

How to Access viSNE & SPADE on Pitt's High Performance Cluster  
(11/08/18)

**Overview:** viSNE and SPADE are installed on the "H2P" (Hail to Pitt!) high performance cluster in the Center for Research Computing (CRC). CRC's mission is to support advanced computing needs of researchers <https://crc.pitt.edu>.

For assistance with...

User access to the H2P cluster Submit a help ticket regarding H2P use	<a href="https://crc.pitt.edu/apply">https://crc.pitt.edu/apply</a> <a href="https://crc.pitt.edu/tickets/">https://crc.pitt.edu/tickets/</a>
Get a Matlab license	my.pitt.edu → software download service
Basic aspects of importing and analyzing fcs files	Lisa Borghesi, Department of Immunology borghesi@pitt.edu
viSNE (developer Dana Pe'er; references below)	<a href="http://www.mybiosoftware.com/visne-cyt-map-high-dimensional-cytometry-data-dimensions.html">http://www.mybiosoftware.com/visne-cyt-map-high-dimensional-cytometry-data-dimensions.html</a>
SPADE (developer Peng Qiu; references below)	<a href="http://pengqiu.gatech.edu/software/SPADE2/">http://pengqiu.gatech.edu/software/SPADE2/</a>

1. Apply for an account on Pitt's H2P cluster - approval takes 1 or 2 days.

<https://crc.pitt.edu/apply>

2. Obtain a Matlab "research" license from Pitt Software Distribution Services at my.pitt.edu. As of 2018, the cost is ~\$100/yr and is renewable annually. Faculty and students are entitled to one free "personal use" version of Matlab. In order to conduct research on the cluster, you need the "research" license. Pay the \$100.

3. On your local computer, open a command line shell and log in to Pitt's H2P cluster. See screen shots below.

Mac users should open Terminal. Terminal is located in Applications Folder → Utilities Folder → Terminal.

Windows users should open Powershell Terminal (i.e., a command prompt terminal).

after you have an approved H2P account, log into H2P

- a. Open the command line window on your local computer
- b. Log into h2p cluster: `ssh -X yourpittusername@h2p.crc.pitt.edu`
- c. enter your password at the key symbol

4. Invoke Matlab.

At the command prompt symbol "\$" type the below commands, sequentially. Hit enter/return after each command. After the second command, Matlab should launch.

```
$ module load matlab
```

```
$ matlab
```

5. From Matlab invoke cyt (for viSNE) or SPADE (for the SPADE algorithm).

After you complete the below command (a) or (b) a new window should launch either cyt or SPADE, respectively.

a) Within Matlab, at the ">>" command symbol type:

```
>>cyt
```

b) or to invoke SPADE:

```
>>SPADE
```

**Troubleshooting:**

You may need to install the X11 Window System to view a basic GUI (graphical user interface). X11 is a remote-display protocol used by Linux/Unix machines.

Mac Users need XQuartz. <https://www.xquartz.org>

Windows Users need PuTTY. <https://www.chiark.greenend.org.uk/~sgtatham/putty/>

6. Transfer fcs files from the local computer to H2P using the Cyberduck file transfer program.

Cyberduck allows you to easily transfer files from local computer to the cluster, and vice versa. Download here: <https://cyberduck.io>

1. Establish a Pitt Pulse Secure Remote Access portal
  - Pulse SecurePIT VPNConnect (enter pw and multifactor authentication)
  - Select: Firewall-SAM-USERS-NetworkConnect
2. Launch Cyberduck on your desktop/laptop
3. Open Connection
4. select SFTP (SSH File Transfer protocol) from drop down menu
  - port should read 22
  - if port reads 21, you've accidentally selected FTP instead of SFTP
4. server: htc.sam.pitt.edu
5. enter your Pitt username and pw
6. freely drag and drop files from desktop/laptop → H2P or from H2P→desktop/laptop

7. Want other algorithms installed on H2P cluster?

Submit a request ticket to CRC <https://crc.pitt.edu/tickets/>

**Example of successful script from Terminal on a Mac.**

```
Last login: Mon Jul 9 11:34:18 on ttys001
cl2-wifi-10-215-43-246:~ lisaborghesi$ ssh -X borghesi@h2p.crc.pitt.edu
borghesi@h2p.crc.pitt.edu's password: (password here)
Last login: Mon Jul 9 11:34:06 2018 from sremote-10-195-58-51.vpn.pitt.edu
#####

Welcome to h2p.crc.pitt.edu!

Documentation can be found at crc.pitt.edu/h2p

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IMPORTANT NOTIFICATIONS

Renewal of CRC allocations requires you to acknowledge and add citations to our
database, login to crc.pitt.edu and navigate to crc.pitt.edu/acknowledge for
details and entry form

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IMPORTANT REMINDERS

Don't run jobs on login nodes! Use interactive jobs: `crc-interactive.py --help`

Slurm is separated into 'clusters', e.g. if `scancel <jobnum>` doesn't work try
`crc-scancel.py <jobnum>`. Try `crc-sinfo.py` to see all clusters.

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#####
```

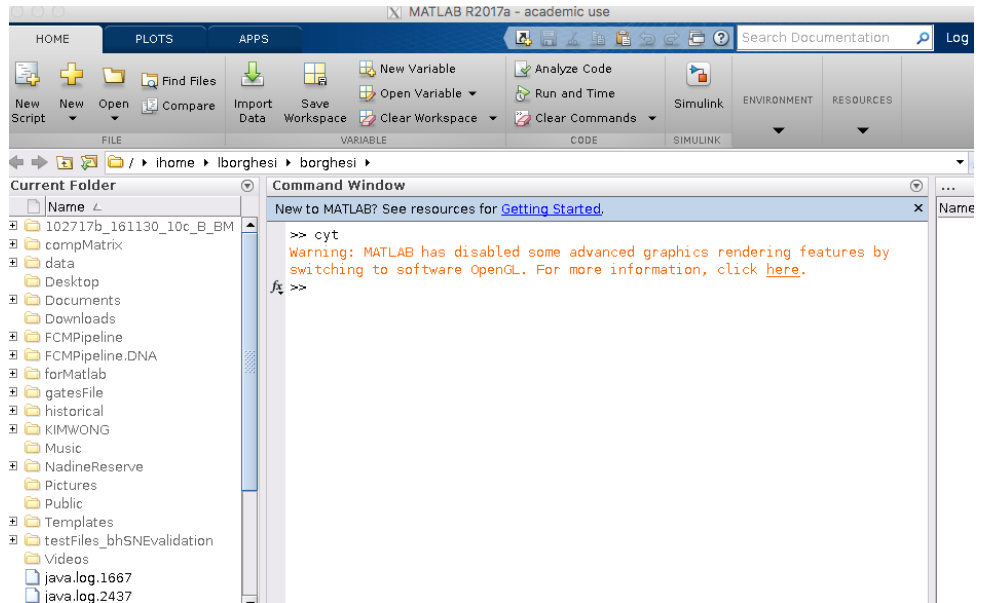
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```
[borghesi@login1 ~]$ module load matlab  
[borghesi@login1 ~]$ matlab  
MATLAB is selecting SOFTWARE_OPENGL rendering.
```

After a pause, a new MATLAB window should open on your local computer.

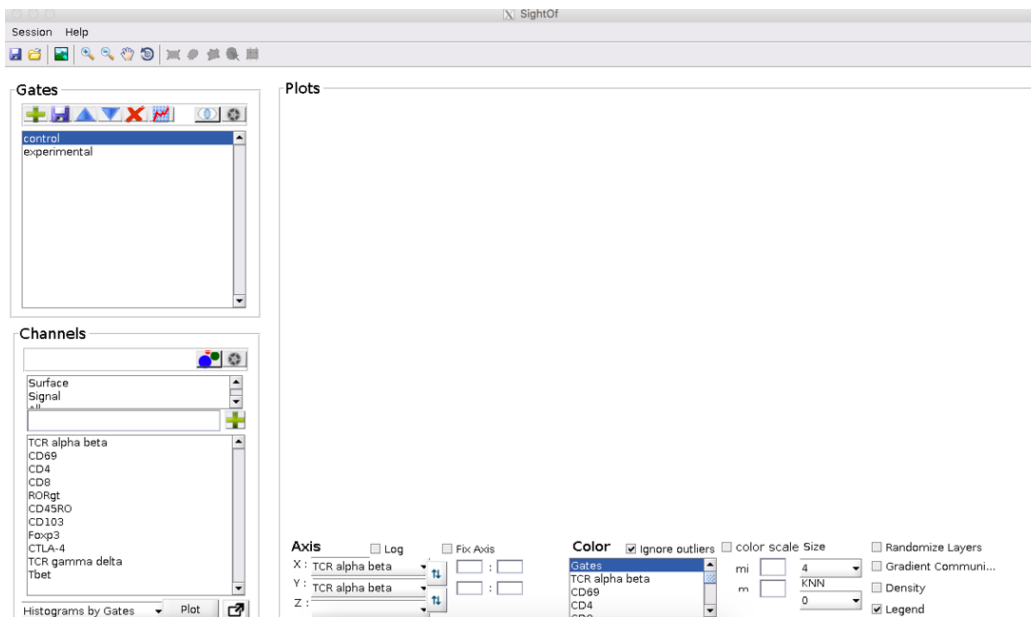
Note the “>>” prompt in the middle panel. Type “cyt”.

```
>> cyt
```



A new Sightof window will appear.

Load your fcs files using the green plus symbol near top left.

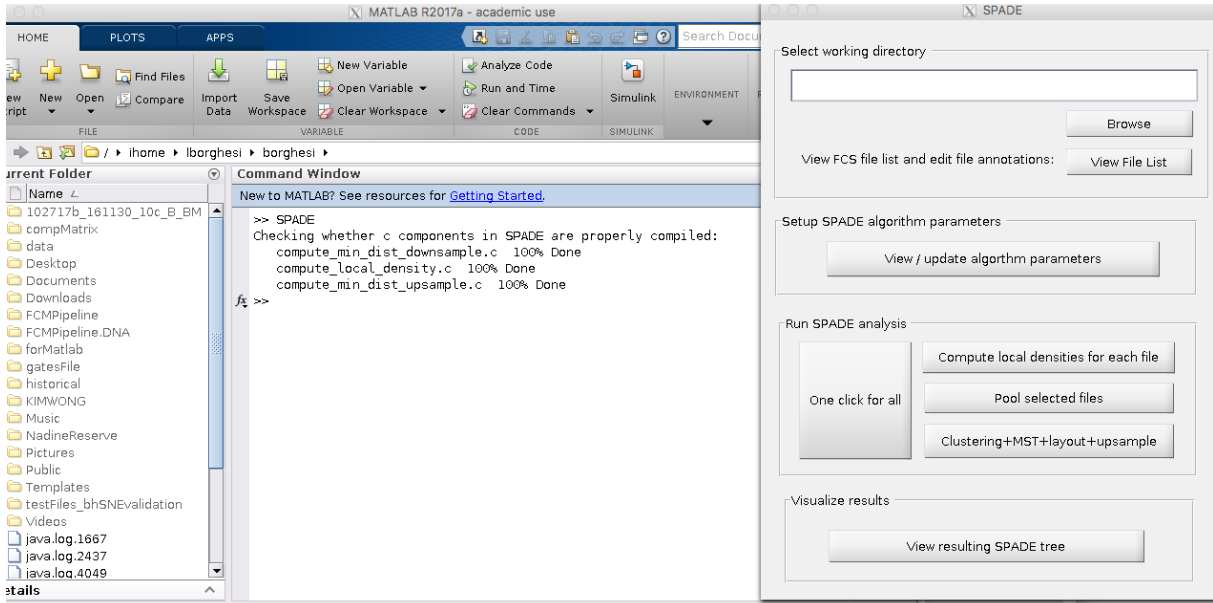


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Or invoke SPADE.

>>SPADE

The SPADE algorithm will open up the window at far right. Use the Browse button to upload fcs files.



### Literature

#### SPADE

Qiu, P., E. F. Simonds, S. C. Bendall, K. D. Gibbs, Jr., R. V. Bruggner, M. D. Linderman, K. Sachs, G. P. Nolan, and S. K. Plevritis. 2011. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nature biotechnology* 29: 886-891.

#### t-SNE and viSNE

van der Maaten, L., and G. Hinton. 2008. Visualizing Data using t-SNE. *J Mach Learning Res* 9: 2579-2605.

Amir el, A. D., K. L. Davis, M. D. Tadmor, E. F. Simonds, J. H. Levine, S. C. Bendall, D. K. Shenfeld, S. Krishnaswamy, G. P. Nolan, and D. Pe'er. 2013. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nature biotechnology* 31: 545-552.

#### Review articles

Chester, C., and H. T. Maecker. 2015. Algorithmic Tools for Mining High-Dimensional Cytometry Data. *Journal of immunology* 195: 773-779.

Mair, F., F. J. Hartmann, D. Mrdjen, V. Tosevski, C. Krieg, and B. Becher. 2016. The end of gating? An introduction to automated analysis of high dimensional cytometry data. *European journal of immunology* 46: 34-43.

Saeyes, Y., S. V. Gassen, and B. N. Lambrecht. 2016. Computational flow cytometry: helping to make sense of high-dimensional immunology data. *Nature reviews. Immunology* 16: 449-462.

Kimball, A. K., L. M. Oko, B. L. Bullock, R. A. Nemenoff, L. F. van Dyk, and E. T. Clambey. 2018. A Beginner's Guide to Analyzing and Visualizing Mass Cytometry Data. *Journal of immunology* 200: 3-22.