

Instructions for using FlowJo Templates and Excel macros:

Perform FlowJo analyses of the FCS files

1. Start FlowJo
2. Open ->FlowJo Templates -> select specific panel template (for example: 2 T-Cell panel template)
3. Drag and drop selected samples
4. Review dotplots and histograms of all samples and adjust regions and quadrants when necessary
5. Save As -> Excel (XLS)
6. When Excel file is opened: Save As -> Excel Workbook (.xlsx)
7. Give name to the File (**should begin with 2 T-Cell panel...**) and choose Folder -> Save
8. Save As -> Workspace (WSP)
9. Give name to the File and choose Folder -> Save
10. Repeat steps 2-9 for all panels
11. Quit FlowJo

Extract useful data from Excel files

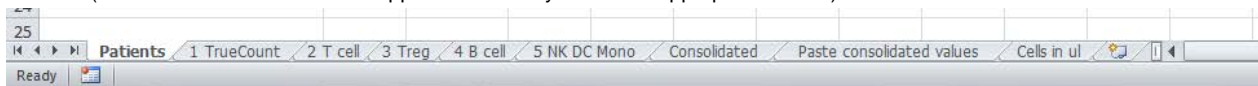
12. Drag HIPc v1 (Excel file) to the folder where you saved excel files from the FlowJo analyses
13. Open HIPc v1->Enable macros (Options -> Enable this content -> OK)
14. Fill the data for the patients on the worksheet "Patients"
15. Open Excel file with data from FlowJo analysis (**for example: 2 T-Cell panel...**)
16. View -> Macros -> View Macros
17. Select appropriate macro (**in this case II_T_cell ->Run**)
18. Close File -> Save changes -> Yes
19. Repeat 15 to 18 for all panels, give appropriate filenames, save in the same folder, use appropriate macros

Integrate data from all workbooks in the HIPc workbook (**works with Win XP to 7 w Excel 2007 and 2010**)

20. Go to HIPc v1-> View -> Macros ->**VI_Integrator** ->Run
21. At question "Do you want to save changes to..." click "No"

Consolidate data from all worksheets in the HIPc workbook*

22. **Make sure that worksheets from left to right are in the following order:**
(worksheet **Consolidated** will appear later after you run the appropriate macro)



Rearrange them if they are not in the specified order (see picture)!

23. View -> Macros -> View Macros -> **VII_Consolidator** -> Run
24. View the values per microliter of blood on the worksheet "Cells in µl"
25. If you have more than 500 samples select range A2 to C12 (yellow cells and expand the formulas to the required number of rows.
26. Copy values and paste (or import them) in SPSS or FileMaker Pro database

*VII_Consolidator will work correctly only if you have all five tubes and if the worksheets are in the correct order (see picture). Otherwise, manually paste data for Truecount tube (including header row) to I1 cell of worksheet "Paste consolidated values" (Q1 for T cell panel, AS1 for Treg, AX1 for B cell and BG1 for NK, DC, Mono tube). You should also provide volume of blood aspirated during acquisition (columns CF-CI of worksheet "Cells in µl") if you don't use Truecount tube