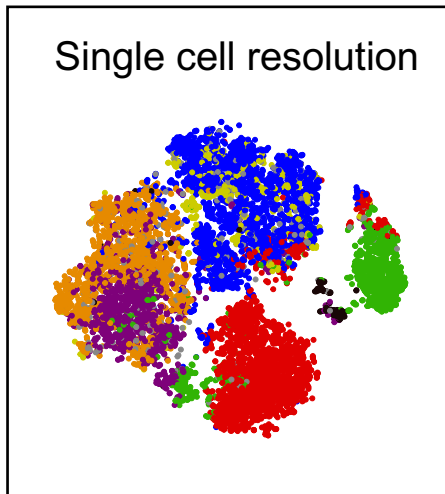
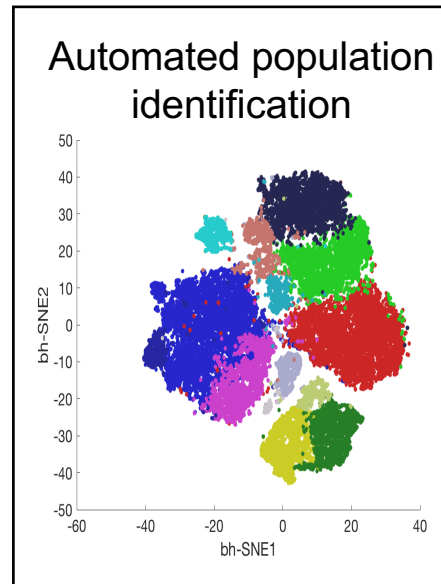


Installing Algorithms

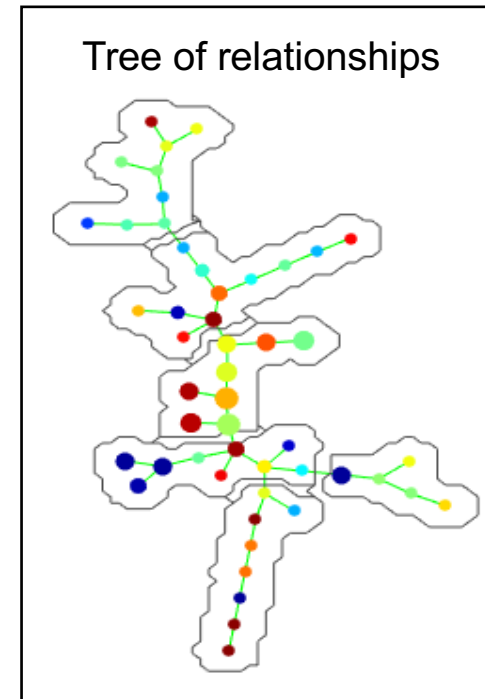
viSNE



PhenoGraph



SPADE



Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

In this tutorial, free or basically free:

- 1) **SPADE** <http://pengqiu.gatech.edu/software/SPADE/> (free)
- 2) **Cyt package: viSNE, PhenoGraph, Wishbone, Wanderlust, PCA, K-means, other statistical tools** (requires Matlab which is free for faculty and students and \$100/yr for everyone else)

Other options, for you to explore:

- 1) FlowJo v.10+: t-SNE, SPADE, FlowMeans (\$200/yr site license)
- 2) ExCYT2 <https://github.com/sidhomj/ExCYT2> (free)
- 3) Cytobank: viSNE, SPADE, CITRUS, Sunburst; FlowSOM beta (\$1,500/yr/user) www.cytobank.org
- 4) FCS Express: t-SNE, SPADE, K-means, PCA uniquely capable of performing t-SNE on ImageStream (\$234-500/yr/license) www.denovosoftware.com

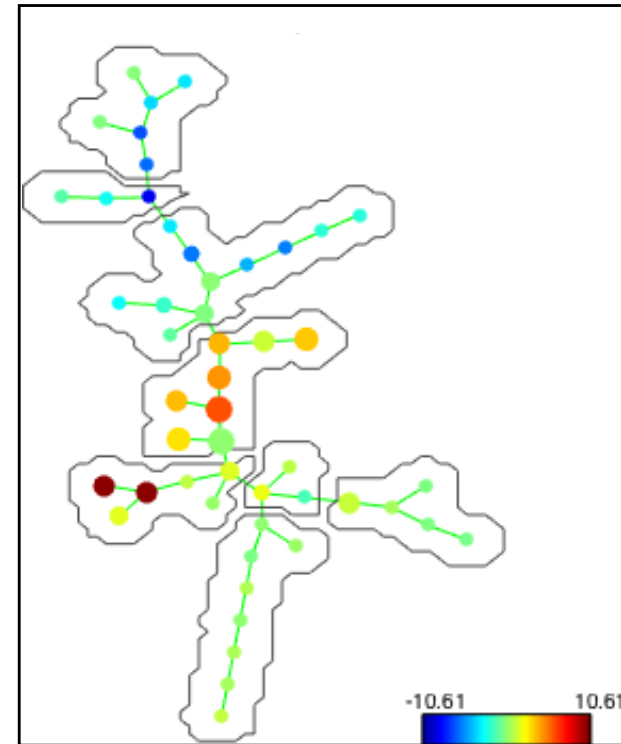
Install SPADE 3.0 on Your Personal Computer

Qiu et al. Nat Biotechnol. 2011 Oct 2;29(10):886-91

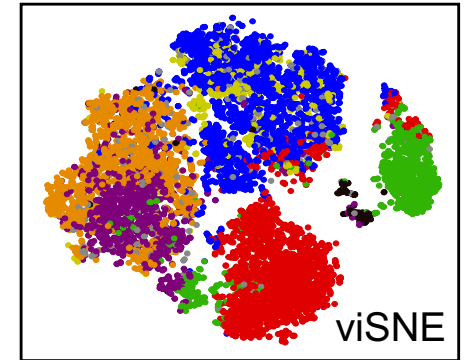
Easy to install and use!

<http://pengqiu.gatech.edu/software/SPADE/>

1. For Mac or Windows
2. Stand-alone (or via Matlab)
3. Great instructions provided at URL
4. Supported by developer Peng Qiu



Install MatLab and the plug-in Cyt3



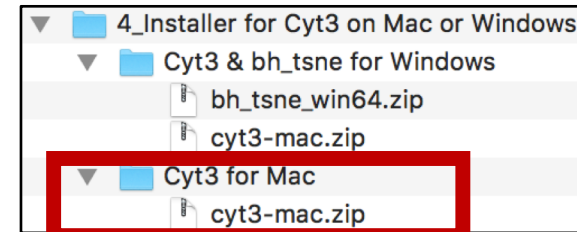
1. Install Matlab from my.pitt.edu software downloads

- Free for students and teaching faculty
- Everyone else, \$100/yr license

2. Install Cyt3, installer located in the PittBox that contains this presentation

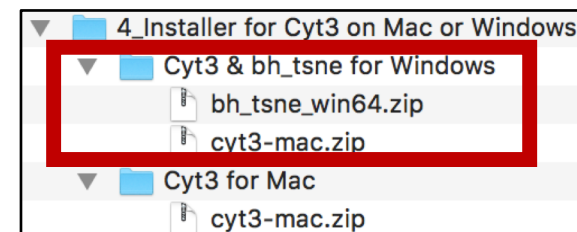
Mac People

- Download .zip to desktop
- Double-click to install
- Cyt3 folder will appear on desktop



Windows People – there is an extra step for you

- Download the two .zip files to desktop
- Double-click cyt3-mac.zip to install
- Double-click bh_tsne_win64.zip to install
- Cyt3 folder will appear on desktop
- In the Cyt3 → src → 3rdparty → bh_tsne folder, *remove* bh_tsne_mac64 and *replace* with bh_tsne_win64



3. Open Cyt3 folder, view powerpoint tutorials provided by developer

Connect Matlab to Cyt3 via “Set Path”

Launch Matlab

1. Click “Set Path”
2. In pop-up window, select the path that leads to the Cyt-3 folder on your desktop
3. Click “Add with Subfolders”
- 4 & 5. Save, Close
6. At the >> prompt type “cyt”
7. A new window titled “Sightof” will appear

The image illustrates the process of connecting Matlab to Cyt3. It shows the Matlab R2016b interface with the 'Set Path' button highlighted (1). The 'Set Path' dialog box is open, showing the path '/Users/lisaborghesi/Desktop/cyt3-mac' selected (2) and the 'Add with Subfolders...' button highlighted (3). The 'Save' (4) and 'Close' (5) buttons are also highlighted. The Command Window shows the command '>> cyt' entered (6). Finally, the 'SightOf' window is shown appearing (7).

**You are ready to perform
computational flow cytometry!**

Resources

Useful starting places - reviews

1. Kimball AK, Oko LM, Bullock BL, Nemenoff RA, van Dyk LF, Clambey ET. A Beginner's Guide to Analyzing and Visualizing Mass Cytometry Data. *J Immunol*. 2018 Jan 1;200(1):3-22.
2. Saeys Y, Gassen SV, Lambrecht BN. Computational flow cytometry: helping to make sense of high-dimensional immunology data. *Nat Rev Immunol*. 2016 Jul;16(7):449-62.
3. Mair F, Hartmann FJ, Mrdjen D, Tosevski V, Krieg C, Becher B. The end of gating? An introduction to automated analysis of high dimensional cytometry data. *Eur J Immunol*. 2016 Jan;46(1):34-43.
4. Chester C & Maecker HT. *J Immunol*. 2015 Aug 1;195(3):773-9. doi: 10.4049/jimmunol.1500633. Algorithmic Tools for Mining High-Dimensional Cytometry Data. *J Immunol*. 2015 Aug 1;195(3):773-9.

Original application of algorithms

1. Qiu P, Simonds EF, Bendall SC, Gibbs KD Jr, Bruggner RV, Linderman MD, Sachs K, Nolan GP, Plevritis SK. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nat Biotechnol*. 2011 Oct 2;29(10):886-91. **SPADE**
2. Amir el-AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, Pe'er D. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Amir el-AD, Davis KL, 3. Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, Pe'er D. *Nat Biotechnol*. 2013 Jun;31(6):545-52. **viSNE**
3. Levine JH, Simonds EF, Bendall SC, Davis KL, Amir el-AD, Tadmor MD, Litvin O, Fienberg HG, Jager A, Zunder ER, Finck R, Gedman AL, Radtke I, Downing JR, Pe'er D, Nolan GP. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. *Cell*. 2015 Jul 2;162(1):184-97. **PhenoGraph**
4. Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. *Proc Natl Acad Sci U S A*. 2014 Jul 1;111(26):E2770-7. **CITRUS**
5. Setty M, Tadmor MD, Reich-Zeliger S, Angel O, Salame TM, Kathail P, Choi K, Bendall S, Friedman N, Pe'er D. Wishbone identifies bifurcating developmental trajectories from single-cell data. *Nat Biotechnol*. 2016 Jun;34(6):637-45. **Wishbone**
6. Bendall SC, Davis KL, Amir el-AD, Tadmor MD, Simonds EF, Chen TJ, Shenfeld DK, Nolan GP, Pe'er D. Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development. *Cell*. 2014 Apr 24;157(3):714-25. **Wanderlust**
7. McInnes L, Healy J, Melville J. UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. <https://arxiv.org/abs/1802.03426> **UMAP (for advanced Matlab Users, no GUI interface)**

ExCYT2

ABOUT JoVE

FOR LIBRARIANS

PUBLISH

VIDEO JOURNAL

SCIENCE EDUCATION

ExCYT: A Graphical User Interface for Streamlining Analysis of High-Dimensional Cytometry Data

John-William Sidhom^{1,2,3}, Debebe Theodros^{1,2,4}, Benjamin Murter^{1,2}, Jelani C. Zarif^{1,2}, Sudipto Ganguly^{1,2}, Drew M. Pardoll^{1,2}, Alexander Baras^{1,2,5}

¹The Bloomberg~Kimmel Institute for Cancer Immunotherapy, **Johns Hopkins University School of Medicine**, ²The Sidney Kimmel Comprehensive Cancer Center, **Johns Hopkins University School of Medicine**, ³Department of Biomedical Engineering, **Johns Hopkins University School of Medicine**, ⁴Department of Immunology, **Johns Hopkins University School of Medicine**, ⁵Department of Pathology, **Johns Hopkins University School of Medicine**

Manuscript & video:

https://www.jove.com/video/57473/excyt-graphical-user-interface-for-streamlining-analysis-high?status=a59479k&fbclid=IwAR2i0i-M51YhAkrEermEwtpinvPLPccc_n88pXmrfZPwhhu9tZLbZTzvQuA

Software:

<https://github.com/sidhomj/ExCYT2>

- interactive gating
- gate directly on tSNE plots
- tSNE and then re-tSNE on gated subsets
- novel high-dimensional flow plots

ExCYT2

