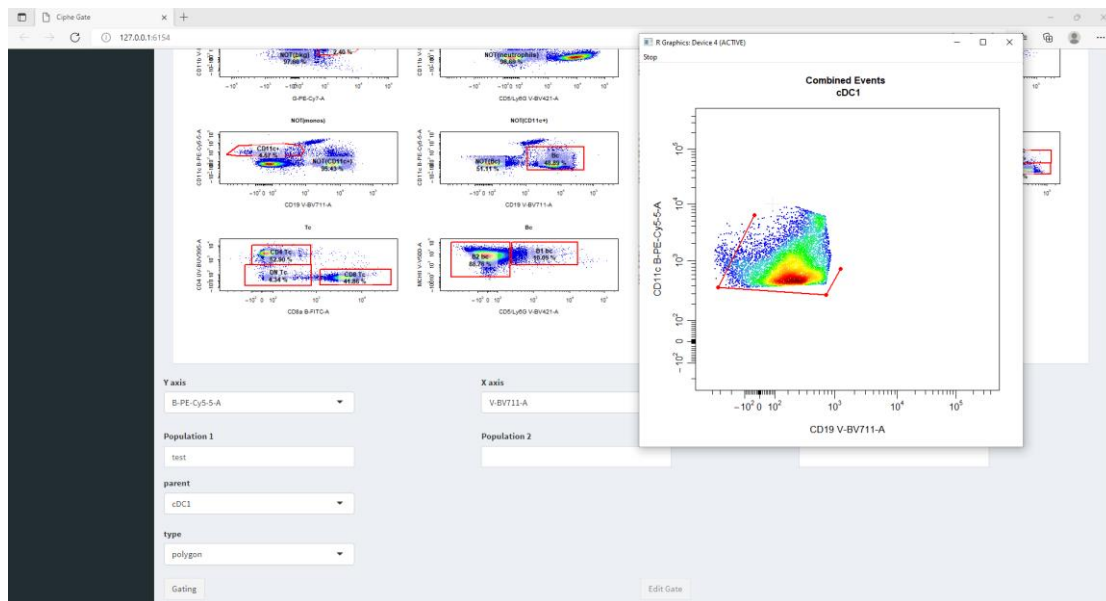


Analysis Tools

- Combinatory ?
- CIPHoLD ?
- BigTiter ?
- Stain Tools ?
- SCRNASeq ?
- CiphelInfinity
- **Ciphe Gate**



Ciphe Gating

AN INTERACTIVE GUIDE

George Alehandro Saad | 13/5/2022

Introduction

“Ciphe Gate” is a R-Shiny web-based tool that serves for multiple purposes.

It’s based, in the majority of it, on the original package of CytoExploreR for setting up and manipulating the different gating strategies in an interactive and intuitive manner.

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 Gate” 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.

Some things could be improved that’s granted, but other stuffs are limited by the original CytoExploreR package over which the creator promised some improvements in their v 2.0.0 release.

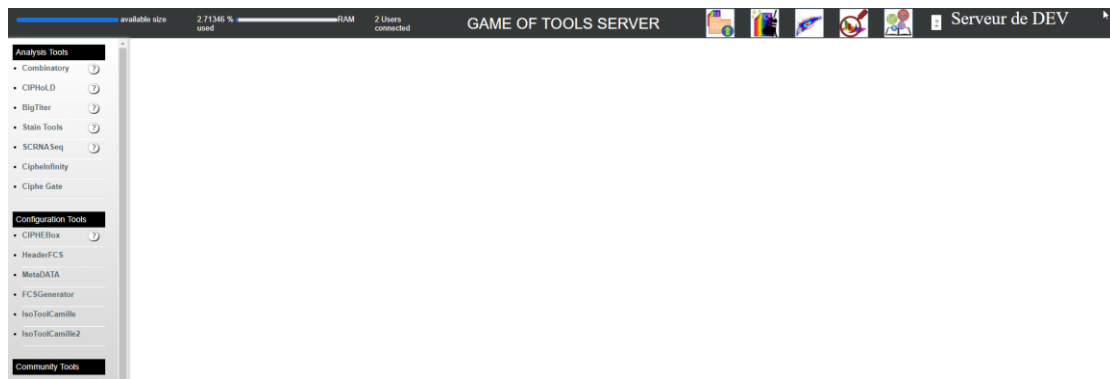
Guide

CONNECTING TO THE TOOL

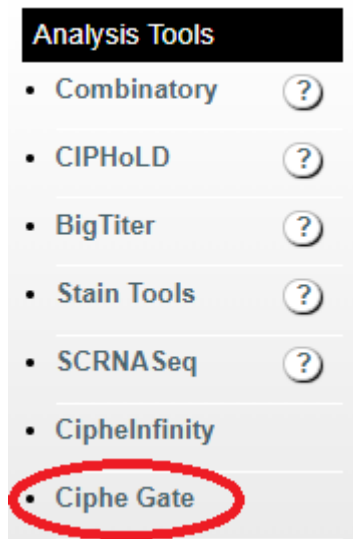
As mentioned in the introduction, the tool is currently available (As of 13/5/2022) in the dev server of GoT (10.71.1.6).

Use the following credentials: hluche1/hluche1

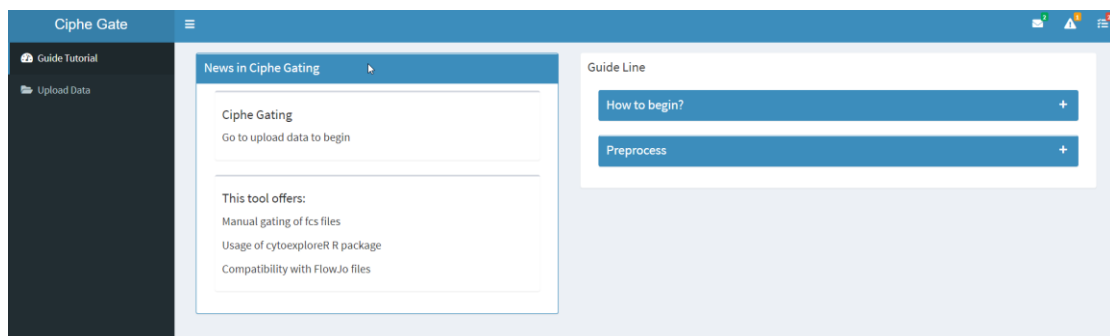
After successfully connecting to the server, the main page should look like this.



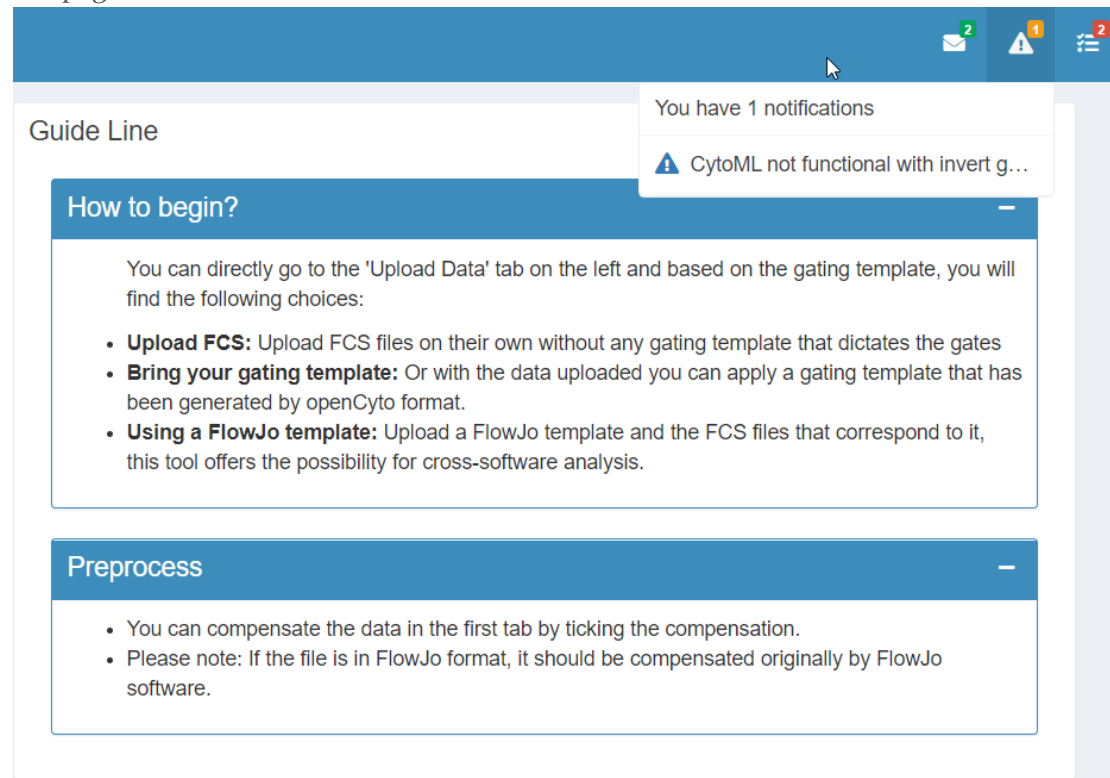
Click on “Ciphe Gate” on the left in order to access tool.



NAVIGATION

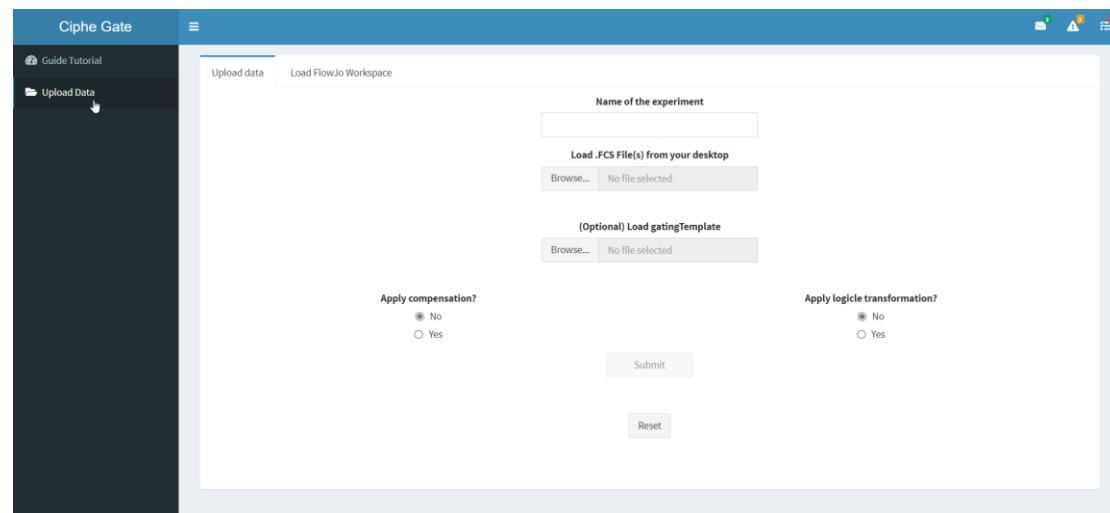


Feel free to read the gentle intro and the notifications that popped up on the head of the page.



Now moving to the next tab “Upload Data”.

UPLOADING DATA

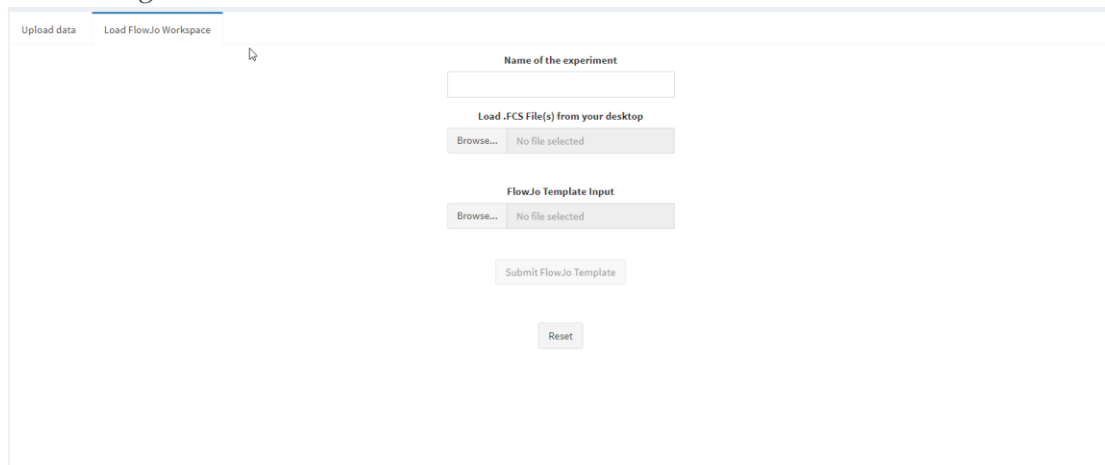


There are two tabs for uploading the files:







- Upload Data
- Load FlowJo Workspace

For both of the ways the experiment name should be specified (because it will be later used when downloading and partitioning the files) and also of course uploading the fcs

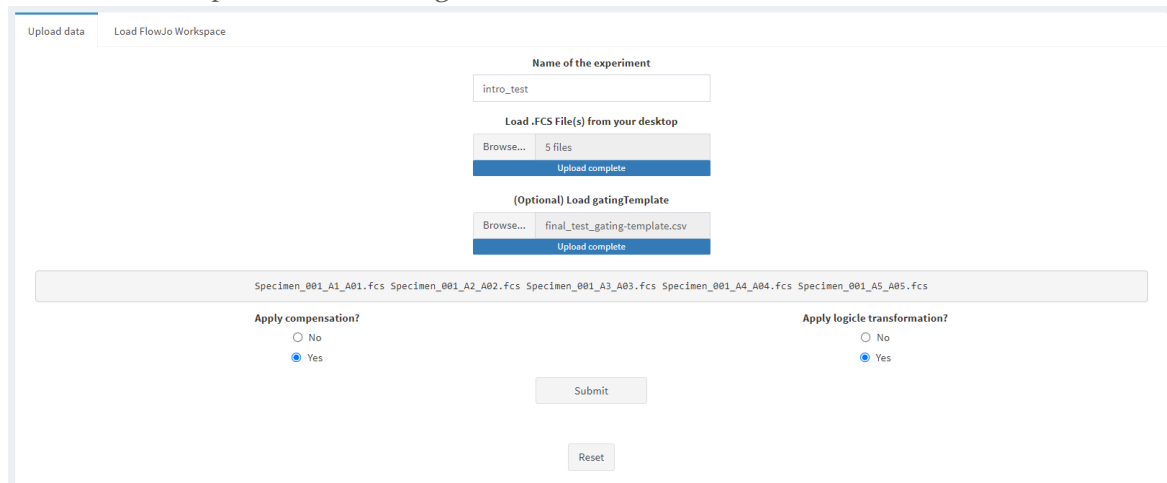
files to be gated.



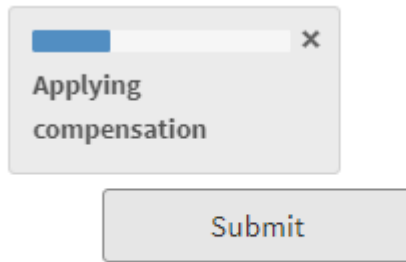
Beside this file, you should receive 5 fcs files of Kevin's dataset with a gating template ready to be applied on the files.

| Name | Date modified | Type | Size |
|--|--------------------|----------------------|-----------|
|  Specimen_001_A1_A01.fcs | 10/23/2018 3:32 PM | FCS File | 21,494 KB |
|  Specimen_001_A2_A02.fcs | 10/23/2018 3:32 PM | FCS File | 22,723 KB |
|  Specimen_001_A3_A03.fcs | 10/23/2018 3:33 PM | FCS File | 19,110 KB |
|  Specimen_001_A4_A04.fcs | 10/23/2018 3:33 PM | FCS File | 16,103 KB |
|  Specimen_001_A5_A05.fcs | 10/23/2018 3:33 PM | FCS File | 15,116 KB |
|  final_test_gating-template | 5/2/2022 3:22 PM | Fichier CSV Micro... | 18 KB |

Go to “Upload data” and upload the files accordingly also in our case we should apply both of the compensations and logic transformation of the data.



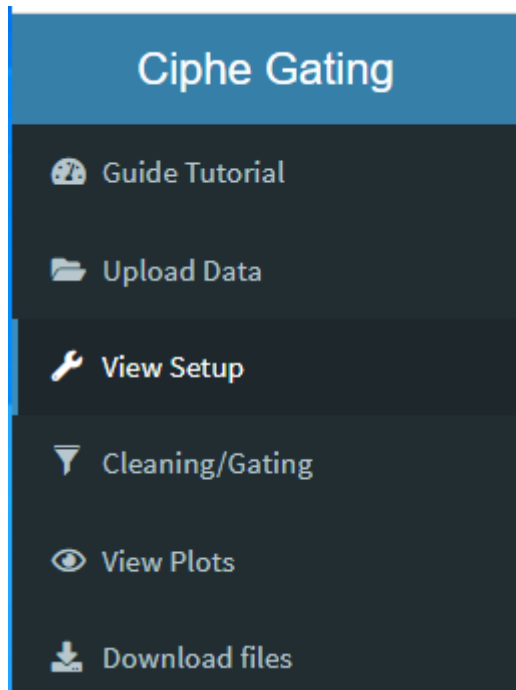
Press “Submit” to proceed.



After uploading the files, new tabs should appear:

- View Setup
- Cleaning/Gating
- View Plots
- Download files

VIEW SETUP



In the “View Setup” the user is able to visualize the channels, markers and name of files uploaded.

Ciphe Gating

Guide Tutorial

Upload Data

View Setup

Cleaning/Gating

View Plots

Download files

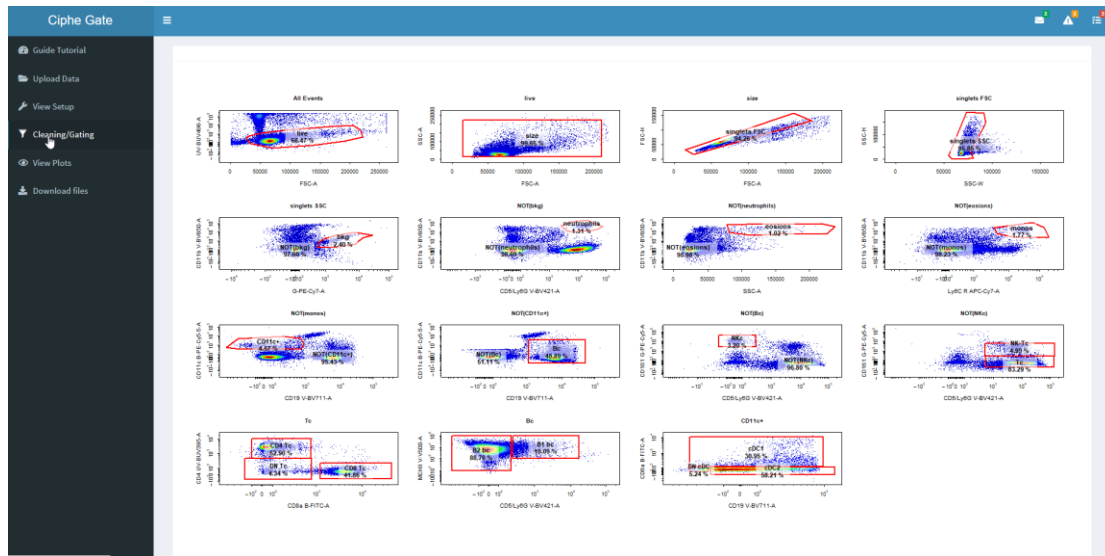
Channels and markers

Files

| | channel | marker |
|----|--------------|----------|
| 1 | FSC-A | |
| 2 | FSC-H | |
| 3 | FSC-W | |
| 4 | SSC-A | |
| 5 | SSC-H | |
| 6 | SSC-W | |
| 7 | UV-BUV395-A | CD4 |
| 8 | UV-BUV496-A | |
| 9 | UV-BUV661-A | |
| 10 | UV-BUV737-A | |
| 11 | V-BV421-A | CD5/Ly6G |
| 12 | V-V500-A | MCHII |
| 13 | V-BV610-A | |
| 14 | V-BV650-A | CD11b |
| 15 | V-BV711-A | CD19 |
| 16 | B-FITC-A | CD8a |
| 17 | B-PE-Cy5-5-A | CD11c |
| 18 | G-PE-A | |
| 19 | G-PE-TR-A | |
| 20 | G-PE-Cy5-A | CD161 |
| 21 | G-PE-Cy7-A | |
| 22 | B-APC-A | |

CLEANING/GATING

Since we provided the gating template for this test, the gating hierarchy will be directly applied to the files.

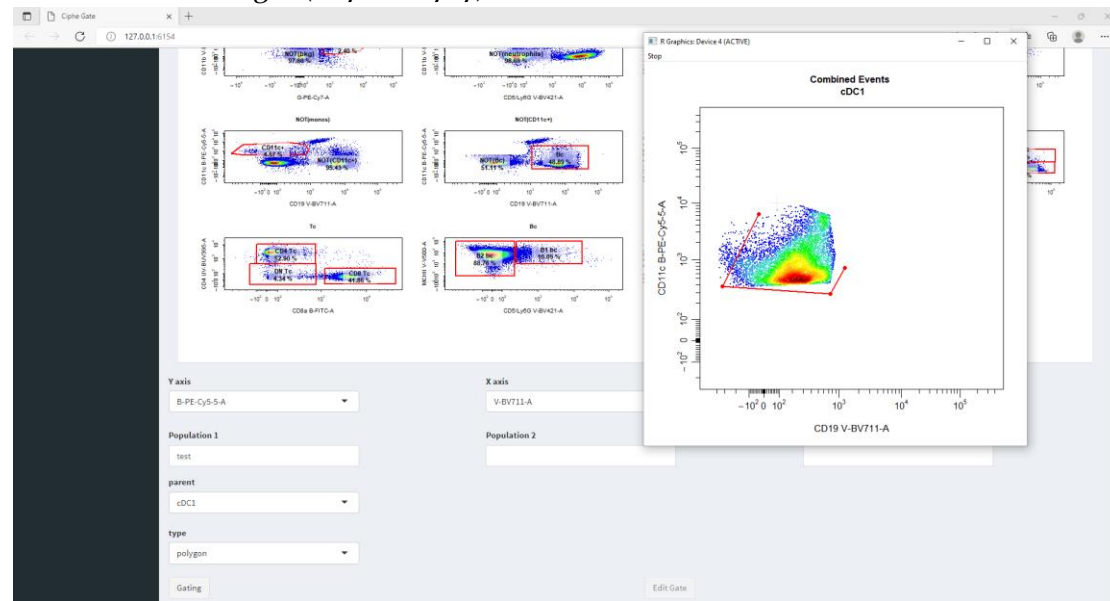


Scrolling a bit down, the different parameters of gating, it's possible to make a new gate or edit what has been already gated.

PLEASE NOTE

As of now, in this version of CytoExploreR it's impossible to make/edit gates on GoT side analysis. Please connect directly to R Studio in server side of the dev server if

interested in testing it (10.71.1.6:8787).



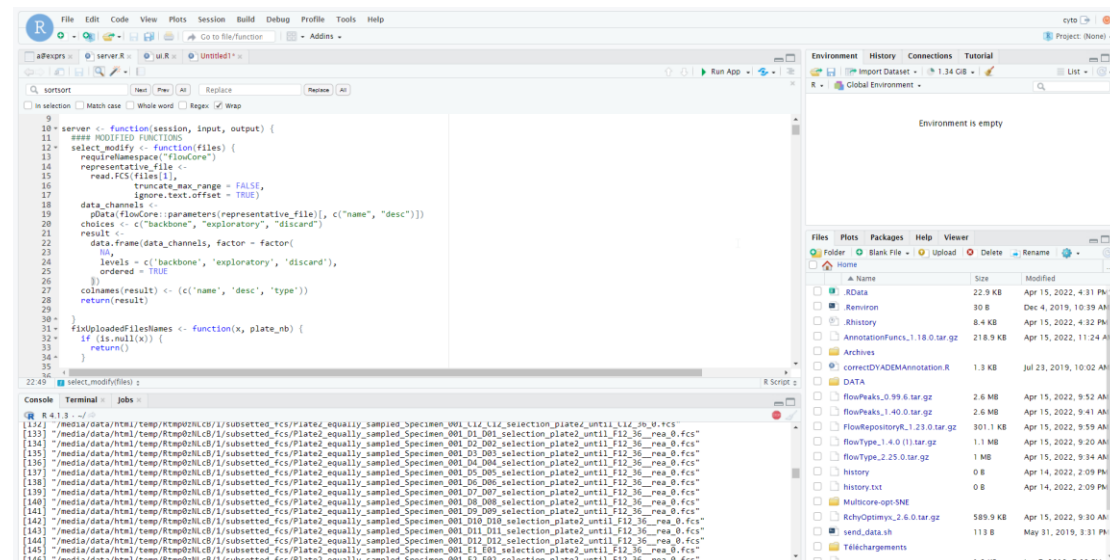
Guide to connect to an interactive R-studio session:

Proceed by accessing this link: <http://10.71.1.6:8787/>

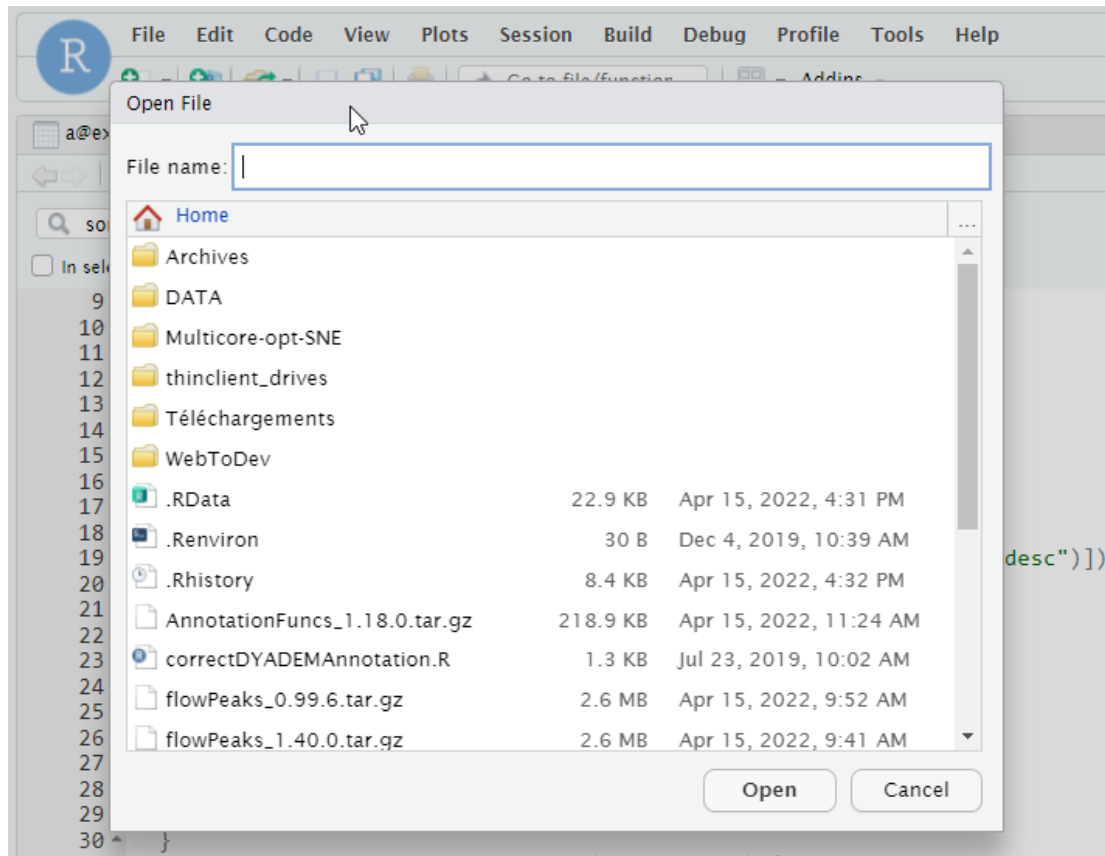
Username: Cyto

Password: P@ssword

The page should look like this (ongoing R-studio session)



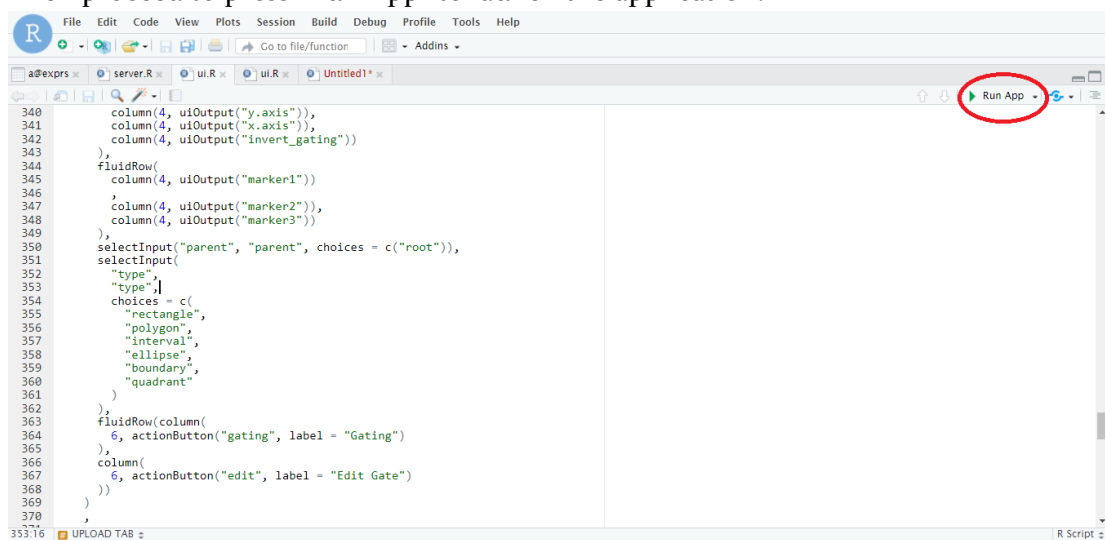
On the top left corner click on: File > Open file name



Copy and paste this: `/media/data/html/source/CIPHEGate/ui.R`

Then press enter to open the file of the script.

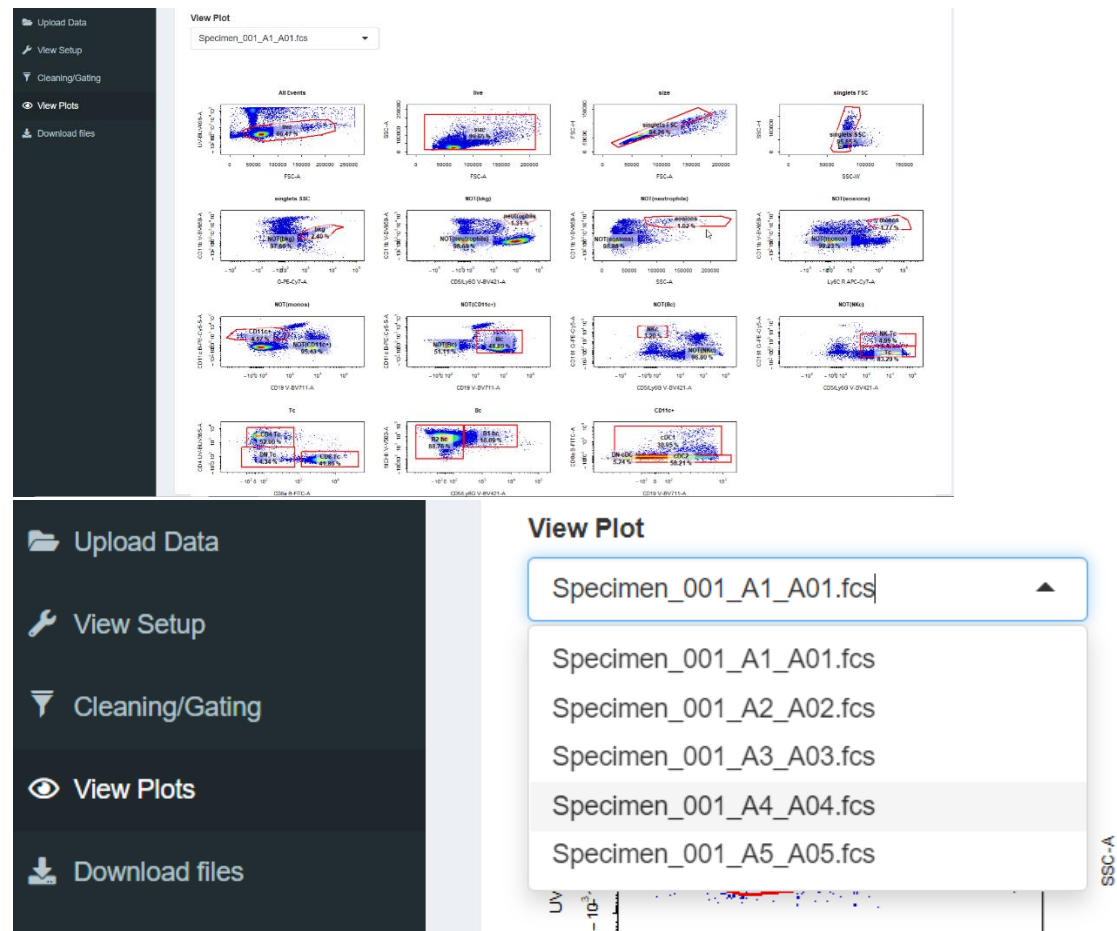
Then proceed to press “Run App” to launch the application.



You can follow the same steps mentioned before and after this part, now you benefit of plotting and editing the gates (will be shown inside the interactive window of R-Studio).

VIEW PLOTS

For visualizing each of the samples uploaded into the server.

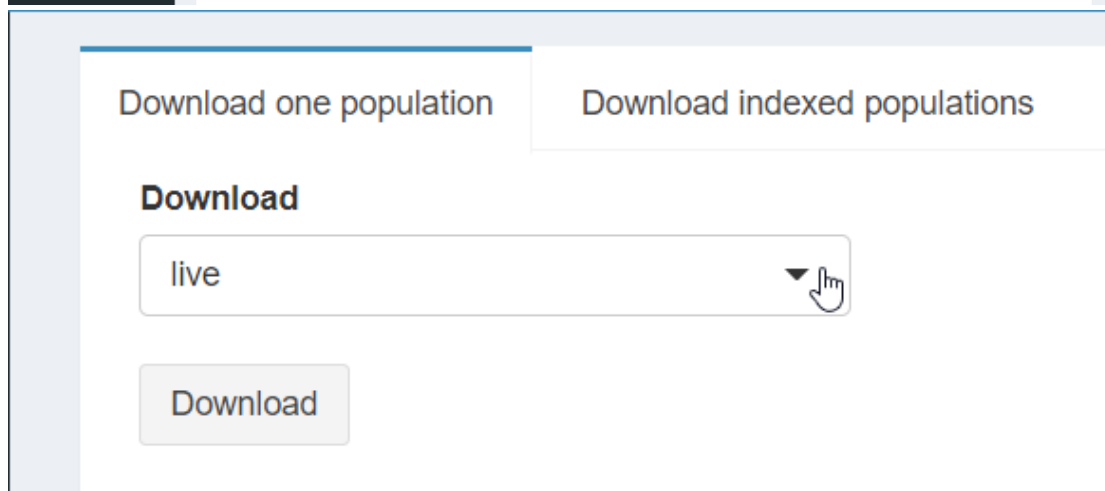
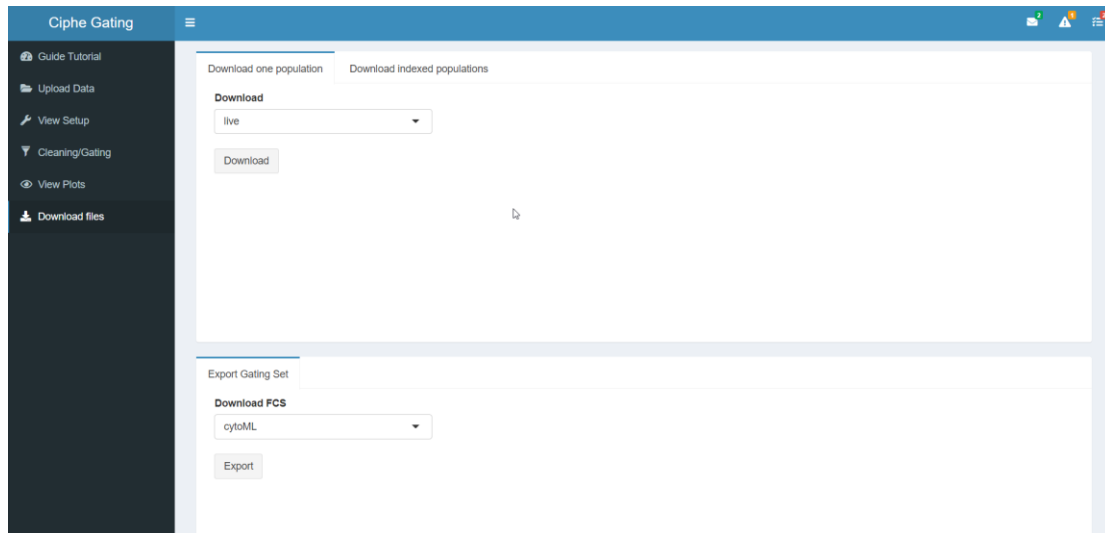


DOWNLOAD FILES

Download files tab should look like this:

- Download one population
- Download indexed population

One thing to mention about “Download indexed population” is that beside the fcs files generated, a csv file will be generated beside them to specify the populations of each index added inside the fcs files.



Download one population

Download indexed populations

Download

live

root
live
size
singlets FSC
singlets SSC
bkg
NOT(bkg)
neutrophils

Download one population

Download indexed populations

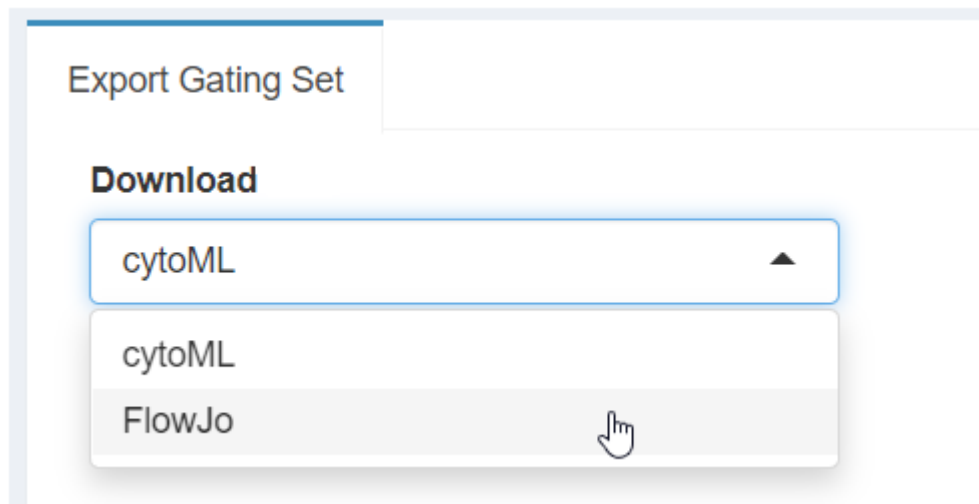
Select populations to index fcs files

cDC2 CD11c+ DN cDC Cd

cDC1
NOT(CD11c+)

Our gating set is automatically saved after each modification, but if we are interested in exporting the gating set of our analysis in a certain format, we have two choices of cytoML (.xml) or FlowJo template.

Please note that it's impossible to gate cytoML with invert gates as of now.



Then by clicking export/download the files will be generated in the working directory in server side which will be further analyzed in other software.