Single-Cell Virtual Cytometer Manual

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0.1 Software presentation

Single-Cell Virtual Cytometer

Single-Cell Virtual Cytometer is a new tool, part of Single-Cell Signature Explorer software package dedicated to high throughput signature exploration in single-cell RNAseq analysis. Single-Cell Virtual Cytometer implements single cell transcriptome analyses with flow cytometry-like analytic tools. It is able to define and gate cell populations from the 2D plot of two antibodies and to display simultaneously the selected cells on a UMAP or tSNE map. The number of antibodies is unlimited. Moreover the software is not limited to antibodies but it is able to combine transcriptomic, proteomic and cellular functions obtained with Single Cell Signature Scorer and display them at the single cell level. Files can be accessed at https://sites.google.com/site/fredsoftwares/products/single-cell-signature-explorer

0.2 Software installation and use

Single-Cell Virtual Cytometer was developed in pure javascript using the graphical library plotly.js [1]. It only needs a web browser with javascript enabled to be executed . We recommend Firefox or Chromium. Single-Cell Virtual Cytometer is not compatible with Internet Explorer or Edge.

0.3 Software basic usage

1. unzip the software
2. scRNAseq data must be stored in a tsv file in which the first row are column names, the first column are cell names and the next columns are the expression values for parameters such as antibodies or genes, or signature scores, or any other parameter, with separator TAB. The table must contains two columns with the x,y coordinates of the UMAP or t-SNE map. By default, the software will use the columns labeled as: tSNE_1 and tSNE_2 to draw the map. This is an example of the table :

id	CD3	CD4	tSNE_1	tSNE_2
AAACCCAAGATTGTGA	3.3	5.3	24.6	-15.1
AAACCCACATCGGTTA	3.6	5.0	25.2	-10.7
AAACCCAGTACCGCGT	2.9	5.3	-10.1	-12.0
AAACCCAGTATCGAAA	3.1	2.7	0.5	36.1

3. To start the software, double click on the Single-Cell Virtual Cytometer HTML file. Your web browser will start with the software interface opened (fig. 1).

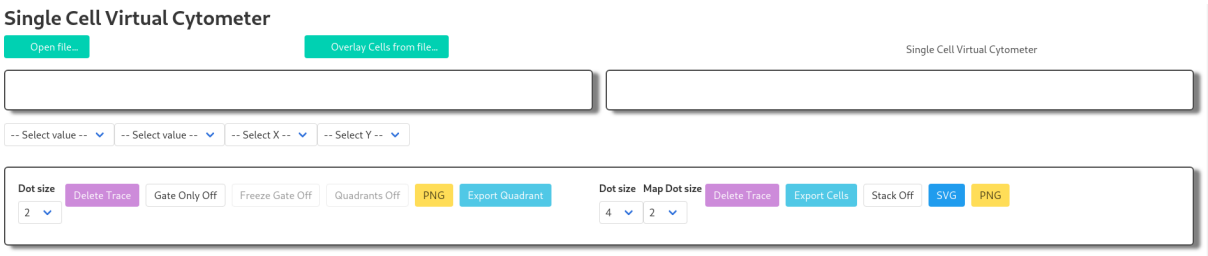

Figure 1: Single-Cell Virtual Cytometer interface displayed in a web browser.

4. Click on the green button "Open file" (top - left) to load the data file (fig. 1).
5. Click on the dropdown lists labeled "Select value" to choose the X, Y parameters of the density plot (fig. 2) .
6. Click on the dropdown lists labeled "Select X/Y" to choose the data columns containing the X,Y coordinates of the map.(fig. 3).
7. Cells can be selected with the lasso or box selection tools (fig. 4).
8. Selected Cell are then displayed on a t-SNE, UMAP.. map (fig. 5).
9. To change the data file, click on the reload page of the web browser and restart the procedure from step 5 .

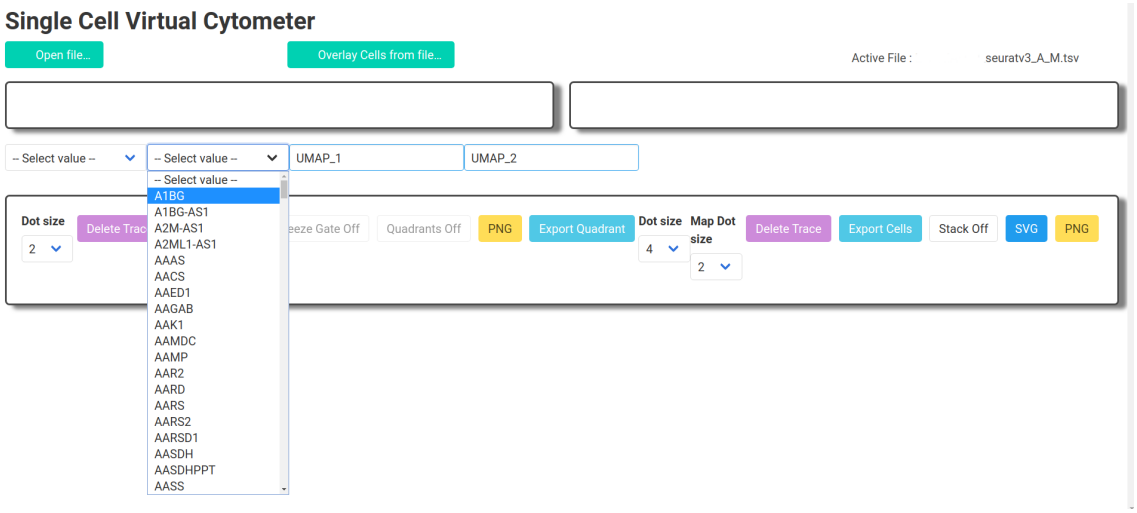

Figure 2: Selection of X, Y parameters using the two dropdown lists labeled "Select value"

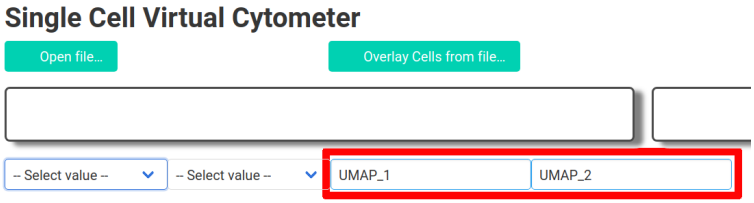

Figure 3: Red box : X, Y data column names should be selected with the dropdown lists labeled "Select X/Y". The figure show how to use UMAP coordinates

0.4 Saving plots

Plots can be saved in :

1. low resolution PNG. For a quick save the plots, click on the camera button of the plot tool bar.

2. high resolution PNG. To obtain a high resolution image click on the PNG yellow button. This save can be very slow because all plots are redrawn at high resolution with a better definition of each cell.

3. SVG vector format. To obtain a SVG image, click on the SVG yellow button. Please not that this possibility is currently only compatible with Firefox and it is very slow because each cell will be drawn as a small circle. For a high number of cells, SVG export can crash the web browser. Use

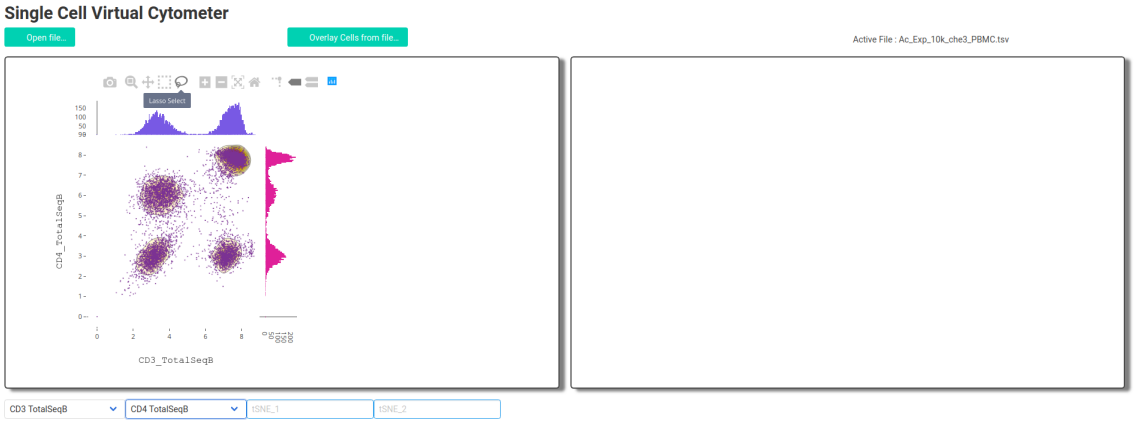

Figure 4: Single-Cell Virtual Cytometer with a cytometry-like 2D plot of the cells labeled with CD3, CD4 or both. The figure shows the plot toolbox : with screen shot tool, selection tools (lasso and box) and scaling tools .

with caution. We recommend Inkscape free software `https://inkscape.org` to work with SVG files.

0.5 Drawing quadrants

1. click on Quadrants Off white button : the button will turn green.
2. place the cursor at the intersection of the quadrants and click
3. quadrants will appears on the left plot with the % of cells in each quadrant. The corresponding 4 cells populations will appear on the right map (fig. 6).
4. Please note that the "Stack On" button will be activated automatically for the right plot. You have to deactivate it manually to continue working without plots overlay.

0.6 Exporting quadrants

To export quadrants, click on the "Export Quadrant" button. A table will be exported in csv format with tab separator. The first column is the name of the quadrant, followed by the cells names and the X, Y cells coordinates. For example :

Q1	AAACCCAAGATTGTGA	3.3	5.3
Q1	AAACCCACATCGGTTA	3.6	5.0
Q1	AAACCCAGTACCGCGT	2.9	5.3
Q1	AAACCCAGTCTACACA	3.1	6.9

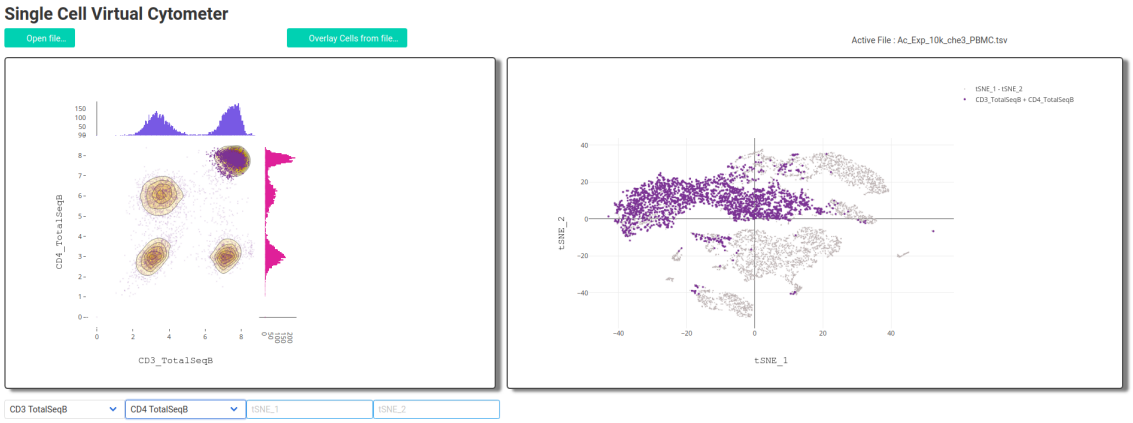

Figure 5: After selection of CD3 CD4 positive cells, this population is displayed on a t-SNE map.

0.7 Exporting Cells

Cells names of the last active population displayed on the t-SNE, UMAP... plots can be exported in a text file using the "Export Cells" blue button.

0.8 Overlaying cell populations on (UMAP, t-SNE...) maps

1. select the first cell population using the lasso or the box select
2. click on the "Stack Off" white button. It will become green and display "Stack On". All the next selected populations will be overlaid on the map. You have to deactivate the "Stack On" button manually to continue working without plots overlayed.

0.9 Using gates

1. select the cell population on the left plot using the lasso or the box select
2. click on the "Gate Only Off" white button. It will become green and display "Gate Only On".
3. Click on the dropdown lists labeled "Select value" to choose another X, Y parameters.
4. select the cell sub-population on the left plot using the lasso or the box select

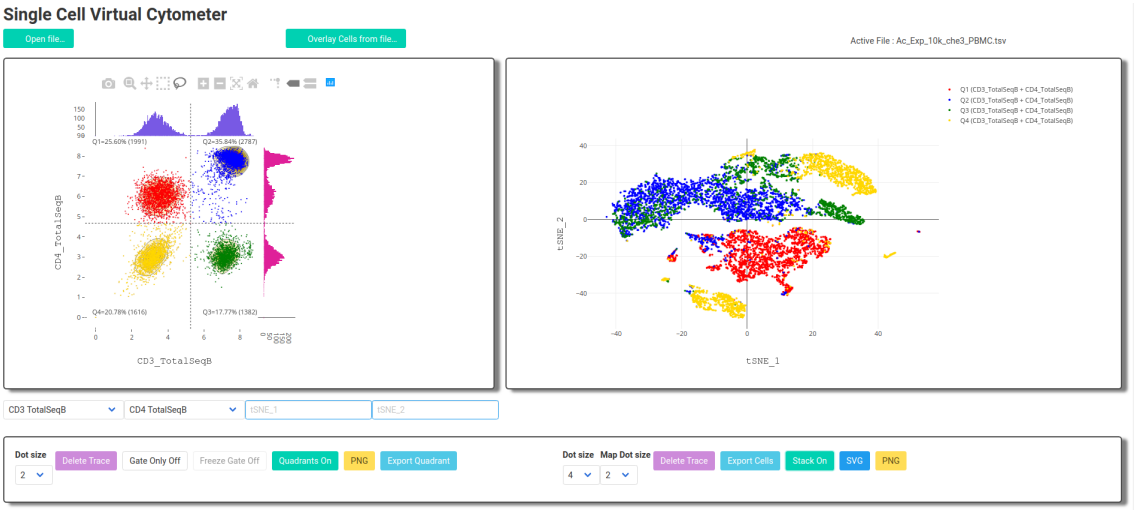

Figure 6: Single-Cell Virtual Cytometer with a cytometry like 2D plot of the cells labeled with CD3, CD4 or both. The figure show the plot toolbox : with screen shot tool, selection tools (lasso and box) and scaling tools .

5. the number of successive gates is unlimited. At any gate level, you can click on the "Freeze Gate Off" button to maintain the same gate level. You have to deactivate the "Freeze Gate On" and the "Gate Only On" buttons manually to continue working without gates.

0.10 Changing dots size

The "Dot size" dropdown lists have to be used to change the cells sizes. The "Map Dot size" dropdown lists on the right map can be used to change the background cells sizes.

0.11 Importing Cells from a file

It is possible to overlay on the left and right plots a set of cells.

1. create a text file containing the cells names, one name by line. For example :

AAACCCATCCGATGTA
AAACCCATCTCAACGA
AAACCCATCTCTCGAC
AAACGAACAATCGTCA
AAACGAACACGATTCA
AAACGAACAGGATCTT

2. click on the "Overlay Cells from file" green button. Imported Cells will be displayed in red on the left plot and in purple on the right map. (fig. 7)

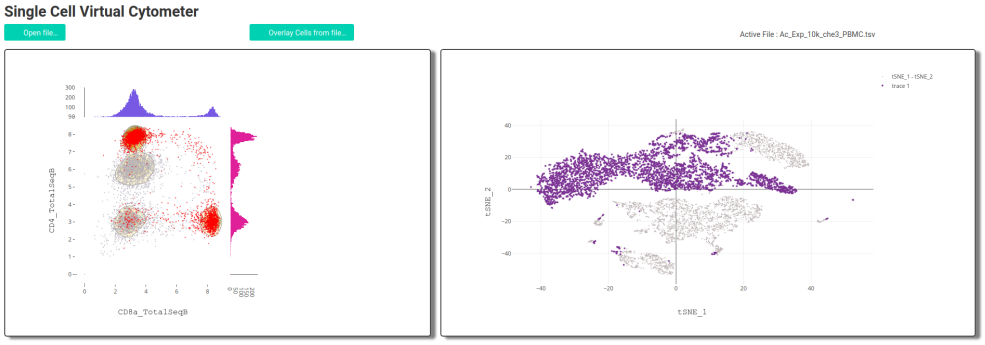

Figure 7: Cells imported from an external file are imported using the "Overlay Cells from file" and displayed in red on the left plot and in purple on the right map.

Bibliography

[1] Inc., P. T. Collaborative data science. (2015).