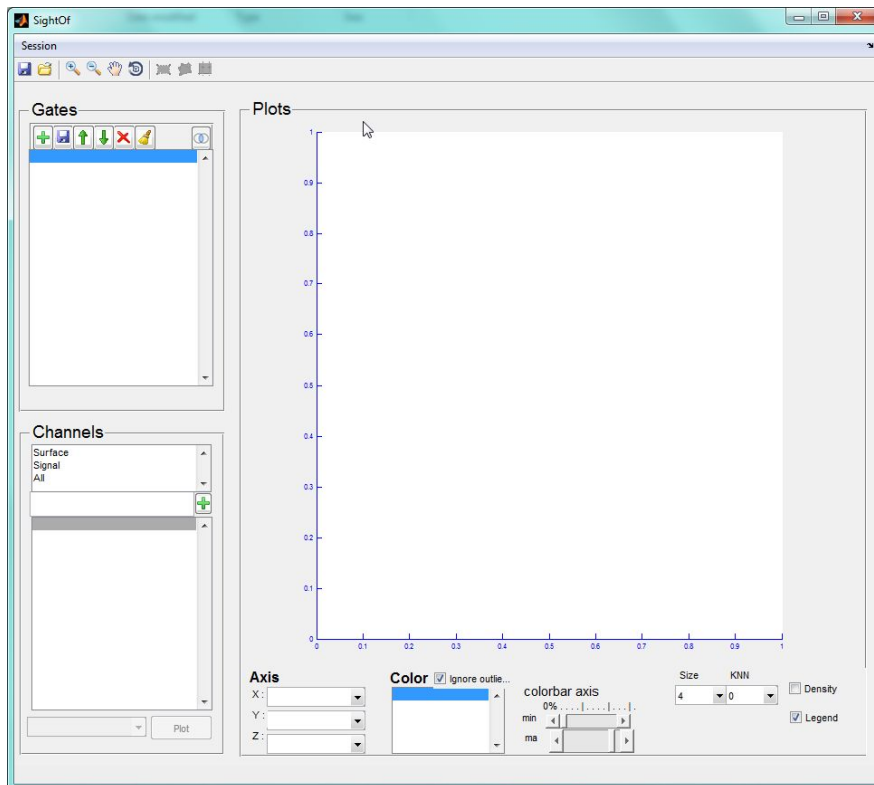


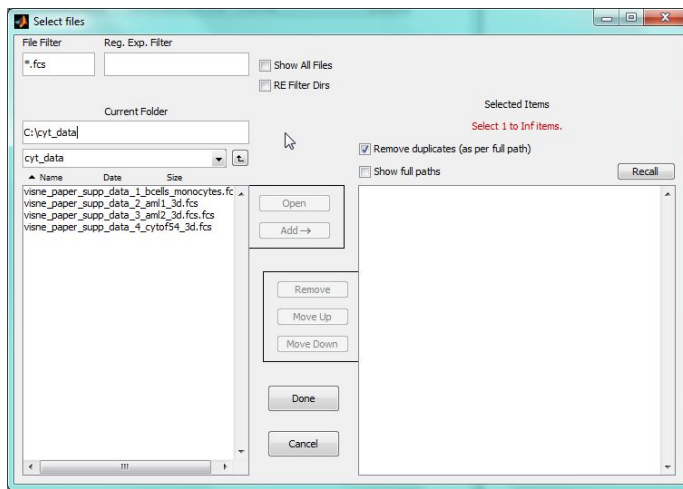
Launching cyt

Unzip cyt.zip to a folder of your choice, launch Matlab, direct it to *cyt*'s folder, and run the script *run_cyt.m*. 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. You will be greeted by the following:

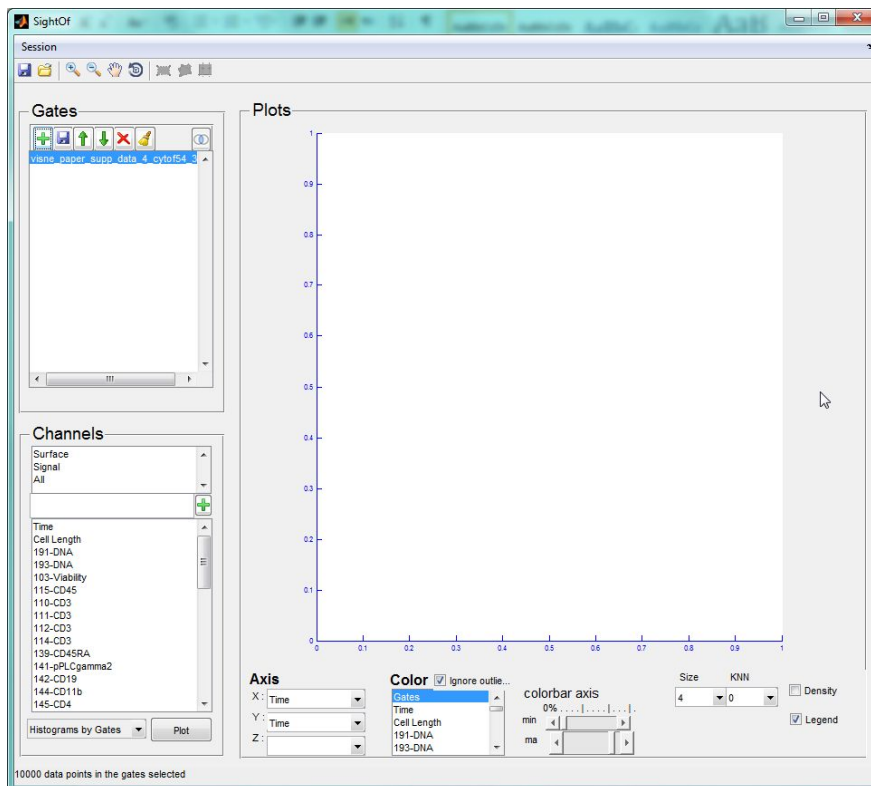


Loading data to cyt

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 (the green plus icon) to load a FCS file:

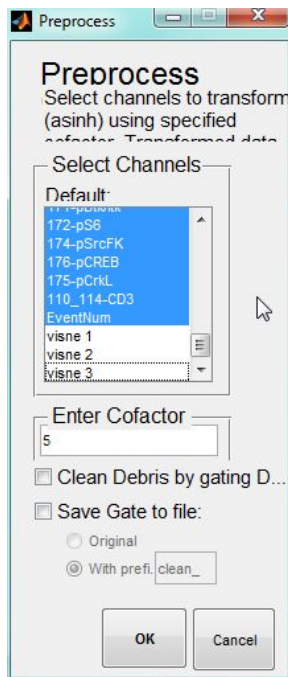


Click on a file's name and click "Add" to add it to the selected items list. For this tutorial, add "visne_paper_supp_data_4_cytof54_3d.fcs". Click Done, and the file will be loaded:



OPTIONAL transformation of data in cyt

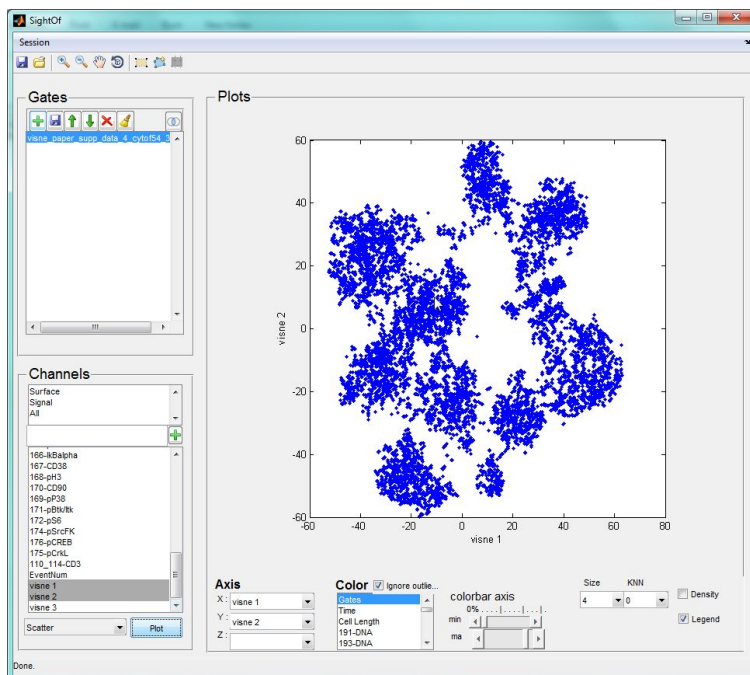
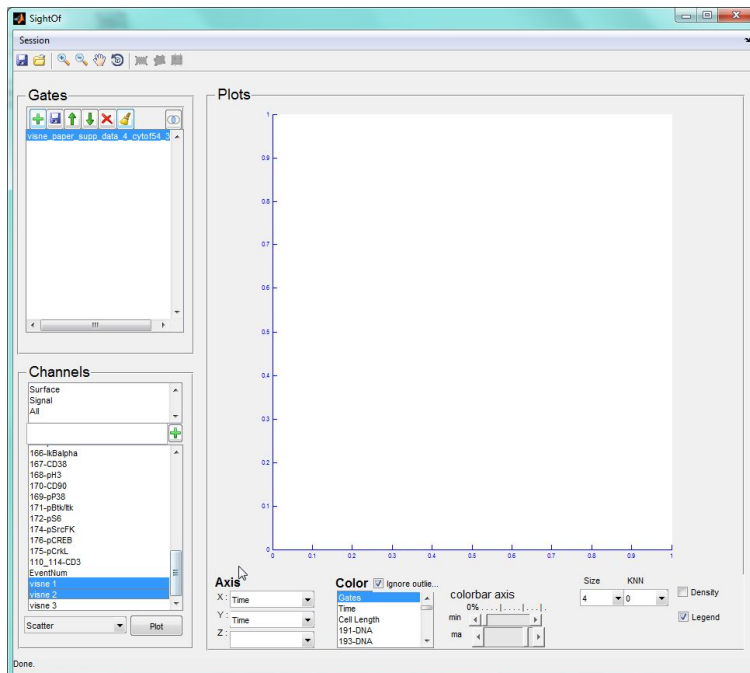
A first (OPTIONAL) step when working with CyTOF data is to transform it using hyperbolic arcsin. Click the brush icon at the top left:



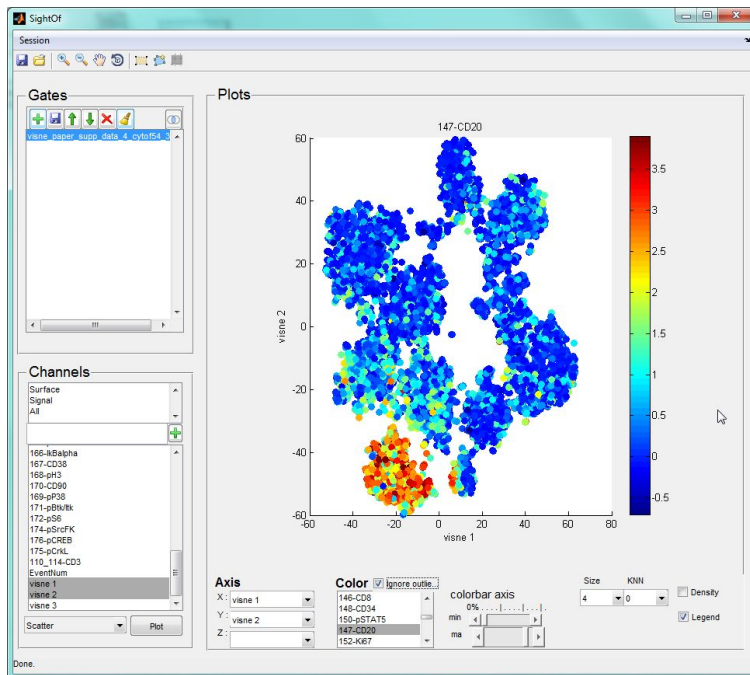
select the the CyTOF channels, perhaps uncheck the three viSNE channels if you're using the example fcs files, by pressing Ctrl and clicking on the channel names. Next, press OK and the data will be transformed.

Using cyt to visualize 2D and 3D viSNE maps.

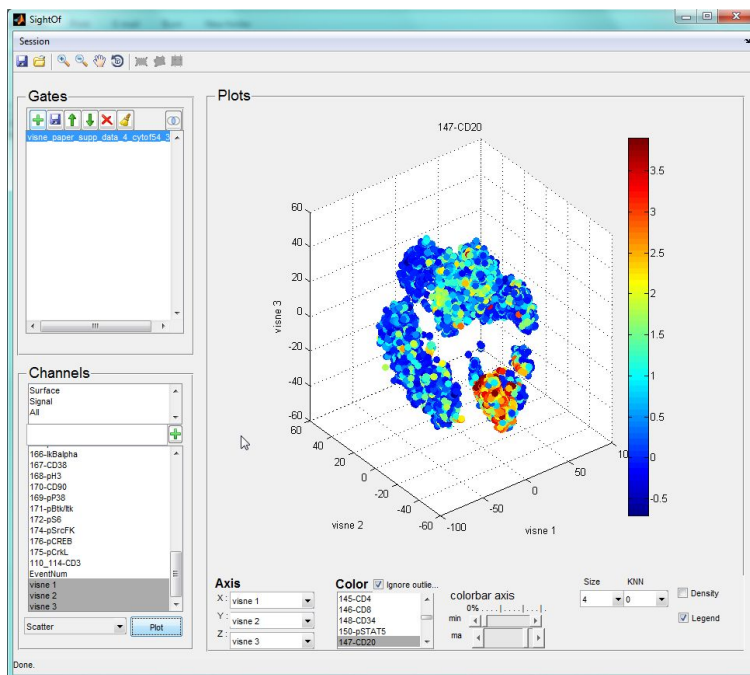
You can now visualize channel intensities over the viSNE channels.
To do so, first use the channel panel to pick the two viSNE channels; then,
click Plot:



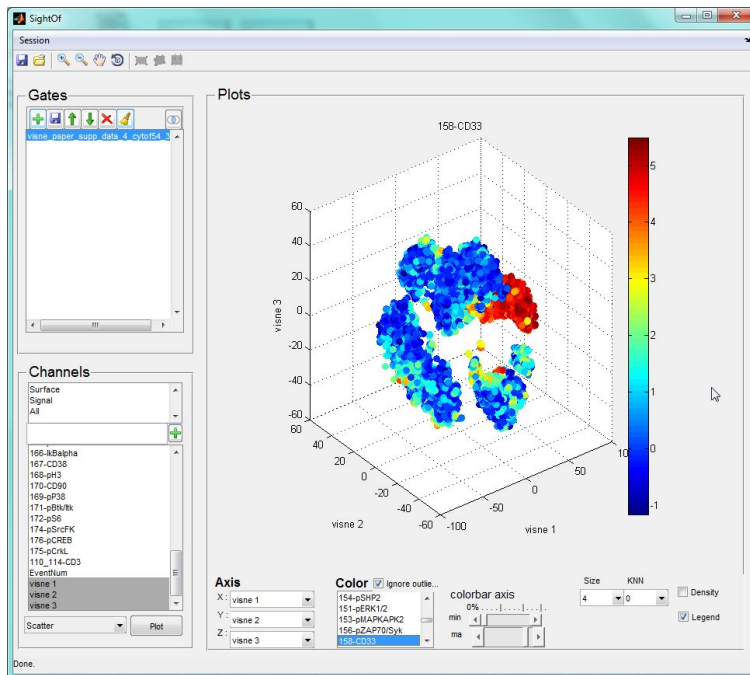
You can now color code cells by channel intensities by picking the channel under the Color panel at the bottom. For example, scroll down and pick CD20:



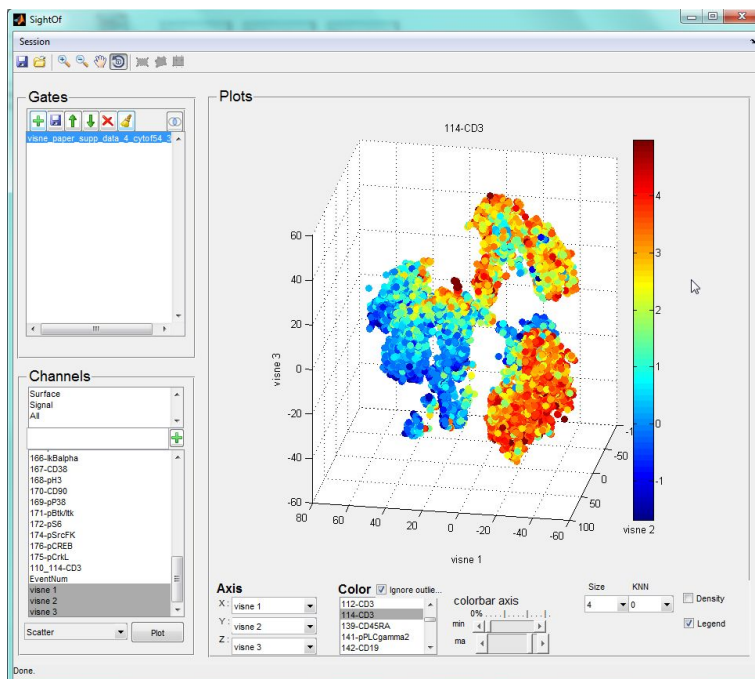
These files include a third viSNE channel, allowing visualization of the data in 3D. Pick all three viSNE channels and click Plot; cyt will retain the CD20 color coding:



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:

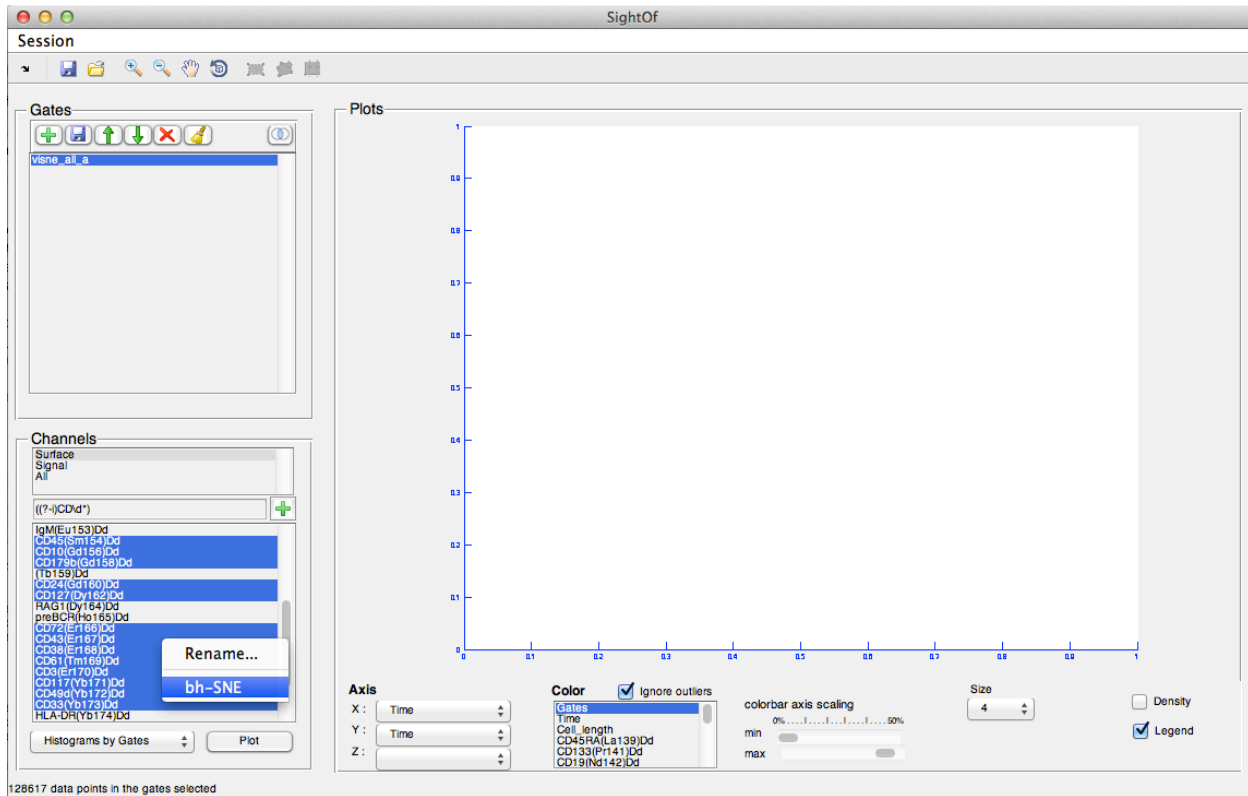


Finally, you can rotate the 3D view using the rotate tool from the top bar (the circular arrow). Here is an example with CD3 coding, slightly rotated to highlight CD4+ and CD8+ T cells:



Using cyt to generate 2D and 3D viSNE maps (compiled for Mac)

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.

Help for cyt

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

cyt.team@gmail.com

