Single-Cell Multilayer Viewer Manual

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April 2020

Contents

1 Software presentation

Single-Cell Multilayer Viewer is a tool for Single-Cell RNA sequencing analysis. This software is able to display cells in a UMAP or tSNE map, from given coordinates. The main goal for this app is to display several parameters at the same time for the cells. You can visualise up to 5 parameters (gene expression, antibodies expression, gene signature... etc) at the same time, by a combination of colours. This tools aims to provides graphics and plots in a dimension of your choice. Single-Cell Mutlilayer Viewer can be downloaded at https://github.com/MarionPerrier/scMLVT

2 Software installation and use

To download this software, go to this page : https://github.com/MarionPerrier/scMLVT. Click on the green "clone or download" button, then "Download .zip". When it's done, unzip the file, and click on the "index.html" file inside the unzipped folder. That's it !

There is no installation needed. However, this software runs on a internet browser. We recommend you to use it on Mozilla Firefox, Chromium or Google Chrome. Single-Cell Multilayer Viewer is not compatible with Safari, Opera, Internet Explorer or Edge.

Once the app is launched, you should have this interface (figure 1) :

Figure 1: Interface when scMLV software is launched in a web browser

3 Functionalities

A user who is analysing a single-cell data set can wishing to visualize a quantitative (one or several gene expressions) or qualitative (sample's type, or patient's name, etc...) parameter. This parameter visualization on a single-cell RNA seq map will constitute a **layer**. Thereby, a user may want to view several of those differents parameters at the same time on the same map. That's what allows the Single-Cell Multilayer Viewer software. Among these proposed features, Single-Cell Mutlilayer Viewer allows to visualize different combinations of layers by clicking on the legend, to the right of the graph.

PLEASE, NOTE : As it can not contain any empty sub-layer : The combination which does not have cells with value considered as positive, will not appears in the legend.

One to five layers with quantitative data can be displayed on the graphic. Up to two layers with qualitative data can be added to the layers, but under a total of five layers. This means you can have all of these configurations :

Single-Cell Multilayer Viewer

1 - Please, load a dataset (.tsv file) :

⬆ Choose a file...   No file uploaded

⬤ Resampling ?

Export graphic :

SVG   PNG

Figure 2: First step : Click on the red circle button, to load your data

Mode 1 layer :	1 quantitative layer	1 qualitative layer	
Mode 2 layers :	2 quantitative layers	1 quantitative + 1 qualitative layers	2 qualitative layers
Mode 3 layers :	3 quantitative layers	2 quantitative + 1 qualitative layers	1 quantitative + 2 qualitative layers
Mode 4 layers :	4 quantitative layers	3 quantitative + 1 qualitative layers	2 quantitative + 2 qualitative layers
Mode 5 layers :	5 quantitative layers	4 quantitative + 1 qualitative layers	3 quantitative + 2 qualitative layers

A quantitative layer is represented by a colourscale starting from a very light gray, to a colour chosen by the user. All the combinations of quantitative layers (i.e : A point having positive values for layer 1 and layer 2 for example, will have an intermediate colour between layer 1 and layer 2 colours). A point with positive values for all layers will have a colourscale between white and black. The intensity of each colour is calculated between the minimum and the maximum value of other points inside the very same layers combination.

4 Software basic usage

For a good use of this software :

4.1 Import a file

Click on "Choose a file" button (circled in red on the picture below : fig 2), and provide a .tsv or .csv file. Please, make sure your data contains coordinates (UMAP or tSNE), and your quantitative data has already been normalised. This is an example of the table you should provide :

id	CD3	CD4	cancerous	tSNE1	tSNE2
AAACCCAAGGGCTAAC	4.65	5.59	yes	17.87	26.59
AAACCCACACAGCGCT	4.64	5.94	yes	- 24.23	10.89
AAACCCAGTTCGTACA	6.14	6.28	no	40.53	- 10.11
AAACGAAAGACAACAT	4.66	5.31	no	- 26.17	- 13.55

4.2 Select coordinates

Select the axes (X and Y) on the drop-down lists labelled "Select X axis" and "Select Y axis" (showed circled in red in the figure 3 below), to display the graphic.

Figure 3: Second step : Select the X and Y coordinates by clicking on these two drop-down lists circled in red

4.3 Select the number of Layers

Select the number of layers you want to visualise (from 1 to 5), by clicking on the "How many layers ?" drop-down list (circled in red in the figure 4 below).

4.4 Select on the drop-down list

Select on the drop-down lists "Select an option" the layers you wish to display. It could be a gene expression, antibody marker, gene signature, or a qualitative variable, such as the type of sample. (Drop-down lists circled in red in the figure 5)

4.5 Modify the plot

Adjust the look of the plot by modifying the colours and/or the size of each layer, by clicking on the colours picker and moving the slider cursor (see figure 6. If a qualitative variable has been selected, the sliders can be moved (for the dots size), the shape of the dots and the colour of each value as well can be changed, as indicates the components circled by red in the figure 7 and figure 8

5 Saving plots

The buttons "PNG" and "SVG" at the bottom of the page is to save an image of the plot, in two different formats. To save the plot made, click on one of the two buttons labelled "PNG" and "SVG" depending on the format wanted.

- PNG format has a lower resolution, but has a faster execution speed to quickly save plot.

- SVG vector format has a higher resolution, but has a lowest execution speed. Please note : For each cell (represented by a dot on the graph) : A 2D form is created (circle, star, etc....), which asks a lot of resources and CPU computing. For this reason, the web browser may crash if there is too many cells in the graph.

For each format, the width and the height can be chosen. Furthermore, the legend can also be saved by clicking "yes" to the pop up asking to save the legend. Please, note that this option is only available for plots with two or more quantitative layers.

Figure 4: Third step : Select how many layers you want to display by clicking on the red circled drop-down list

Figure 5: Fourth step : Select the layers you wish to display by clicking on the red circled drop-down lists

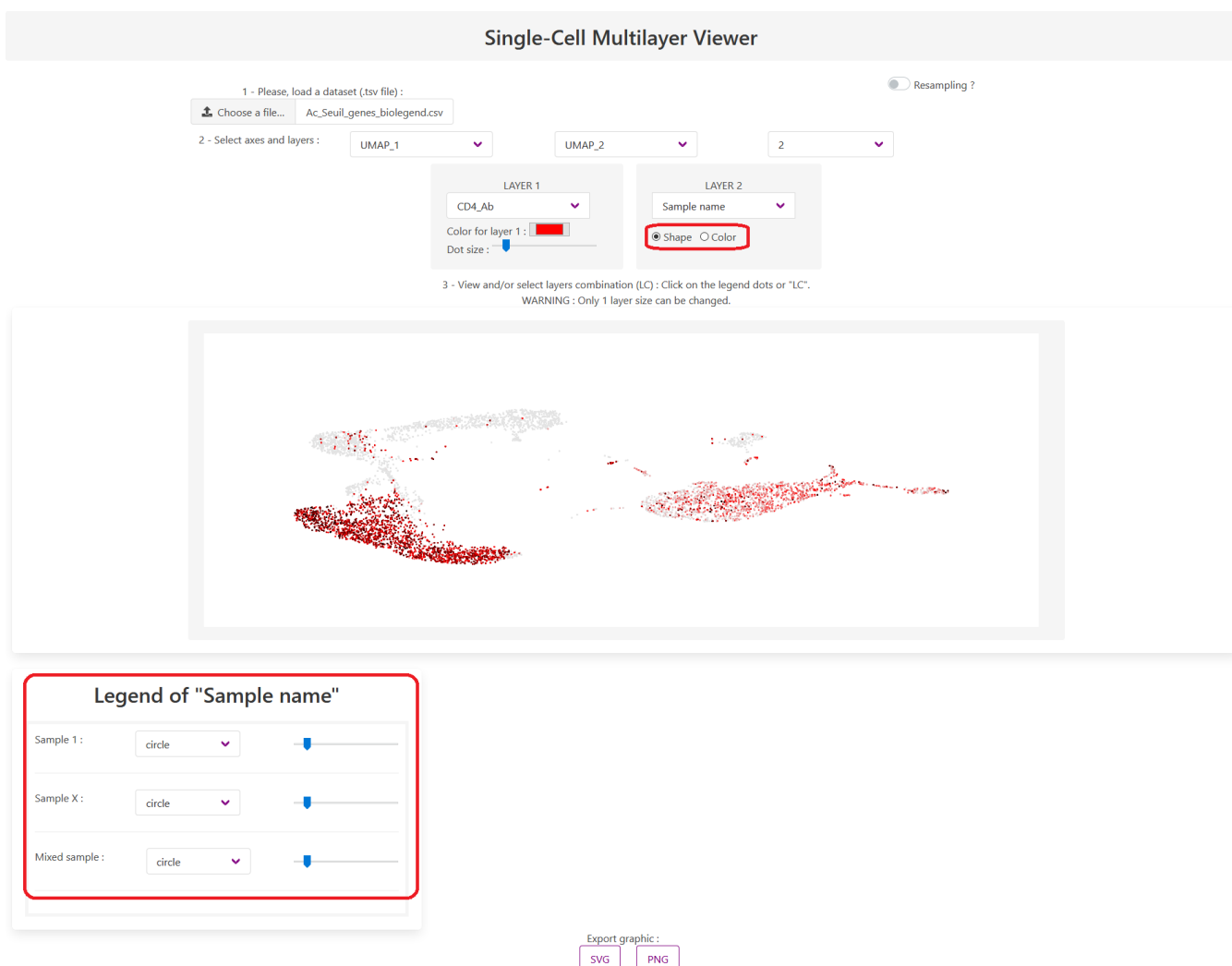Figure 6: Fifth step : Slider cursor and colours pickers to modify, circled in red.

Figure 7: Sliders and colour pickers to modify the colours of dots for qualitative variables.
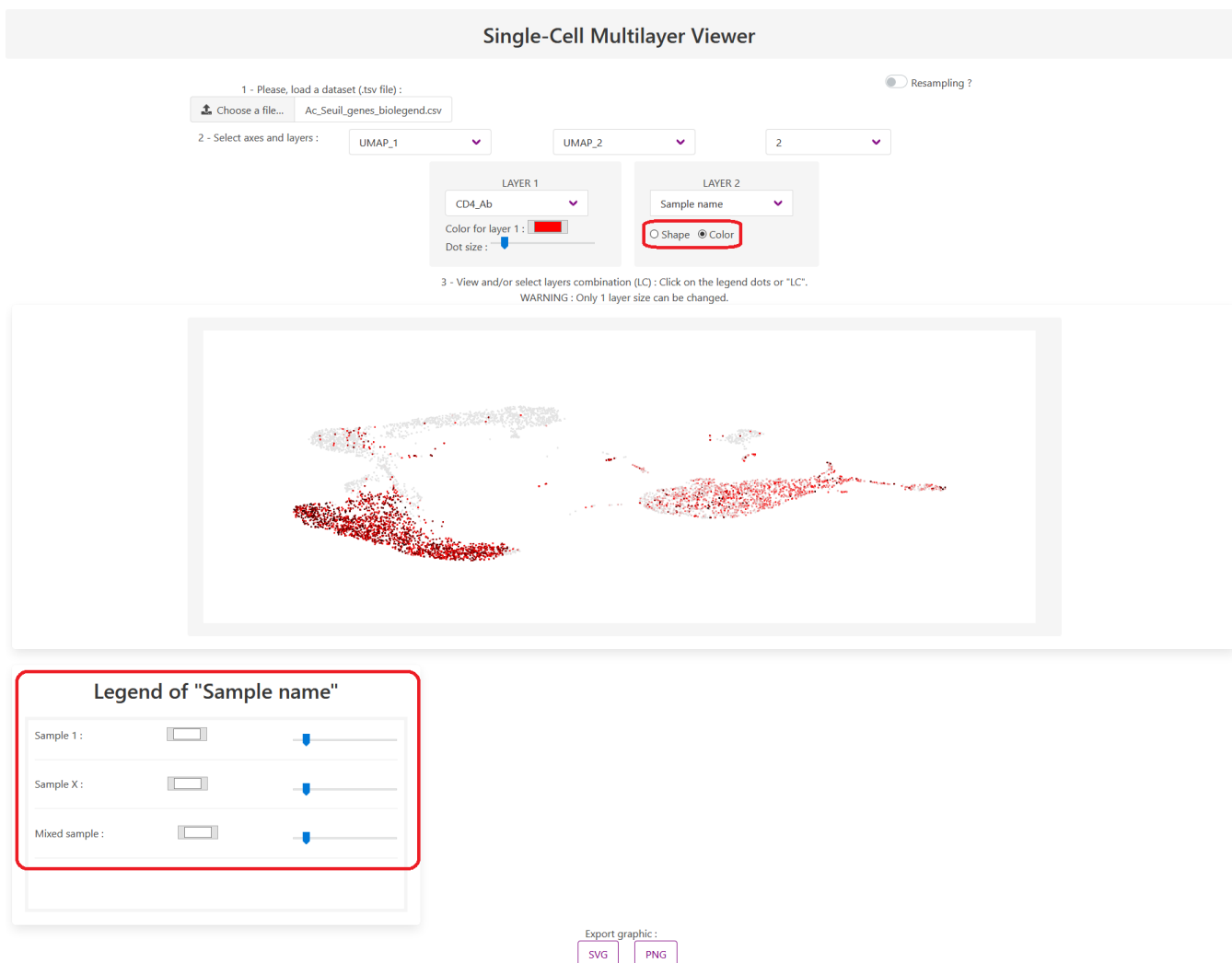
Figure 8: Sliders and drop-down select to modify the shapes of dots for qualitative variables.