Single-Cell Virtual Cytometer Manual

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February 7, 2020

Contents

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 hallmarks computed by Single Cell Signature Scorer, and to display both of 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 any parameter such as antibody (ADT) intensity or gene expression, or signature scores, or any other parameter, with separator TAB. The table must contain 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).

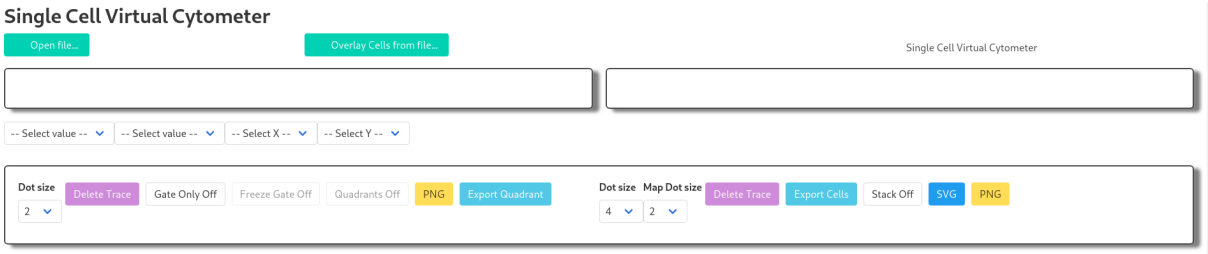

Figure 1: Single-Cell Virtual Cytometer interface displayed in a web browser.

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).

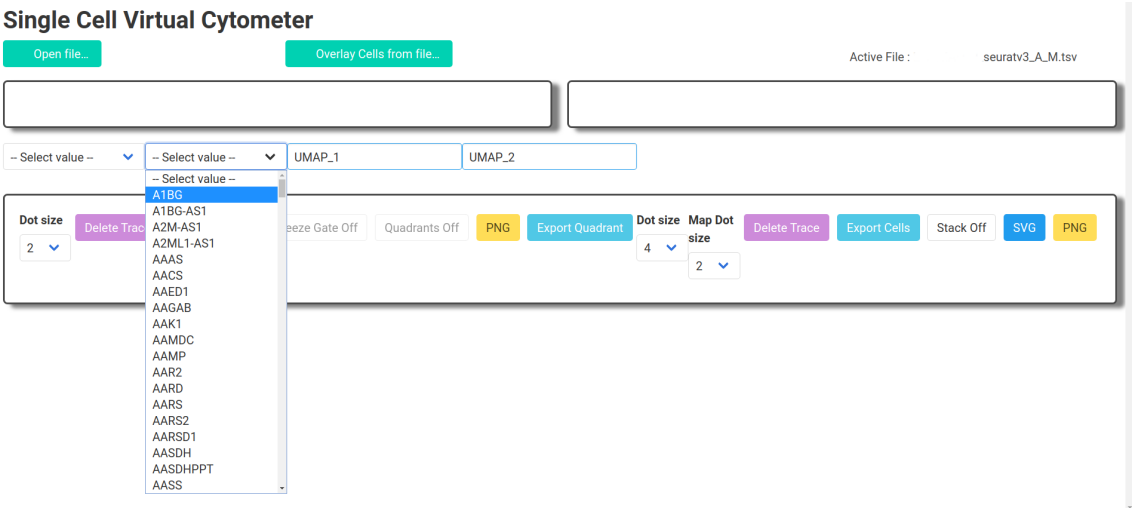

Figure 2: Selection of X, Y parameters using the two dropdown lists labeled "Select value"

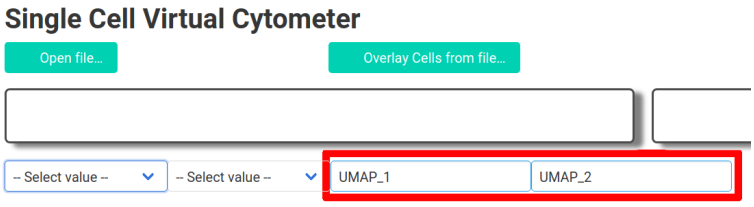

Figure 3: Red box : X, Y data column names should be selected with the dropdown lists labeled "Select X/Y". The figure show how to use UMAP coordinates

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 .

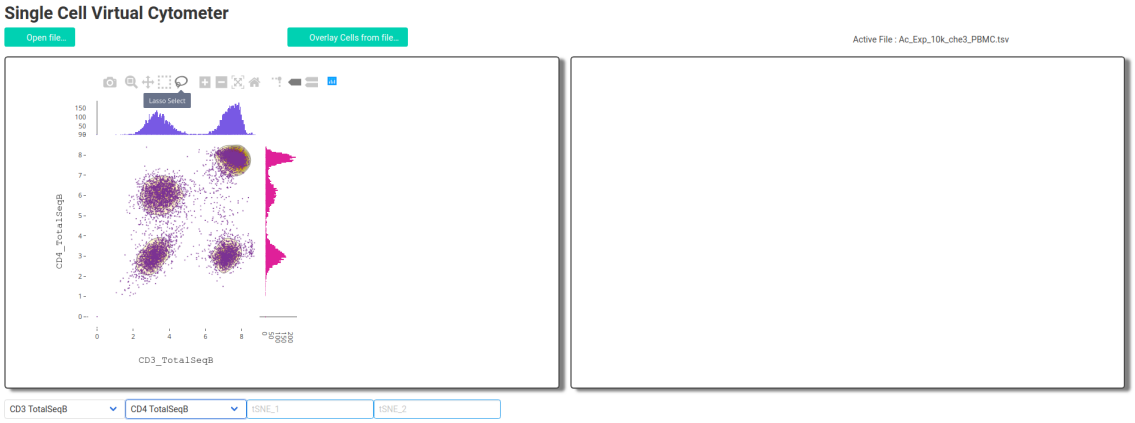

Figure 4: Single-Cell Virtual Cytometer with a cytometry-like 2D plot of the cells labeled with CD3, CD4 or both. The figure shows the plot toolbox : with screen shot tool, selection tools (lasso and box) and scaling tools .

0.4 Saving plots

Plots can be saved in :

1. low resolution PNG. For a quick save of 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 note that this possibility is currently only compatible with Firefox, and is very slow because each cell will be drawn as a small circle. For a high number of cells, SVG export can eventually crash the web browser. To be used with caution. We recommend Inkscape free software `https://inkscape.org` to work further 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 resulting four cell populations will appear as correspondingly colored cells on the right transcriptome-based map (fig. 6).

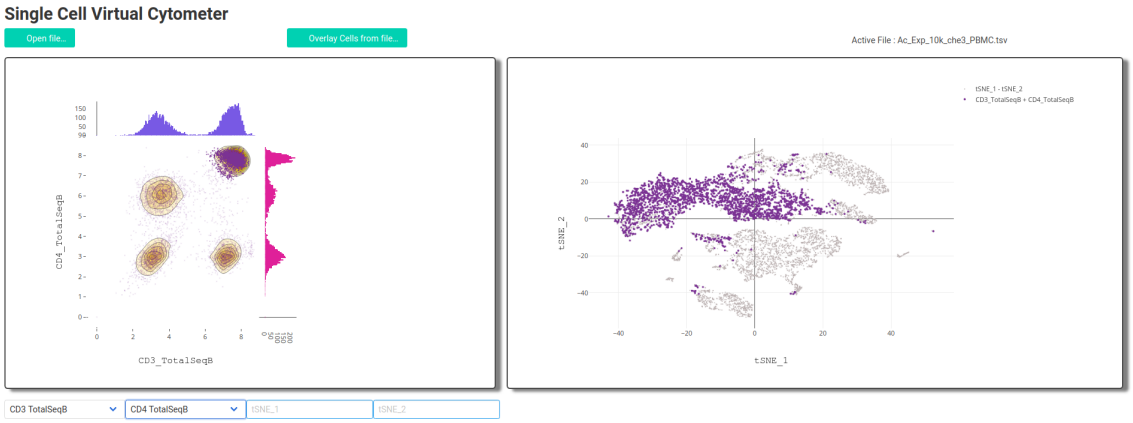

Figure 5: After selection of CD3 CD4 positive cells, this population is displayed on a t-SNE map.

4. Please note that the "Stack On" button will be activated automatically for the right plot. User has to manually deactivate it 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

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

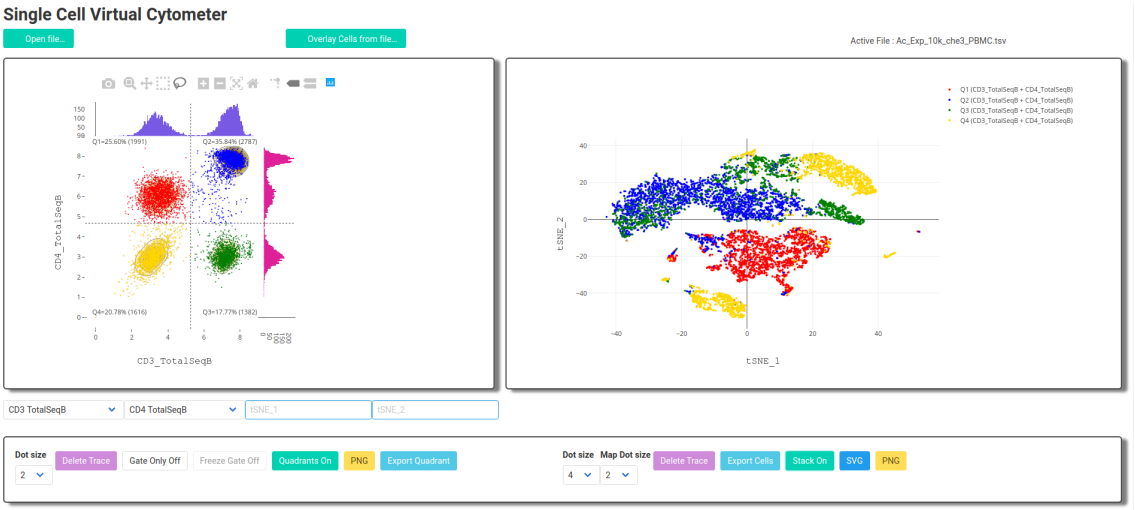

Figure 6: Single-Cell Virtual Cytometer with a cytometry-like biaxial plot of cells labeled with CD3 and CD4. The figure show the plot toolbox : with screenshot tool, selection tools (lasso and box) and scaling tools .

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. User has to manually deactivate the "Stack On" button to continue working without overlaid plots.

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) set of parameters.
4. select the cell sub-population on the left plot using the lasso or the box select
5. the number of successive gates is unlimited. At any gate level, you can click on the "Freeze Gate Off" button to switch it to "Freeze Gate On" such as to maintain the same gate level. You have to manually deactivate both the "Freeze Gate On" and the "Gate Only On" buttons 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 an additional file

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

1. create a text file containing the cells codes, 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)

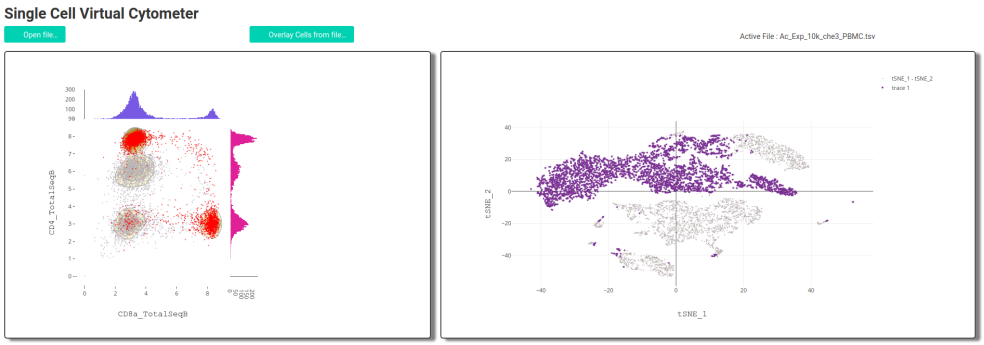

Figure 7: Cells imported from an external file are imported using the "Overlay Cells from file" and displayed in red on the left plot and in purple on the right map.

0.12 Display clusters in map

Clusters can be displayed using the drop-down lists labeled "Select cluster". It is possible to display all clusters (cf fig. 8) or a single one (cf fig. 9). Very

importantly, the type of data for the cluster column must be integers. Cluster numbers such as [0,1,2,3] are valid whereas [0.0,1.0,2.0,3.0] are not valid.

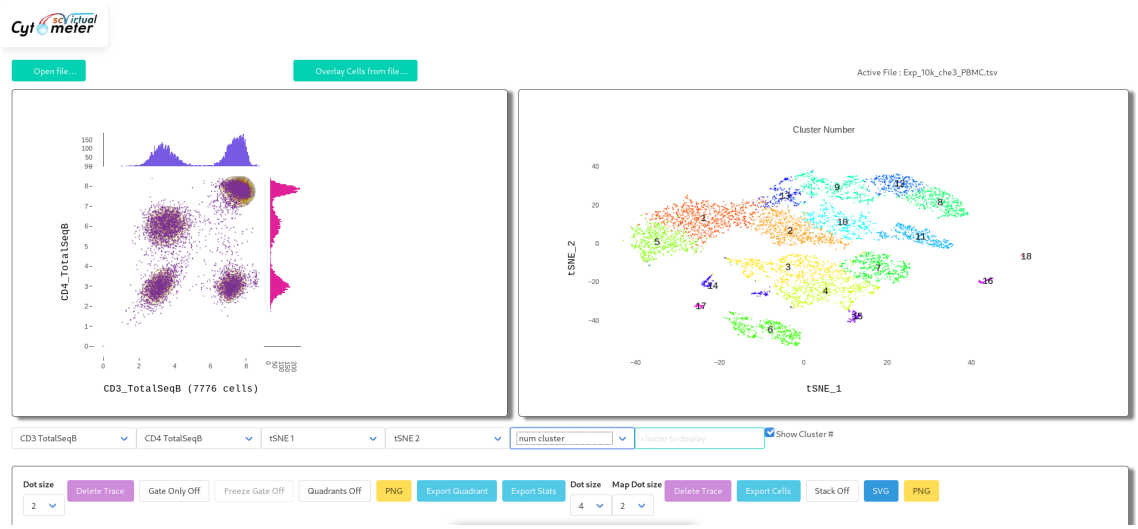

Figure 8: Clusters display with numbers on the t-SNE map

0.12.1 Display all clusters

All clusters are displayed by default when the "cluster to display" text box is empty. If a cluster number is entered in the "cluster to display" text box, to show again all the clusters, type -1 in the "cluster to display" text box.

0.12.2 Display one cluster

To display one cluster, enter the cluster number in the "cluster to display" text box (cf fig. 9).

0.12.3 Remove clusters numbers

To remove the cluster numbers, uncheck the "Show Cluster #" check box before typing a cluster number in the "cluster to display" text box to show one cluster, or -1 to show all clusters.

0.12.4 Tip : cluster number of a dot.

The cluster number of a dot can be obtained by positioning the mouse pointer over a dot. (cf. fig. 10).

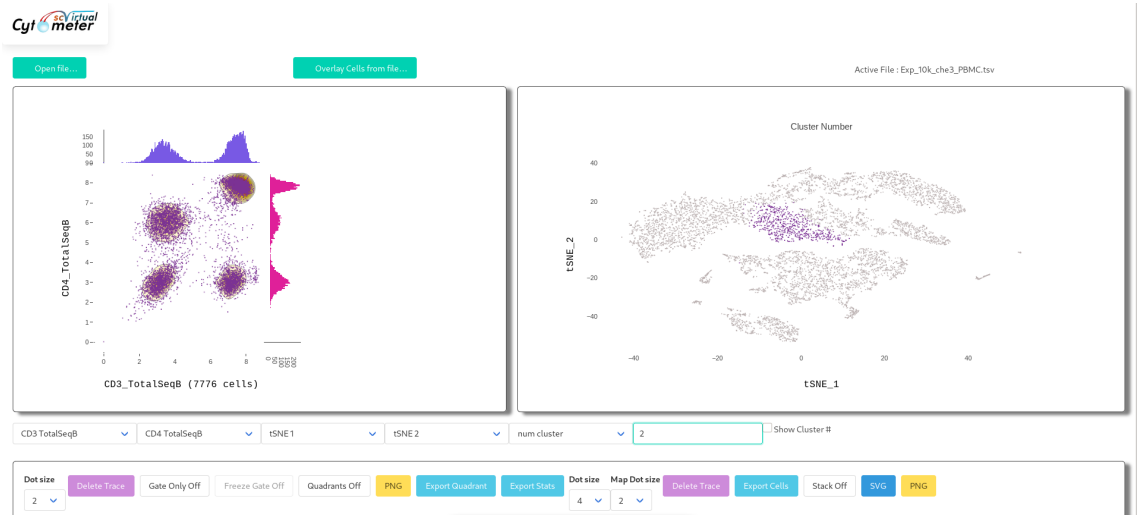

Figure 9: Cluster 2 is displayed without number on the t-SNE map

0.13 Display antibodies signal in map

Antibodies can be displayed using the dropdown lists labeled "Select cluster" (cf. fig. 11). The software distinguish antibodies from clusters using the colum type. The type of the genes columns must be floats. Genes values such as [0.0,1.0,2.0,3.0] are valid whereas [0,1,2,3] are not valid.

0.14 Display gene expression in map

Genes can be displayed using the drop-down lists labeled "Select cluster" (cf. fig. 7). The software distinguish genes from clusters using the column type. The type of the genes columns must be floats. Genes values such as [0.0,1.0,2.0,3.0] are valid whereas [0,1,2,3] are not valid.

0.15 Set Dot size of cluster/gene maps

The dot size can be changed using the "Map dot size" button. (cf. fig. 10)

0.16 Export statistics

To export a summary of the descriptive statistics for one gate or quadrants, press the "Export Stats" buttons. These descriptive statistics will be saved in a text file (see below).

Figure 10: Cluster 2 is displayed without number on the t-SNE map. The dot size has been increased using the "Map Dot size" button and the cluster number is displayed by positioning the mouse pointer over a dot.

Figure 11: Cells expressing the CD3 epitope on the t-SNE map

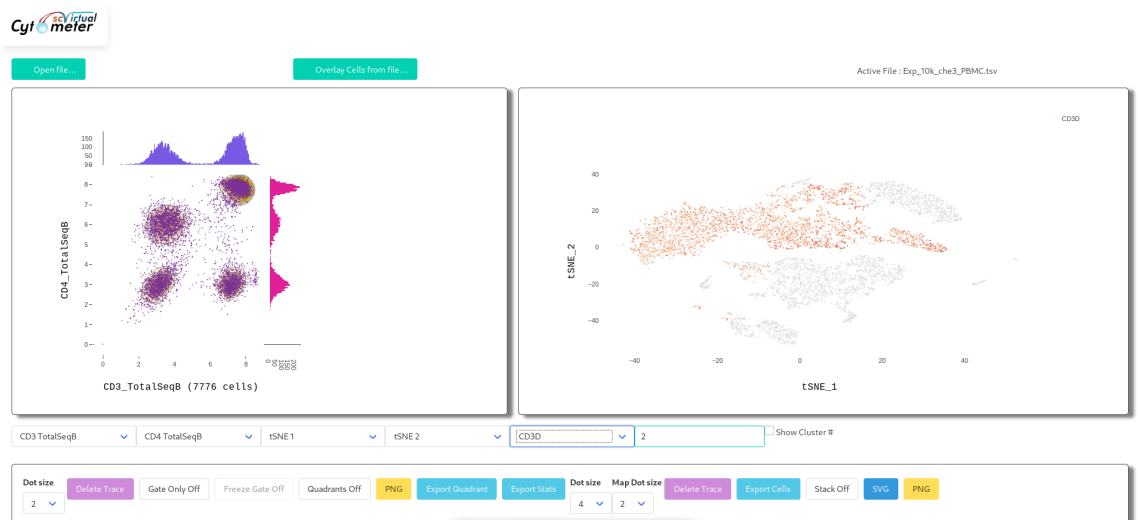

Figure 12: Expression of the gene CD3 on the t-SNE map

```
scVirtual Cytometer Statistics
------------------------------

User parameters
---------------
X parameter:    myeloid cell score
Y parameter:    CD3_Ab

Number of cells
---------------
Total number of cells                          :   5559
Number of gated cells shown in density plot :   5559
Number of cells in quadrant 1 : 2876
Number of cells in quadrant 2 : 220
Number of cells in quadrant 3 : 1320
Number of cells in quadrant 4 : 1143

% of cells in density plot
--------------------------
% cells in quadrant 1 : 51.74
% cells in quadrant 2 : 3.96
% cells in quadrant 3 : 23.75
% cells in quadrant 4 : 20.56

X Arithmetic Means
------------------
X Mean for cells in quadrant 1 :        0.4
X Mean for cells in quadrant 2 :        2.08
X Mean for cells in quadrant 3 :        2.61
X Mean for cells in quadrant 4 :        0.47

X Medians
---------
X Median for cells in quadrant 1 :      0.38
X Median for cells in quadrant 2 :      2.02
X Median for cells in quadrant 3 :      2.54
X Median for cells in quadrant 4 :      0.45

X Standard deviation
--------------------
X SD for cells in quadrant 1 :  0.16
X SD for cells in quadrant 2 :  0.54
X SD for cells in quadrant 3 :  0.77
X SD for cells in quadrant 4 :  0.19

X Variance
----------
X VAR for cells in quadrant 1 : 0.03
X VAR for cells in quadrant 2 : 0.3
```

```
X VAR for cells in quadrant 3 : 0.59
X VAR for cells in quadrant 4 : 0.04

X Coefficient of Variation
--------------------------

X CV for cells in quadrant 1 :  0.4
X CV for cells in quadrant 2 :  0.26
X CV for cells in quadrant 3 :  0.29
X CV for cells in quadrant 4 :  0.4

Y Arithmetic Means
------------------

Y Mean for cells in quadrant 1 :        4.67
Y Mean for cells in quadrant 2 :        4.31
Y Mean for cells in quadrant 3 :        2.27
Y Mean for cells in quadrant 4 :        2.09

Y Medians
---------

Y Median for cells in quadrant 1 :      4.67
Y Median for cells in quadrant 2 :      4.36
Y Median for cells in quadrant 3 :      2.3
Y Median for cells in quadrant 4 :      2.12

Y Standard deviation
--------------------

Y SD for cells in quadrant 1 :  0.52
Y SD for cells in quadrant 2 :  0.73
Y SD for cells in quadrant 3 :  0.34
Y SD for cells in quadrant 4 :  0.36

Y Variance
----------

Y VAR for cells in quadrant 1 : 0.27
Y VAR for cells in quadrant 2 : 0.53
Y VAR for cells in quadrant 3 : 0.12
Y VAR for cells in quadrant 4 : 0.13

Y Coefficient of Variation
--------------------------

Y CV for cells in quadrant 1 :  0.11
Y CV for cells in quadrant 2 :  0.17
Y CV for cells in quadrant 3 :  0.15
Y CV for cells in quadrant 4 :  0.17
```

Bibliography

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