the Menu Bar (C) of **Infinicyt™**. We can control all software functions from this window; here we can configure statistics, graphs, profiles etc., and access to Batch Analysis and Compass tools.

The Population Tree may contain as many labels and colours as we need to classify the different cell populations. The design of a proper Population Tree is very important in order to proper classify of our events.

The Population Tree from Figure 5 is the one linked to the default profile, but it can be completely customised by the user.

→ 1st OPEN Profile :

→ "click" Profile & select

→ "Load Profile from Folder"

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→ select "default profile v2.0"

→ click "select"



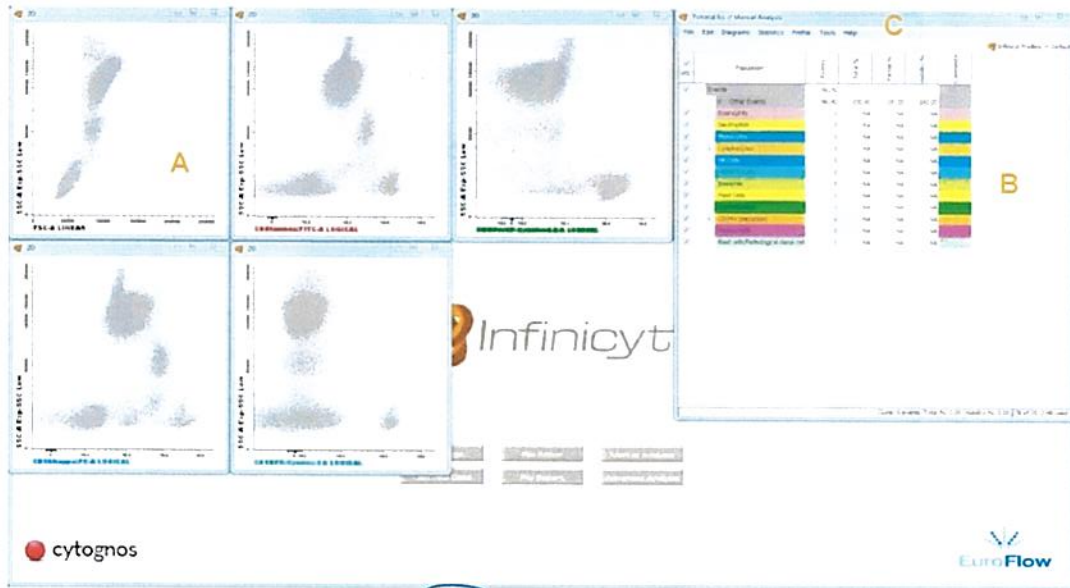


Figure 5. Default Manual Analysis Window

If, at some point, by clicking somewhere else in the screen, all the diagrams and the Analysis Window are hidden, you only need to click on **Manual Analysis** button and data will appear on the screen again. You can also use the keyboard shortcut **Ctrl+A**. ✓

EDIT A POPULATION TREE

As mentioned before, the default profile shows five DotPlots and an already created Population Tree. In this chapter we will learn how to edit the Population Tree in order to better classify our populations according to sample type.

1. Remove all populations

First of all, we will remove all populations from default Population Tree to configure our Tree from the beginning. By right-clicking on any of the coloured labels the contextual menu will be displayed.

Two options to remove populations are available:

- a) Remove populations one by one. We select a population and choose “**Delete Selected**” (we can also select several populations at the same time using Ctrl+click).
- b) Remove all populations at the same time. We choose the option “**Delete All Populations**”.

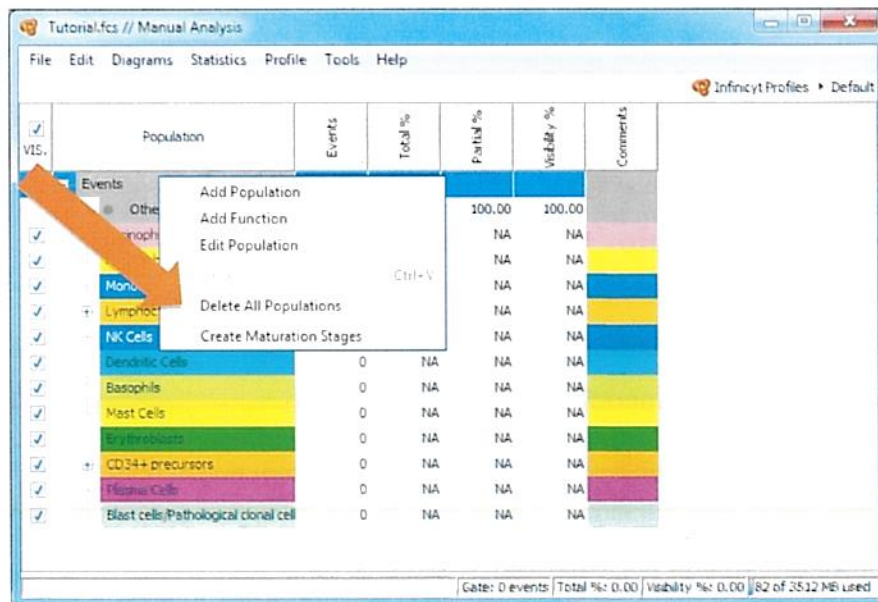


Figure 6. Default Population Tree

If populations are removed by mistake, the option **Edit → Undo (Ctrl+Z)** will recover them.

2. Add and edit a population

Now, we are going to create a new Population Tree. In this case, we will create a short tree including “Leucocytes” population, which includes “Lymphocytes” population; besides, three subpopulations (“B cells”, “T cells” and “NK cells”) will depend on “Lymphocytes” population.

First of all, right-click on the grey population named “Events” and choose “Add Population”.

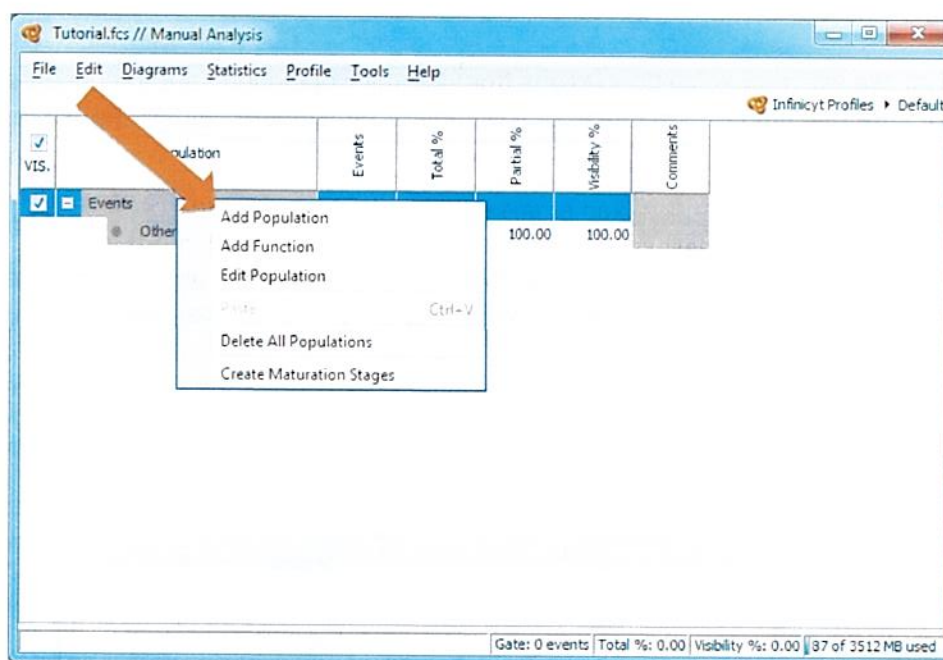


Figure 7. Add a new population

2.1 Give name and color to a population

When selecting “Add Population” a dialogue box will appear:

- Write the name “**Leucocytes**” in the Name box.
Select the colour of the population among the available colours in “**Basic**” tab (e.g. blue). Additional colours can be found in “**Swatches**”, “**HSB**”, “**RGB**” tabs .
- **Highlight** the population if you want to visualize the dots in a larger size (**Visualisation Highlight**). In this case, we use **Visualisation Normal**.

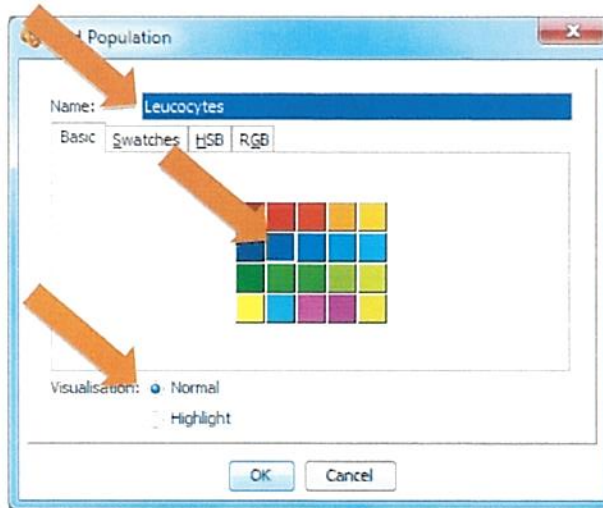


Figure 8. Add Population window

Once the visualisation is customised, click “OK”. A new population, named “Leucocytes”, in blue will appear in the Population Tree.

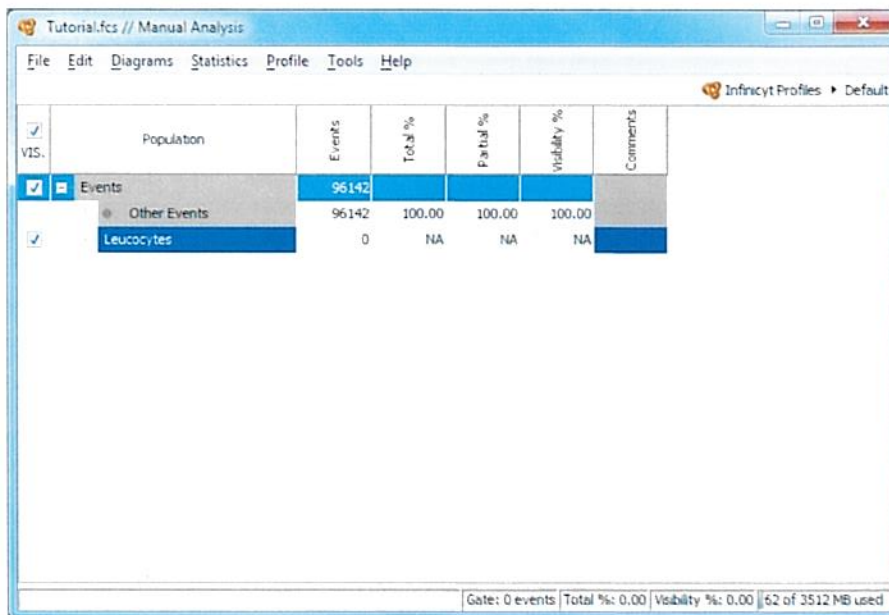


Figure 9. Population Tree with the new population added

Note:

If you create a population tab by mistake, you can delete it by right-clicking on the population to be deleted and selecting “Delete Selected”. If you want to edit a certain label, right-click on the label and choose “Edit Population” or double-click on the corresponding label.

See User’s Manual chapter 3, Population Tree, for more information.

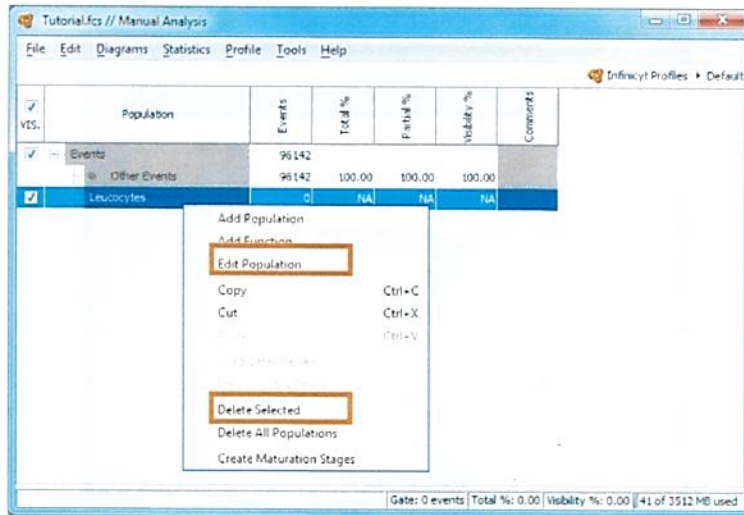


Figure 10. "Remove" or "Edit Population"

3. Add and edit subpopulations

Now, we will create a new population dependent on the population "Leucocytes". Right click on the parent population; in this case "Leucocytes", and select "Add Population".

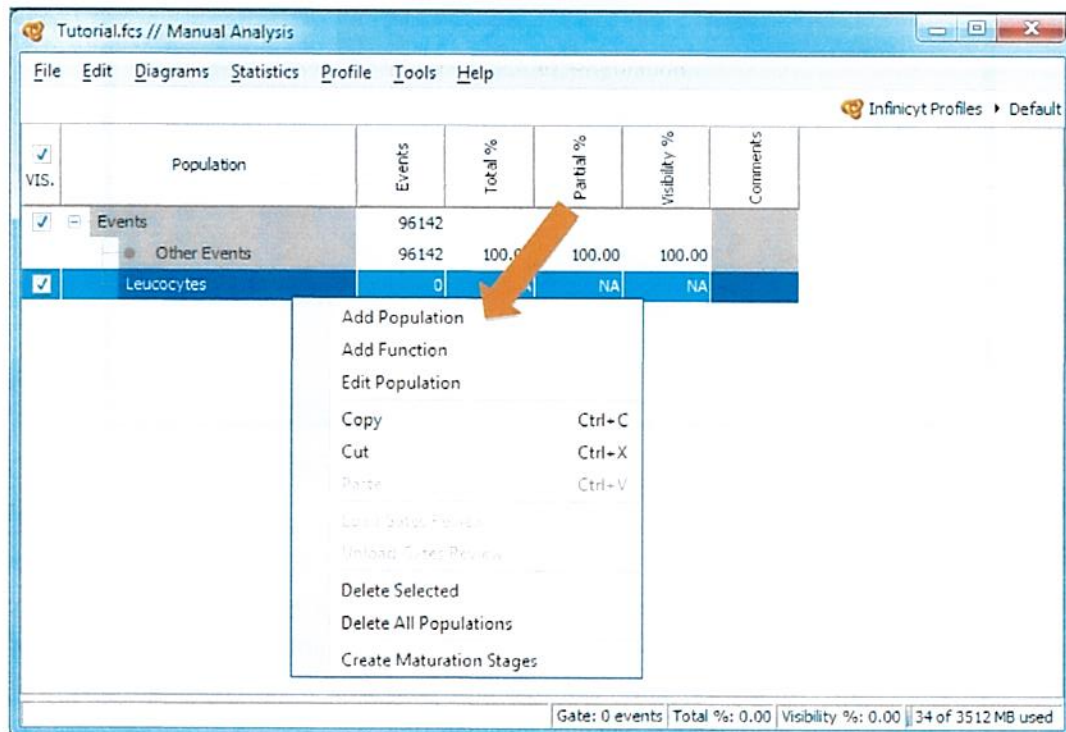


Figure 11. "Add Population"

Name the new population as “**Lymphocytes**” for example. Write the name of the label in the appropriate box, choose a colour (in our example, green) and click “OK”.

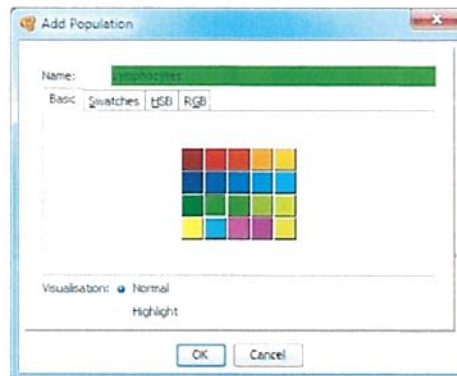



Figure 12. Add Population window

3.1 Visualize a subpopulation

“Lymphocytes” population will be subordinated to “Leucocytes” population.

We can hide the subpopulations by clicking on the  symbol located on the left side of the label “Leucocytes” and show them by clicking on the symbol.

VIS.	Population	Events	Total %	Partial %	Visibility %	Comments
<input checked="" type="checkbox"/>	Events	96142				
<input checked="" type="checkbox"/>	Other Events	96142	100.00	100.00	100.00	
<input checked="" type="checkbox"/>	Leucocytes	0	NA	NA	NA	
<input checked="" type="checkbox"/>	Other Leucocytes	0	NA	NA	NA	
<input checked="" type="checkbox"/>	Lymphocytes	0	NA	NA	NA	

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00 | 64 of 3512 MB used

Figure 13. New population created dependent on Leucocytes

VIS.	Population	Events	Total %	Partial %	Visibility %	Comments
<input checked="" type="checkbox"/>	Events	96142				
<input checked="" type="checkbox"/>	Other Events	96142	100.00	100.00	100.00	
<input checked="" type="checkbox"/>	Leucocytes	0	NA	NA	NA	

Gate: 0 events | Total %: 0.00 | Visibility %: 0.00 | 63 of 3512 MB used

Figure 14. New population created

4. Create the rest of populations

Follow the steps previously shown in order to create the rest of the populations required: “**T Cells**”, “**B Cells**” and “**NK Cells**”, all of them dependent on “**Lymphocytes**”.

Right click on the label “**Lymphocytes**” and click on “**Add Population**”, assign a name and a color to the new population (**T Cells**) and click “**OK**”. Repeat the same steps to create B Cells and NK Cells populations dependent on “**Lymphocytes**”.

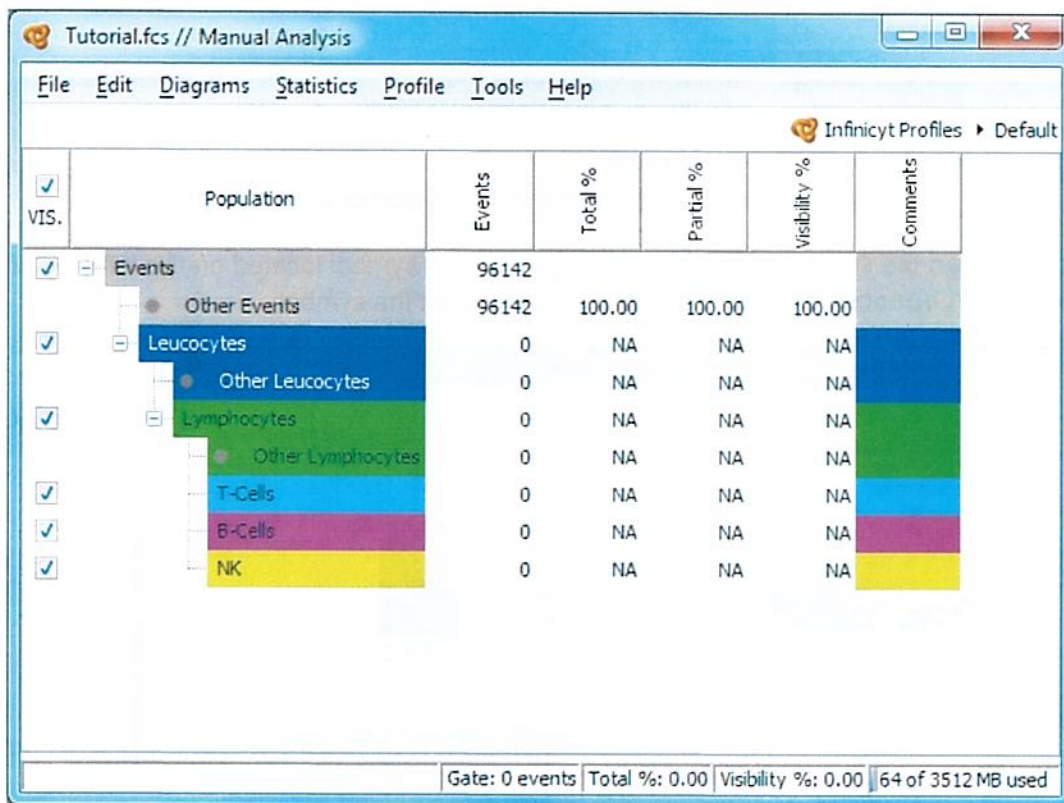


Figure 15. Population Tree

5. Summarize

We have learned how to configure the Population Tree, how to add populations, how to remove populations and how to create subpopulations (dependent on a parent population).

A proper Population Tree configuration will be necessary not only for identifying and classifying sample cells, but for optimising the statistics we can extract from this configuration. By using the right Population Tree we can obtain the total number of events for each population and also partial percentages of subpopulation events with regard to parent population (more information about statistics will be provided in the tutorial "How to configure statistics in Manual Analysis").

After completing this tutorial, the Manual Analysis workspace will show the visual aspect displayed in Fig. 16. You can continue with the next tutorial ("How to create and configure diagrams") using the same file.



Figure 16. Manual Analysis workspace after following the tutorial

Thank you for following this tutorial.

To follow learning how to use Manual Analysis module you can consult the tutorials available in our web site:

- How to create and configure Diagrams.
- How to analyze a sample.
- How to configure the Statistics.
- How to configure a Report.
- How to save the Profile and an Analyzed file.

If you need further information about the present tutorial or any other aspect of Infinicyt™ do not hesitate to contact us and ask for the assistance of our Technical Support Team.



End of Tutorial ①

Cytognos S.L. Technical Support

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