CYT Usage Instructions

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Install and Launch cyt

Unzip cyt.zip to a folder of your choice.

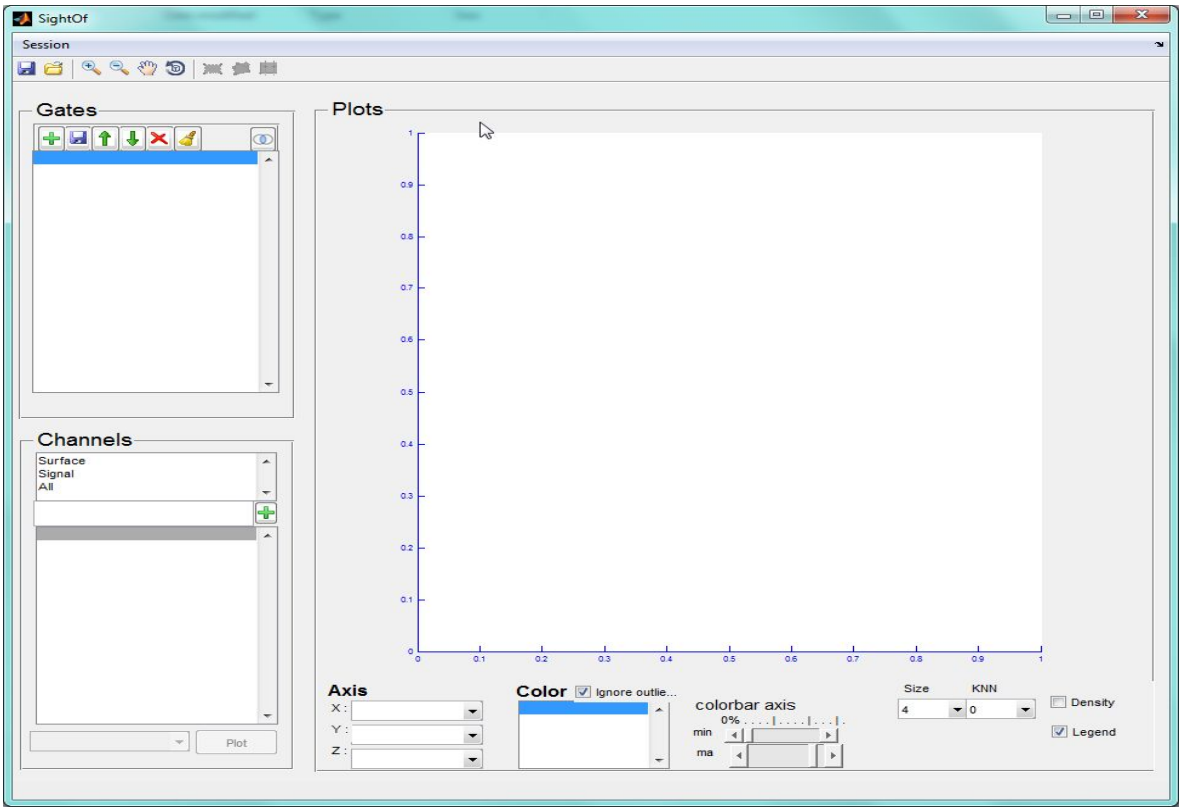
The path should not contain spaces. For example:

```
/usr/myname/documents/MATLAB/cyt
```

*You might wish to copy the FCS files (there are example FCS files (data files) on the website on the cyt download page) to that folder as well. Alternatively, you can load them into *cyt* from a different folder.

Launch Matlab, and navigate to *cyt*'s folder.

Run the script *run_cyt.m*. You will be greeted by the following:



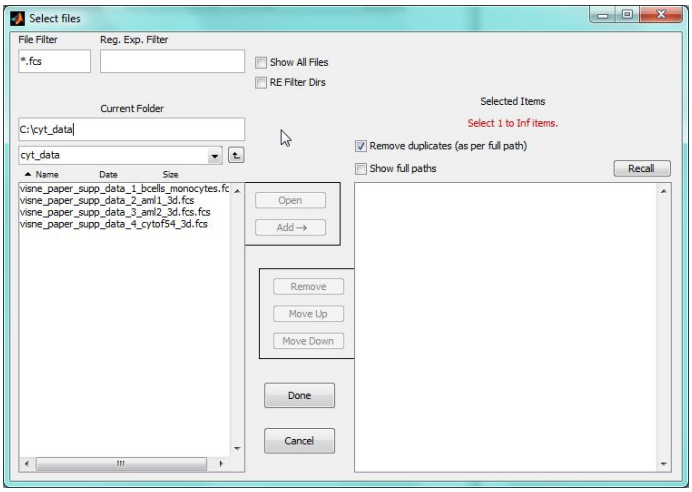
Minimum requirements for cyt

MatLab 2010b or higher

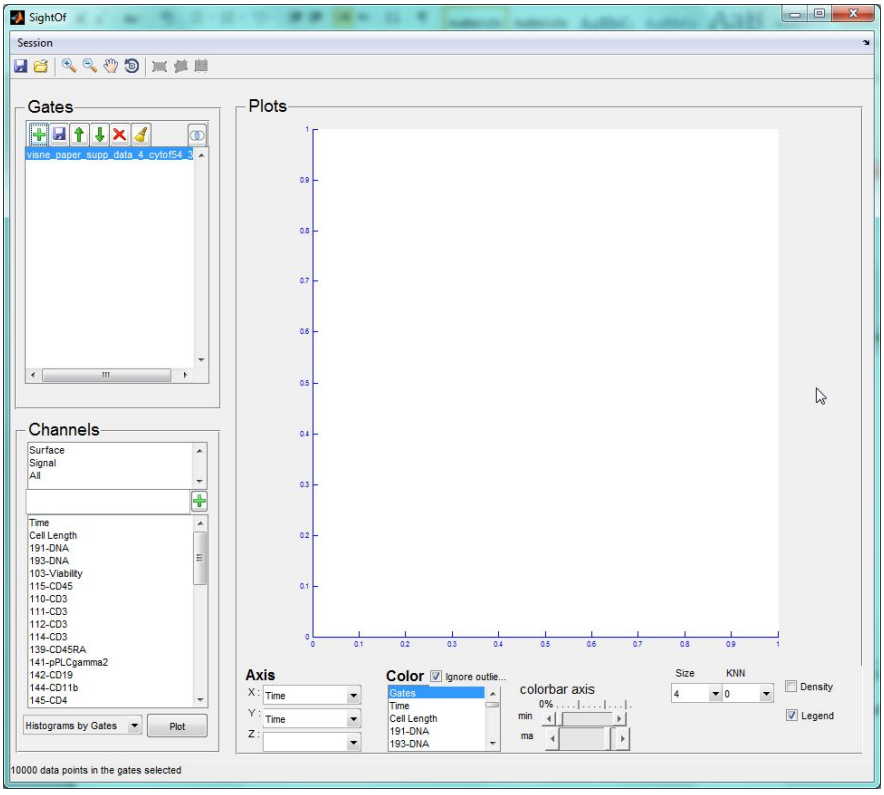
Parallel computing toolbox.

Loading data to cyt

In the "**Gates**" panel, you can load and export FCS files, rearrange the order of FCS files and gates, remove gates, and transform the data using hyperbolic arcsin (the brush icon). Click the load button (+) to load a FCS file:

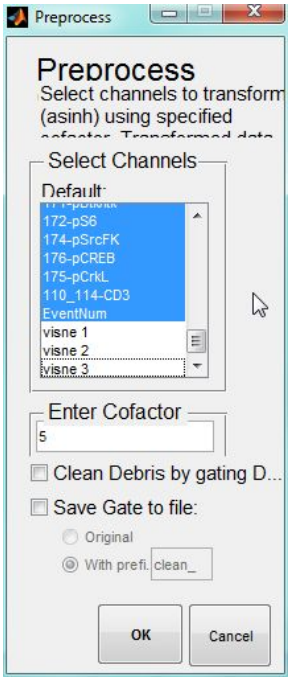


Click on a file's name and click "Add" to add it to the selected items list. For this tutorial, add "visne_paper_supp_data_4_cytof54_3d.fcs". Click Done, and the file will be loaded:



OPTIONAL transformation of data in cyt

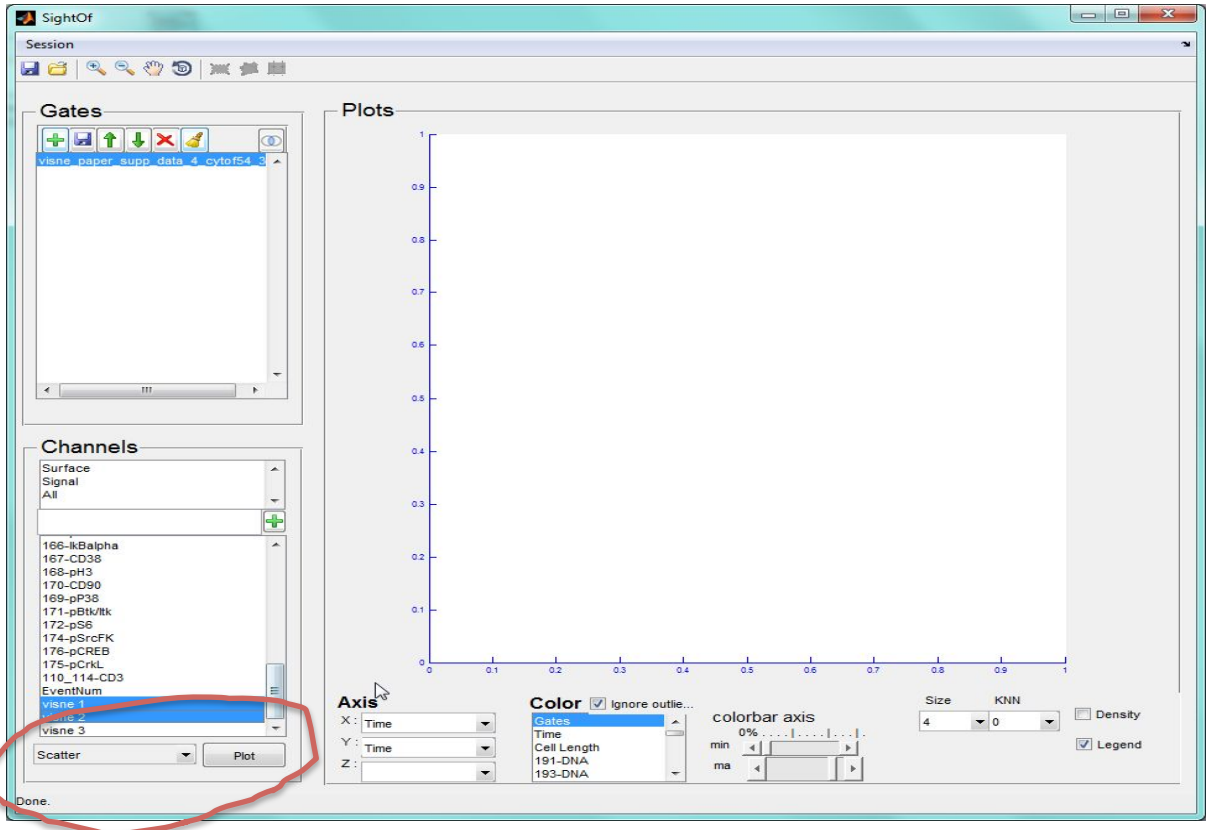
A first (OPTIONAL) step when working with CyTOF data is to transform it using hyperbolic arcsin. Click the brush icon at the top left:

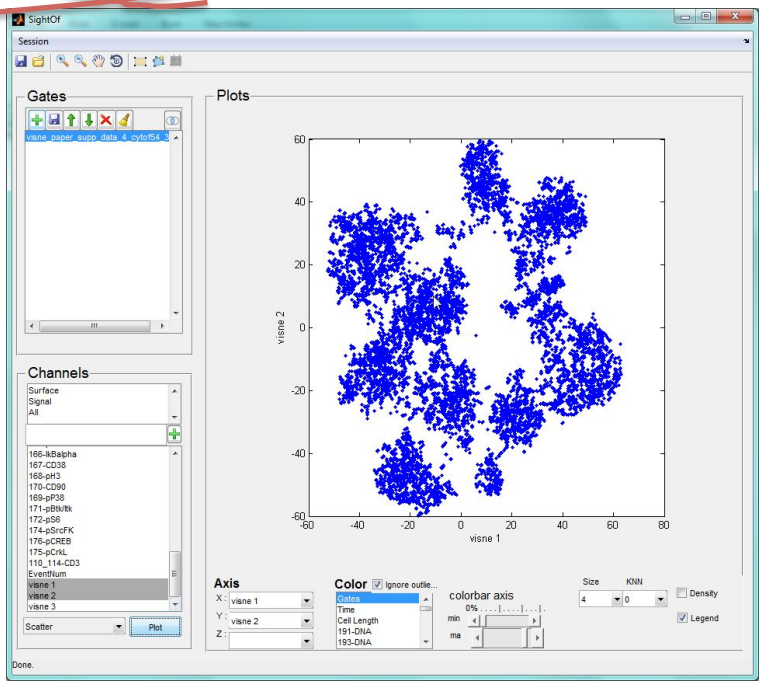


select the the CyTOF channels, perhaps uncheck the three viSNE channels if you're using the example fcs files, by pressing Ctrl and clicking on the channel names. Next, press OK and the data will be transformed.

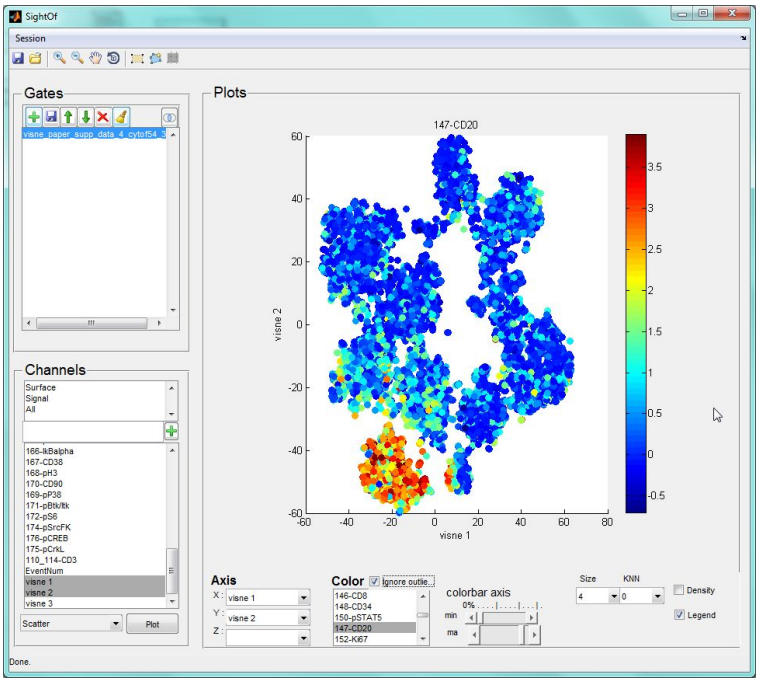
Using cyt to visualize data.

You can now visualize channel intensities over the other channels (for example visualize CD20 over ViSNE channels). To do so, first use the channel panel to pick two channels (in this example with will choose two viSNE channels); select 'scatter' from the combo box under the channel list and click '`Plot`':

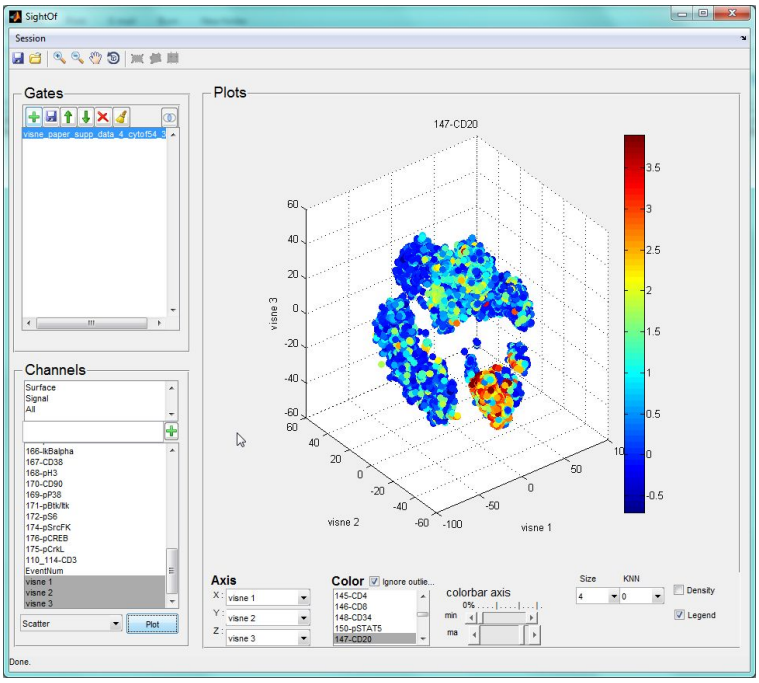




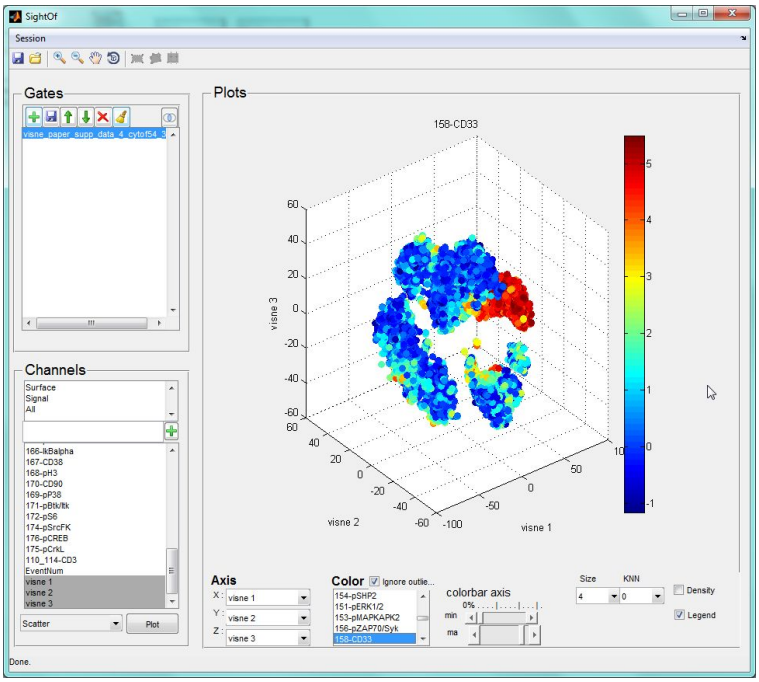
You can now color code cells by channel intensities by picking the channel under the Color panel at the bottom. For example, scroll down and pick CD20:



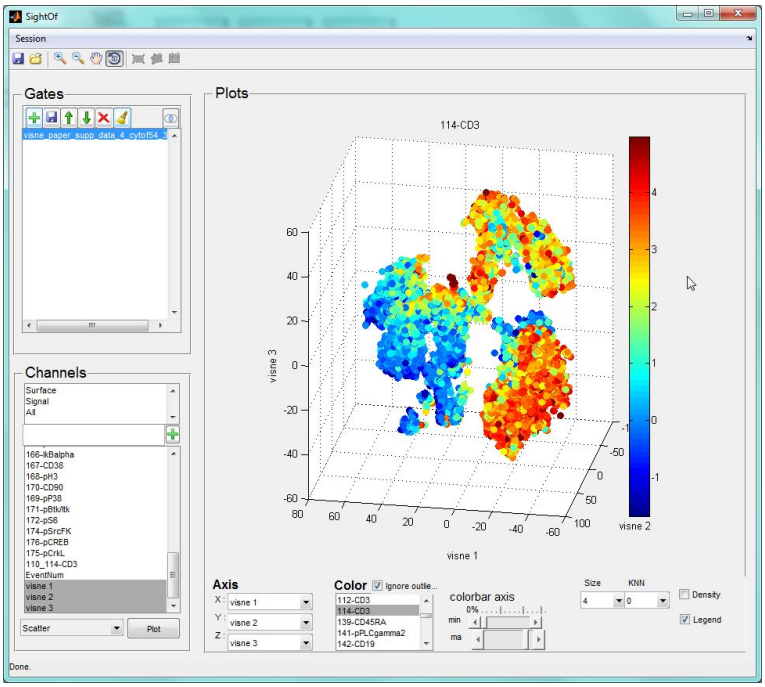
These files include a third viSNE channel, allowing visualization of the data in 3D. Pick all three viSNE channels and click Plot; *cyt* will retain the CD20 color coding:



You can visualize the other channels by picking another channel from the Color panel. For example, scroll and pick CD33 to see the separation between B cells and myeloids:

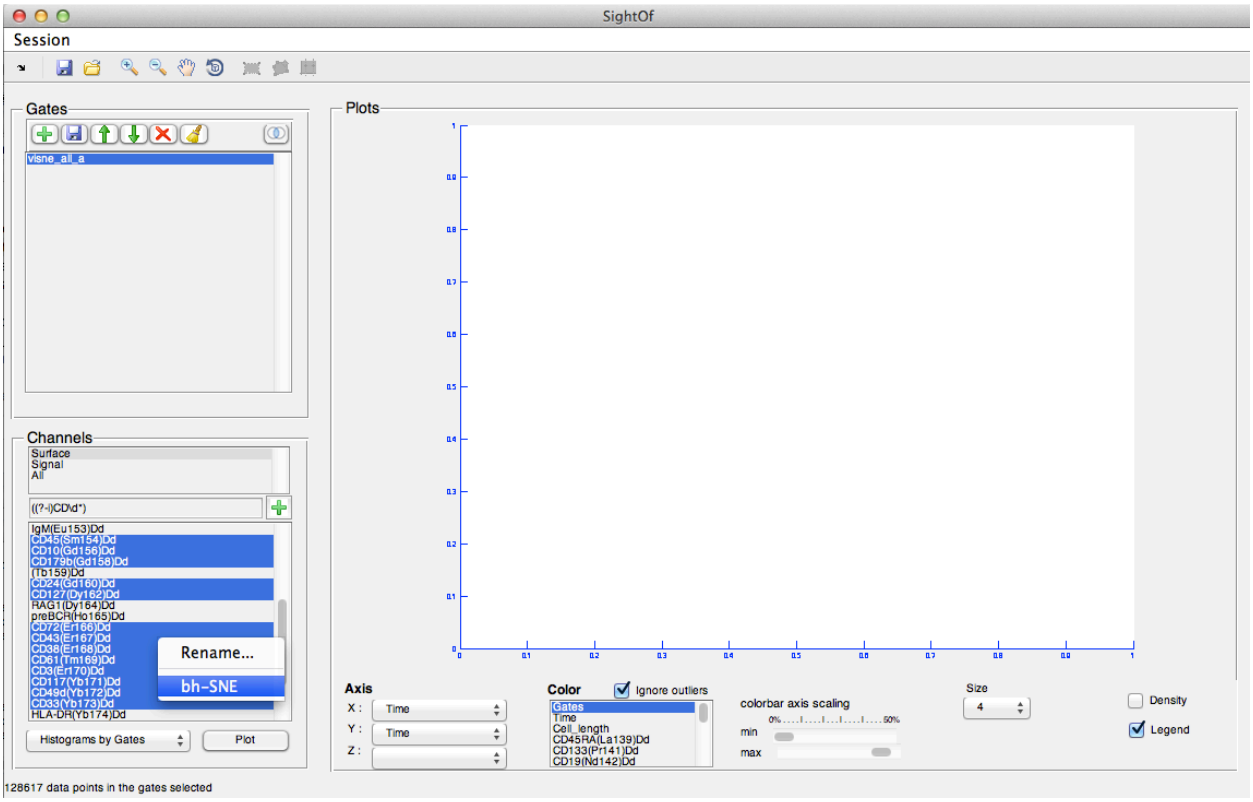


Finally, you can rotate the 3D view using the rotate tool from the top bar (the circular arrow). Here is an example with CD3 coding, slightly rotated to highlight CD4+ and CD8+ T cells:



Using cyt to generate 2D ViSNE maps

To generate a ViSNE map, select the set of channels you would like to create the ViSNE map for and use 'Ctrl'+click to open the context menu and select the bh-sne option:



A please wait message will appear while the results are being computed. 100k points takes approx. 5 min. 1 million points is approx. 15m. The results will appear as new channels in the channel list and can be visualized as described in the preceding section. Please check out MATLAB's terminal to see if there are any error messages.

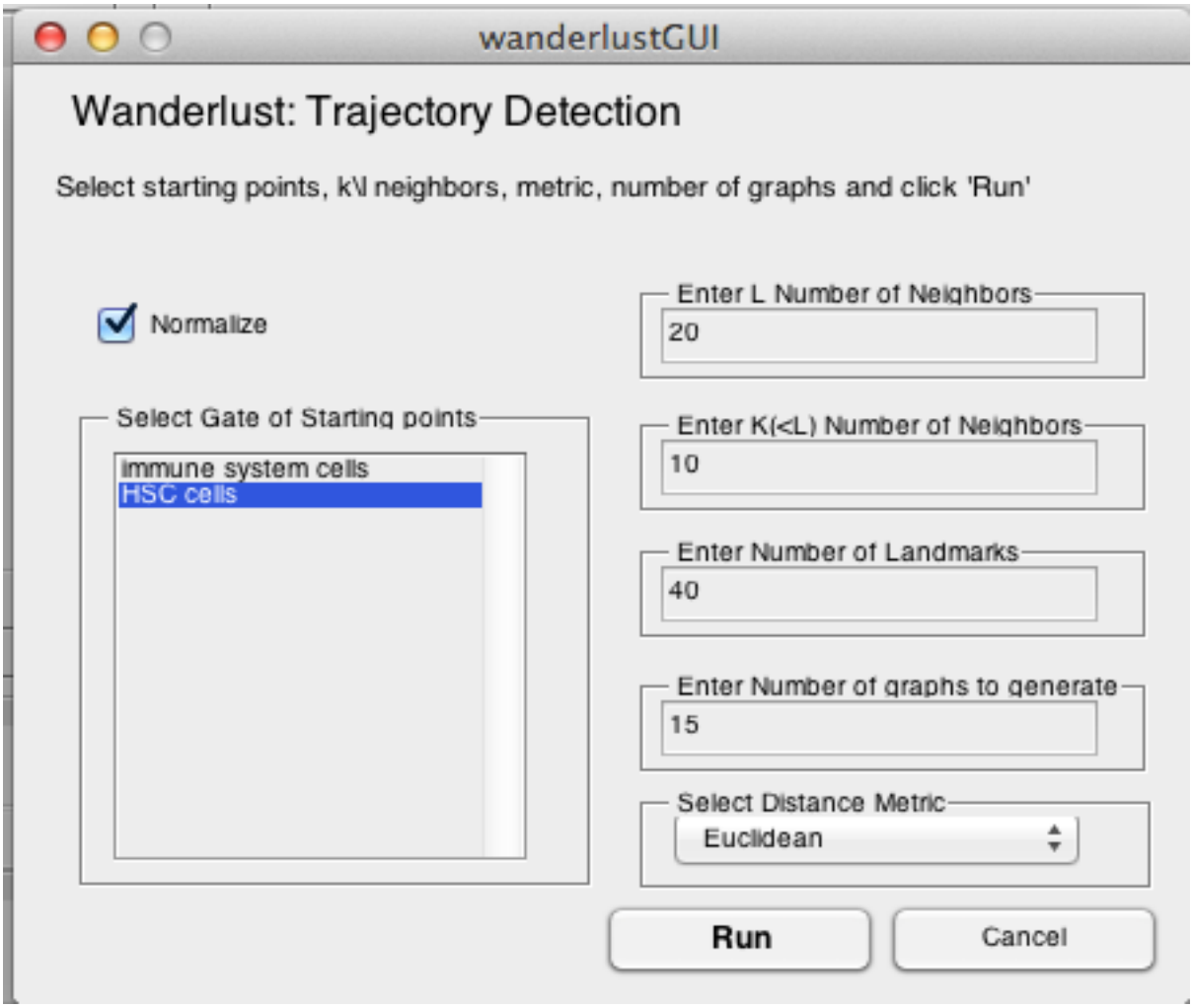
Wanderlust:

Use cyt for trajectory detection (i.e. find a progression component)

First, gate a small population of cells that you would like wanderlust to 'start' from. These would be the 'youngest' cells if you were tracing development along features.

In the Channels list box, select the set of channels for whom you would like to trace their progression and use 'Ctrl'+click to open the context menu and select the 'wanderlust…' option.

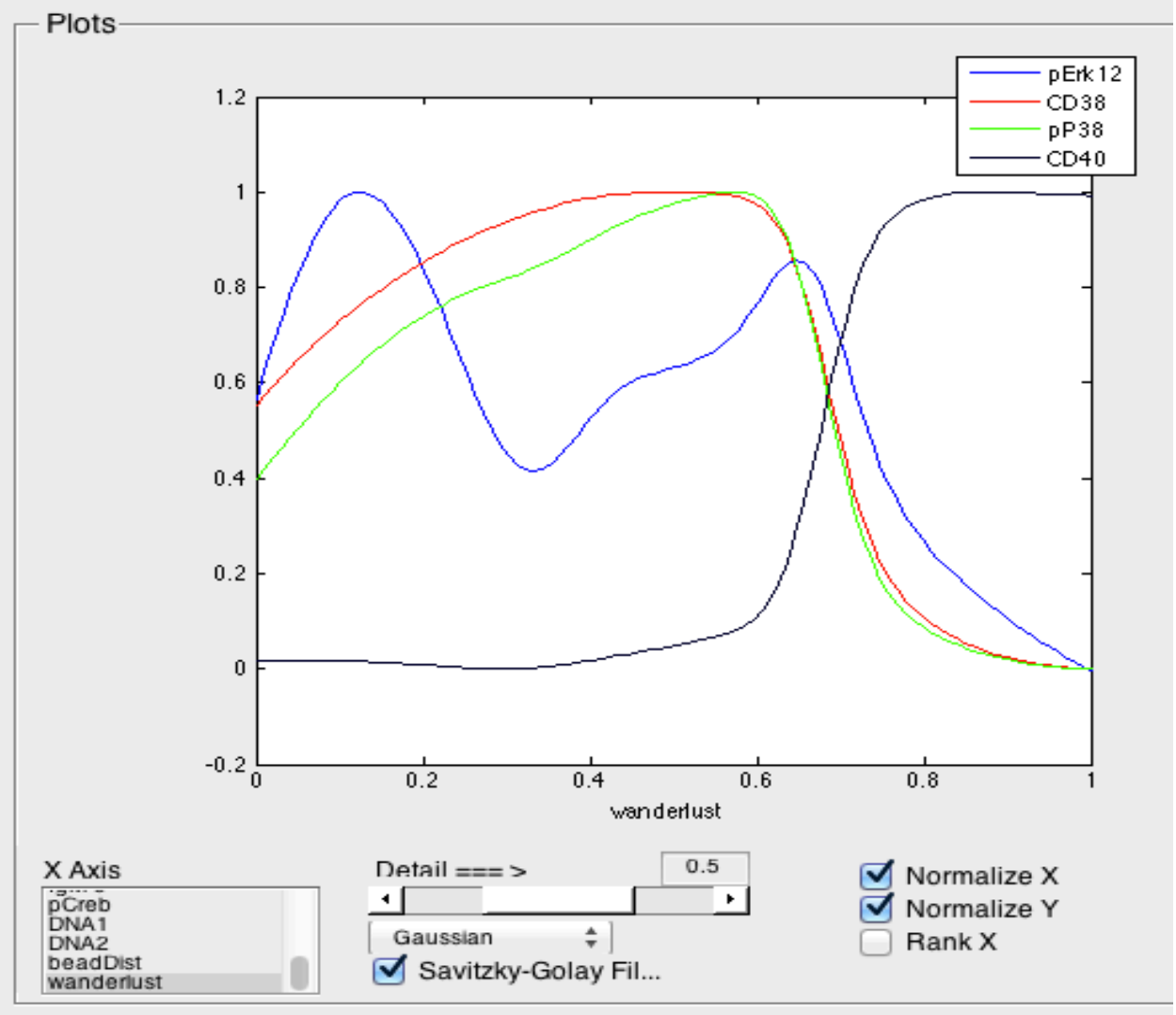
You will be greeted by a dialog to select parameters.



Most important, select the Gate of the starting points that you have gates out in step one.

Visualizing wanderlust

In the Channels listbox, select the channels you would like to view along wanderlust. Now select ‘plot along time’ in the combo box beneath the channel list and click ‘plot’. You will be greeted by a view like this



Select the wanderlust channel for ‘X Axis’.

‘Rank X’ sorts the values in the wanderlust channels and ignores the actual values or distances between the cells.

Help for cyt

Please email us with any questions, comments, and feedback at:

cyt.team@gmail.com