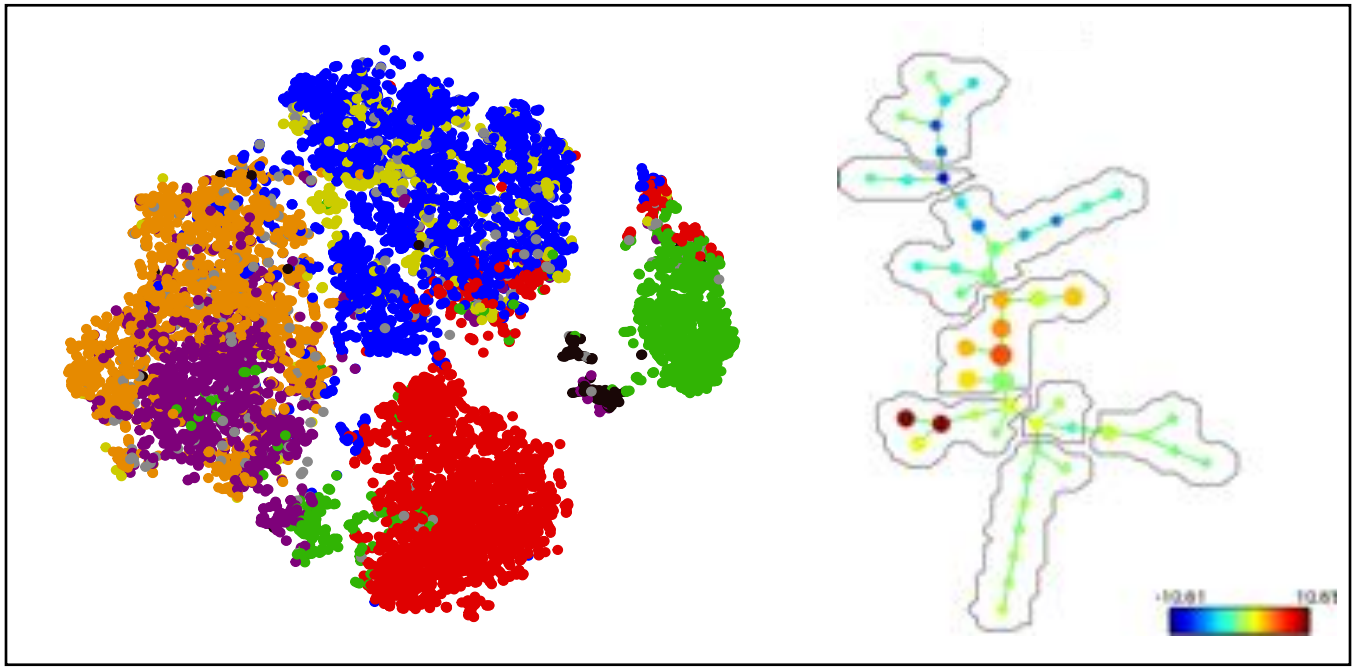
Intro to Computational Flow Workshop: viSNE, PhenoGraph, SPADE



Lisa Borghesi
Associate Professor of Immunology
Director, Unified Flow Core

Outline

viSNE, PhenoGraph, SPADE

how the algorithms work

maximizing comparable results across experiments

Overview of Analysis Workflow

FJ → export populations to cluster → algorithm

Go for it!

Cyt (viSNE, PhenoGraph) then SPADE

Systems Immunology

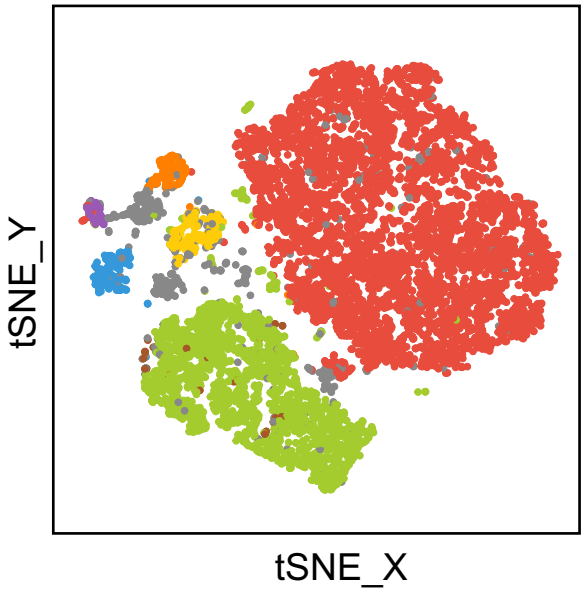
Allows for many simultaneous measurements to be made in a given sample, allowing the variation to be taken into account and used in order to make new discoveries

Imagine 20+ color high dimensional flow cytometry space…

t-SNE – dimensionality reduction algorithm

Goal: find a low dimensional visualization that best reflects population structure in high dimensional space

→ colloquially, get a feel for how objects are arranged in data space



Laurens van der Maaten explains t-SNE (UCSD seminar) – fun and informative!!

https://www.youtube.com/watch?v=EMD106bB2vY

t-SNE vs viSNE

Van der Maaten

t-SNE: t-distributed stochastic neighbor embedding

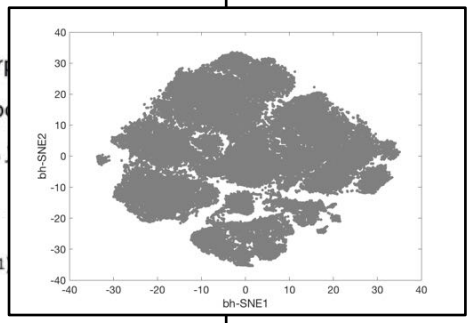
bh-SNE: Barnes-Hut modification of t-SNE (computationally faster)

Dana Pe'er

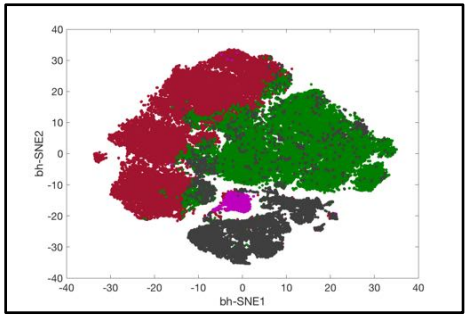
viSNE: visualization of t-distributed stochastic neighbor embedding

t-SNE → bh-SNE

t-SNE algorithm:
begin
1. Compute pairwise affinities $p_{j|i}$ with per
2. Set $p_{ij} = \frac{p_{i|j}+p_{j|i}}{2n}$ (n - Number of data p
3. Sample initial solution $Y^{(0)} = y_1, y_2, \ldots,$
   for t=1 **to** T **do**
   1. Compute low-dimensional affinities q_{ij}
   2. Compute gradient $\frac{\delta C}{\delta y}$
   3. Set $\mathcal{Y}^{(t)} = \mathcal{Y}^{(t-1)} + \eta\frac{\delta C}{\delta y} + \alpha(t)(\mathcal{Y}^{(t-1}$
   end
end



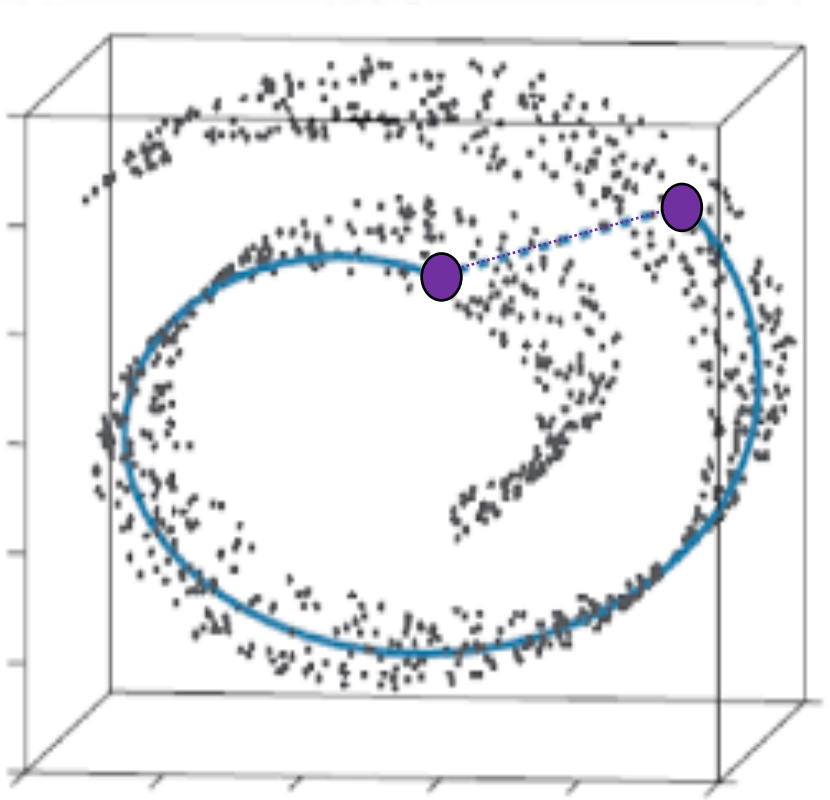
viSNE



Why not just use PCA?

PCA

Swiss roll problem



- Principal Components Analysis (PCA) preserves large pairwise distances
- Euclidean distance between two points on the Swiss roll does not accurately reflect local structure

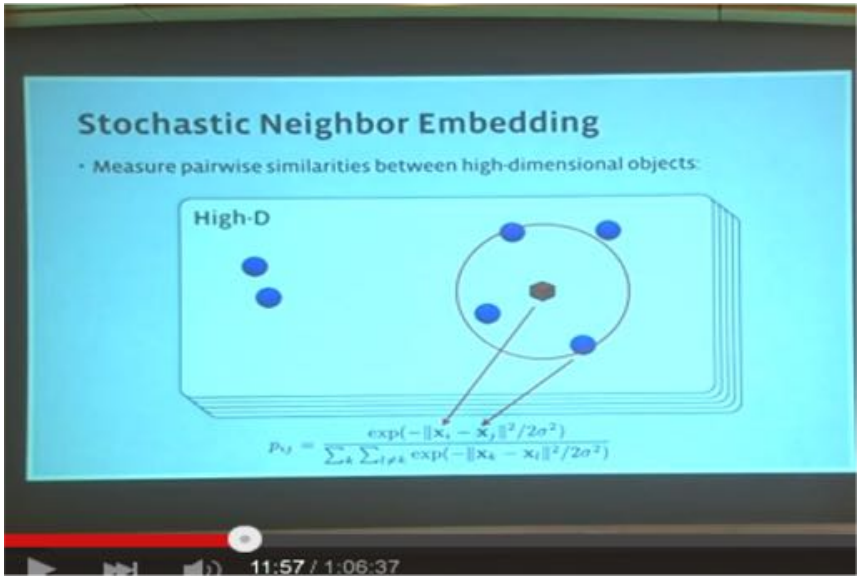
t-SNE preserves local distances and global distances

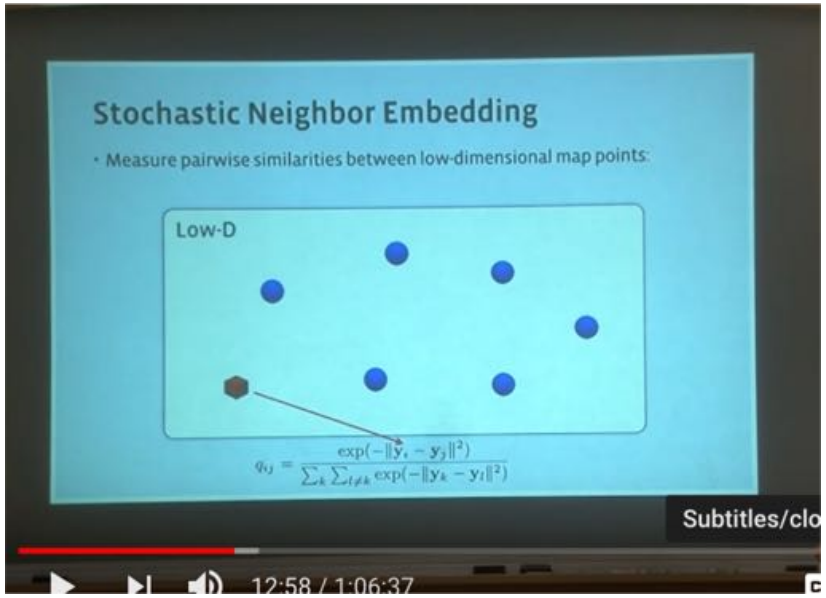
PCA

tSNE

Amir 2013 Nature Biotech, Suppl.

t-SNE operation



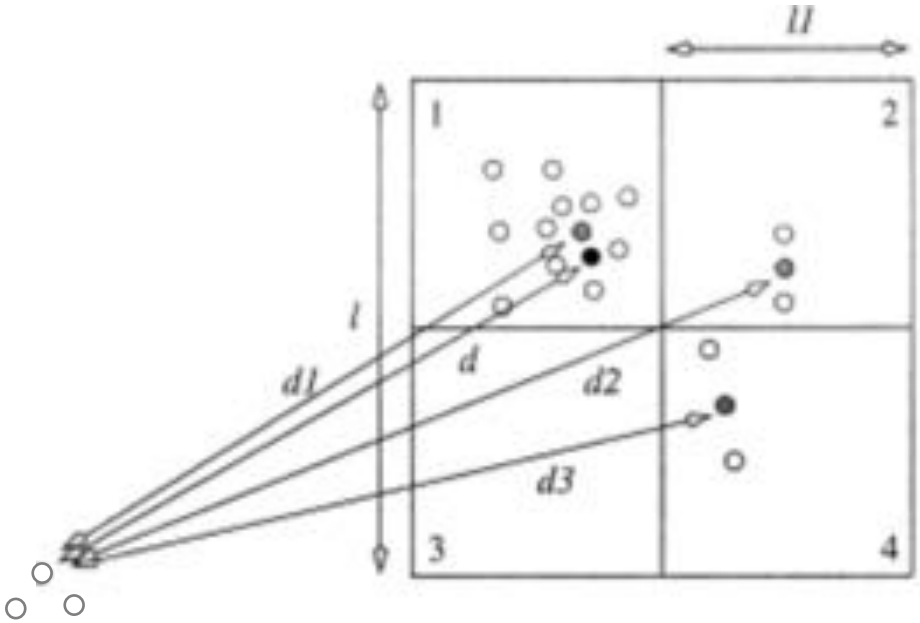


High-D data space. Draw Gaussian bell (circle) around data point. Measure density of all other points relative to that Gaussian bell, and establish probability distribution that represents their similarity. Computes local densities to get a distribution of pairs of points.
→ **Pij**

Low-D 2D map. Repeat above.
→ **Qij**

Mathematically minimize **P||Q** difference. Zero would be if two points were the same.

Barnes-Hut Modification of t-SNE



- Center of mass of domain
- Centers of mass of subdomains
- Source particle

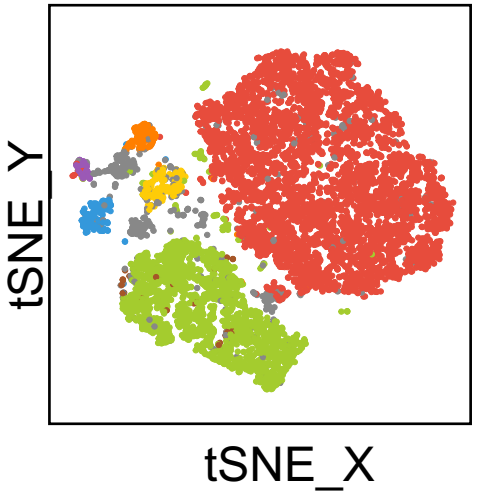
if (l/d < a)
compute direct force interaction
with the center of mass of domain.
else
if (ll/d1 < a)
compute direct force computation
with center of mass of subdomain 1
else
expand subdomain 1 further

Apply similar criteria to domains 2, 3, and 4

t-SNE

Advantages

- single cell information
- non-linear assumptions (as opposed to PCA)
- preserves local and global structure



Limitations

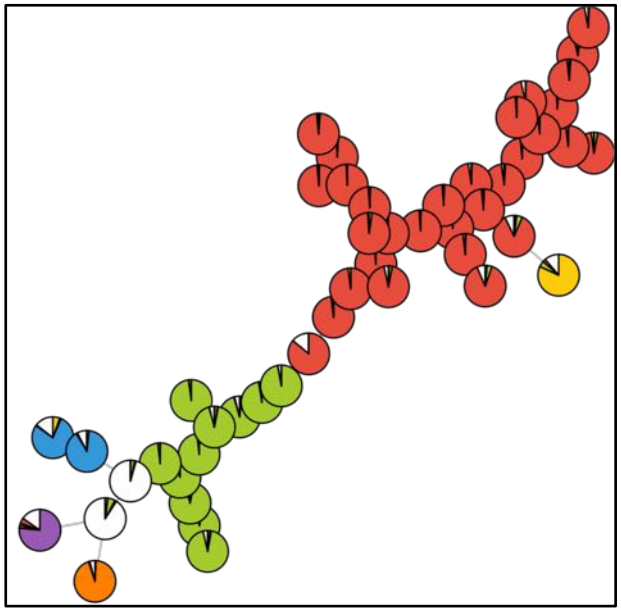
- computationally expensive; obligate downsampling means data are discarded
- plot axes are arbitrary and have no intrinsic meaning
- no population identification; follow up approaches required to assign identity to clusters and cells
- distance between clusters is not meaningful; no hierarchy

SPADE: Hierarchical clustering algorithm

spanning tree progression of density normalized events

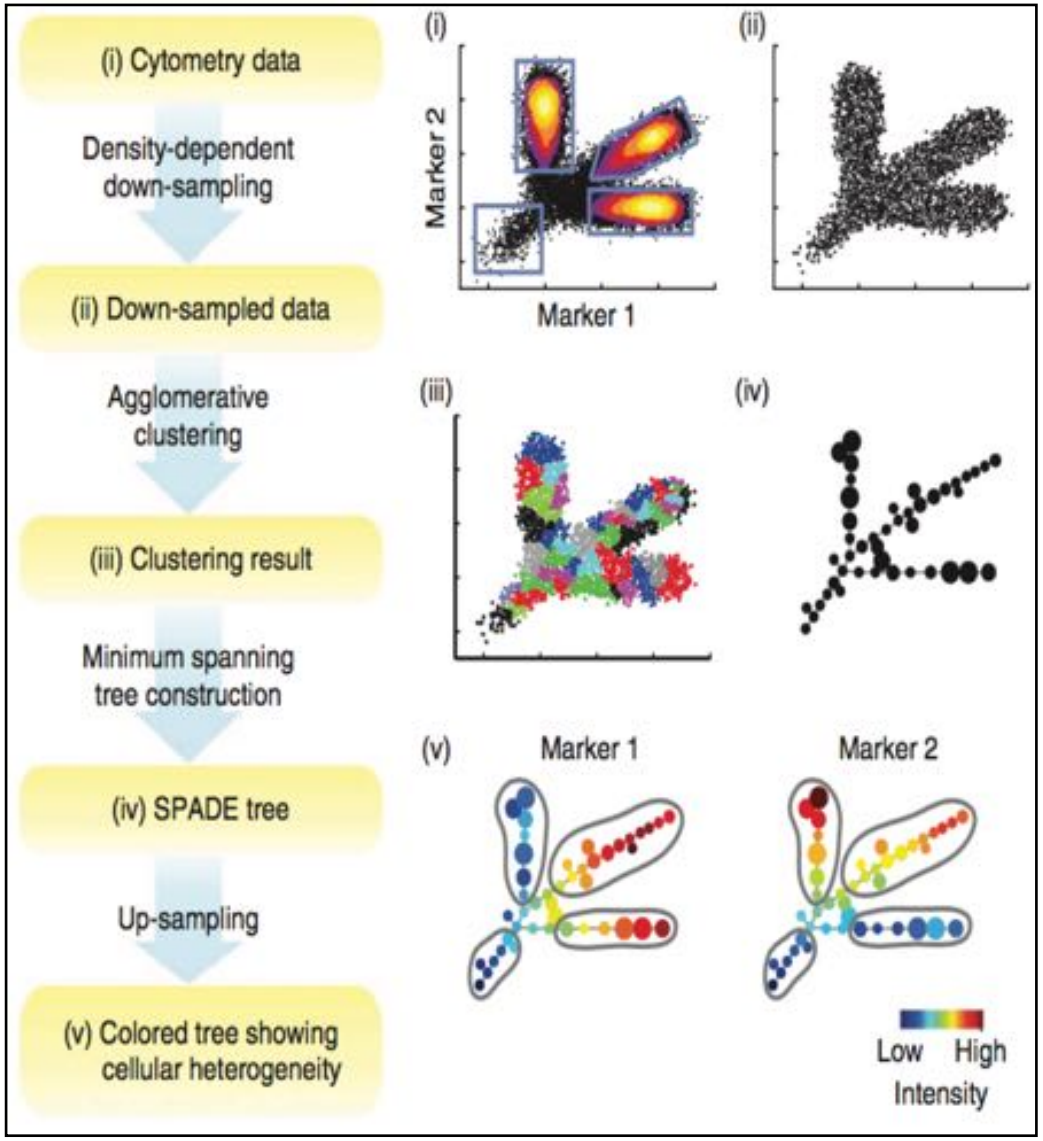
Goal: organize cells into a **hierarchy** using unsupervised approaches

→ colloquially, generate a tree of relationships



Output minimal spanning tree (MST) highlights the relationships between most closely related cell type clusters

SPADE



SPADE views **data as a cloud** of points (cells) where the dimensions = # markers

Density-dependent downsampling to equalize density in different parts of cloud, ensures rare cells not lost

Agglomerative clustering based on marker intensity

Connect clusters in minimal spanning tree that best reflects geometry of the original cloud

Upsampling, map each cell in the original data set to the clusters

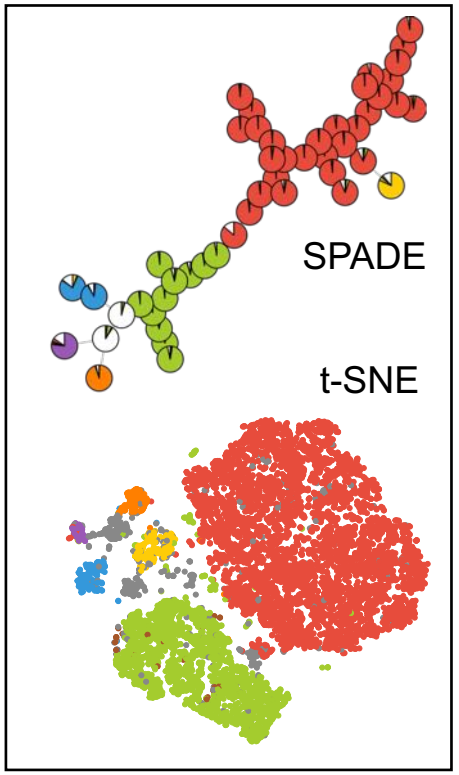
SPADE

Advantages

- rare pops preserved through density-dependent downsampling
- enables visualization of continuity of phenotypes
- can combine data sets that share common markers, and then co-map any markers unique to each data set (see orig. paper)

Limitations

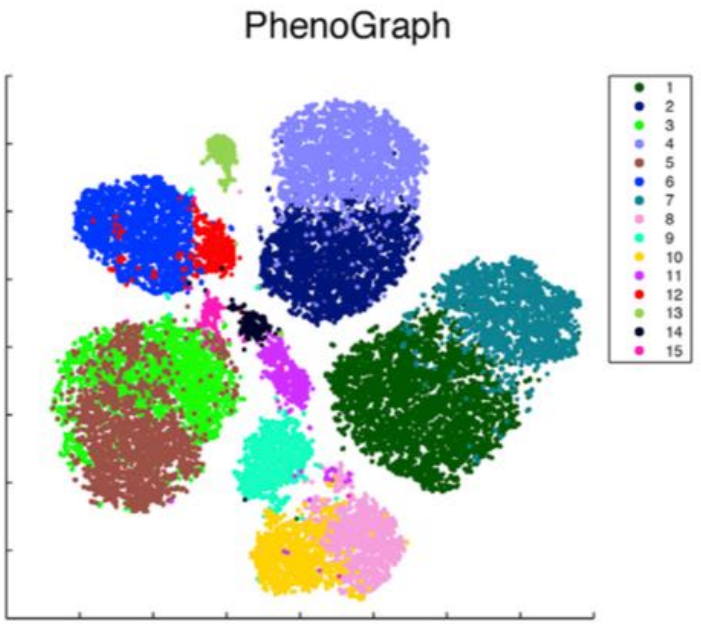
- loss of single cell information
- user chooses cluster number
- MSTs are non-cyclic and paths can be artificially split



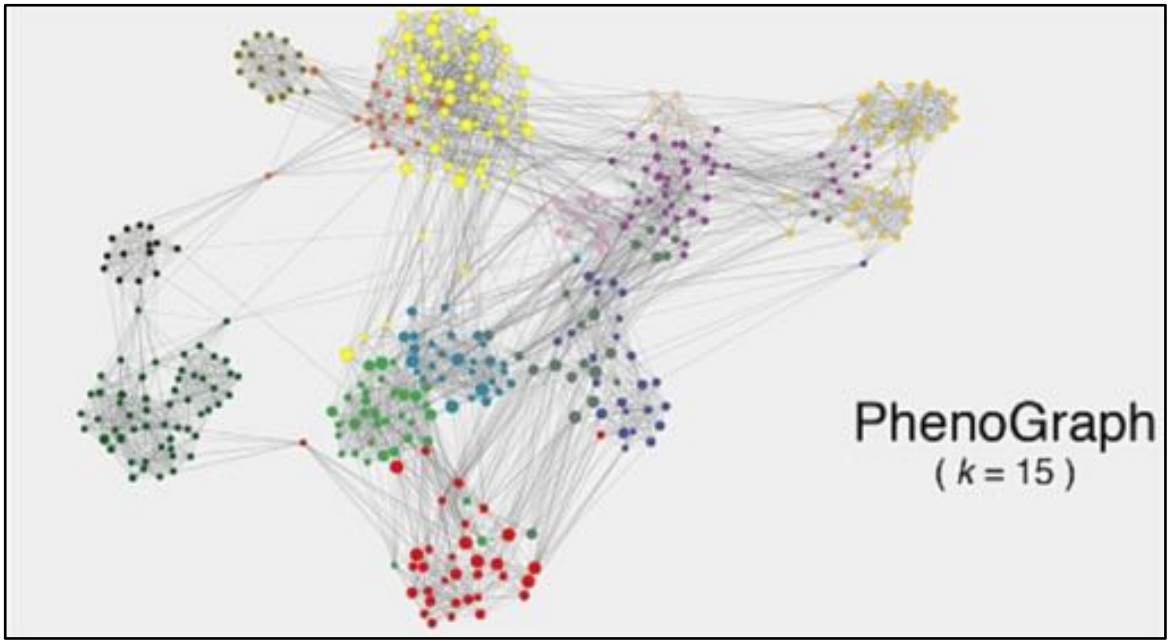
PhenoGraph

Goal: automated partitioning of high-dimensional single-cell data into subpopulations

→ colloquially, map nearest neighbors



PhenoGraph



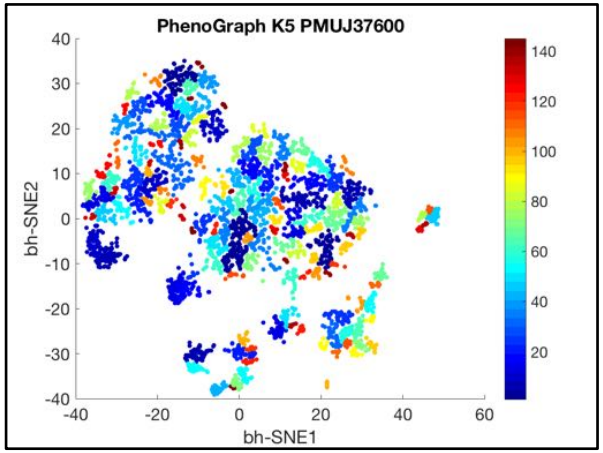
First order relationship – find the *k* nearest neighbors for each cell using Euclidean distance

Second order relationships – cells with shared neighbors should be placed near one another

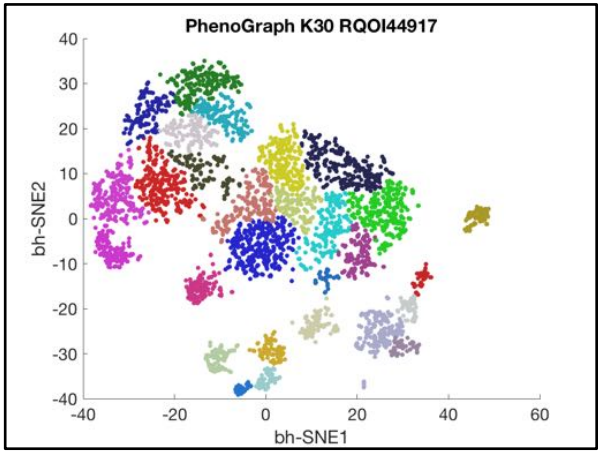
Third, identify communities – Louvain method that measures the density of edges inside communities to edges outside communities

PhenoGraph: Number of neighbors

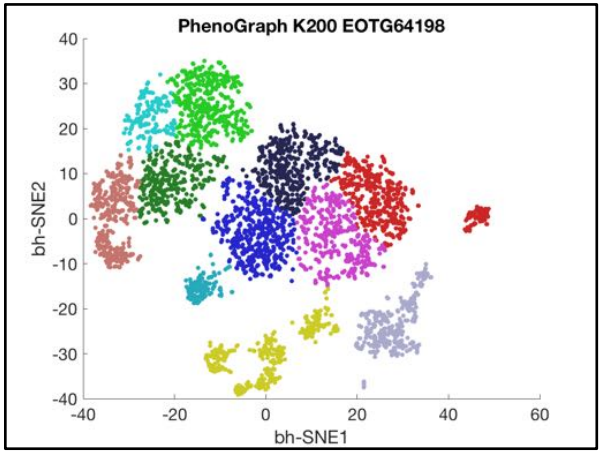
Neighbors = 5



Neighbors = 30

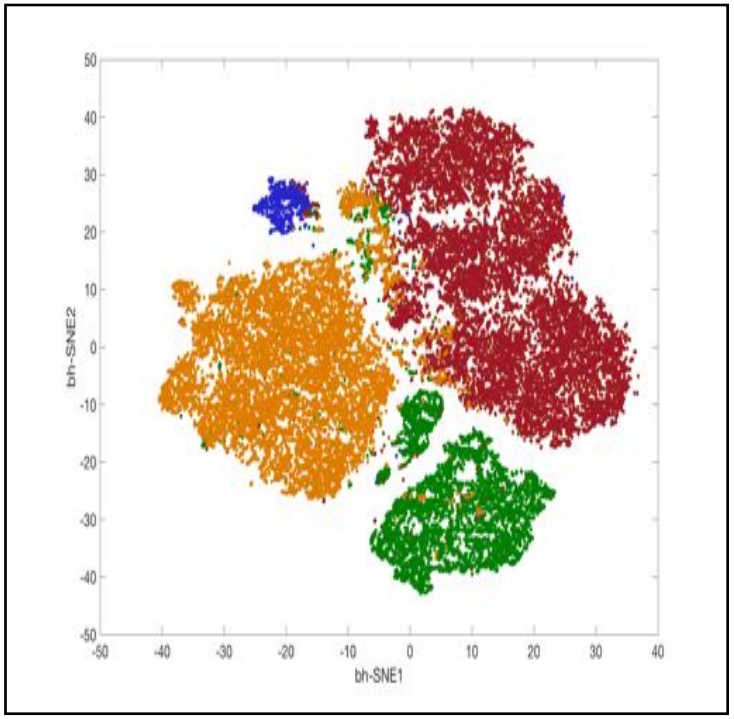


Neighbors = 200



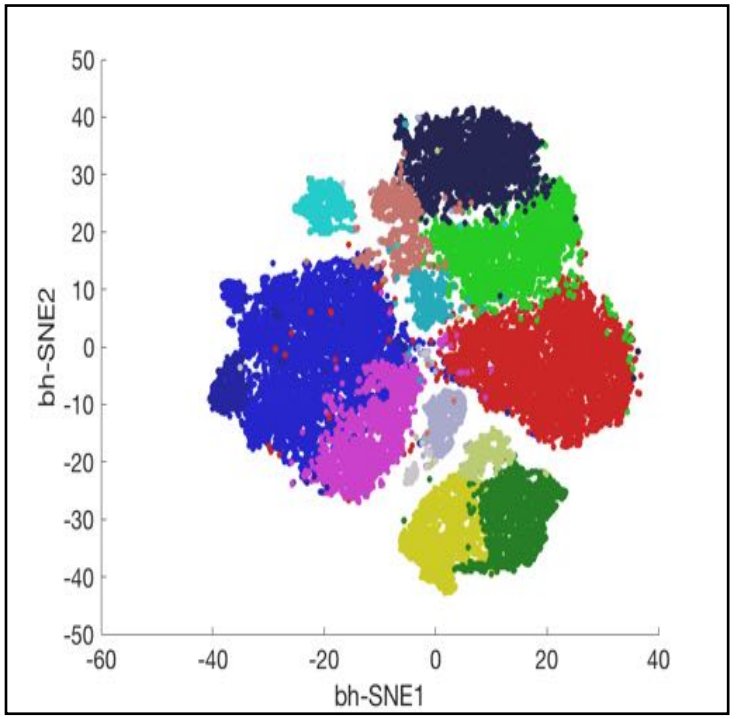
PhenoGraph – population discovery

Manual gate overlays



- Naive B
- ASC
- MBC (total)
- Ag-exper.

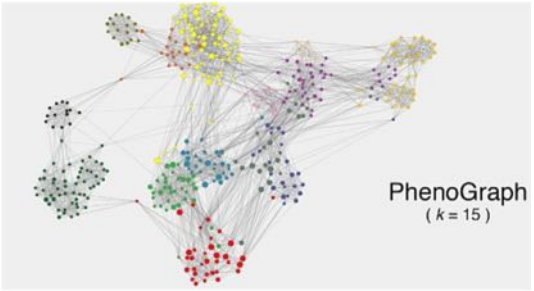
PhenoGraph



PhenoGraph

Advantages

- opportunity for population discovery
- can resolve subpopulations as rare as 1 in 2000 cells
- robust to cluster shape (e.g., need not be spherical)



Limitations

- user specifies number of neighbors
- ideal cluster number, or biologically relevant cluster number, is largely unconstrained

Pooling data across experiments

Standardize fluorescence intensities when performing experiments over time

→ **Use Rainbow Beads to adjust PMT voltages for each fluorescence channel to target MFI values**

Leukemia (2012) 26:1986-2010
Nature Protocols (2012) 7:2067-2079

Outline

viSNE, PhenoGraph, SPADE

how the algorithms work

maximizing comparable results across experiments

Overview of Analysis Workflow

FJ → export populations to cluster → algorithm

Go for it!

Cyt (viSNE, PhenoGraph) then SPADE

Basic Workflow

export gated
fcs files

viSNE, PhenoGraph

SPADE

Gate on live singlets, population
to cluster

Ex. Live single CD45+ cells
Ex. Live single CD19+ B cells
Ex. Live single CD4+Foxp3+ Tregs

Goal: Multidimensional profiling of human skin T cells

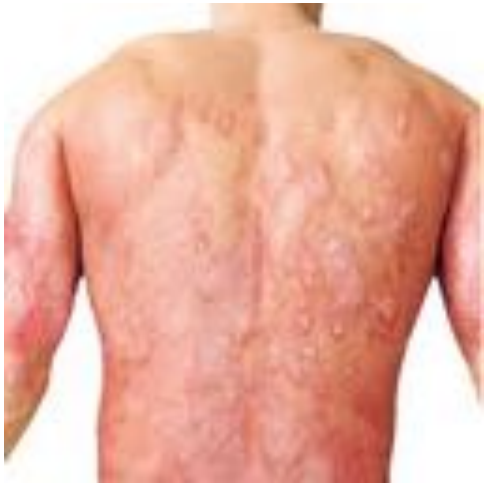
→gate on dump^{neg}CD3^{pos}

Two specimens

1. Normal skin “lib 1mg”
2. Psoriasis (PsO_stelara)

Two compensation matrices

1. Normal
2. Psoriasis



Antibody Panel:

CD4 BUV 395
CD8 BUV 737
CD3 FITC = population to cluster
TCRab APC (A647)
TCRgd PerCP/Cy5.5

CD45RO BV510
CTLA4/CD152 PE-TxRed
CD69 AF700
CD103 PE-Cy7

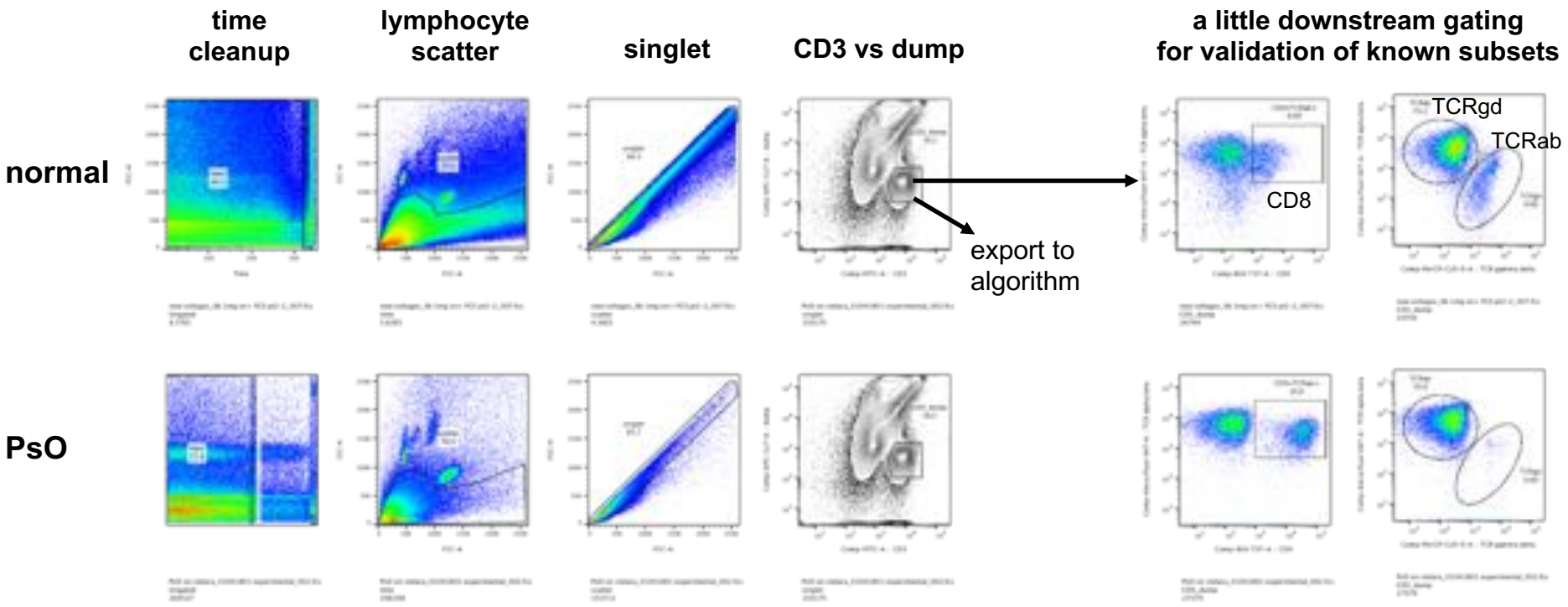
Foxp3 PE
Tbet BV605
RORgt BV421

Dump: **live/dead, CD11c, CD19, CD14 APCCy7**

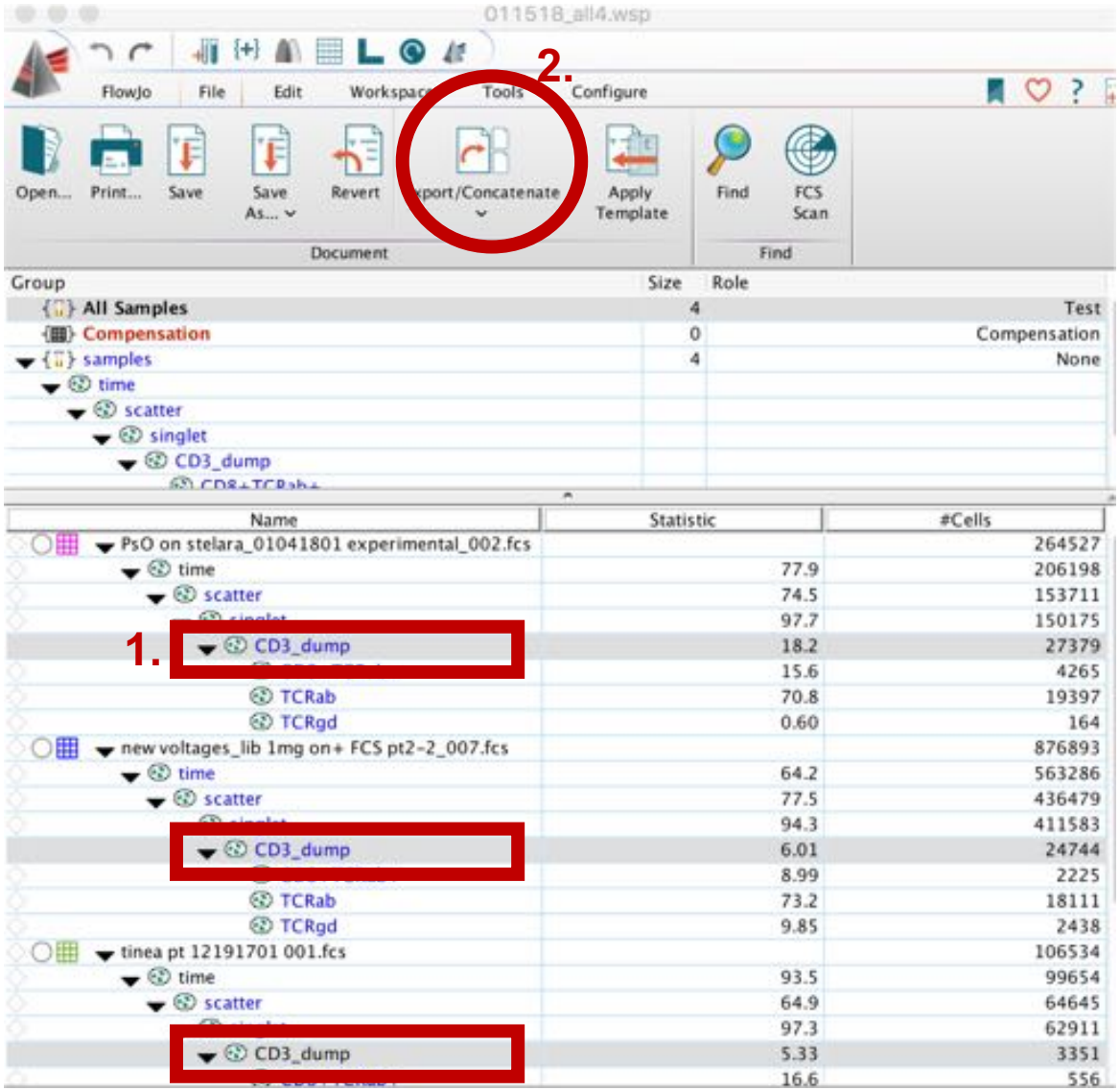
Courtesy: fcs data Sarah Whitely

Go to FlowJo → import files, gate

FlowJo gating, live single CD3+ T cells



Export gated population that you want to cluster



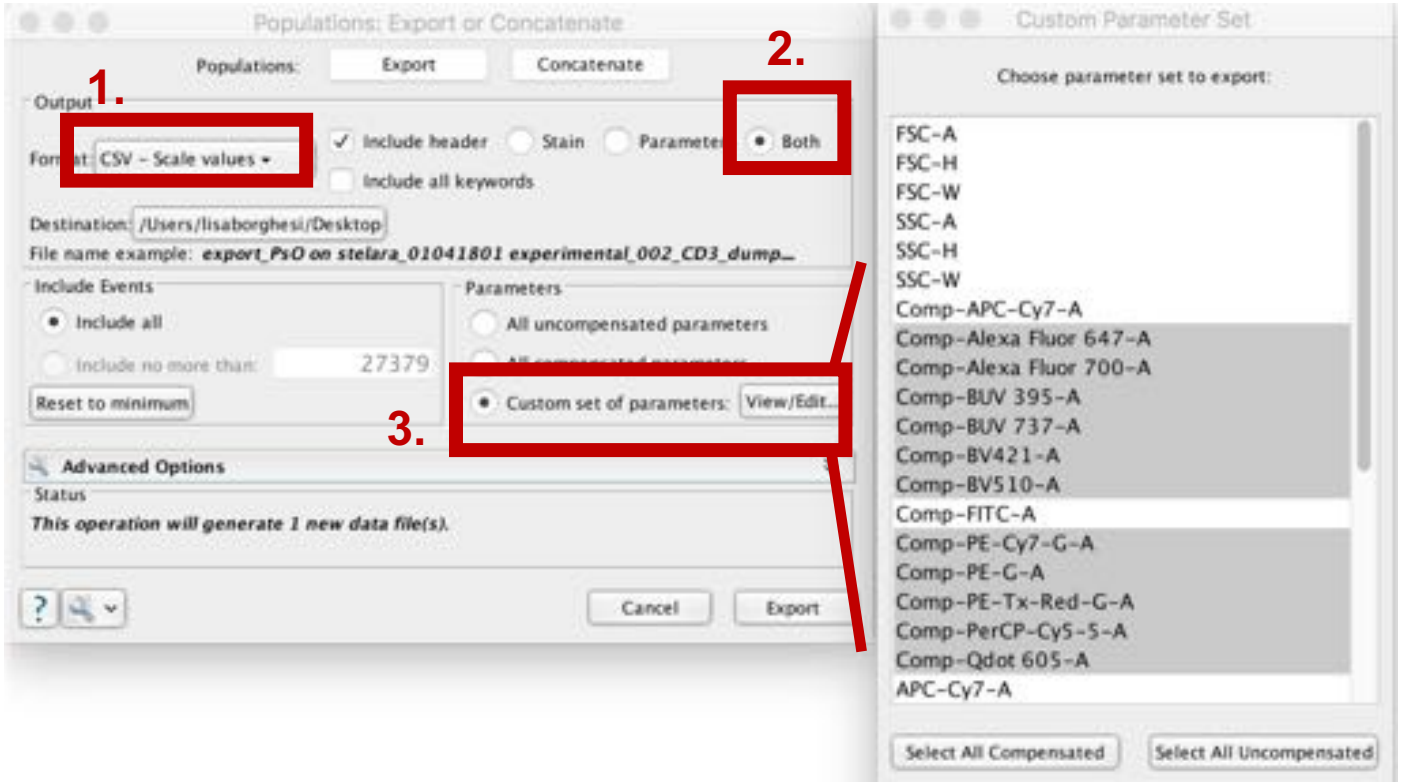
1. Highlight gates to export

2. Select Export → Export/Concatenate Populations

3. You'll get a pop-up window (next slide)

Export custom parameters that you want to cluster

1. Under Format choose “CSV – Scale values”
2. Select “both” stain & parameter
3. Select the compensated fluors you wish to export for clustering
   - leave behind: viability dye APC-Cy7 (you’ve already excluded dead cells)
   - leave behind: CD3 FITC (you’ve already gated on CD3)
   - leave behind: uncompensated parameters

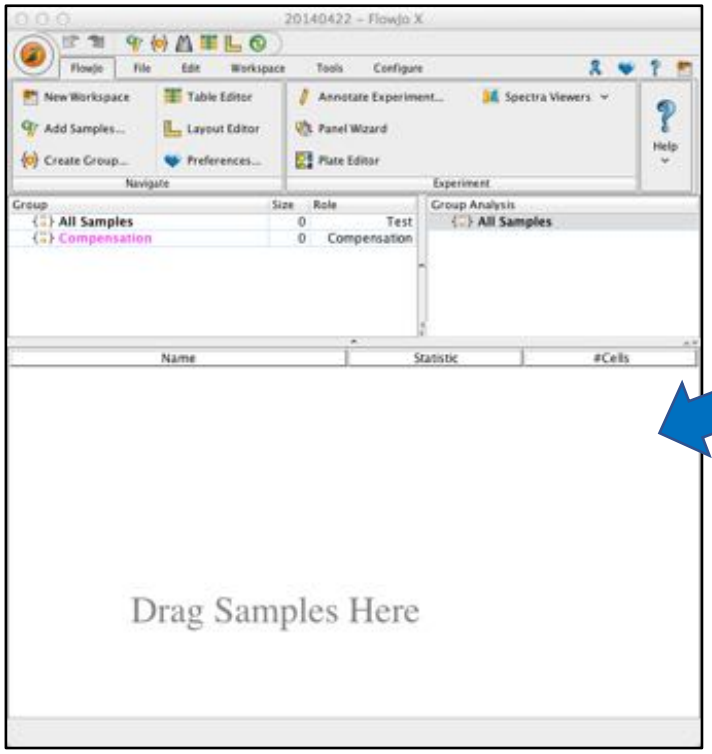


Convert CSV → FCS

1. Open a new FlowJo workspace

2. Drop the CSV file onto the workspace

3. a new FSC file will appear in the same location where you saved the CSV file
   - conversion may take a minute or two so be patient



File.csv

File.fcs –
will appear in same
folder as your .csv

Launch Matlab/Cyt3

Steps for viSNE analysis in Matlab/Cyt3

1. **Import** fcs data gated on the population you wish to cluster

2. **Transform**, arcsinh 150

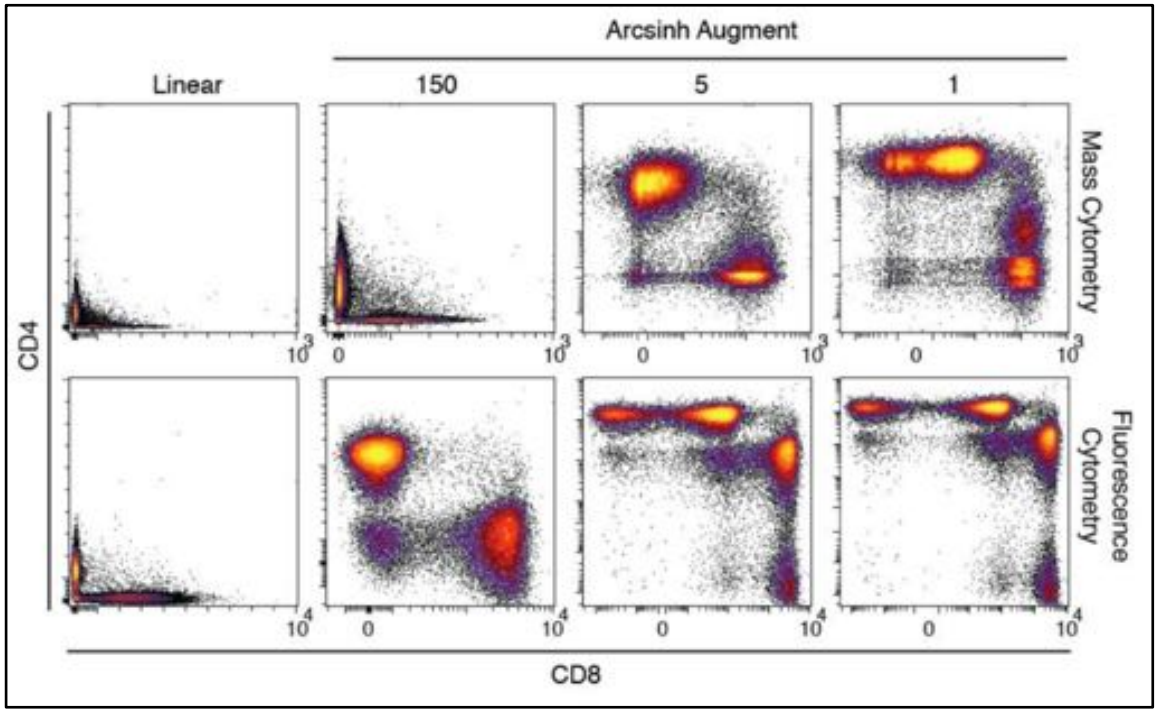
3. **Downsample** (i.e., subsample a portion of total events)
   - to reduce computational burden
   - to select a small subset of events for a quick first-pass analysis
   - to normalize events across comparative analyses

4. Invoke **bh-SNE**

Transformation, value 150 for flow cytometry data

- fcs data can have negative numbers due to compensation correction and instrument baseline correction. Algorithms can't handle negative numbers.

- hyperbolic arcsine (arcsinh) transformation is similar to biexponential transformation in FlowJo. See http://docs.flowjo.com/d2/graphs-and-gating/gw-transform-overview/



Bendall 2011 Science 332:687, Fig S2

Import gated populations into Cyt3 “SightOf”

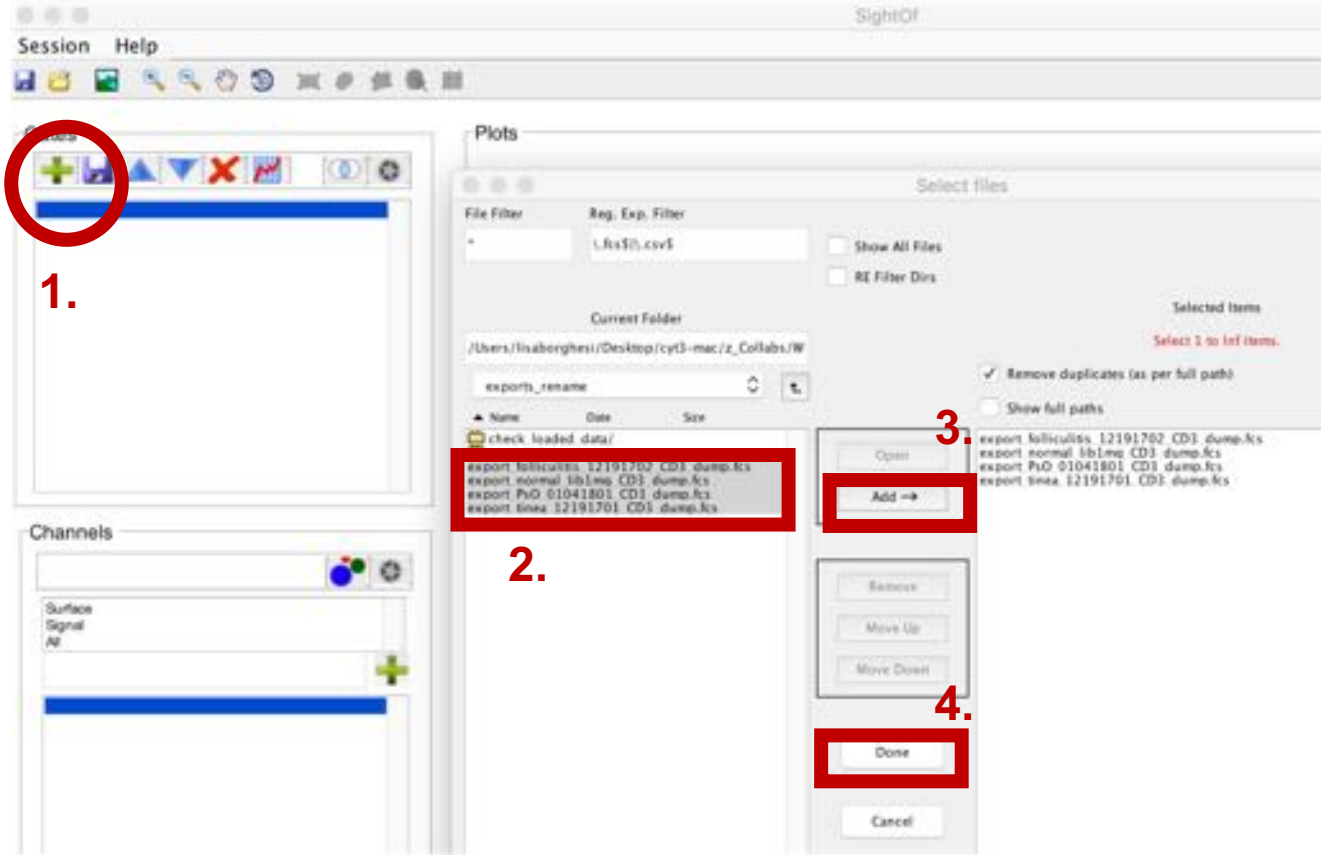
Cyt3

1. Click the “+” sign to import fcs files

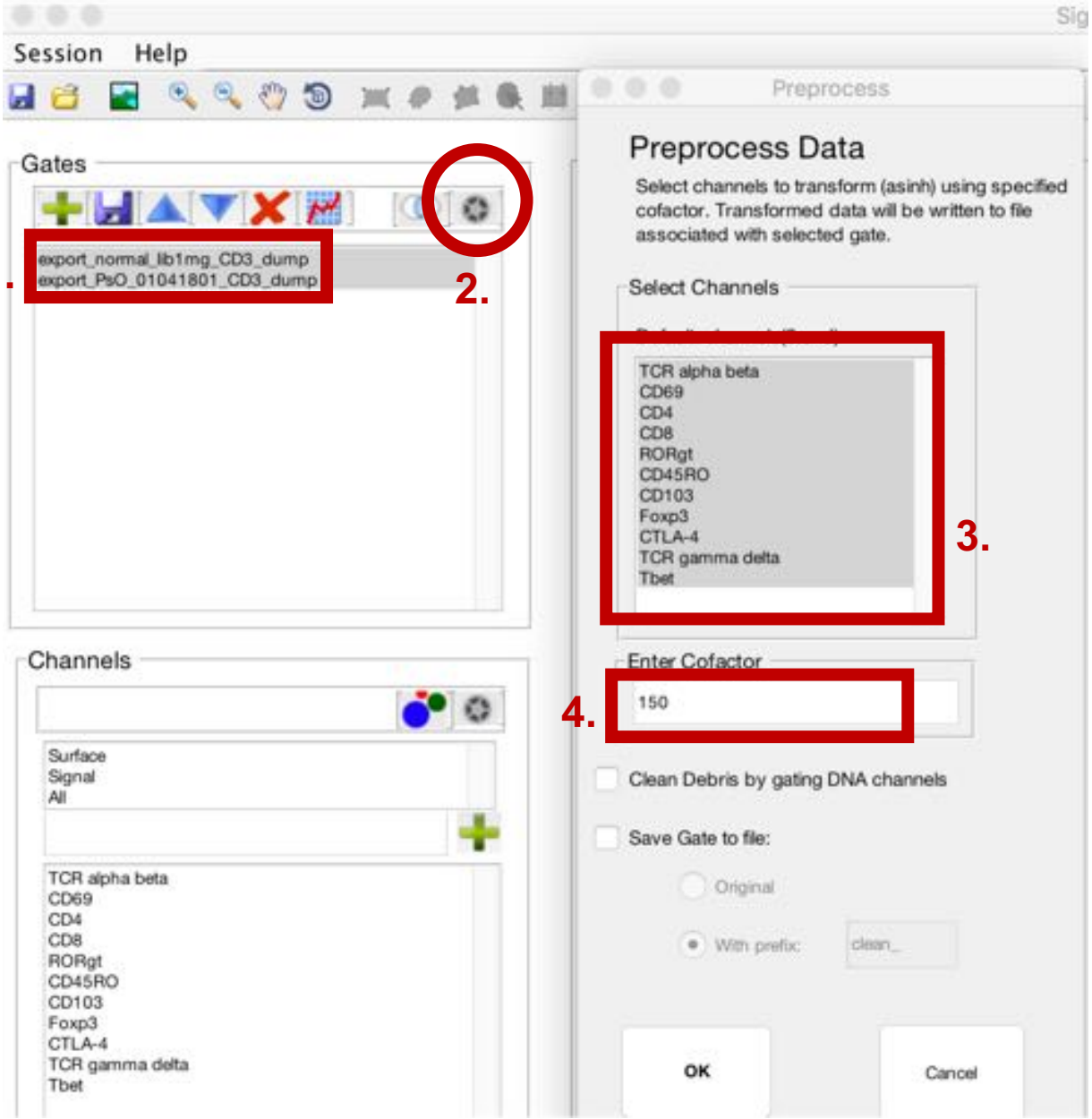
2. Navigate folder and select files

3. Select “Add”

4. Click “Done”



Pre-processing steps for viSNE analysis: Transform



1. Import fcs files

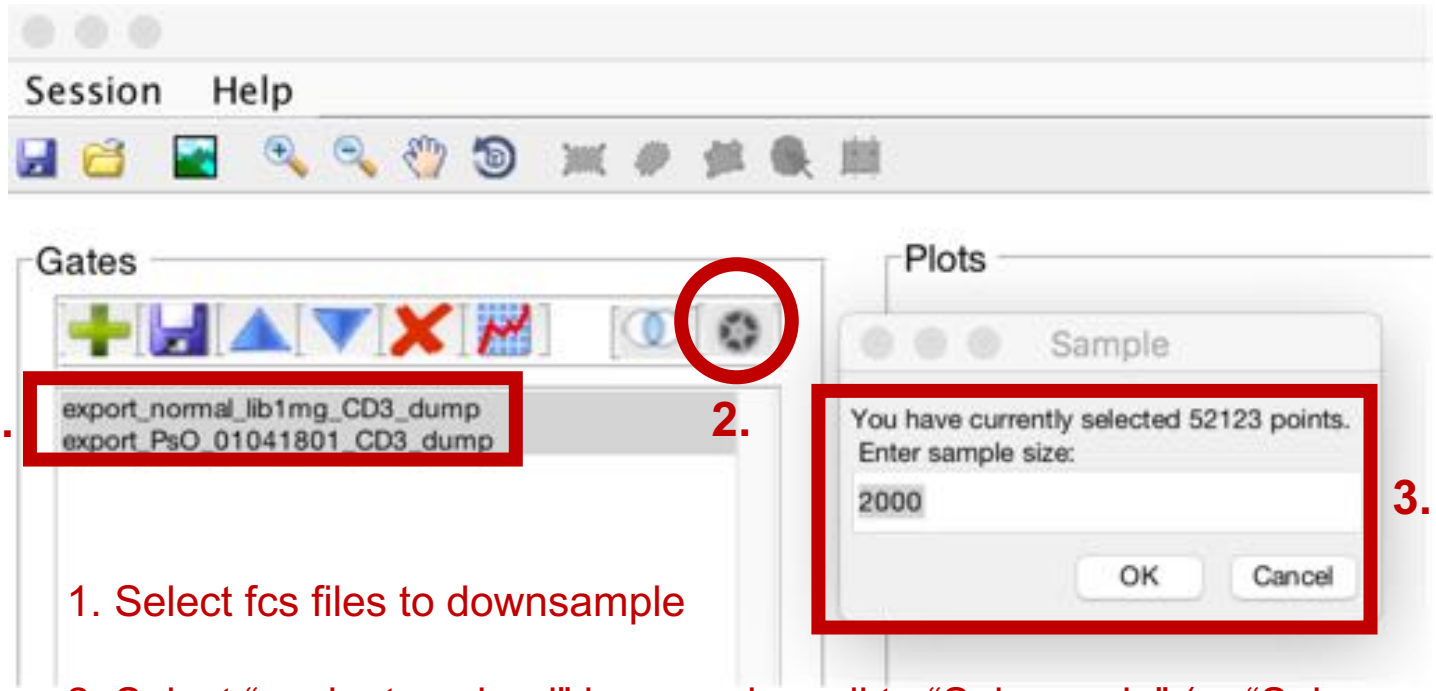
2. Select “navigator wheel” icon, and scroll to Transform

3. In popup window Select Channels to transform

4. Enter Cofactor 150 for flow cytometry (or 5 for mass cytometry)

5. Click “OK”

Pre-processing steps for viSNE analysis: Downsample



1. Select fcs files to downsample

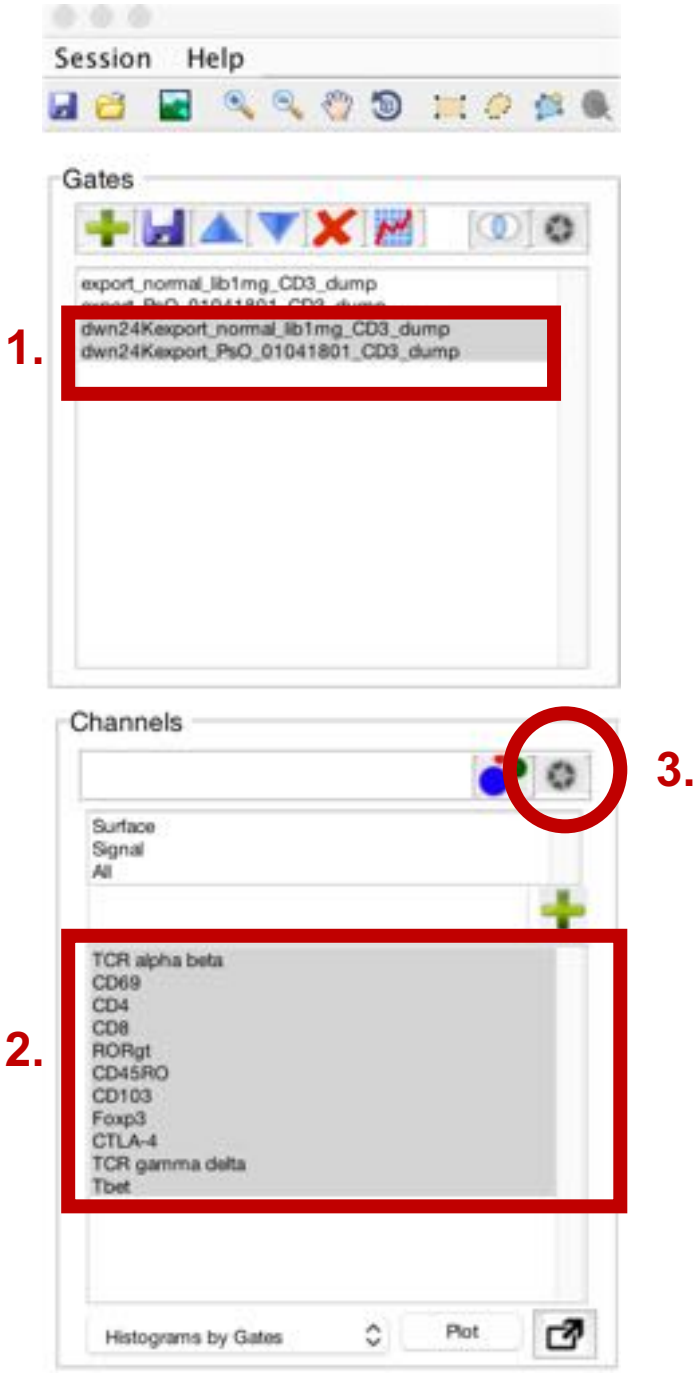
2. Select “navigator wheel” icon, and scroll to “Subsample” (or “Subsample Each” if you have multiple samples to simultaneously downsample)

3. In popup window specify the number of events

4. Click “OK”. Then type a short prefix to be appended to sample name.

5. The new subsampled files you created should appear in the list

Invoke bh-SNE



1. Select the (transformed, downsampled) fcs files to cluster

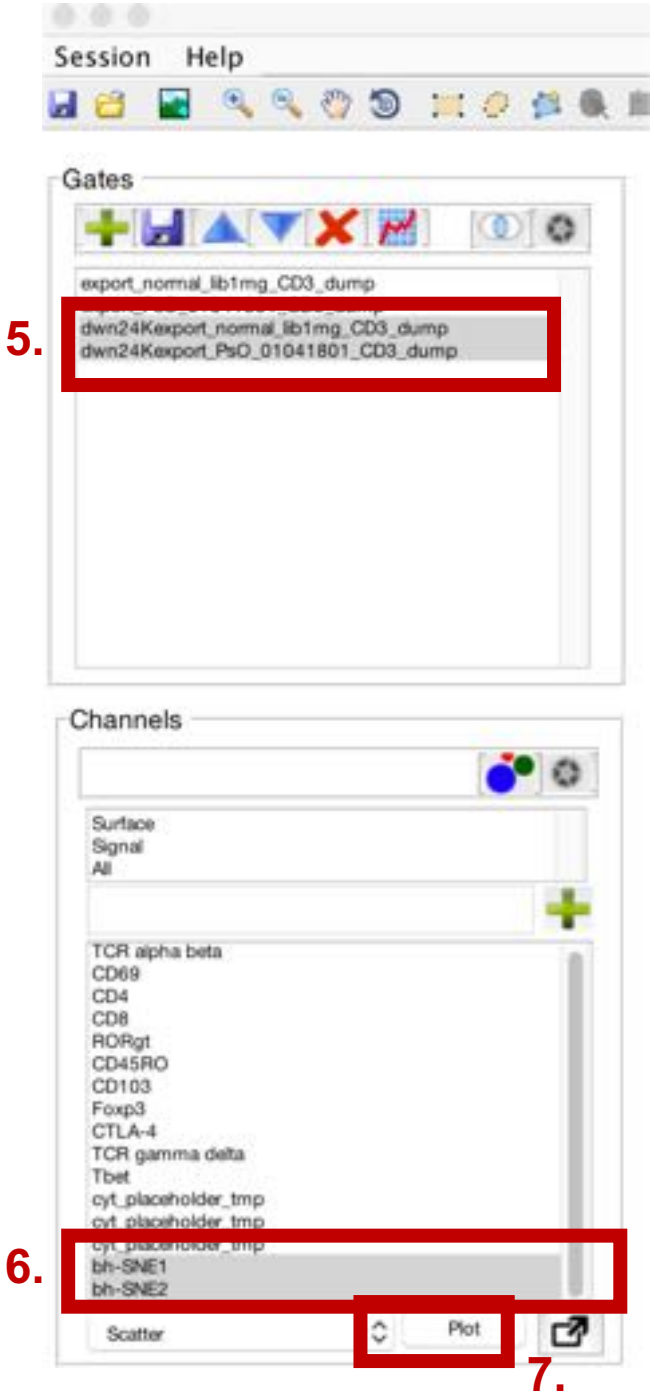
2. Select fluors to include in clustering

3. Under “lower navigator wheel” select bh-SNE

4. Check original Matlab window (remember, you are currently in Cyt window) for algorithm progress

→Once algorithm has finished, the two new derived parameters will appear in lower pane: bh-SNE1 and bh-SNE2

View your bh-SNE plot

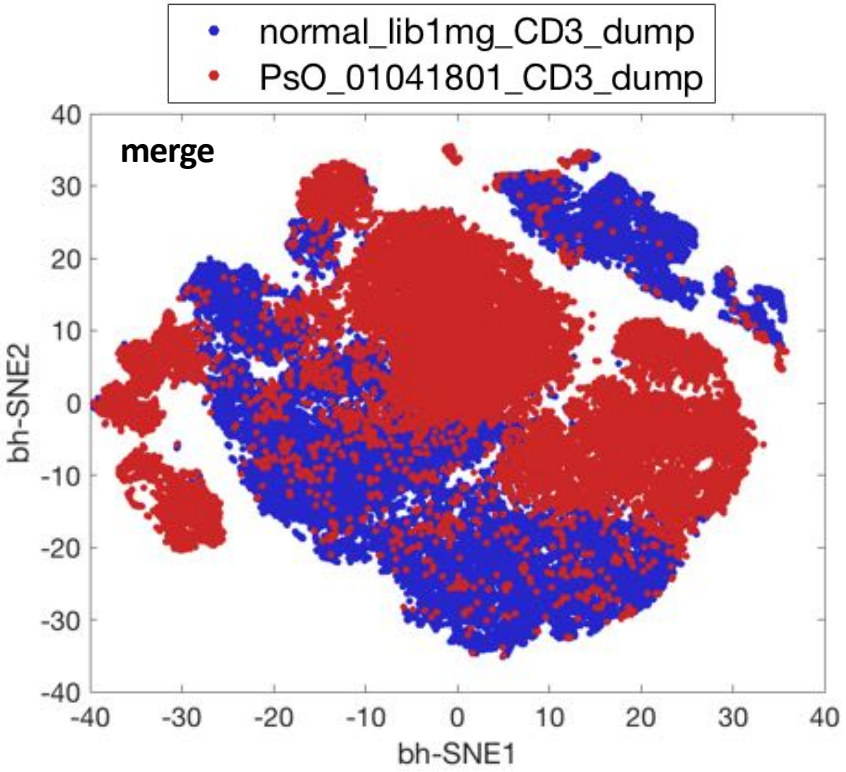


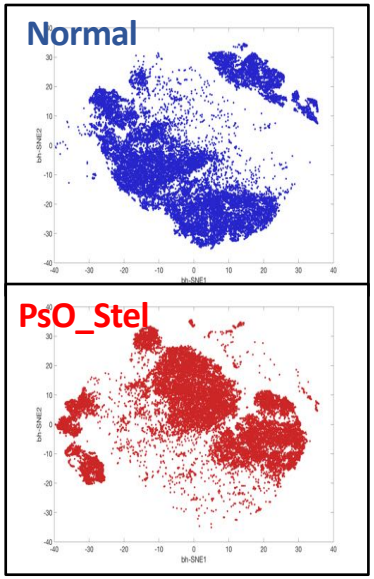
5. Make sure the files you clustered are still selected.

6. Select the two new derived parameters bh-SNE1 and bh-SNE2

7. Select “Plot”

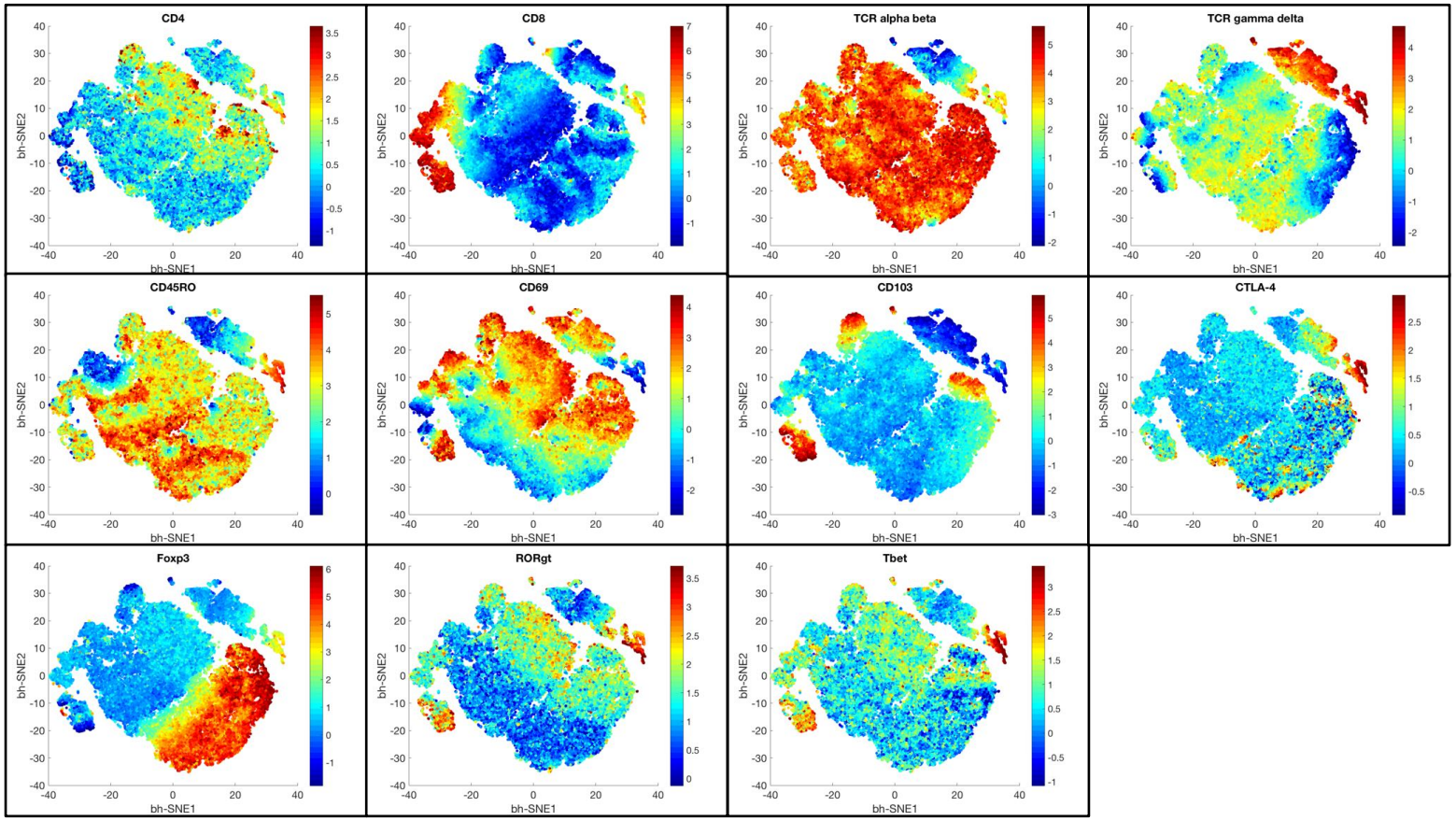
viSNE: normal vs PsO



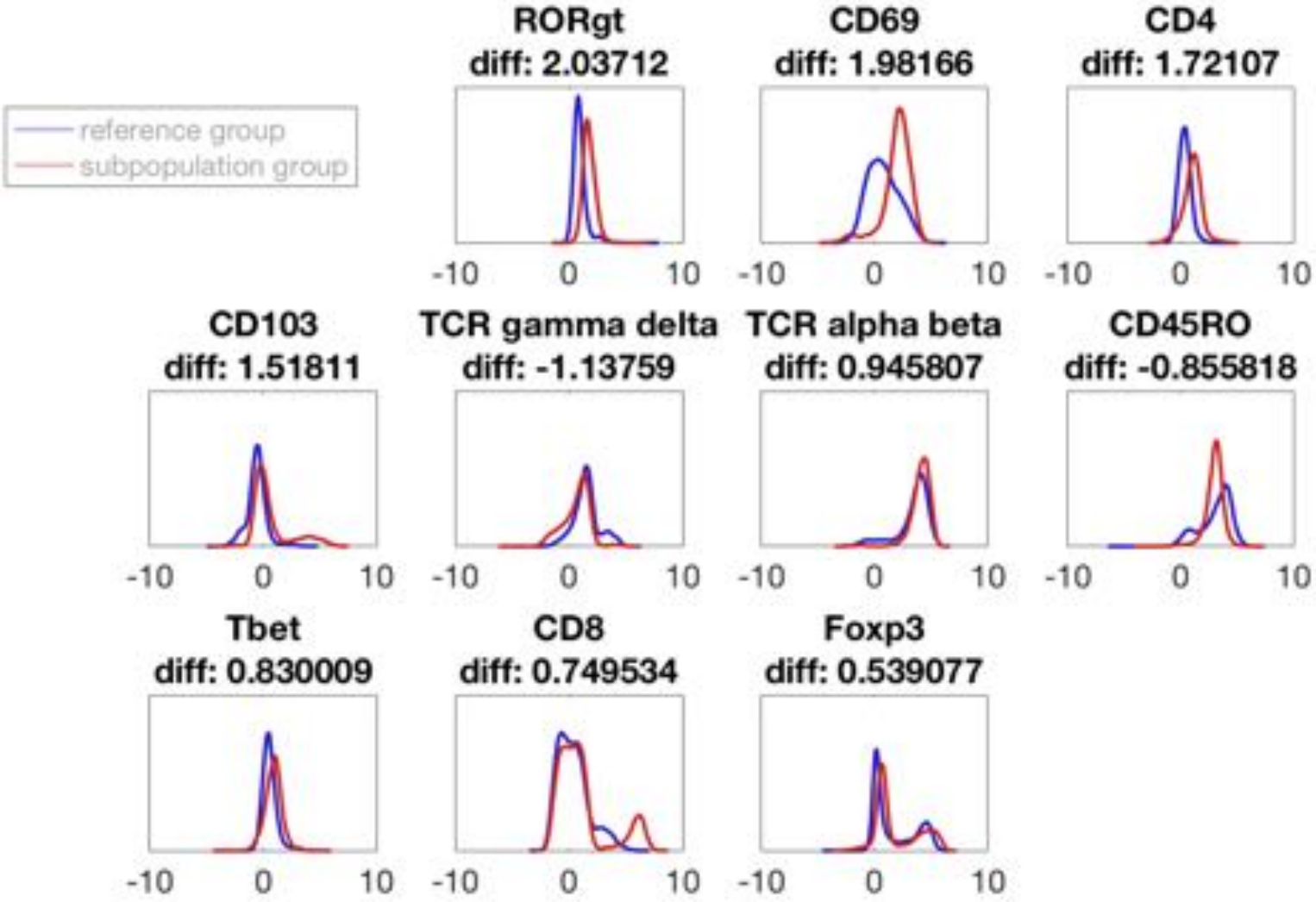


- downsample Normal, PsO_stel to 24K/ea
- cluster all markers except for pre-gated channels (CD3, dump)

Marker heatmaps

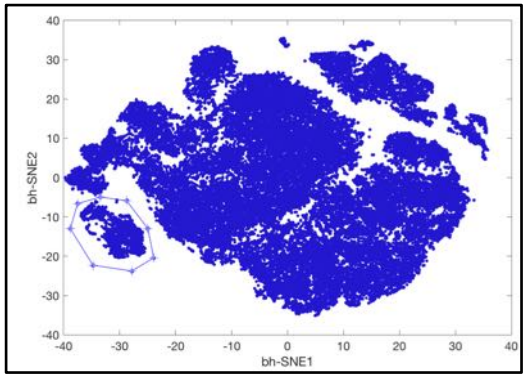


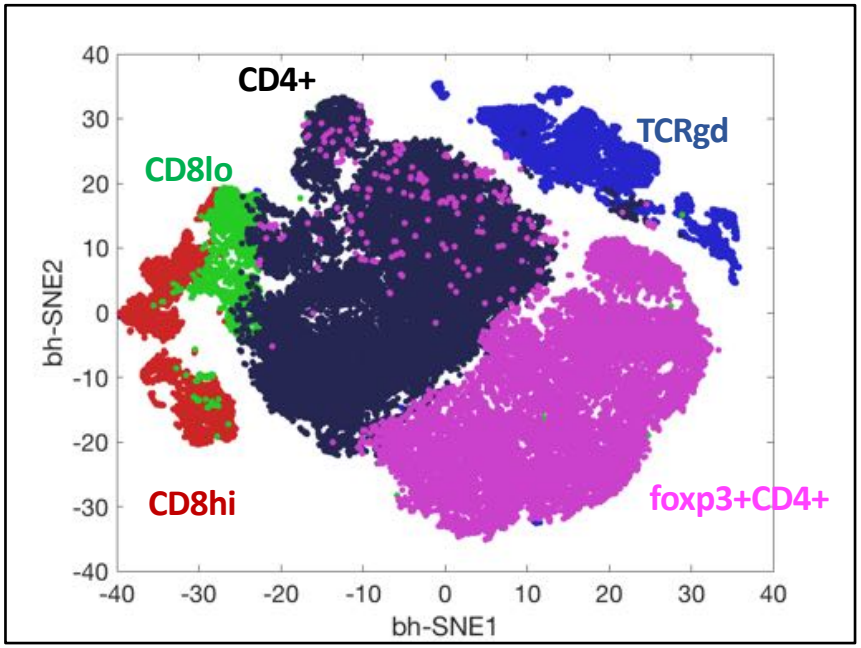
L1 statistic – difference between marker distributions



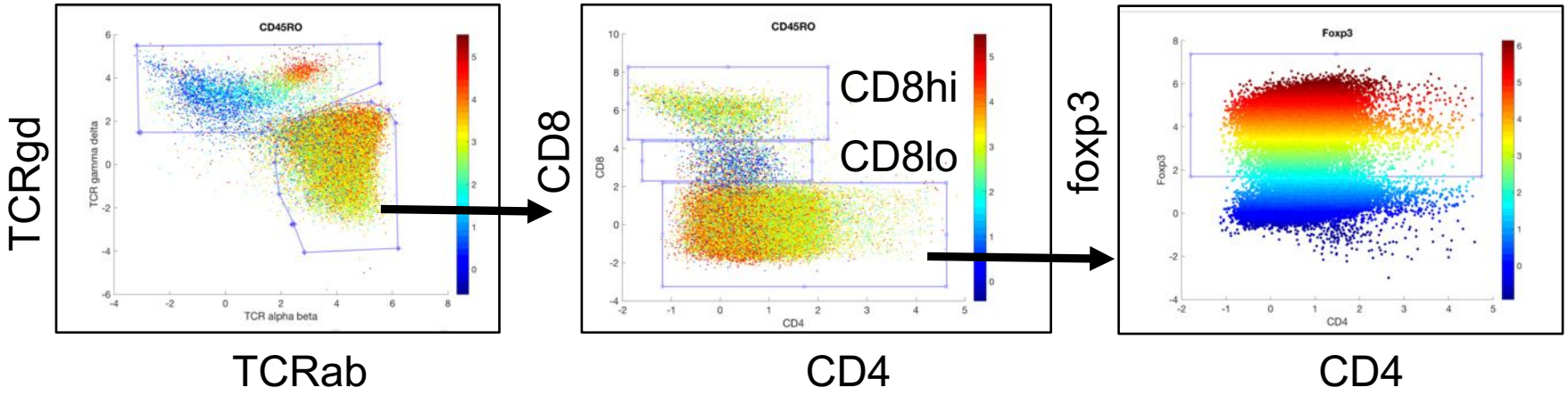
Manual gating overlays

Gate on tSNE clusters

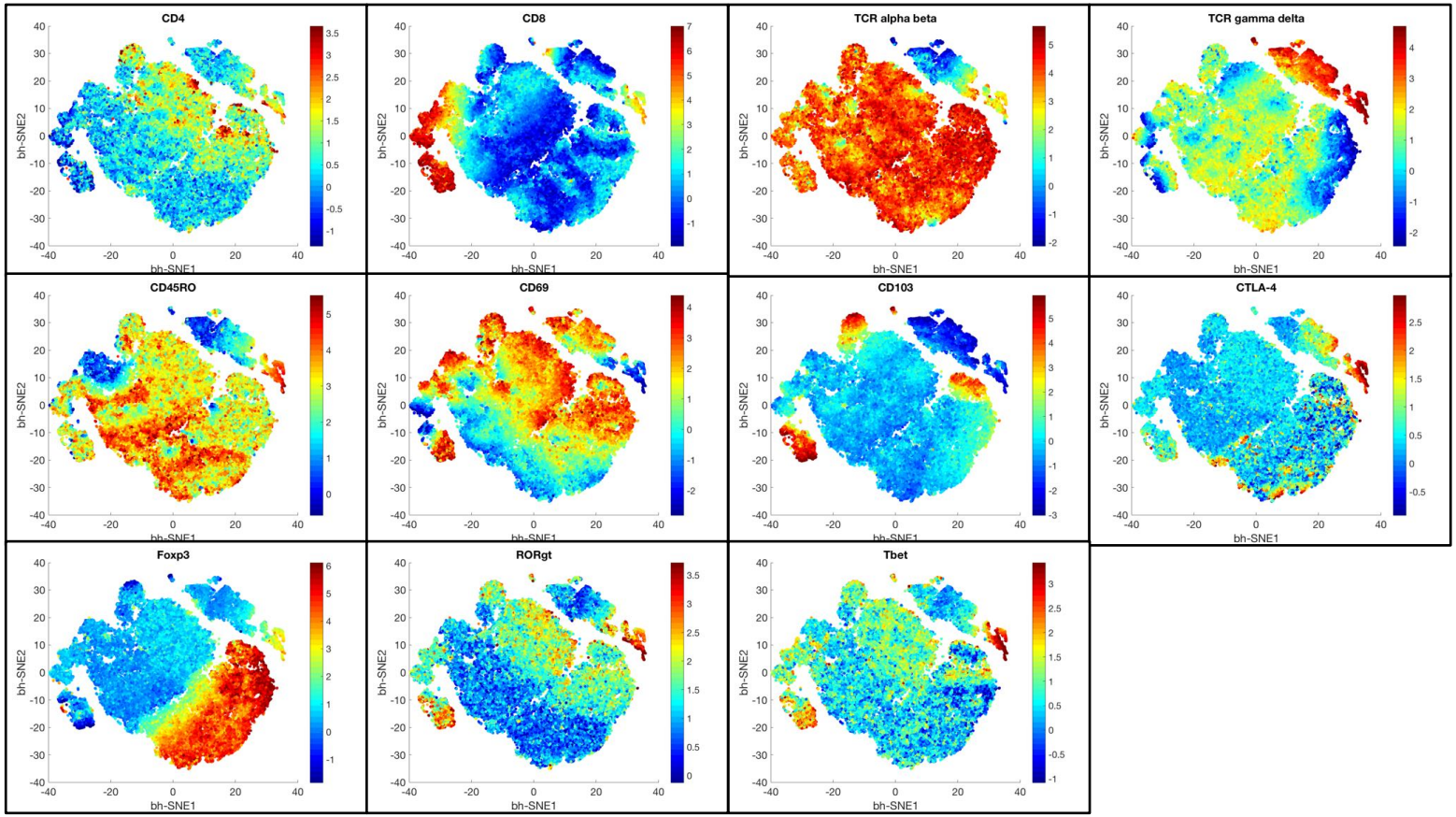


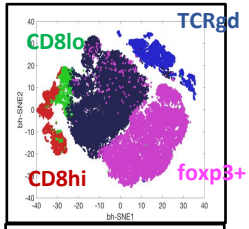


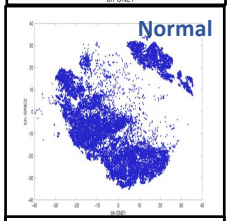
Gate on fluors

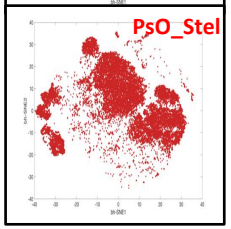


Summary: Multidimensional profiling of human skin T cells









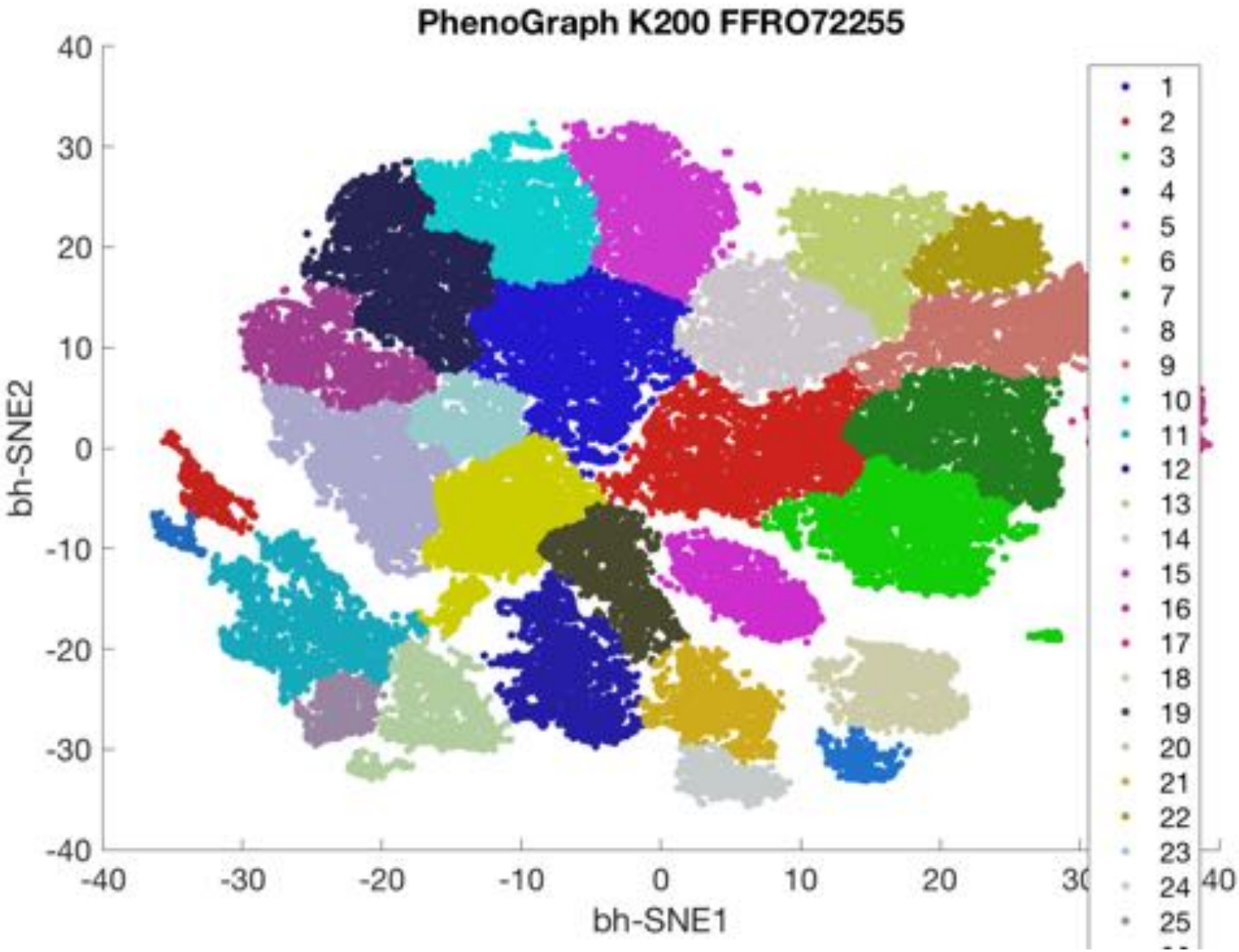
PhenoGraph

1. Make sure the files you clustered are still selected.

2. Select the two new derived parameters bh-SNE1 and bh-SNE2

3. Under navigation wheel select “PhenoGraph”

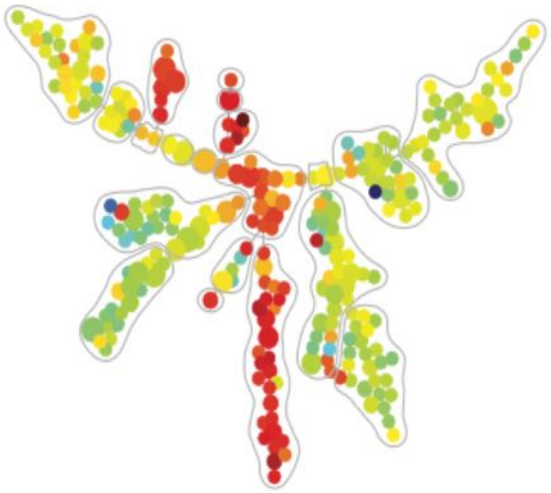
PhenoGraph – automated clustering



SPADE

SPADE developer provides great instructions

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

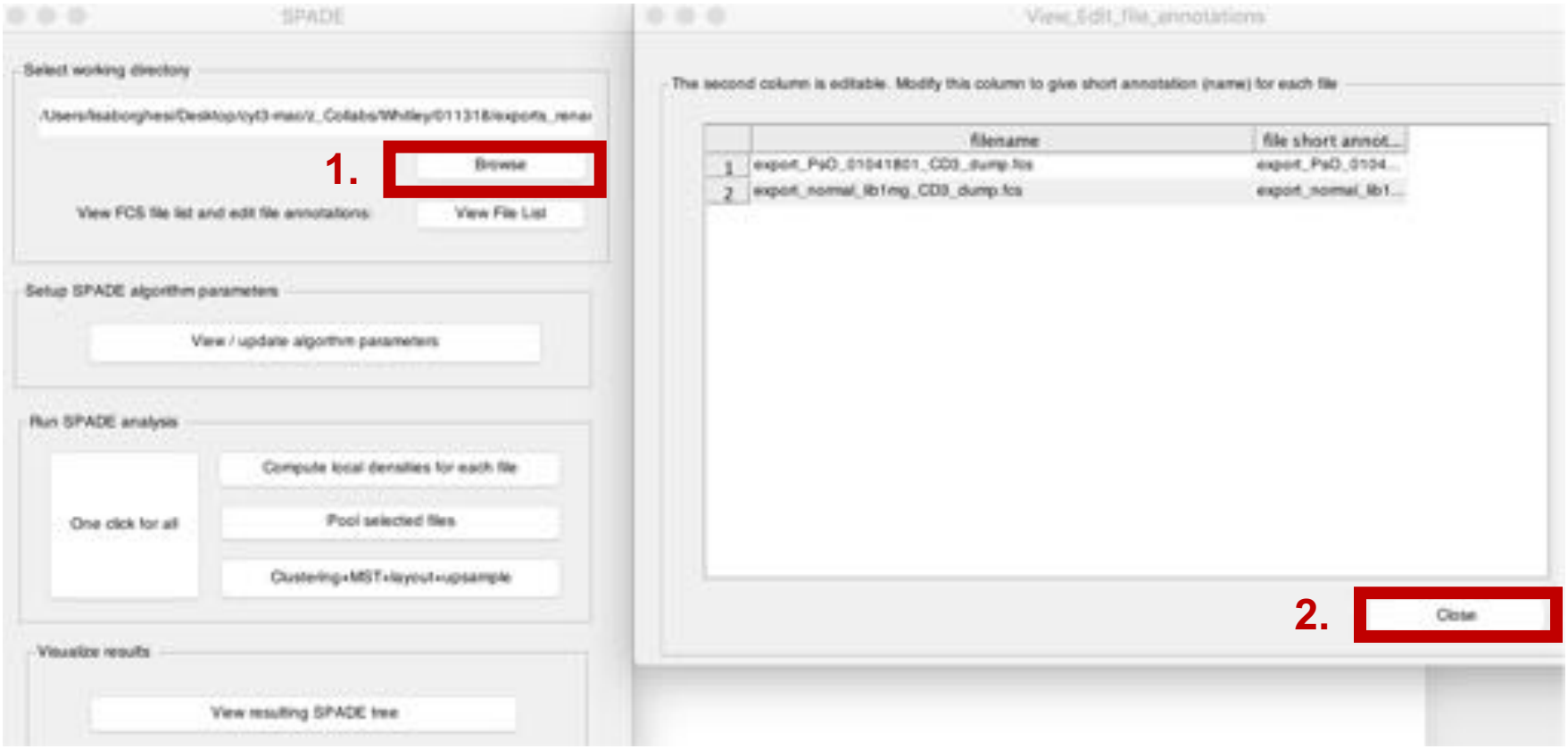


Import gated populations into SPADE

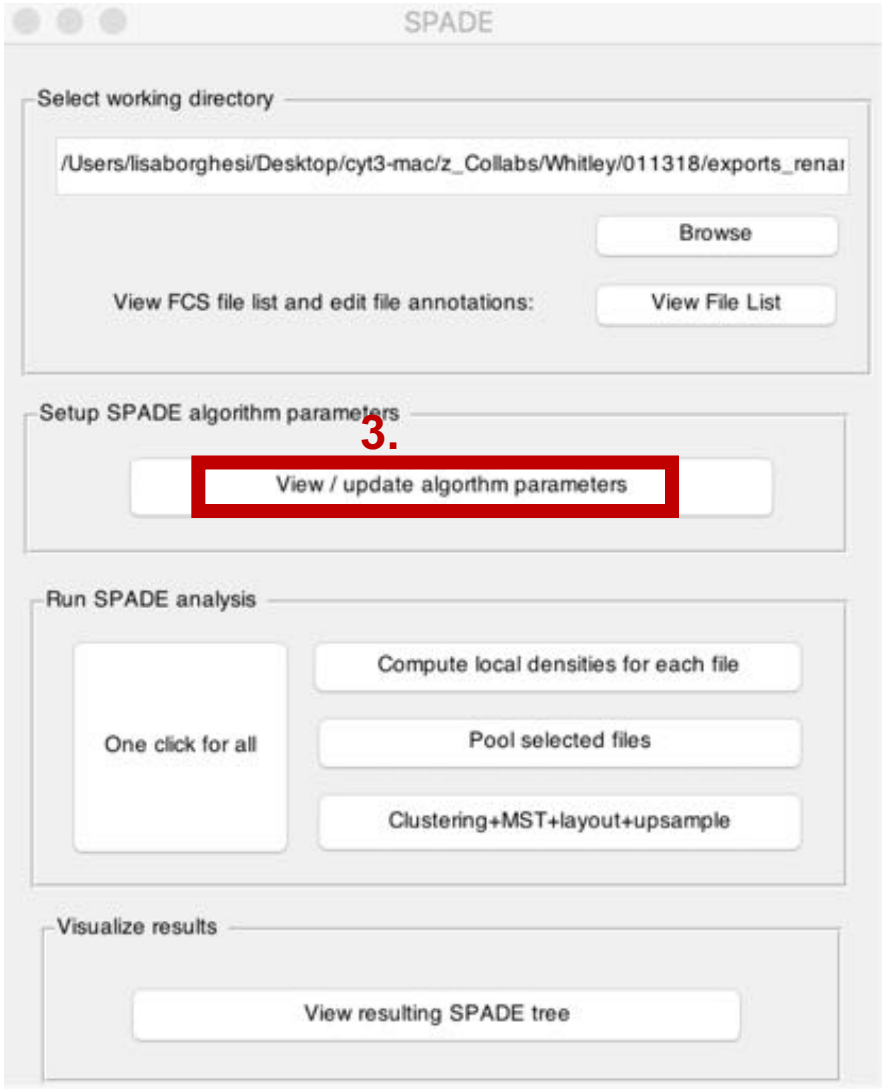
1. Click “Browse” and navigate to folder that contains gated fcs files

2. Select the folder and click “Close”

SPADE

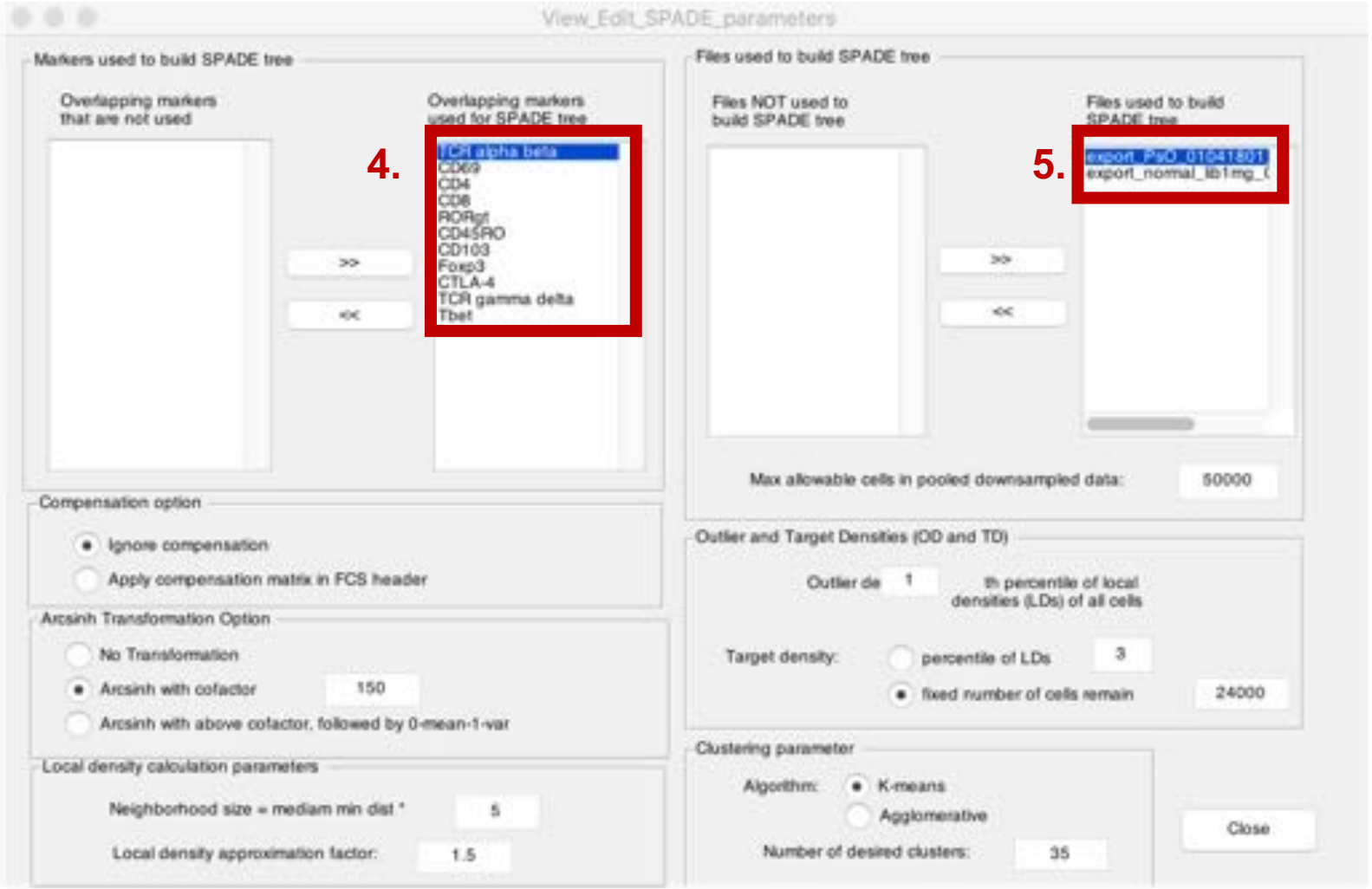


Setup SPADE parameters



3. Click “View/update algorithm parameters”

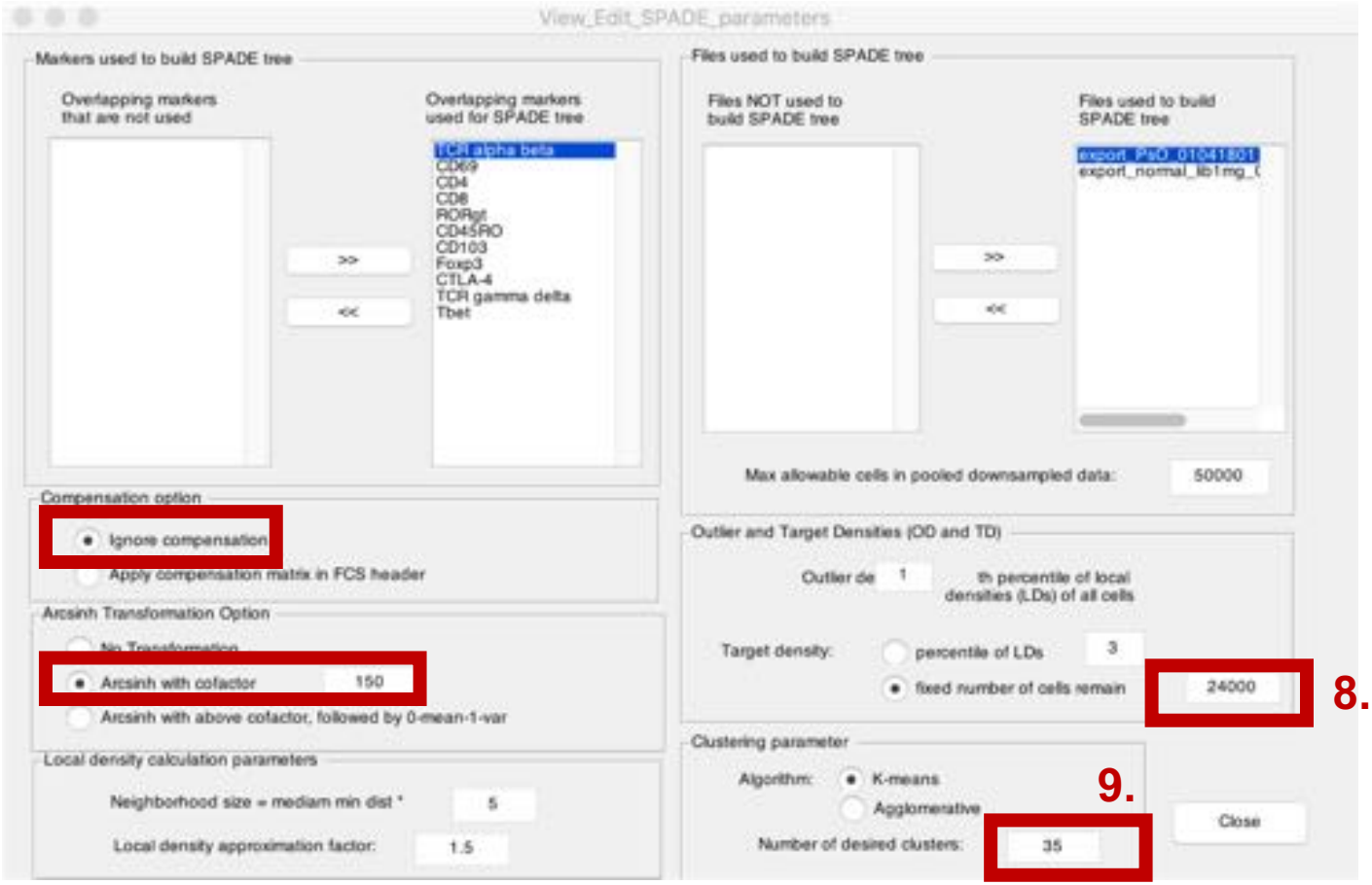
Setup SPADE parameters



4. Select markers for building SPADE tree (move left >> right)

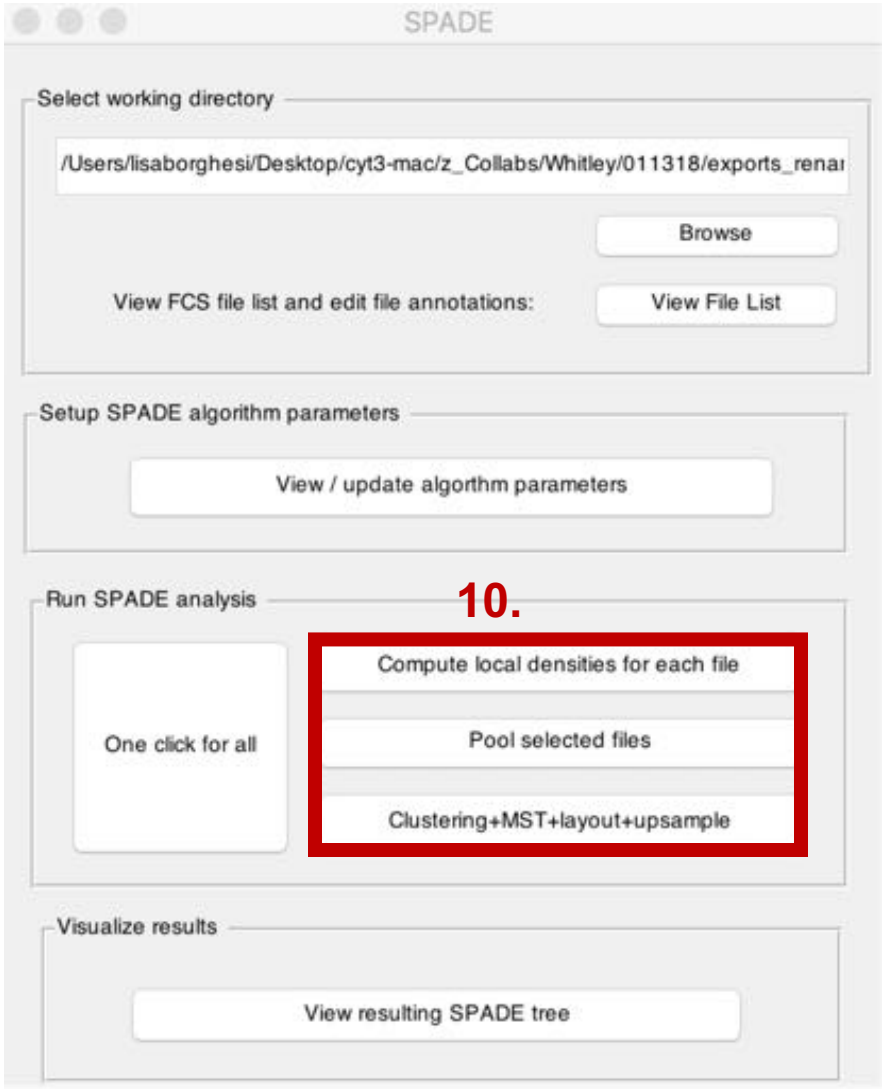
5. Select files to use for building SPADE tree (ditto)

Setup SPADE parameters



6. Select ”Ignore compensation” since we are using compensated data from FlowJo
7. Arcsinh transform, cofactor 150
8. Assign target density such that a fixed number of cells survive the downsampling process
9. Set desired number of clusters

Run SPADE analysis



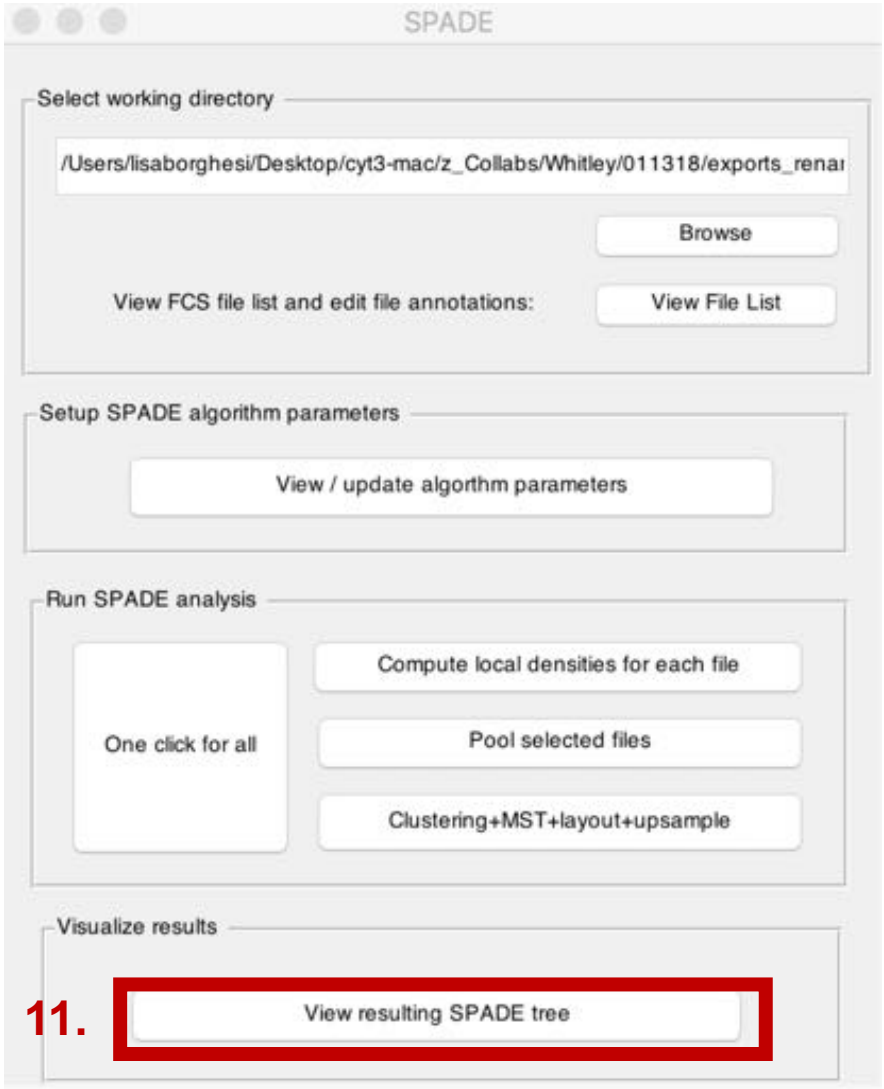
10. Today we'll sequentially walk through each step in the algorithm.

a) Compute local densities for each file
→ Fast. Feedback is "100% Done"

b) Pool selected files
→ Takes a bit longer. Feedback is "Done!"

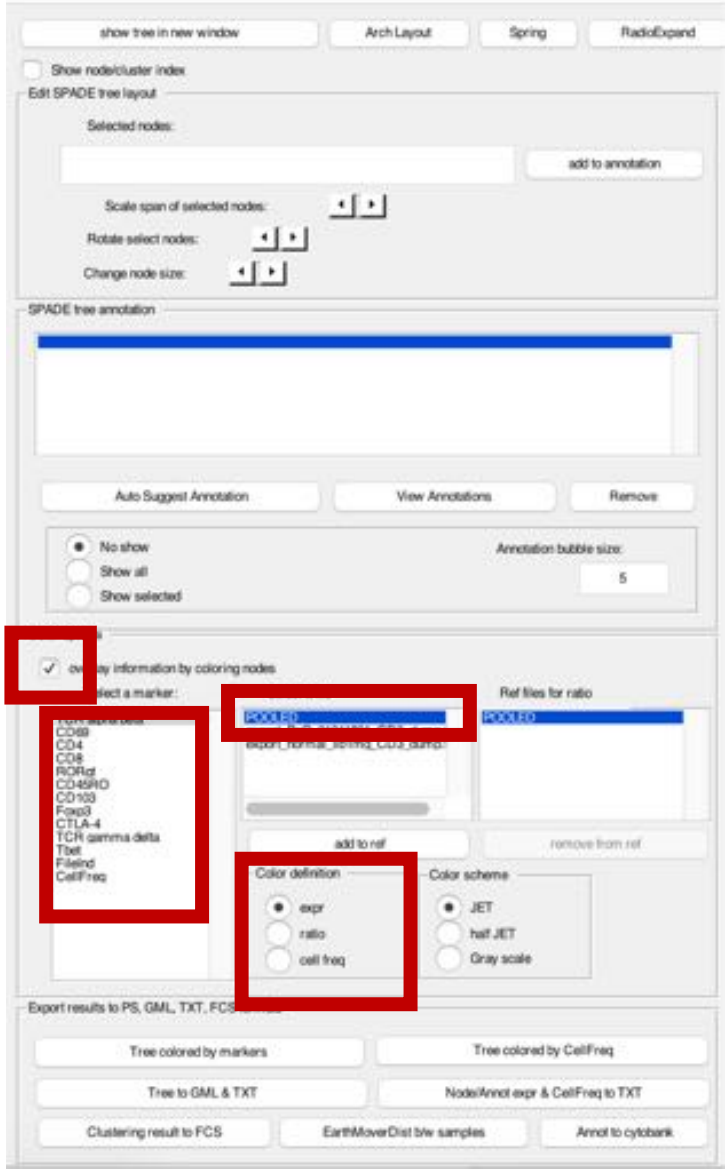
c) Clustering
→ Fast. Feedback is "Done"

Run SPADE analysis

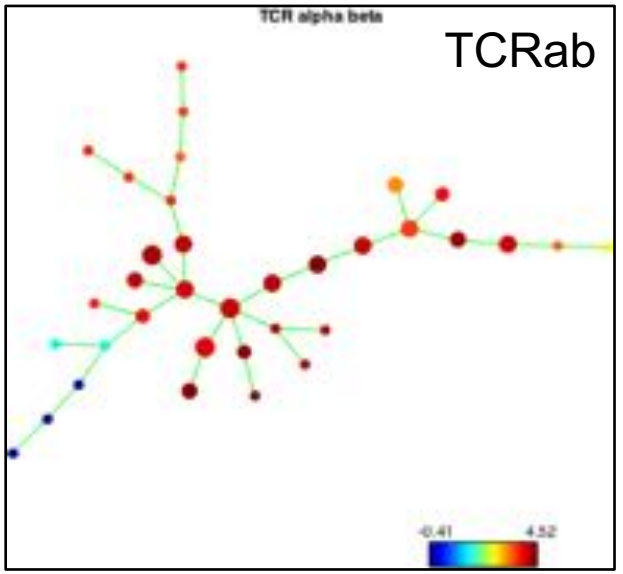


11. View resulting SPADE tree

Color tree by marker

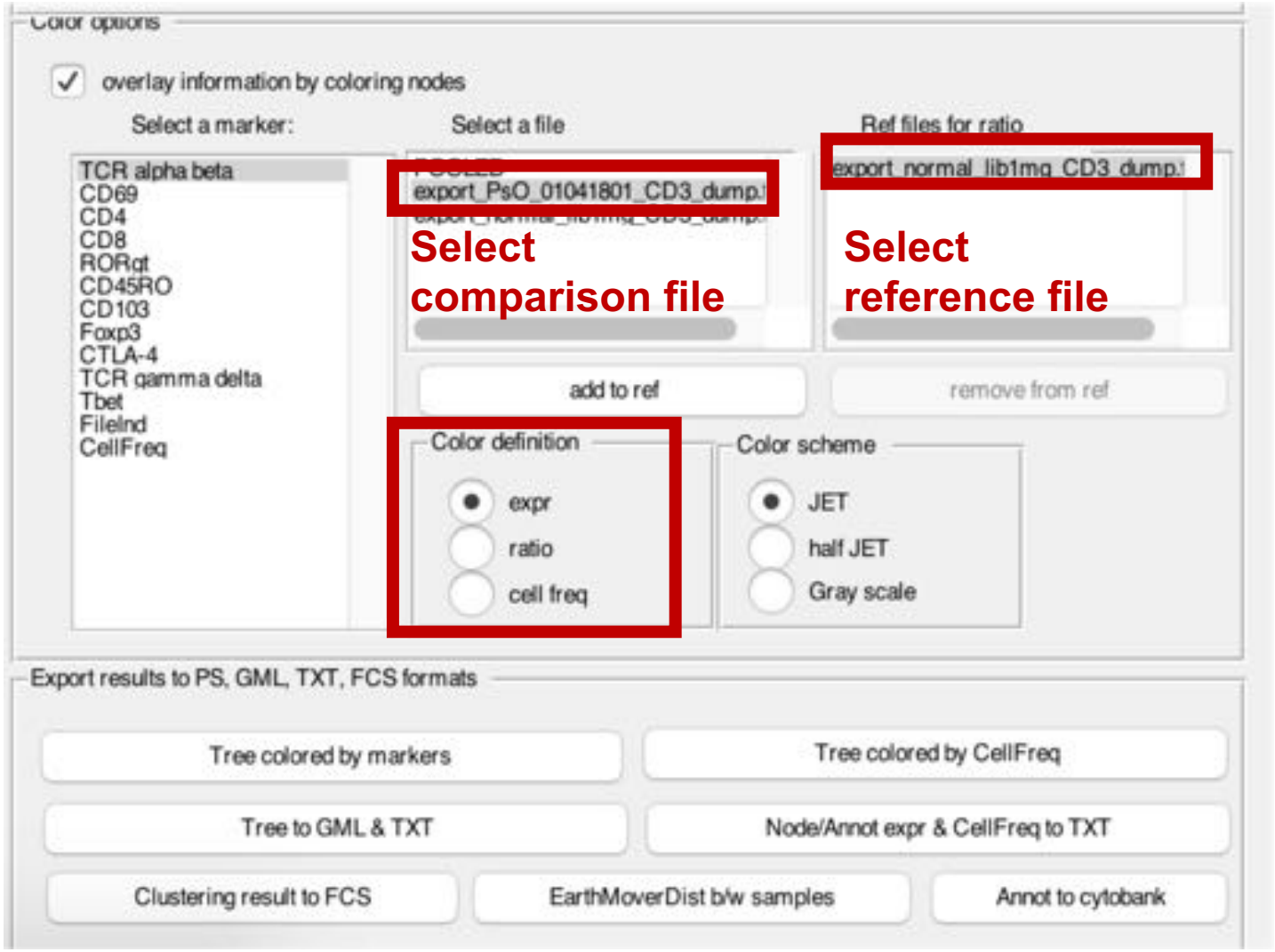


→ Circle size proportional to pop frequency



- Check the overlay box
- Select marker (e.g., TCRab)
- Select file (e.g., pooled)
- Select “expr” = median fluor intensity of cells in the cluster

Compare samples



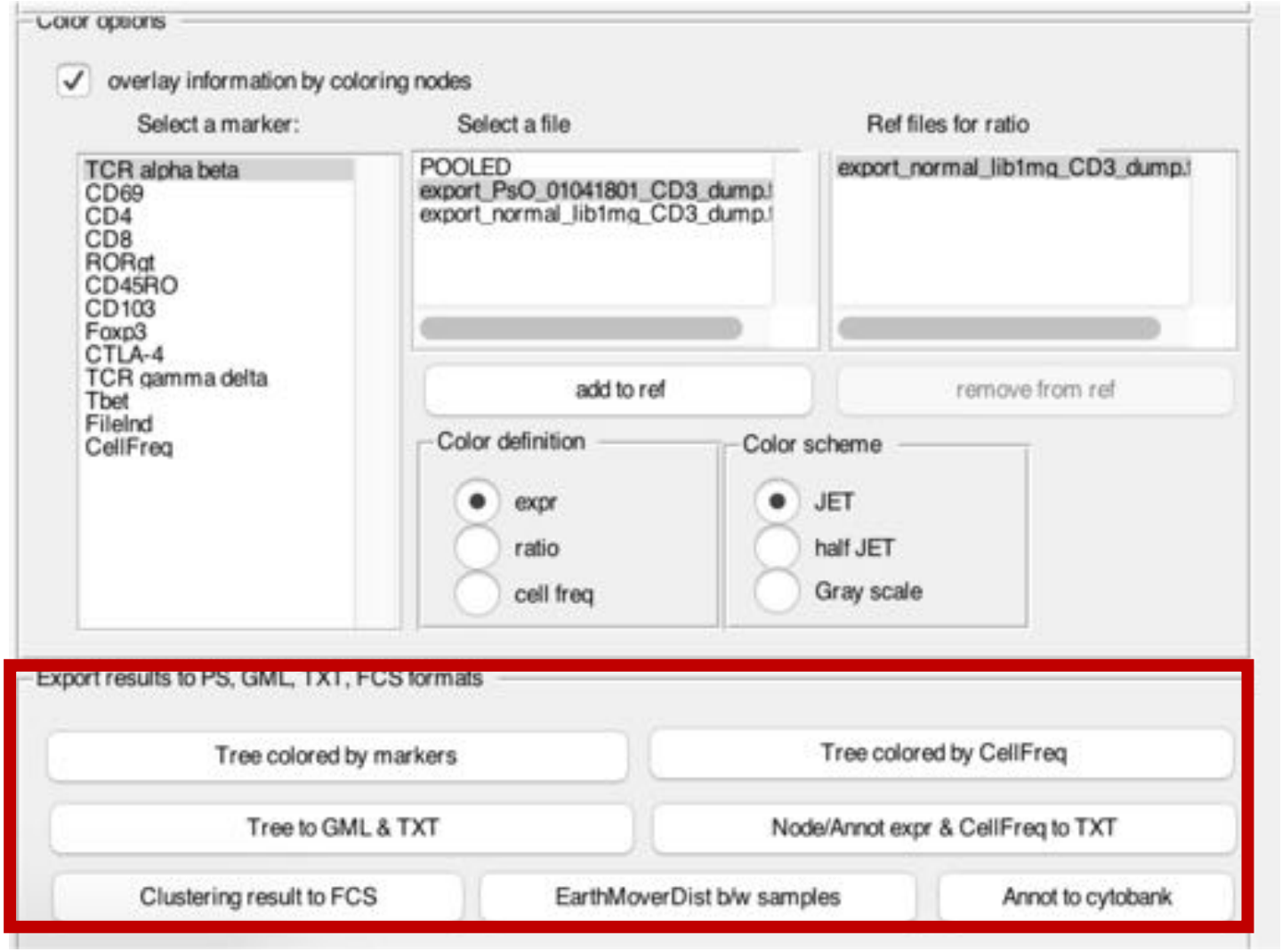
Or compare samples:

Expr = median fluor intensity of cells in that cluster

Ratio = the difference b/w two samples

Cell freq = freq. of cells in cluster

Compare samples

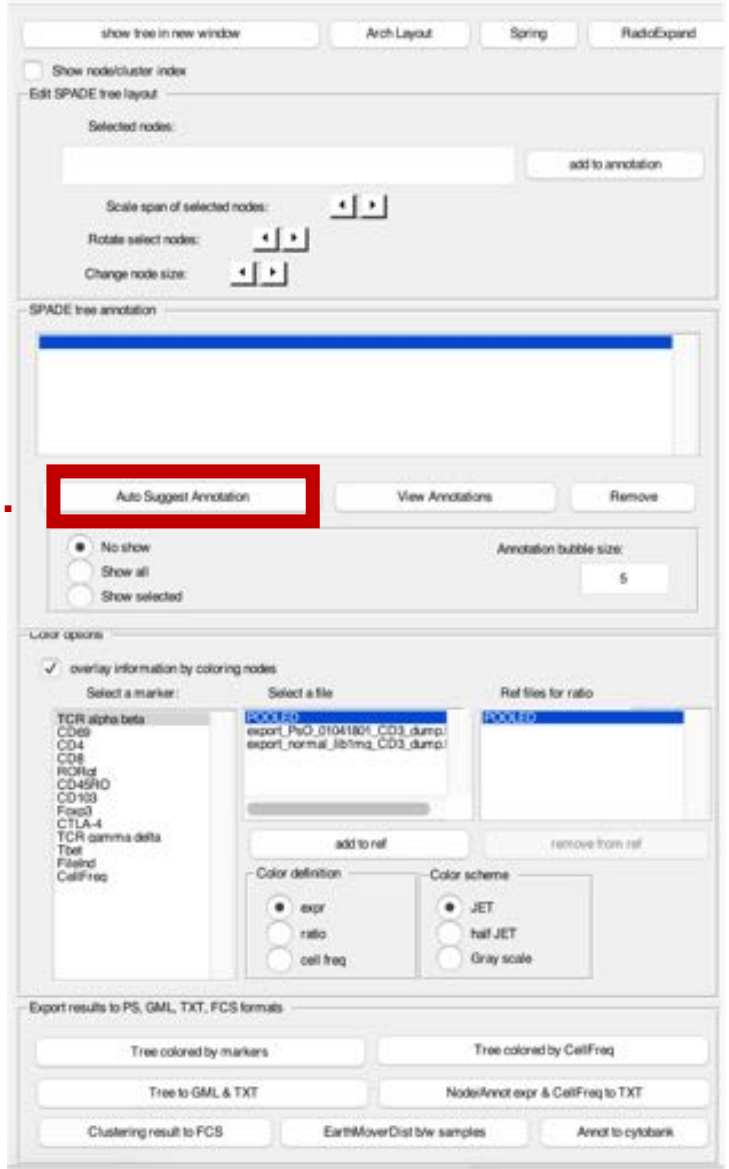


Export SPADE trees or files

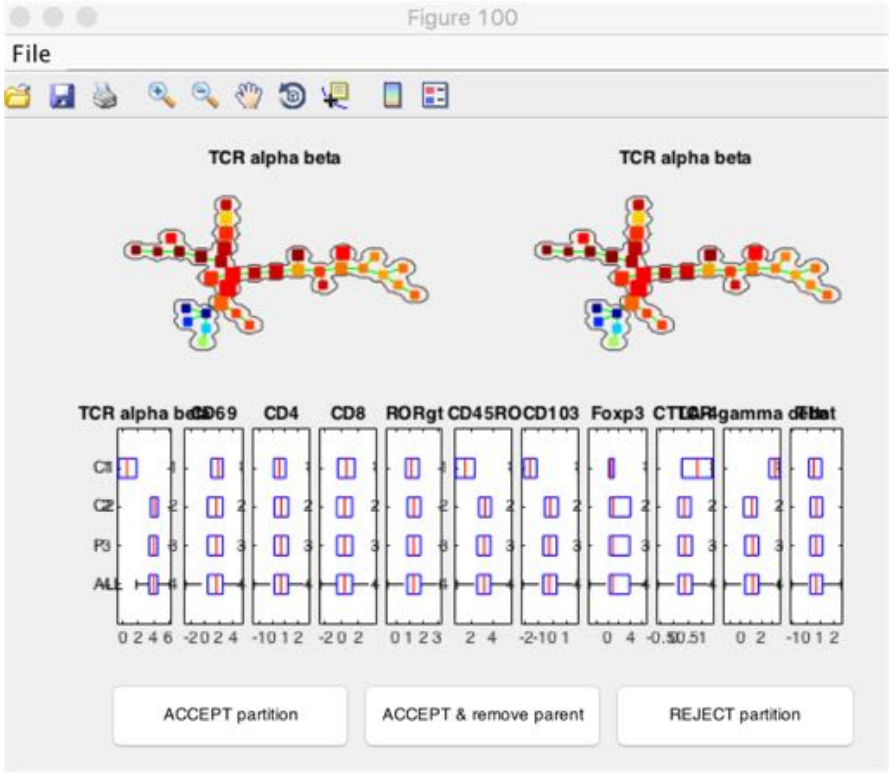
Annotate SPADE tree (unbiased)

1. Select “Auto Suggestion Annotation”
2. In popup window, accept/reject proffered partition

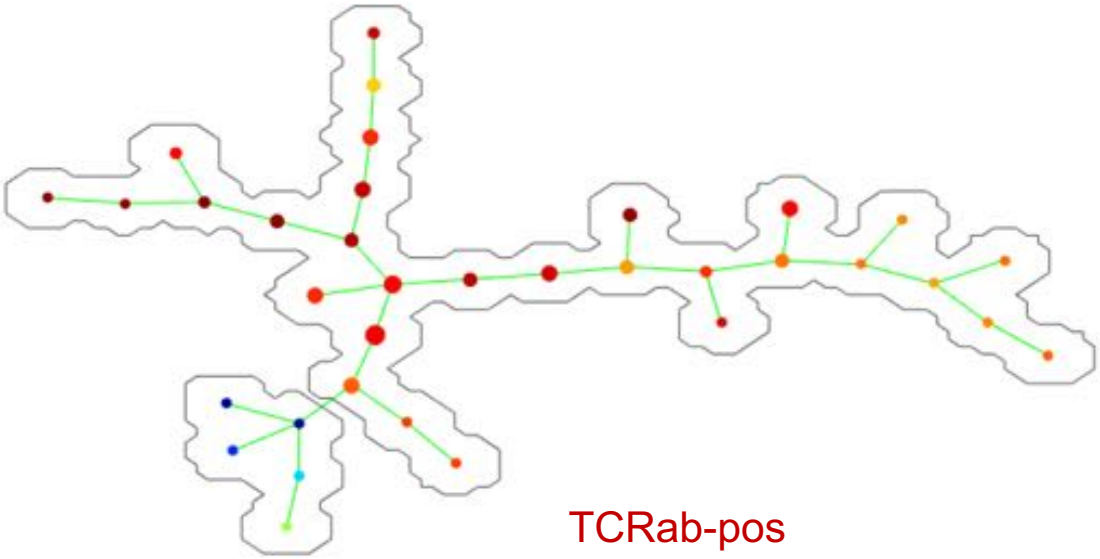
1.



2.



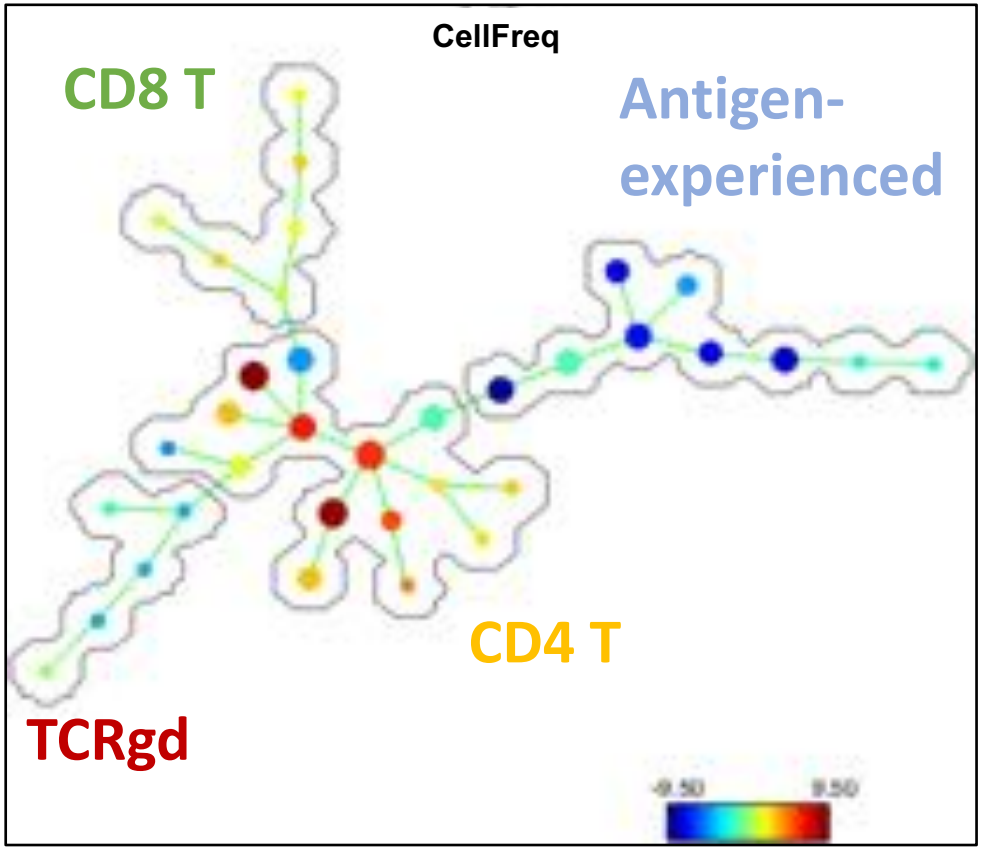
Annotations (unbiased)



TCRab-pos

TCRab-neg

Changes in population frequency PsO_Stelara relative to Normal



Outline

viSNE, PhenoGraph, SPADE

how the algorithms work

maximizing comparable results across experiments

Overview of Analysis Workflow

FJ → export populations to cluster → algorithm

Go for it!

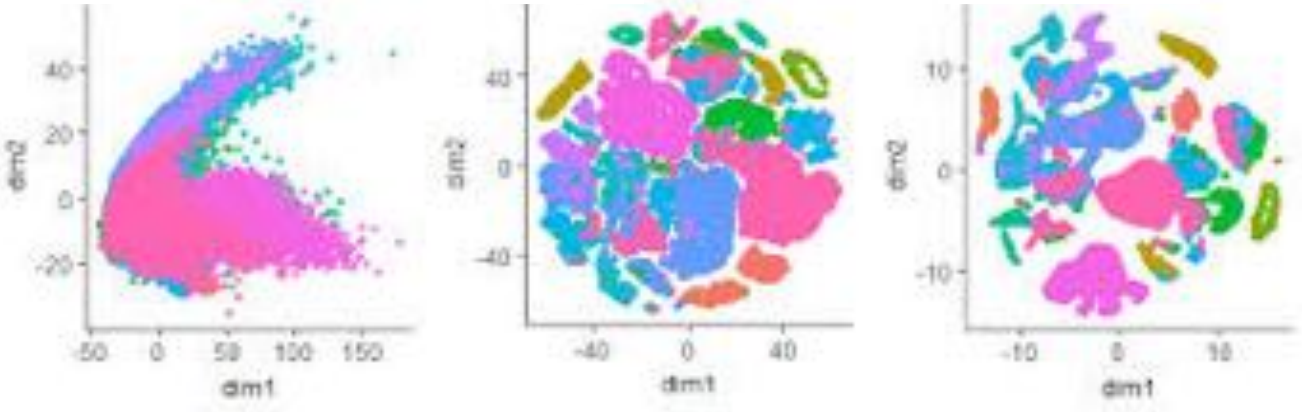
Cyt (viSNE, PhenoGraph) then SPADE

UMAP*

Uniform Manifold Approximation and Projection for Dimension Reduction

https://arxiv.org/abs/1802.03426

PCA **tSNE** **UMAP**



PCA – preserves largest pairwise difference, loss of local structure

tSNE – dimensionality reduction algorithm that preserves local and global structure but NOT cluster distance

UMAP – dimensionality reduction algorithm that preserves local and global structure, including cluster distance

*for MatLab savvy users who don't need a GUI; available in MathWorks as Add On

References

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.

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**

Acknowledgements

Unified Flow Core

Dewayne Falkner
Director of Operations

Aarika Yates MacIntyre
Senior Technologist

Ailing Liu
Applications Specialist

Center for Research Computing

Kim Wong

MBIS HSLS

Ansuman Chattopadhyay
Carrie Iwema
Srilakshmi Chaparala

Unified Core, Board

Mark Shlomchik
Fadi Lakkis
Mark Gladwin
Larry Moreland
John McDyer