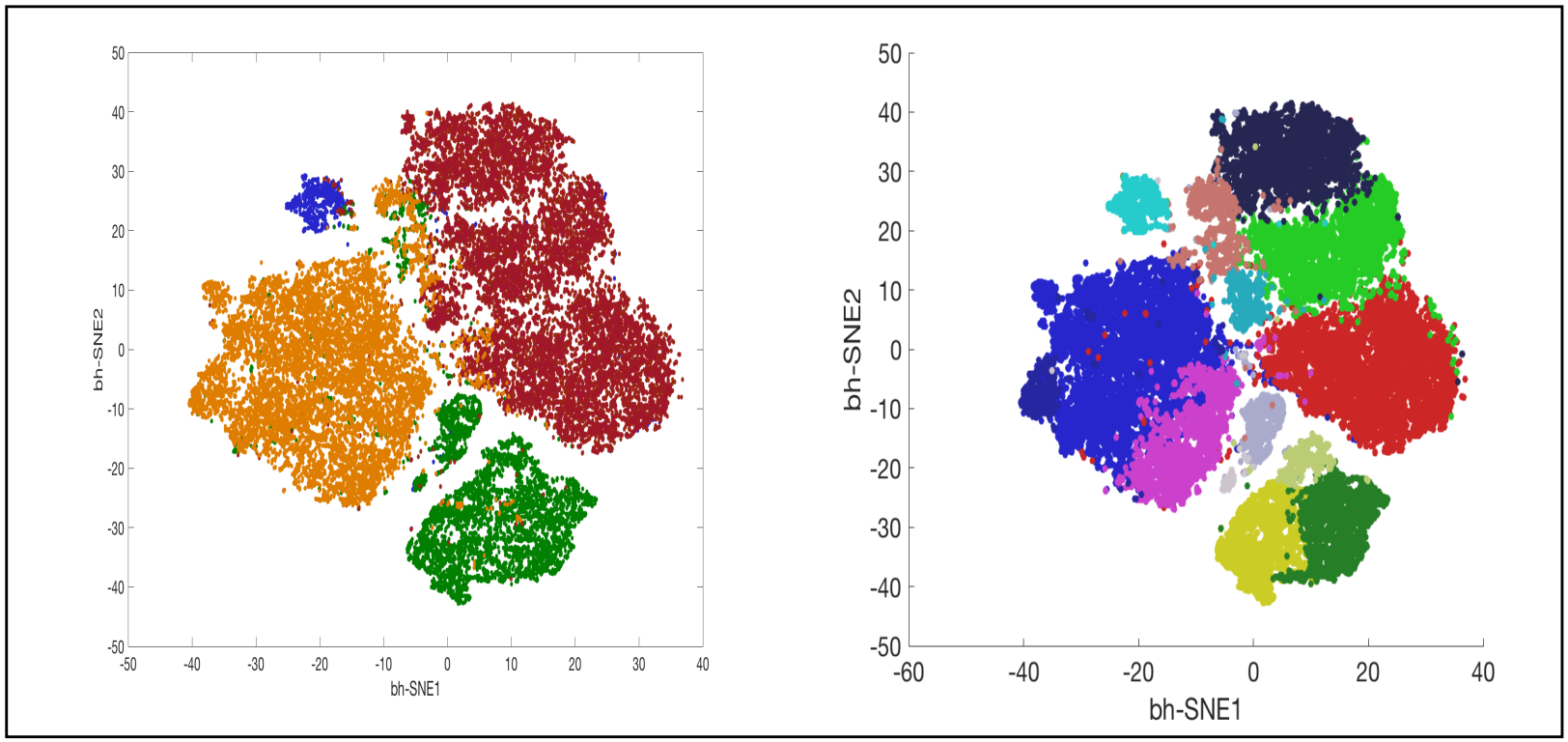
viSNE & PhenoGraph Step-By-Step

viSNE **PhenoGraph**



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Steps for viSNE analysis in Matlab/Cyt3

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

2. **Transform**, arcsinh 150

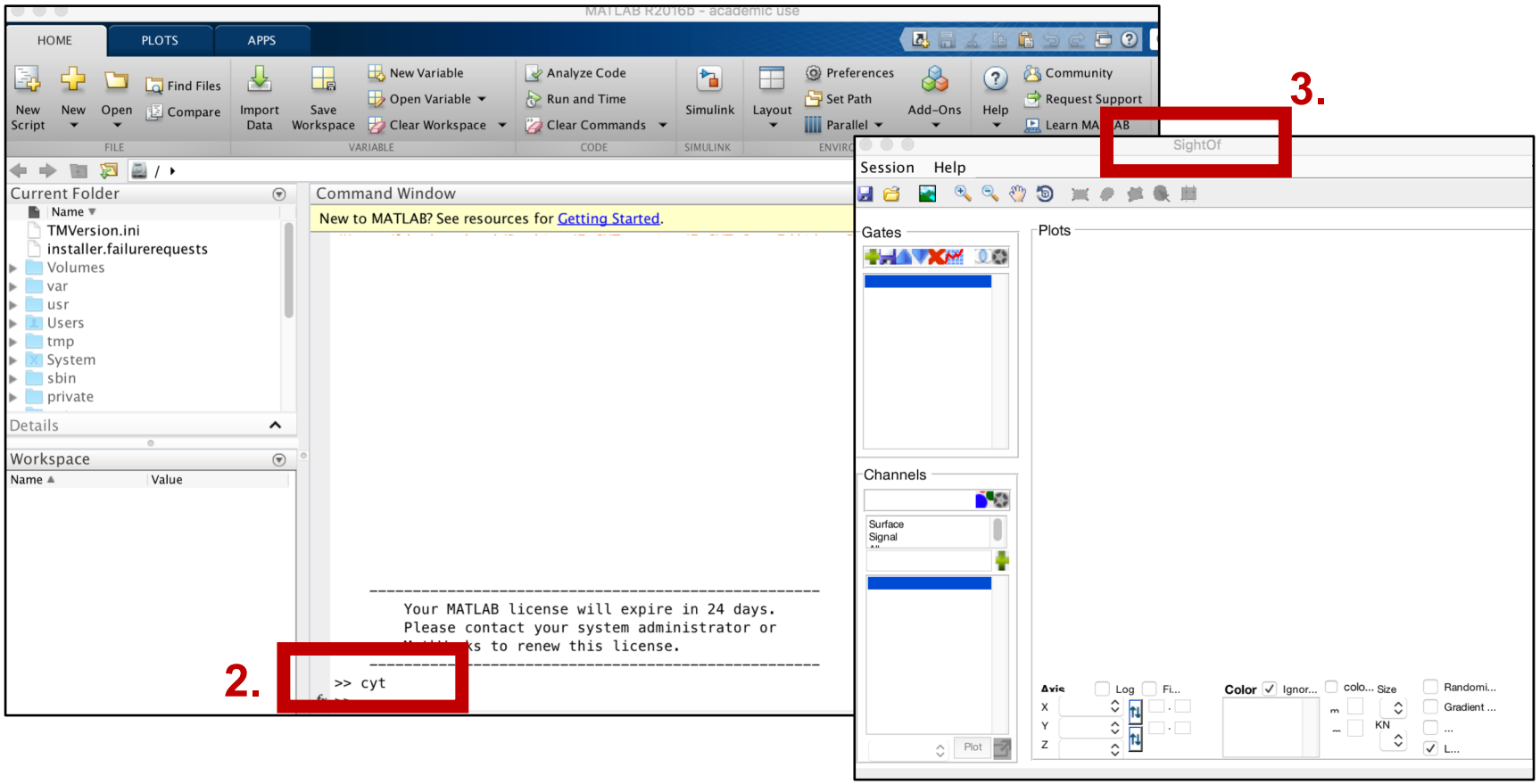
3. **Downsample** (i.e., subsample a portion of total events)
   - to reduce computational burden
   - to select a small subset of events for a quick first-pass analysis
   - to normalize events across comparative analyses

4. Invoke **bh-SNE**

Launch Matlab/Cyt3

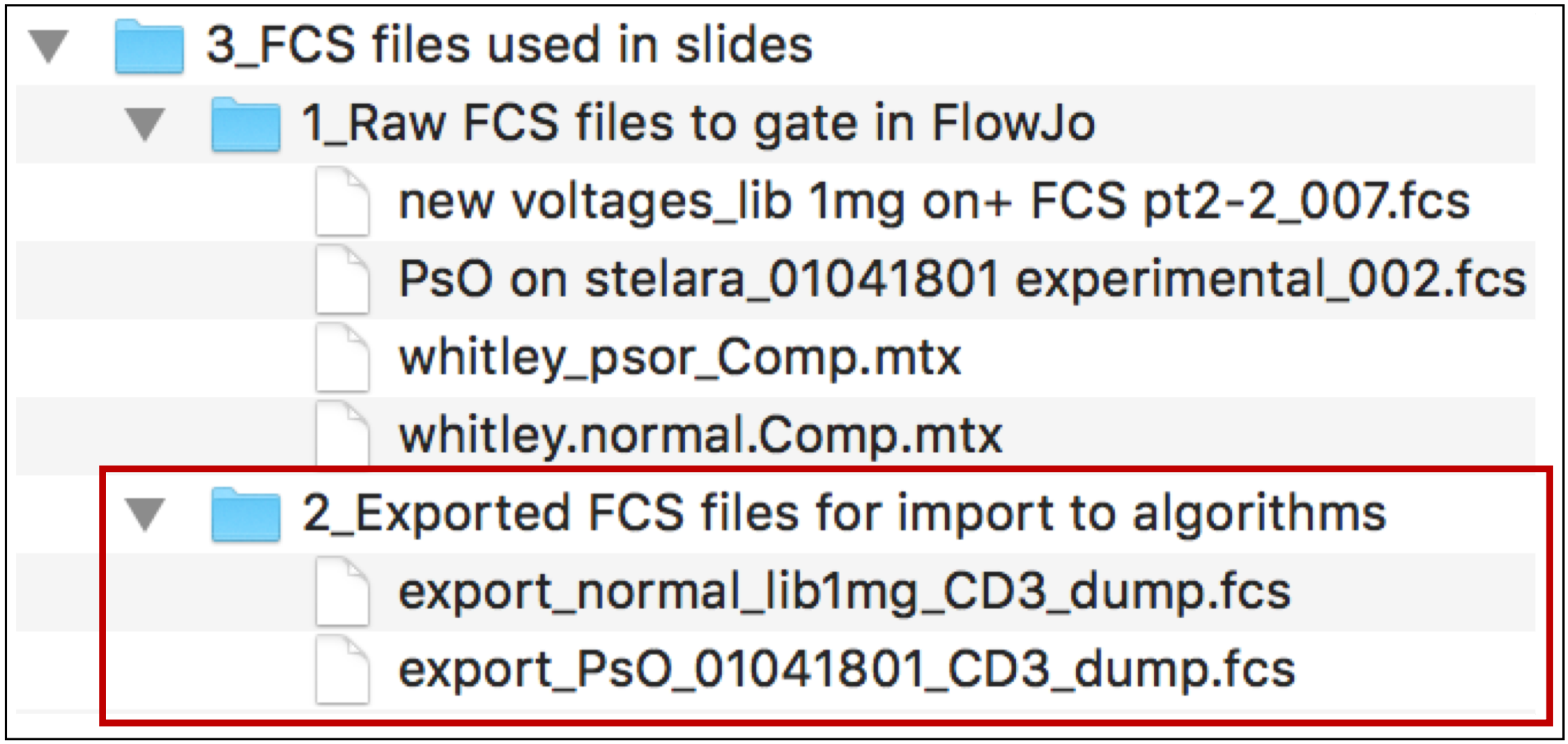
1. Launch Matlab
2. At the >> prompt type “cyt”
3. A new window should appear name ”SightOf” at the top

1.



Import pre-gated fcs files (live, singlet, gated on population to cluster) into Cyt3 “SightOf”

Use your fcs files or choose the “Exported FCS files” below.
Files are located in the same PittBox as this ppt.



Import pre-gated fcs files (live, singlet, gated on population to cluster) into Cyt3 “SightOf”

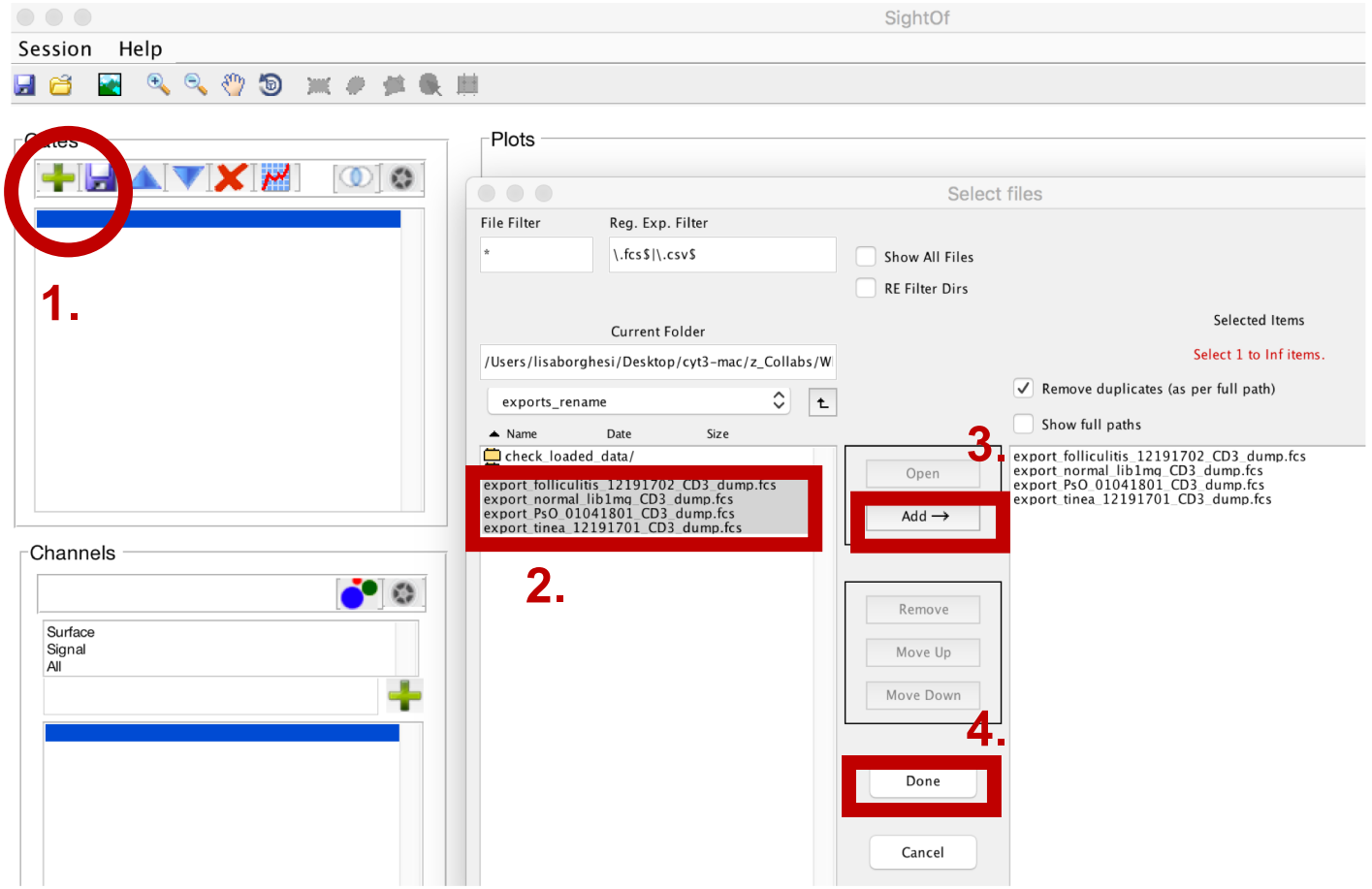
Cyt3

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

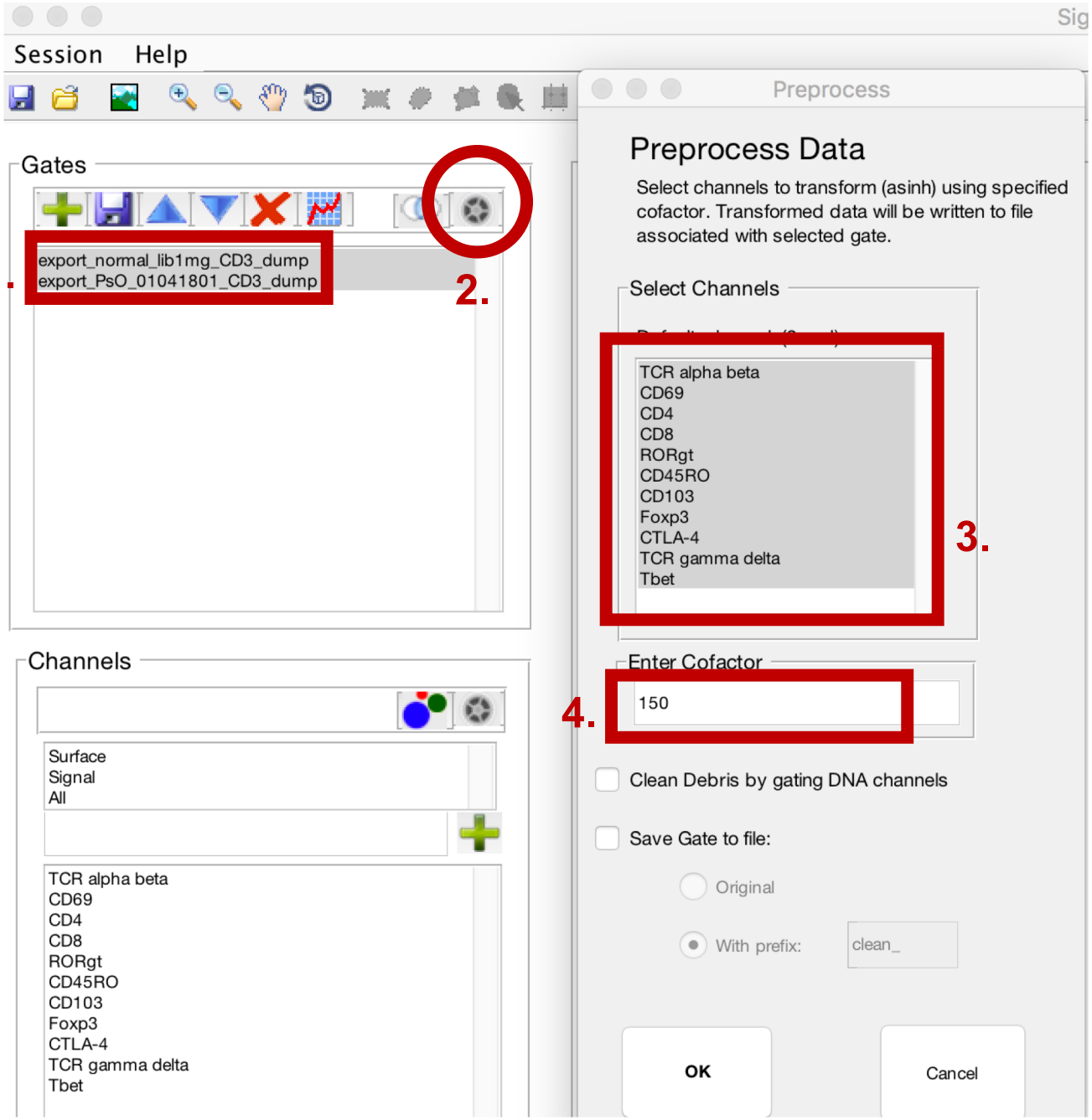
2. Navigate folder and select files

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

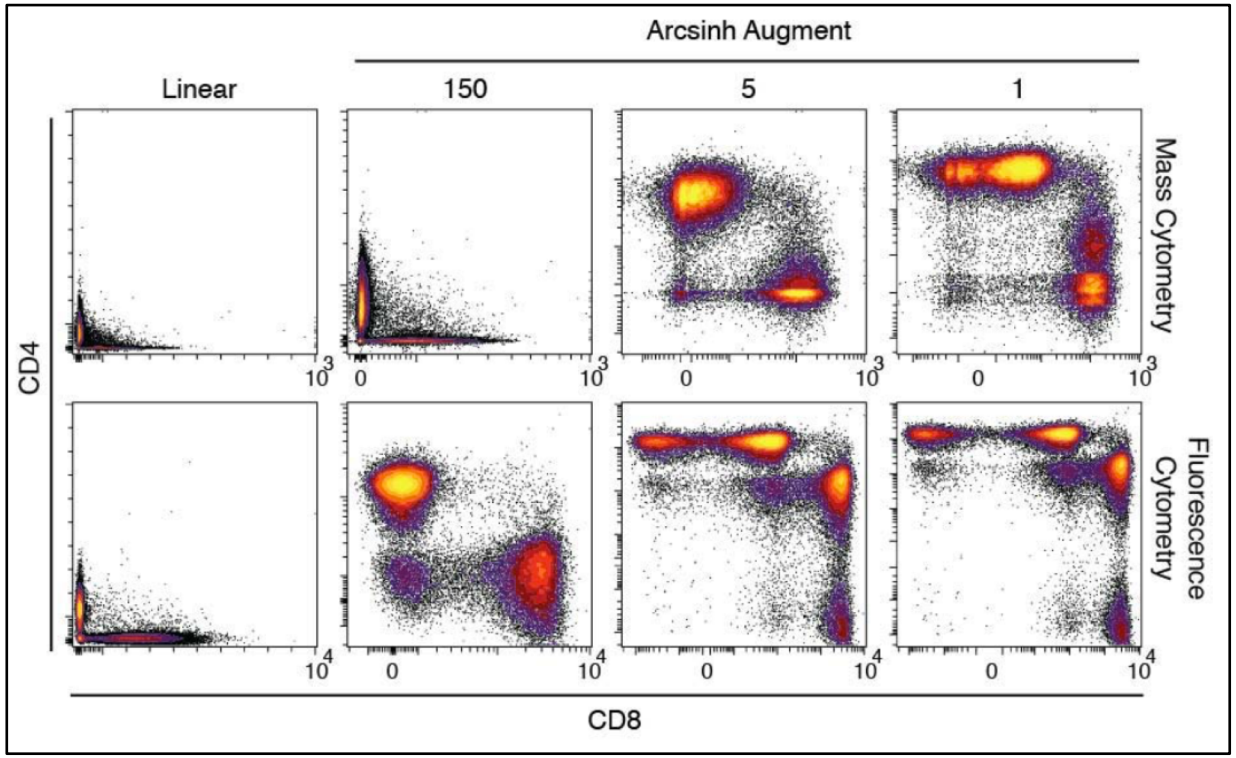
4. Enter Cofactor 150 for flow cytometry (or 5 for mass cytometry)

5. Click “OK”

Why transformation value of 150 for fcs files?

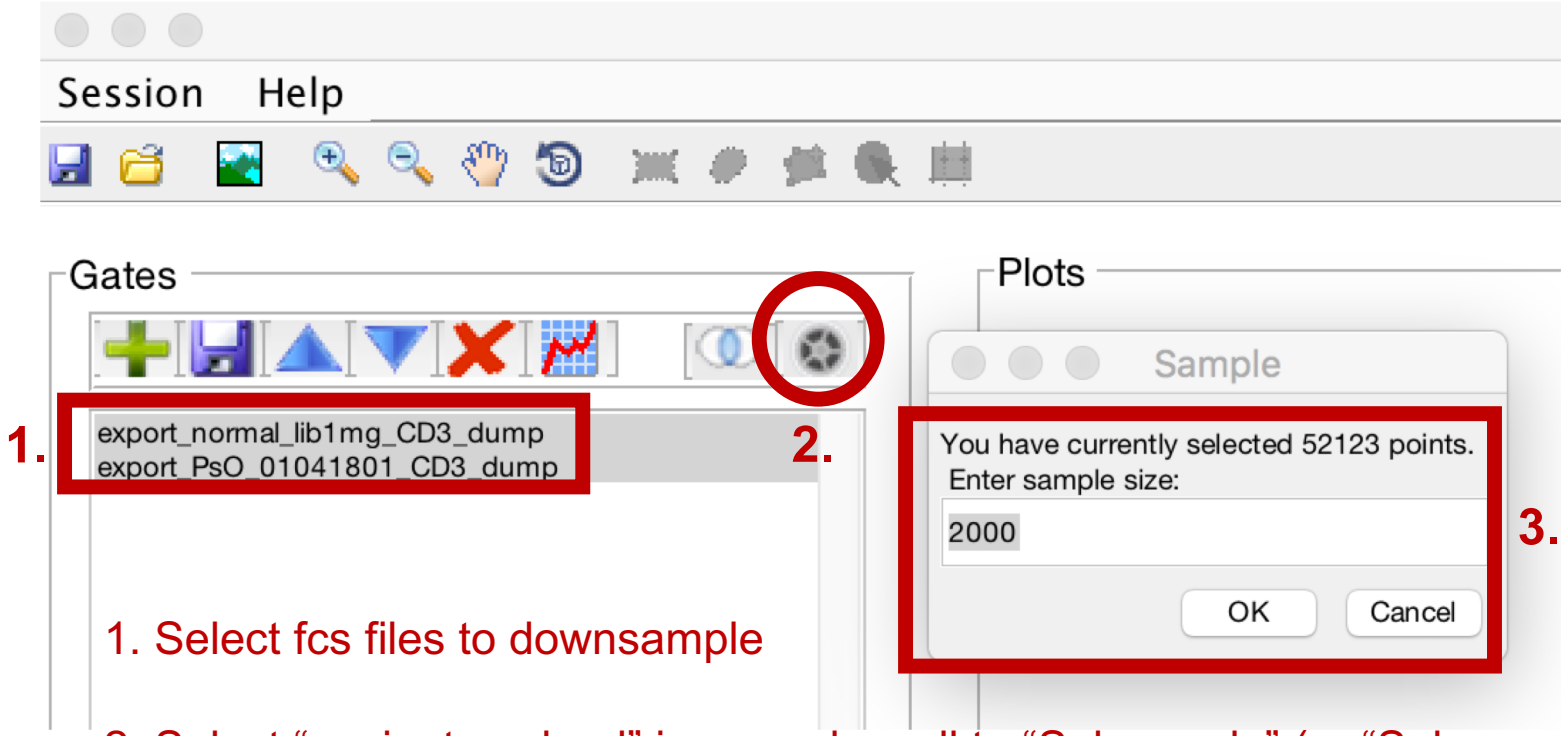
- fcs data can have negative numbers due to compensation correction and instrument baseline correction. Algorithms can't handle negative numbers.

- 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

Pre-processing steps for viSNE analysis: Downsample



1. Select fcs files to downsample

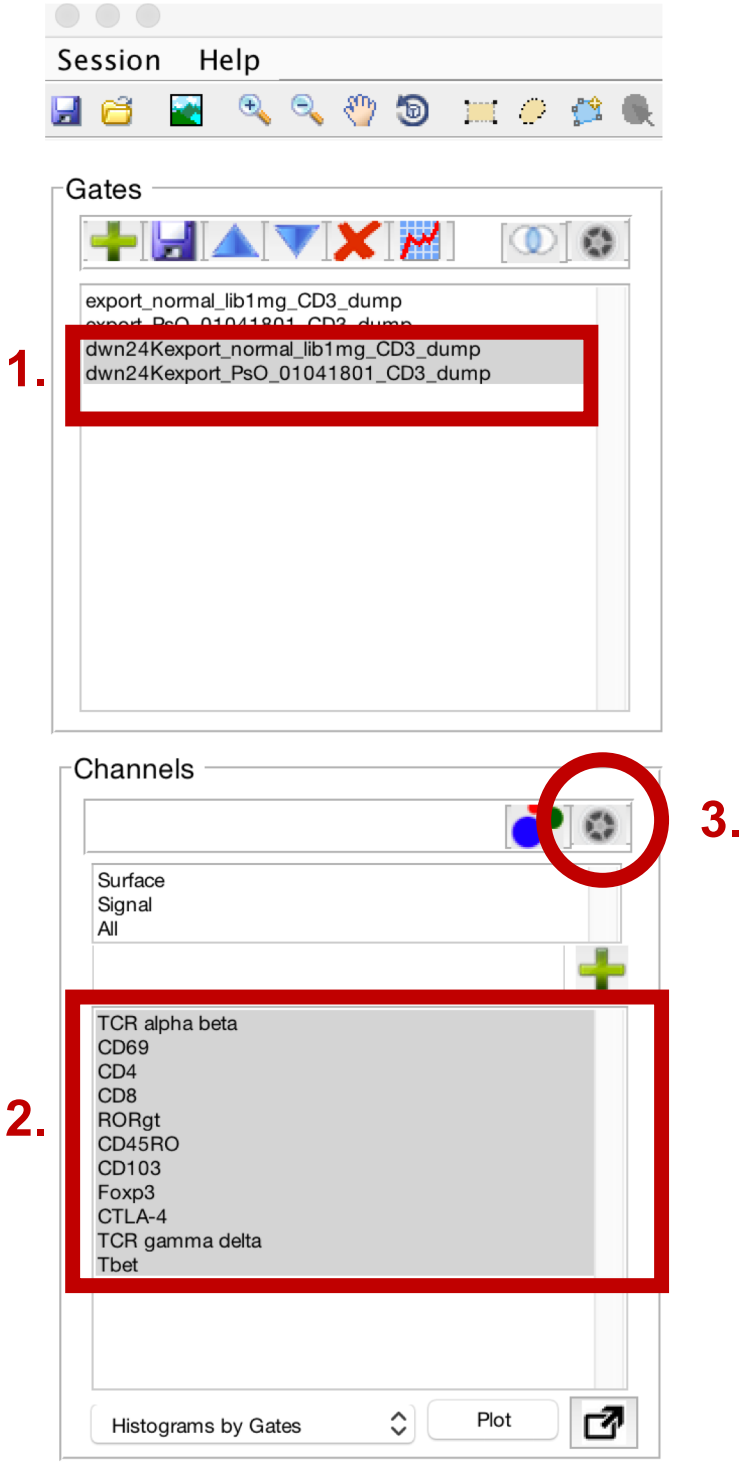
2. Select “navigator wheel” icon, and scroll to “Subsample” (or “Subsample Each” if you have multiple samples to simultaneously downsample)

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

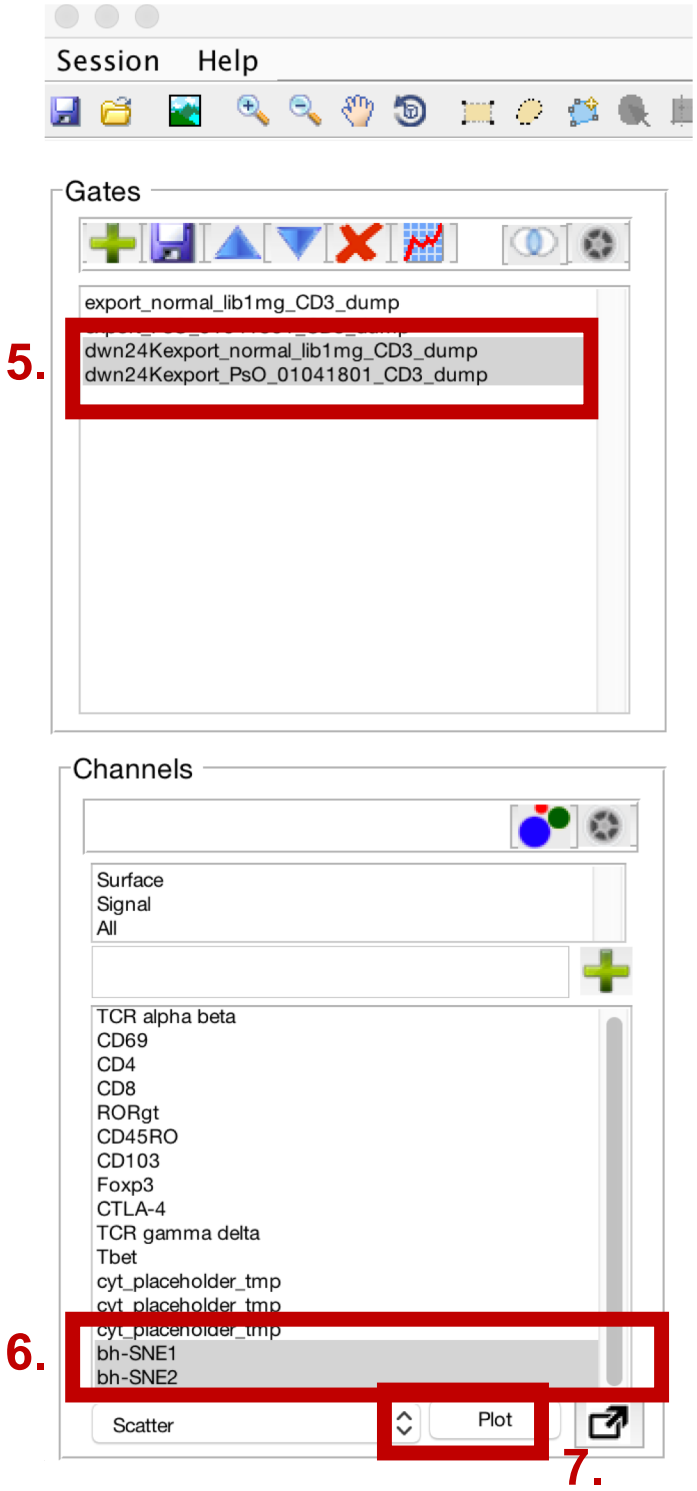
2. Select fluors to include in clustering

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

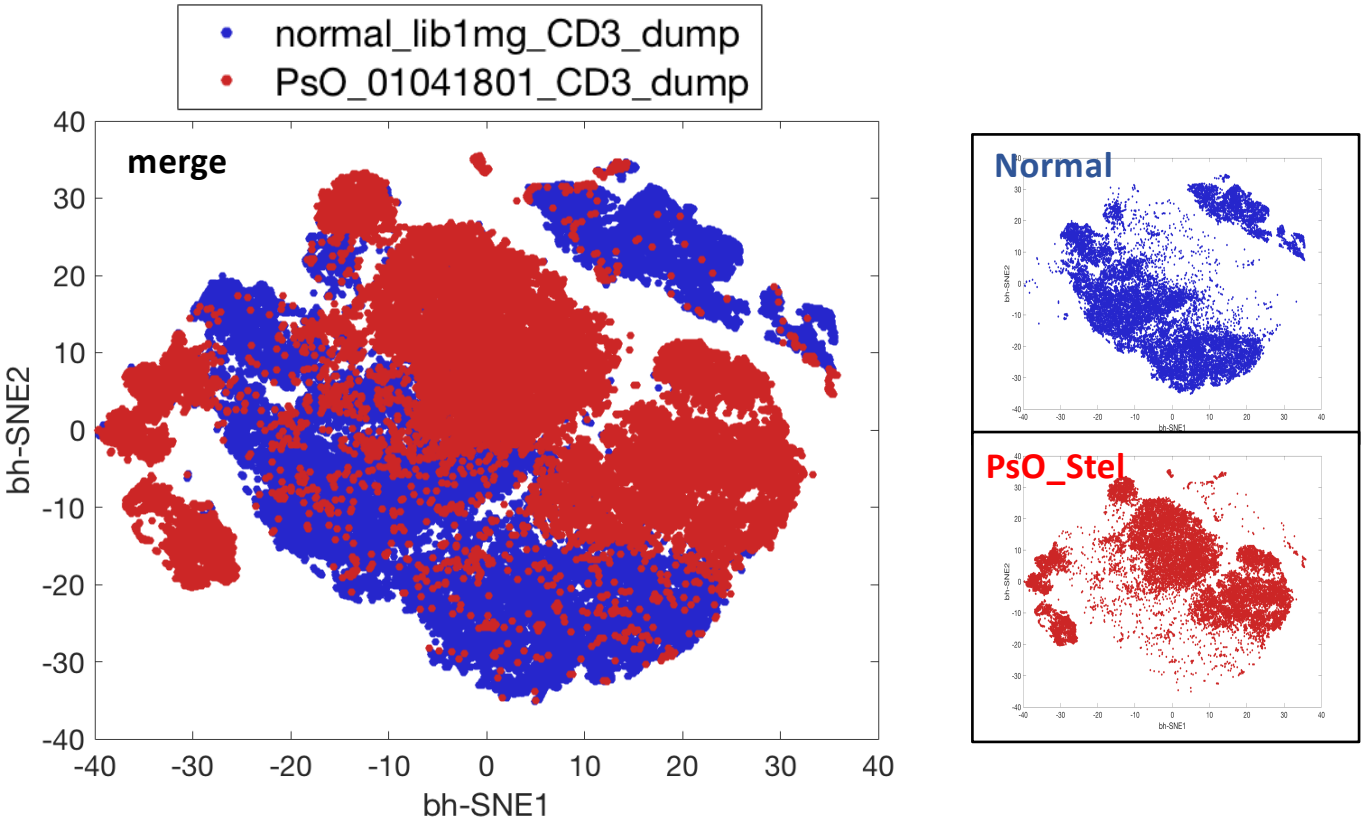


5. Make sure the files you clustered are still selected.

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

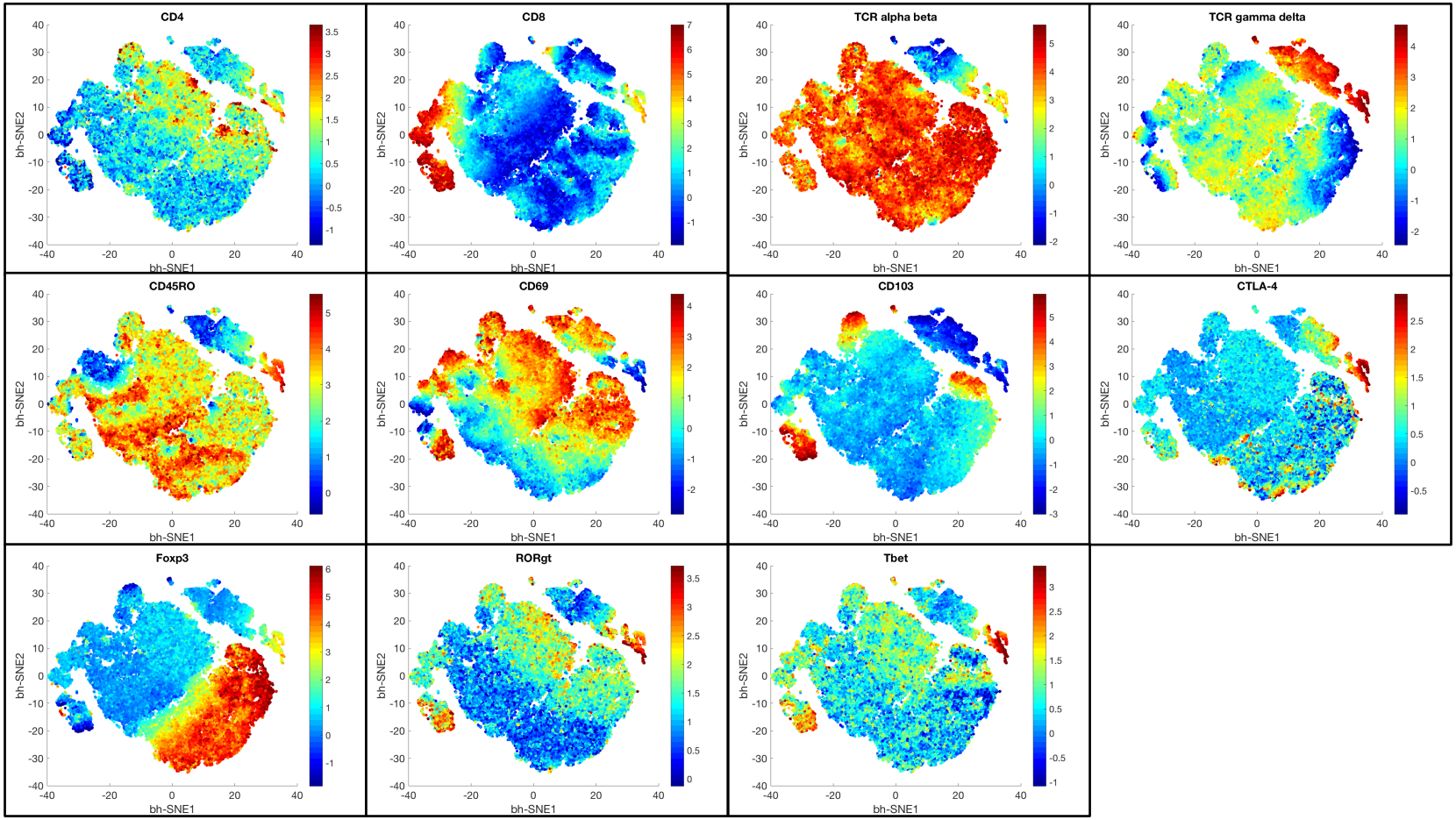
7. Select “Plot”

viSNE: normal vs PsO

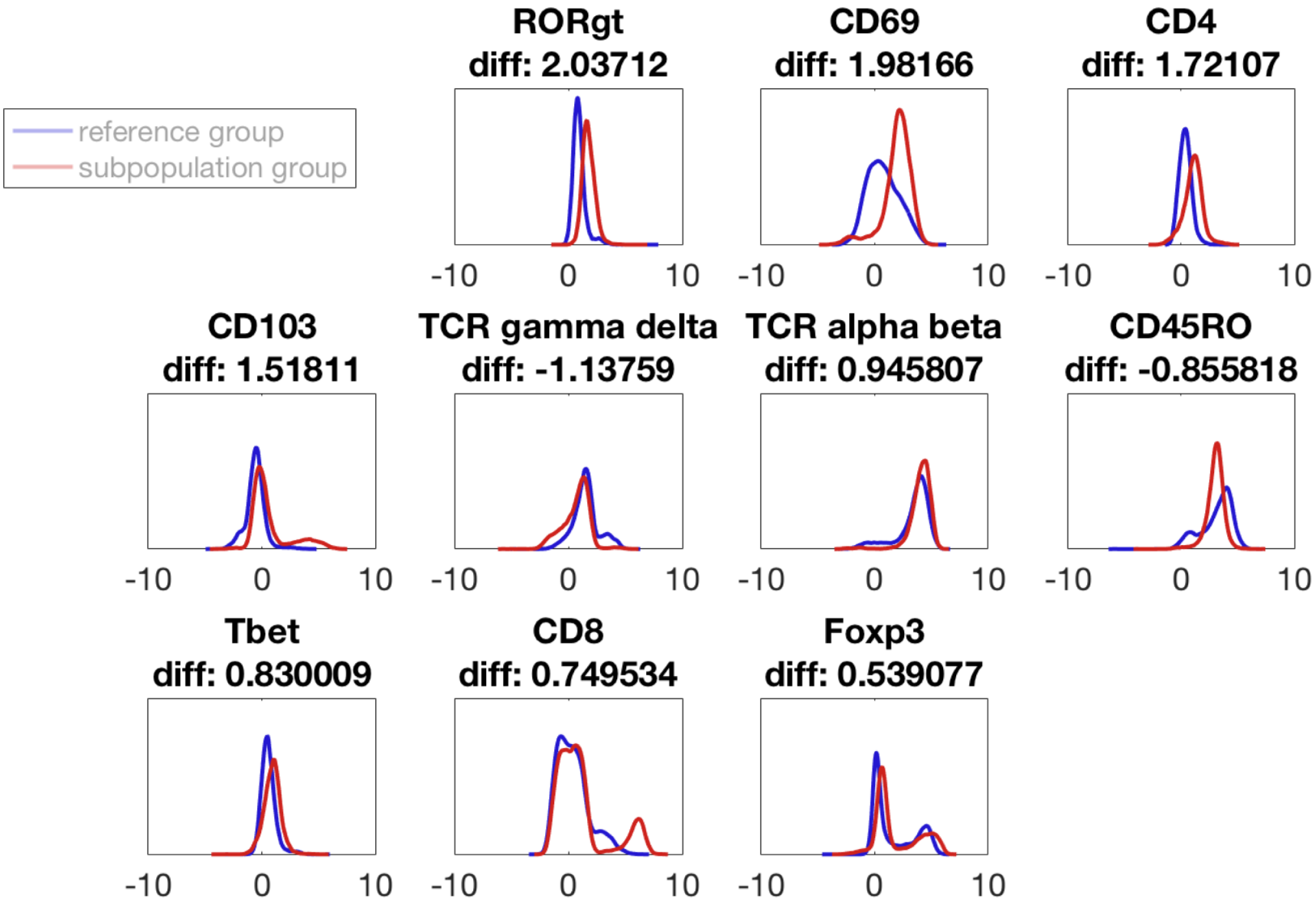


- downsample Normal, PsO_stel to 24K/ea
- cluster all markers except for pre-gated channels (CD3, dump)

Marker heatmaps

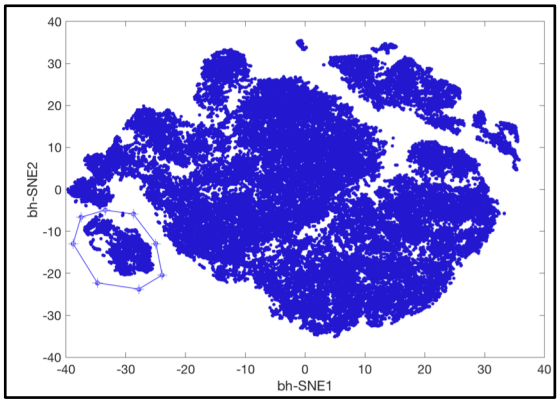


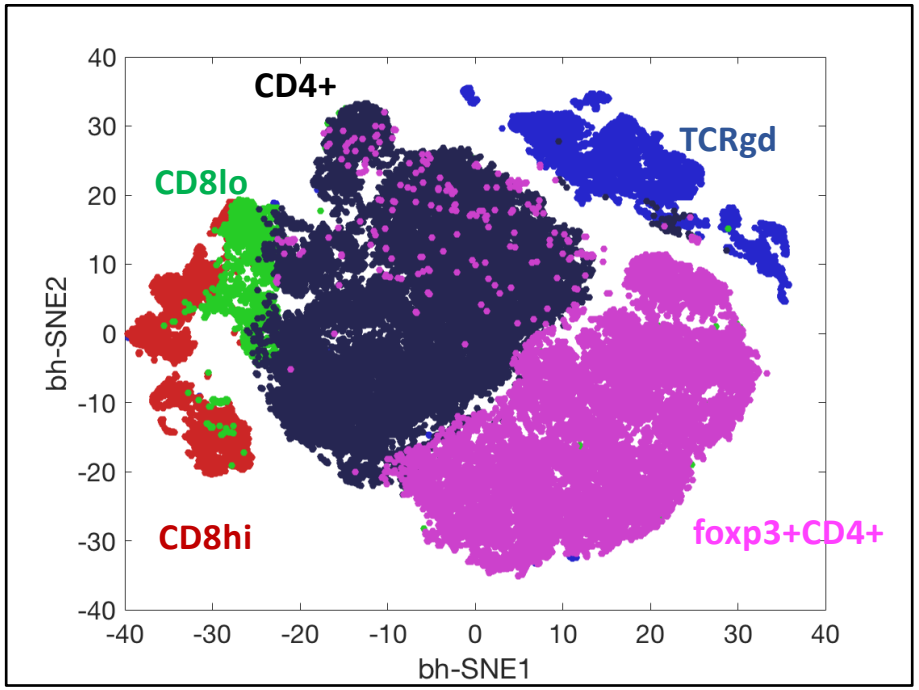
L1 statistic – difference between marker distributions



Manual gating overlays

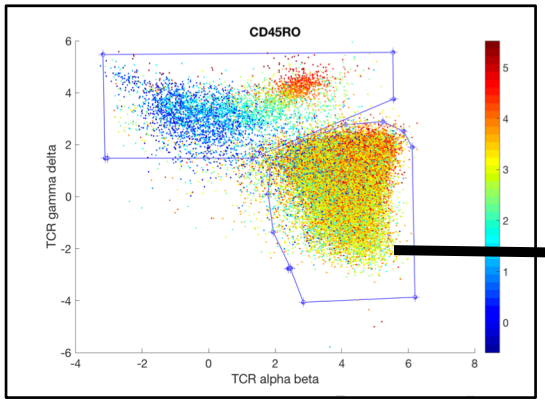
Gate on tSNE clusters





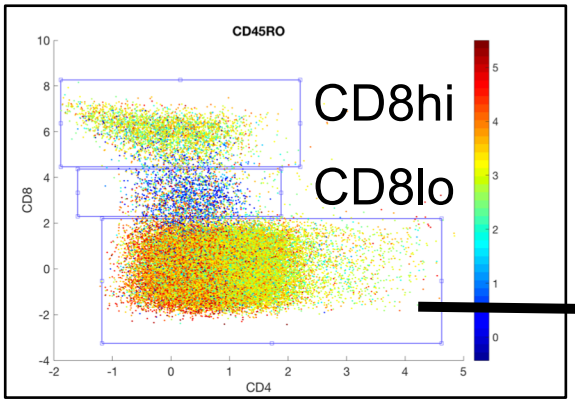
Gate on fluors

TCRgd



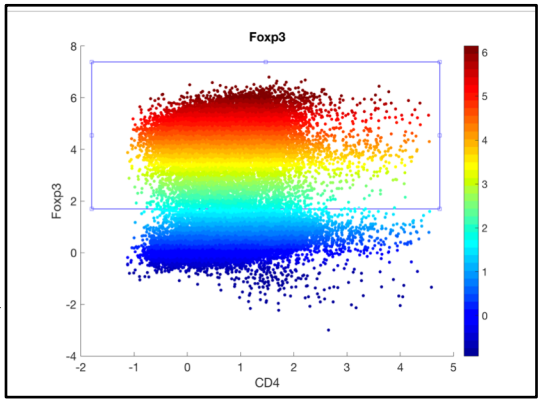
TCRab

CD8



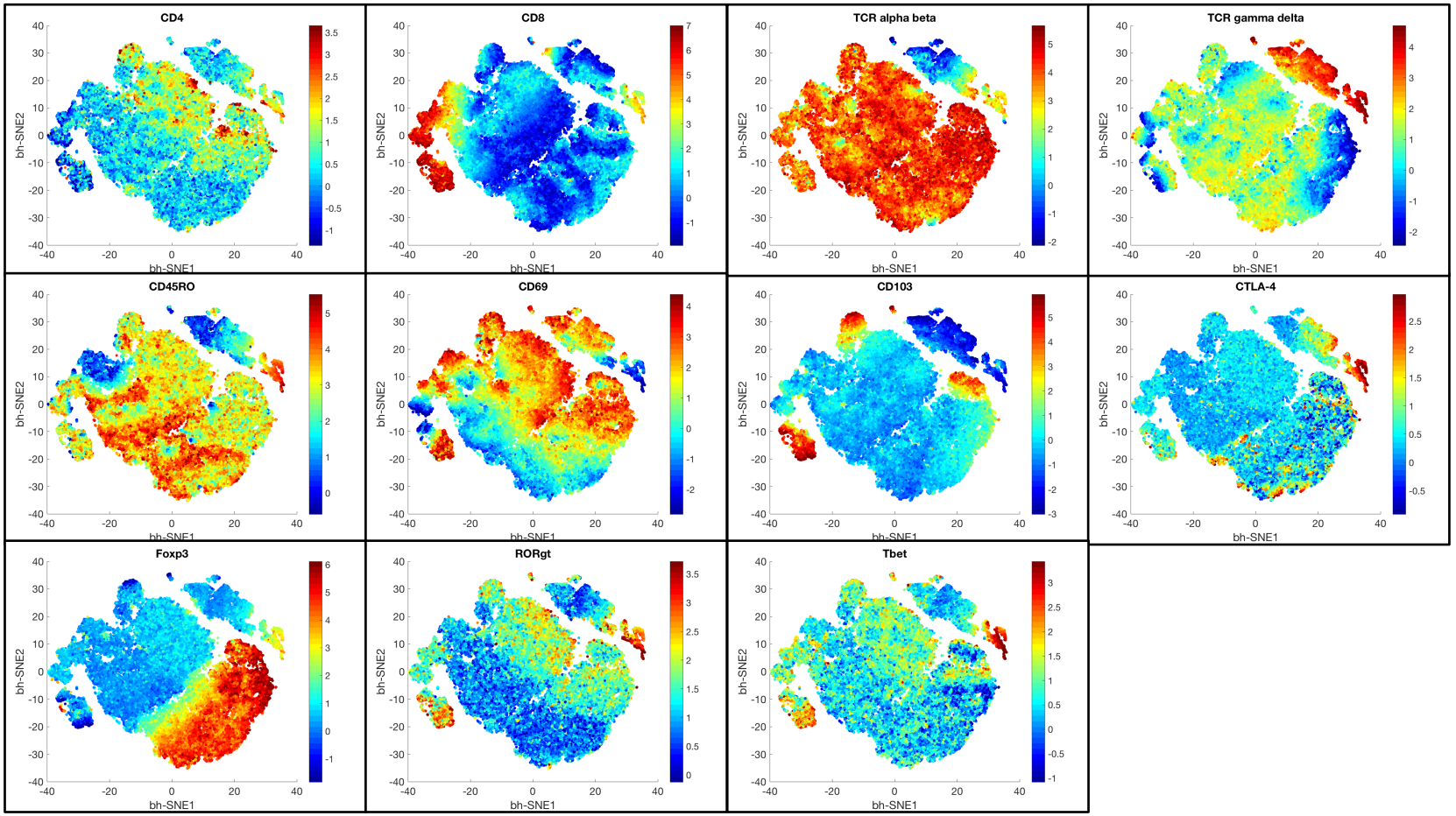
CD4

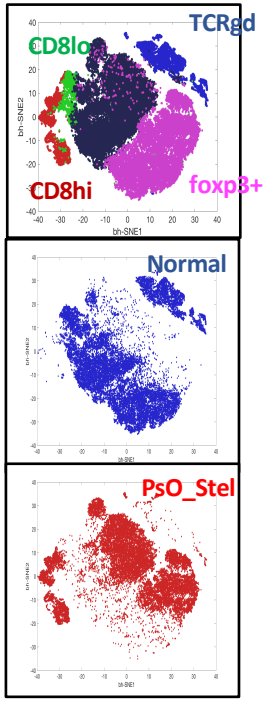
foxp3



CD4

Summary: Multidimensional profiling of human skin T cells



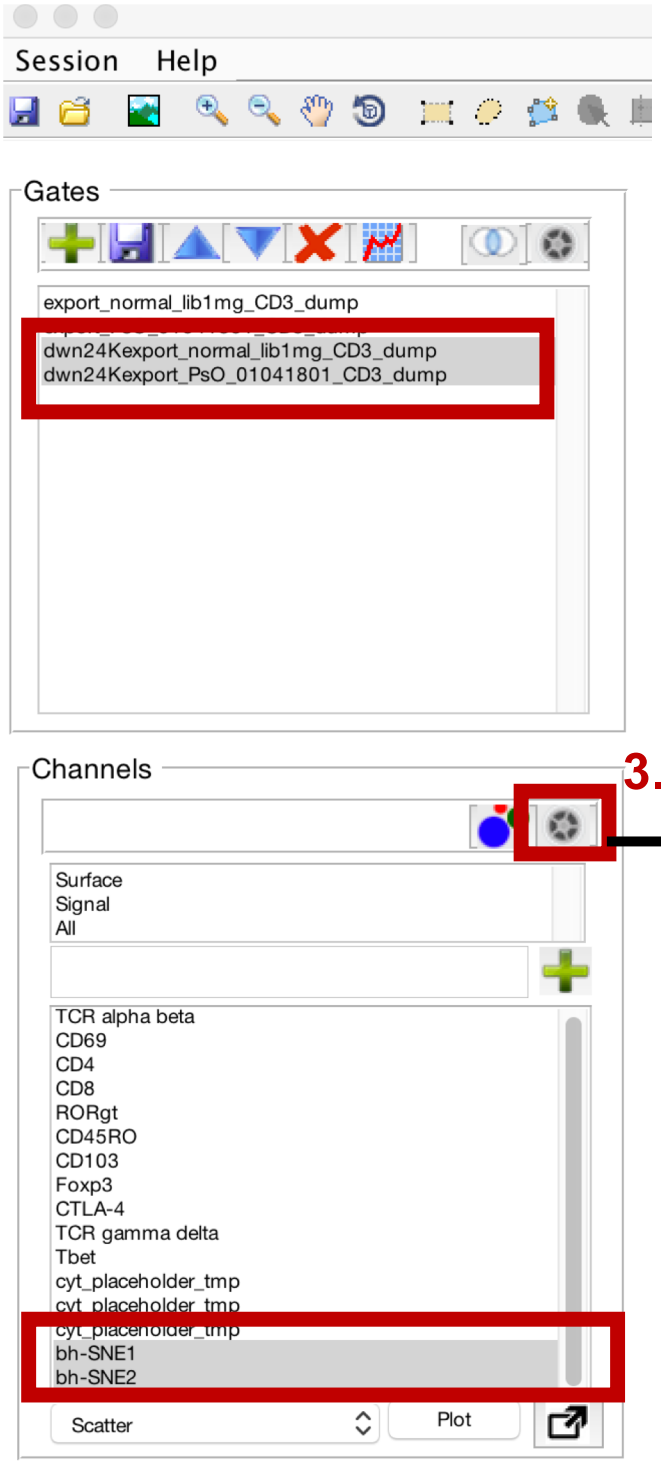


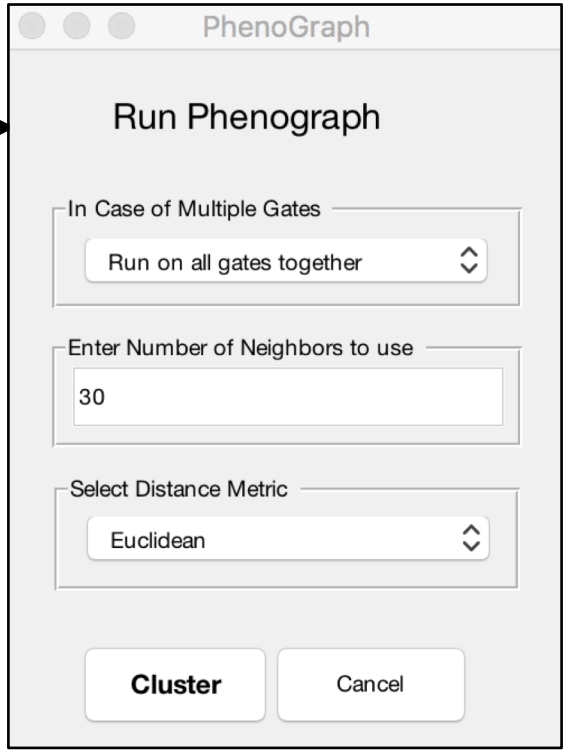
PhenoGraph

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

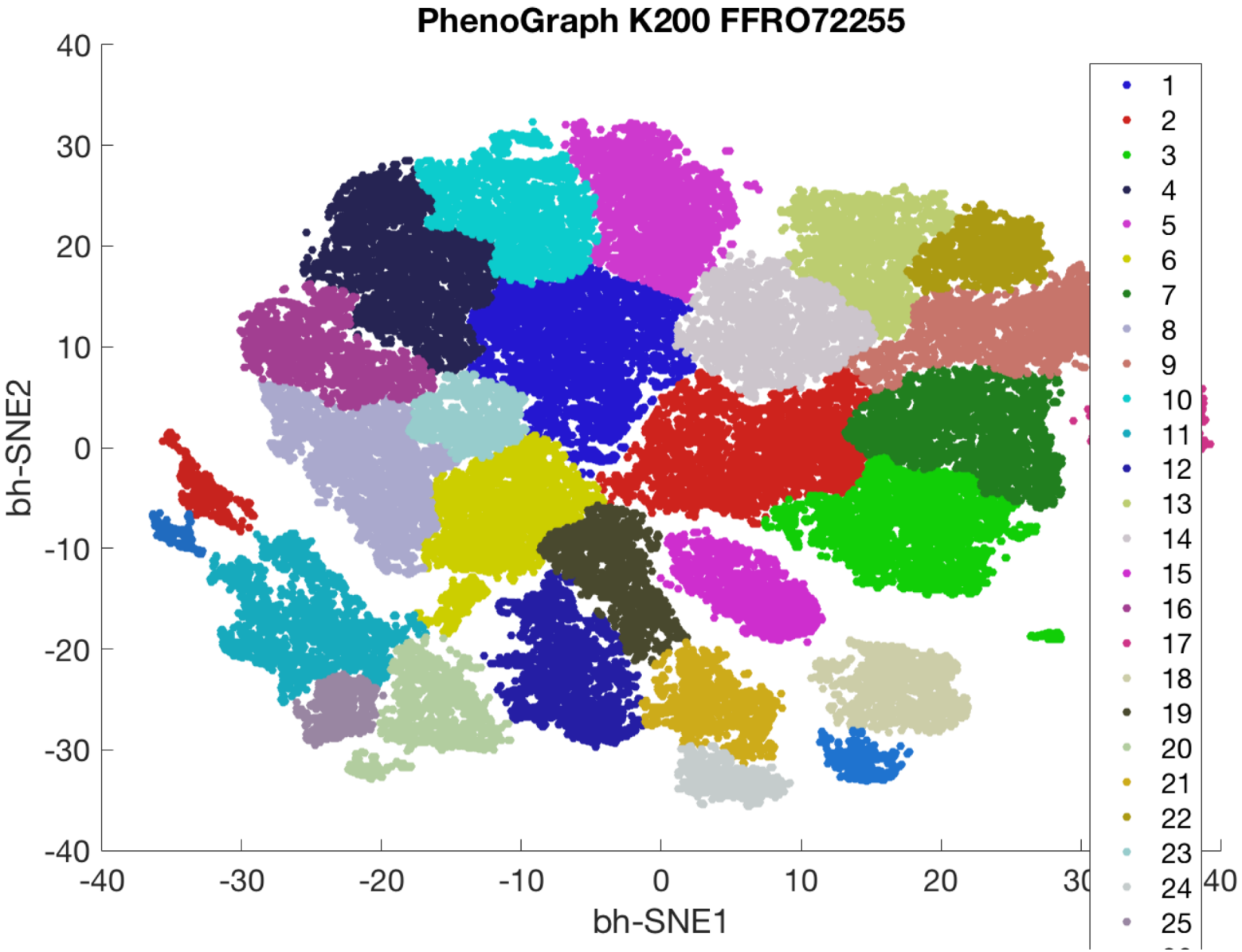
2. Select the two new derived parameters bh-SNE1 and bh-SNE2

3. Under navigation wheel select “PhenoGraph”





PhenoGraph – automated clustering



References

Reviews

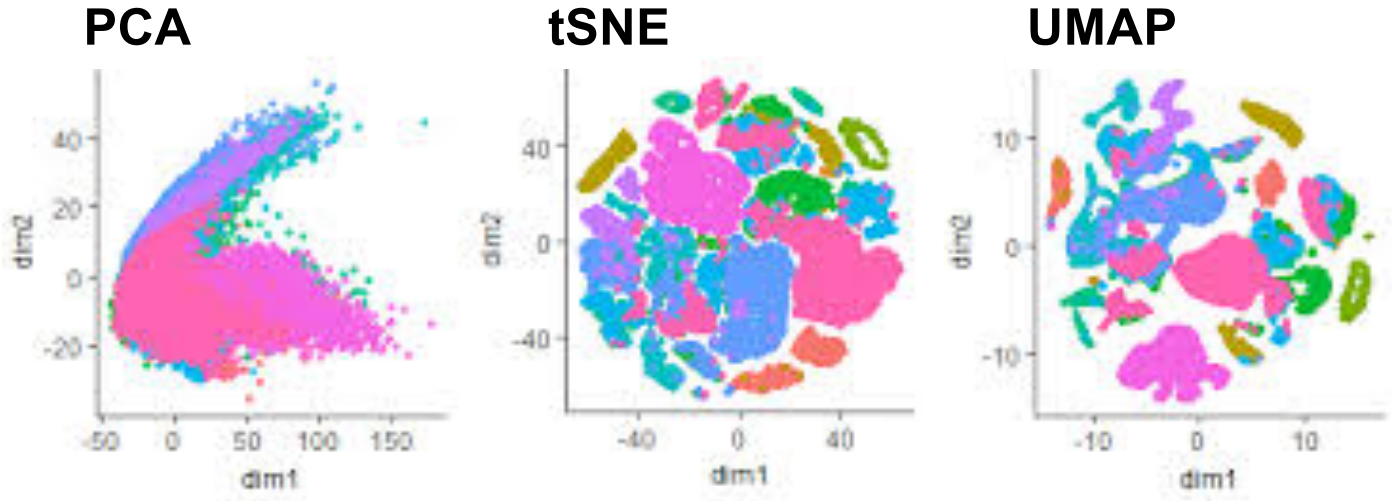
1. Kimball AK, Oko LM, Bullock BL, Nemenoff RA, van Dyk LF, Clambey ET. A Beginner's Guide to Analyzing and Visualizing Mass Cytometry Data. J Immunol. 2018 Jan 1;200(1):3-22.
2. Saeys Y, Gassen SV, Lambrecht BN. Computational flow cytometry: helping to make sense of high-dimensional immunology data. 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**

UMAP

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



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, https://arxiv.org/abs/1802.03426