

Analysis Tools

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Ciphe Infinity

AN INTERACTIVE GUIDE

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Introduction

“Ciphe Infinity” is a R-Shiny web-based tool that serves for the purpose of running “infinity Flow” analysis for cell-surface markers quantification in an intuitive way that doesn’t require any prior coding knowledge.

It’s based on the original package (“infintyFlow”) for running the analysis and (“ggplot2”) and (“complexHeatmap”) for visualizing the results.

This tool is available for the moment on the GoT’s dev server of CIPHE (soon should be implemented in the prod server).

Purpose of the guide

“Ciphe Infinity” is in the early stages of release, this guide is mainly directed towards the other members of the team in order to provide some feedback over some stuffs that they may not find intuitive or understandable enough inside the UI.

Guide

CONNECTING TO THE TOOL

As mentioned in the introduction, the tool is currently available (As of 13/5/2022) in the dev server of GoT (10.71.1.6).

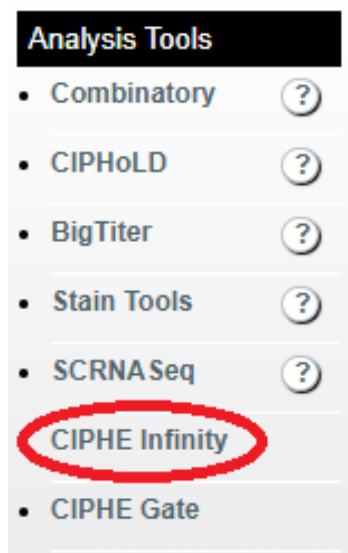
The screenshot shows a web interface with a light blue background. At the top, it says "Welcome to Game of Tools, [CIPHE](#)" in bold, with "CIPHE" as a blue link. Below this, it says "a CIPHE server that contains several tools in RShiny for the analysis of flow cytometry data". In the center, there is a login form with labels "Login :" and "Password :", each followed by a white input box. Below the password box is a "Connexion" button. Underneath the login form is a blue link "Cytometry Public Web Portal". At the bottom, there are four logos: "Inserm" (Institut national de la santé et de la recherche médicale), "cnrs", "Ciphe" (Designing genes Profiling immunity), and "phenomin" (Phenotyping Immune Responses). There are also small "img" icons below the Inserm and phenomin logos.

Use the following credentials: hluche1/hluche1

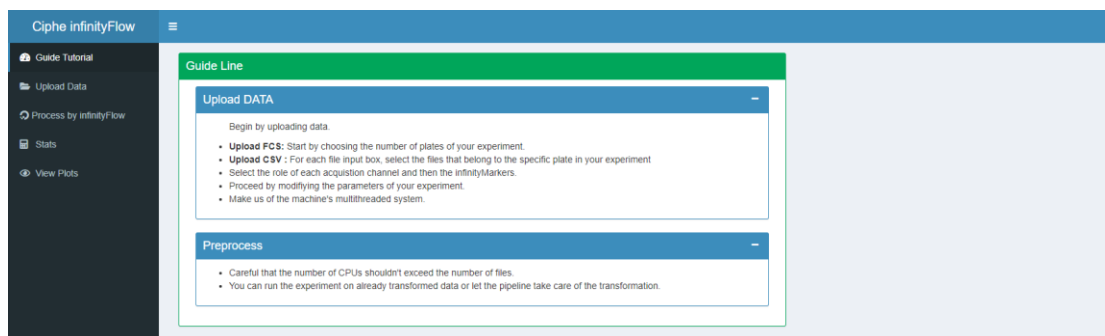
After successfully connecting to the server, the main page should look like this.



Click on “Ciphe Infinity” on the left in order to access tool.



NAVIGATION



Feel free to read the gentle intro that indicates the general use and steps of the tool.

Now moving to the next tab “Upload Data”.

UPLOADING DATA

Cipe infinityFlow

Upload plates data

Number of plates for the experiment: 2

Load .FCS/.TXT/.CSV File(s) from your desktop

Browse... No file selected

Load .FCS/.TXT/.CSV File(s) from your desktop

Browse... No file selected

Submit

Currently there is only one possibility for uploading files. (Soon to be implemented a shinyDirChoose that allows for direct server-side files selections, if files are saved in the analysis server.)

The user should choose how many plates are there in his experiment, in our example we will use an an experiment of two plates with 5 files in total.

Upload plates data

Number of plates for the experiment: 2

Load .FCS/.TXT/.CSV File(s) from your desktop

Browse... 2 files

Upload complete

Plate2_Specimen_001_A3_A03_003.fcs Plate2_Specimen_001_A09_009.fcs

Load .FCS/.TXT/.CSV File(s) from your desktop

Browse... 5 files

Upload complete

Plate3_Specimen_001_C6_C06_030.fcs Plate3_Specimen_001_C11_C11_035.fcs Plate3_Specimen_001_C11_C11_035.fcs

Submit

Submitting data will generate a new tab that will allow for user selection of grouping of columns of acquisition under three labels: Discarded (not used in analysis), Backbone (over which the ML model will run the analysis) and Exploratory (which will be used to infer the values of expression for the markers).

Background/Exploratory Selection

	name	desc	type
\$P1	FSC-A		▼
\$P2	FSC-H		▼
\$P3	FSC-W		▼
\$P4	SSC-A		▼
\$P5	SSC-H		▼
\$P6	SSC-W		▼
\$P7	FJComp-APC-A	CD69-CD301b	▼
\$P8	FJComp-APC-eFlour780-A	Zombie	▼
\$P9	FJComp-Alexa Fluor 700-A	MHCII	▼
\$P10	FJComp-BUV395-A	CD4	▼
\$P11	FJComp-BUV737-A	CD44	▼
\$P12	FJComp-BV421-A	CD8	▼
\$P13	FJComp-BV510-A	CD11c	▼
\$P14	FJComp-BV605-A	CD11b	▼
\$P15	FJComp-BV650-A	F480	▼
\$P16	FJComp-BV711-A	Ly6C	▼
\$P17	FJComp-BV786-A	Lineage	▼
\$P18	FJComp-GFP-A	CD45a488	▼
\$P19	FJComp-PE(yg)-A	Legend	▼
\$P20	FJComp-PE-Cy7(yg)-A	CD24	▼
\$P21	FJComp-PerCP-Cy5-5-A	CD103	▼
\$P22	Time		▼

Confirm Background Exploratory Selection

Background/Exploratory Selection			
	name	desc	type
\$P1	FSC-A		discard
\$P2	FSC-H		discard
\$P3	FSC-W		discard
\$P4	SSC-A		discard
\$P5	SSC-H		discard
\$P6	SSC-W		discard
\$P7	FJComp-APC-A	CD69-CD301b	backbone
\$P8	FJComp-APC-eFlour780-A	Zombie	discard
\$P9	FJComp-Alexa Fluor 700-A	MHCII	backbone
\$P10	FJComp-BUV395-A	CD4	backbone
\$P11	FJComp-BUV737-A	CD44	backbone
\$P12	FJComp-BV421-A	CD8	backbone
\$P13	FJComp-BV510-A	CD11c	backbone
\$P14	FJComp-BV605-A	CD11b	backbone
\$P15	FJComp-BV650-A	F480	backbone
\$P16	FJComp-BV711-A	Ly6C	backbone
\$P17	FJComp-BV786-A	Lineage	backbone
\$P18	FJComp-GFP-A	CD45a488	exploratory
\$P19	FJComp-PE(yg)-A	Legend	discard
\$P20	FJComp-PE-Cy7(yg)-A	CD24	
\$P21	FJComp-PerCP-Cy5-5-A	CD103	
\$P22	Time		

After validating our selection, another table will also show up to choose the labels and isotypes of the studied markers in the experiment. Initially, preset LEGENDScreen plates will be shown to make the selection easier for the user (with the possibility to add new presets for later use).

Background/Exploratory Selection

Infinity Markers selection

Choose .xl or CSV file for Infinity panel (1)

Choose .xl or CSV file for Infinity panel (1)

Browse...

infinity_panel_2.txt

Upload complete

Or Choose a preset Infinity panel:

infinity_isotypes_LEGENDSCREEN_plate_1

	Infinity_target	Infinity_isotype
1	KLRG1	SHlgG
2	Ly-49c/F/I/H	SHlgG

Choose .xl or CSV files for Infinity panel (2)

Choose .xl or CSV files for Infinity panel (2)

Browse...

infinity_panel_3.txt

Upload complete

Or Choose a preset Infinity panel:

infinity_isotypes_LEGENDSCREEN_plate_1

	Infinity_target	Infinity_isotype
1	Podoplanin	SHlgG
2	SSEA-3	rlgM
3	TCR Vg3	SHlgG
4	SHlgG	SHlgG
5	rlgM	rlgM

11	Delta-like 4	Armenian Hamster IgG
12	CD195	Armenian Hamster IgG
13	Notch 4	Armenian Hamster IgG
14	CD229 (Ly-9)	Armenian Hamster IgG
15	CD69	Armenian Hamster IgG
16	Notch 3	Armenian Hamster IgG

11	Delta-like 4	Armenian Hamster IgG
12	CD195	Armenian Hamster IgG
13	Notch 4	Armenian Hamster IgG
14	CD229 (Ly-9)	Armenian Hamster IgG
15	CD69	Armenian Hamster IgG
16	Notch 3	Armenian Hamster IgG

Alternatively, the user can upload his CSV file (like the case in this example). Or also by pressing “Create custom input” an empty table will be initialized which will be then customized.

Press “Confirm Infinity markers” to proceed.

Create custom input

Confirm Infinity markers

PROCESS BY INFINITYFLOW

After successfully uploading all the necessary input for the pipelines, the last step consists of specifying the parameters that will be used for the pipeline run.

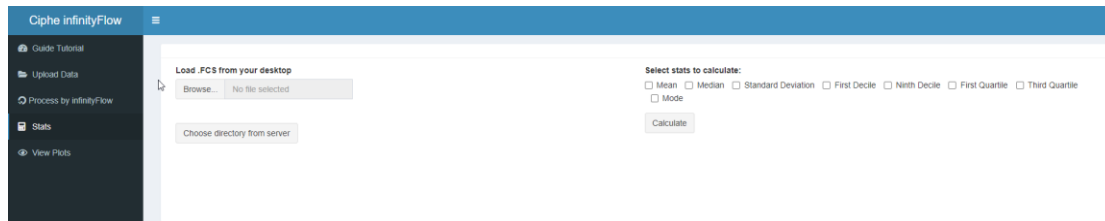
The screenshot shows the 'Process by infinityFlow' interface. On the left is a sidebar with navigation links: 'Guide Tutorial', 'Upload Data', 'Process by infinityFlow' (active), 'Stats', and 'View Plots'. The main area is titled 'infinityFlow pipeline'. It contains two sliders for 'Input Events Downsampling %' and 'Prediction Events Downsampling %', both set to 10. Below these is a dropdown menu for 'Cores/Threads to be used:' set to 1. A radio button group for 'Are the fcs files transformed?' has 'No' selected. A 'Submit pipeline' button is at the bottom.

Based on the average number of events per file, the % of sampling will be applied to all the input files. Moreover, the user has the possibility to opt for multithreaded analysis (\leq the number of cpu's that shouldn't be higher than the number of files chosen), also if the files are already transformed, the user can choose to skip the transformation step.

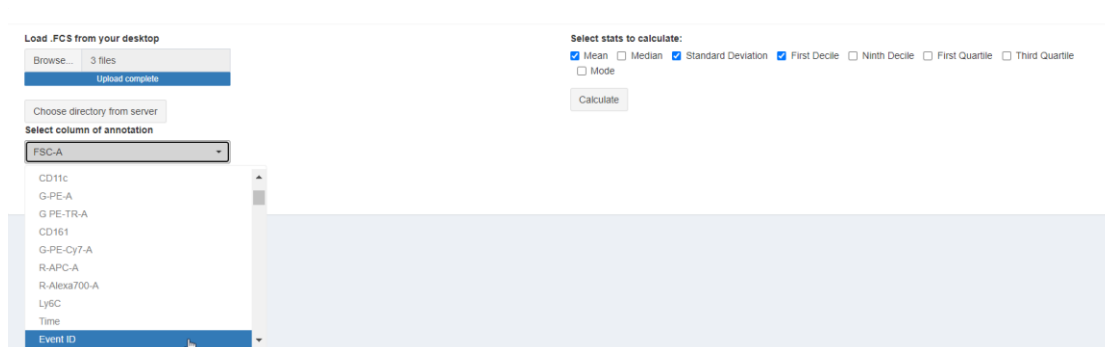
This screenshot shows the same 'Process by infinityFlow' interface as above, but with the 'Cores/Threads to be used:' dropdown menu set to 4. The sliders for downsampling and the 'Are the fcs files transformed?' radio buttons remain unchanged. A mouse cursor is pointing at the 'Submit pipeline' button.

GENERATION OF STATISTICS

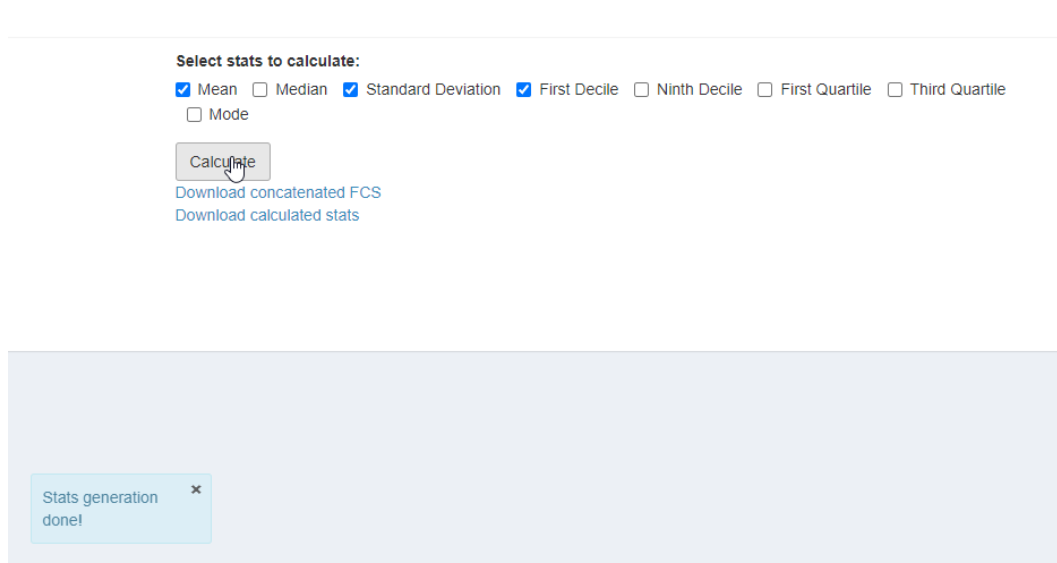
The user can choose to generate statistics for FCS files uploaded from their local PC or from the server he is working on.



There are different choices of statistics. This tool offers the flexibility of being used with files that are from tools outside the pipeline.

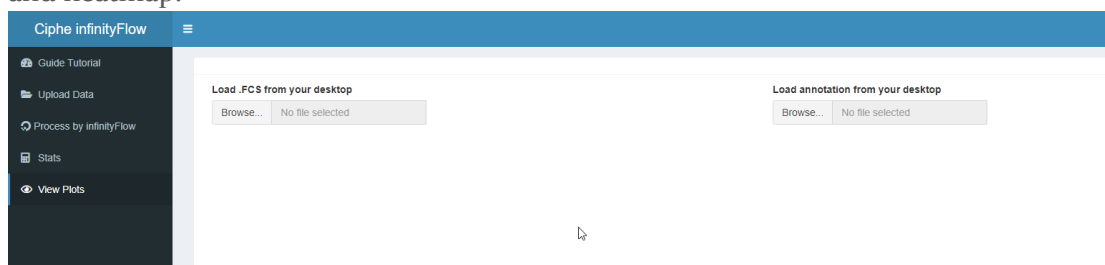


When the calculation is done, the files can be downloaded alongside the concatenated sampled FCS files resulting from the selection.



VISUALIZING PLOTS

Finally, Ciphe Infinity also offers the possibility of visualizing the data using UMAP and heatmap.



In this example, three FCS files were provided alongside a CSV indicating the annotation of the populations (generated by another tool, CIPHE Gate).

Load FCS from your desktop

Browse...

3 files

Upload complete

Select files for plot

Nothing selected

Select column of annotation

FSC-A

Load annotation from your desktop

Browse...

testing_population_index.csv

Upload complete

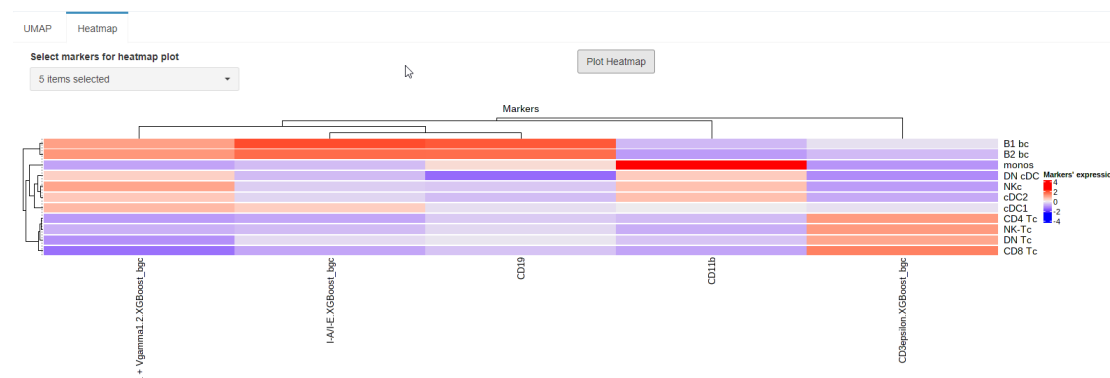
Select populations for heatmap plot

☐ CD4 Tc
 ☐ CD8 Tc
 ☐ DN Tc
 ☐ NK-Tc
 ☐ NKc
 ☐ B1 bc
 ☐ B2 bc
 ☐ cDC1
 ☐ cDC2
 ☐ DN cDC
 ☐ monos

The annotation ID in this example is called “Event ID”, and as we can see we decide to plot a UMAP based on the values inferred to CD3epsilon.



Also for heatmaps, we can choose more than one column (marker). Two hierarchical clustering methods are applied on populations and markers.



We can choose to visualize all the populations, or a group of the populations annotated by selecting the buttons underneath the annotation file box.

