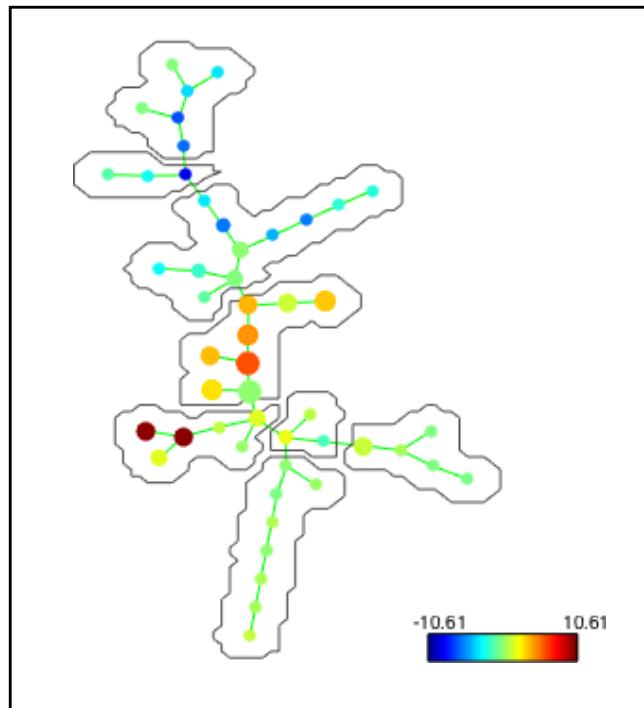


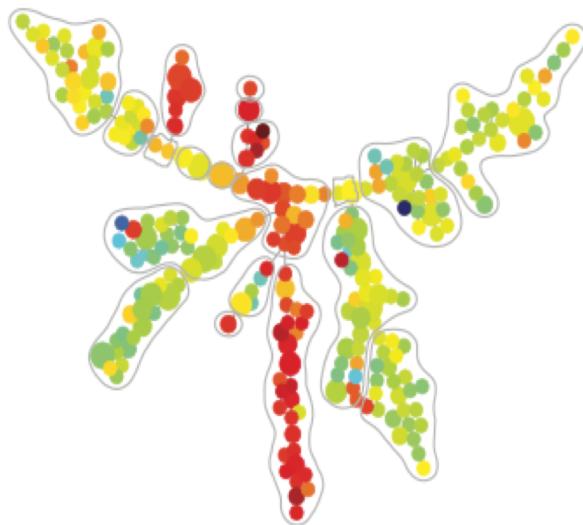
SPADE Step-By-Step



Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

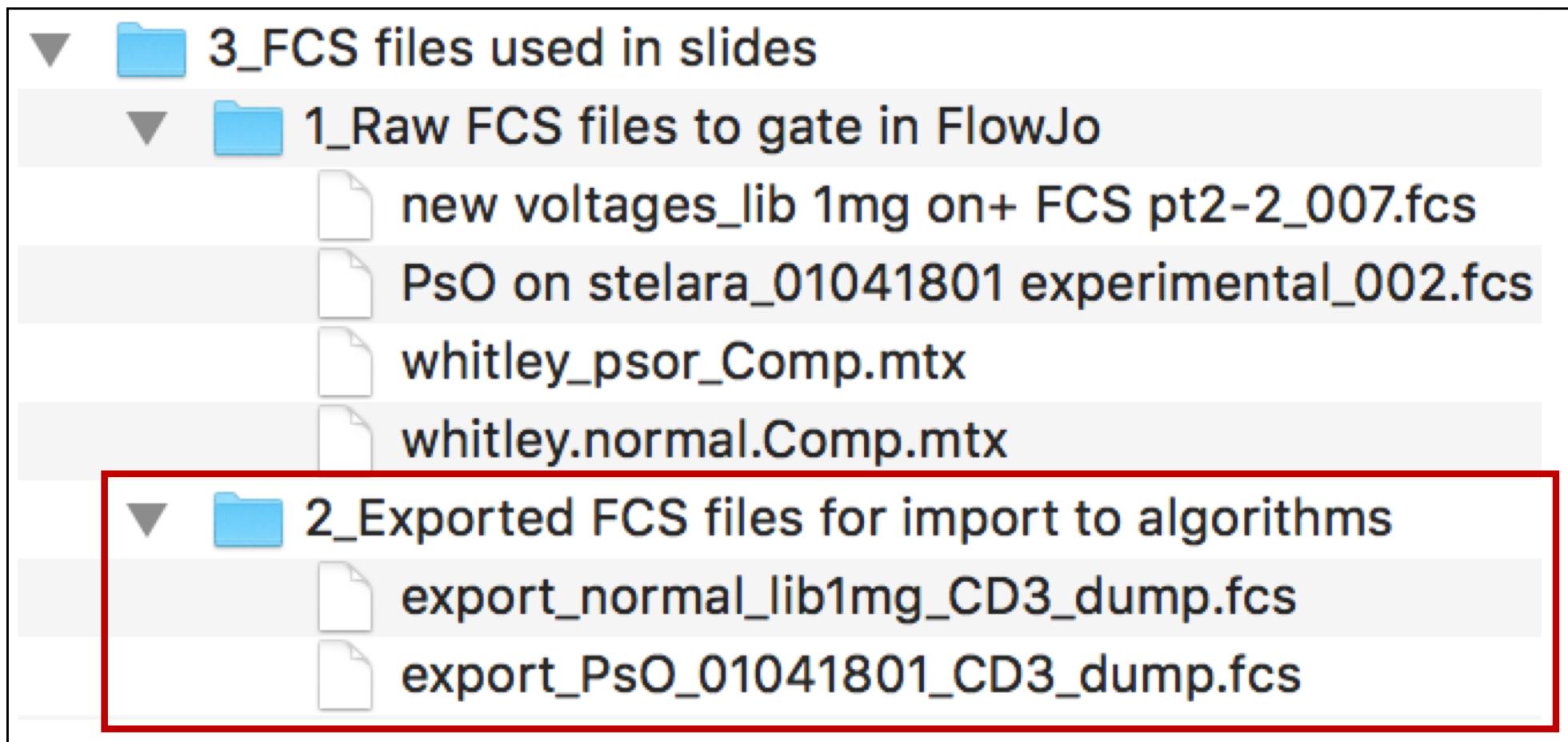
SPADE developer provides great instructions including screenshots

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



Import pre-gated fcs files (live, singlet, gated on population to cluster) into SPADE

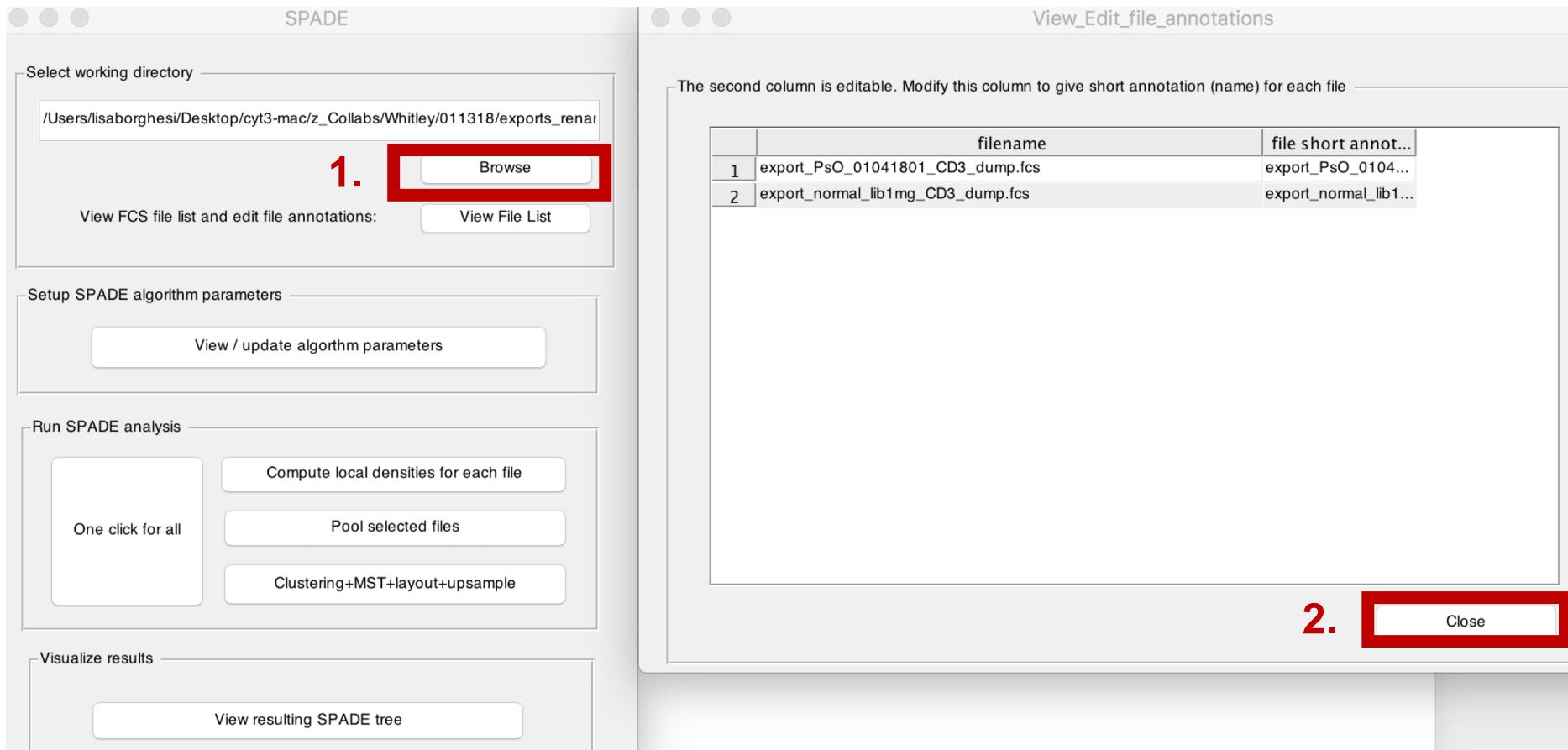
Use your fcs files or choose the “Exported FCS files” below.
Files are located in the same PittBox as this ppt.



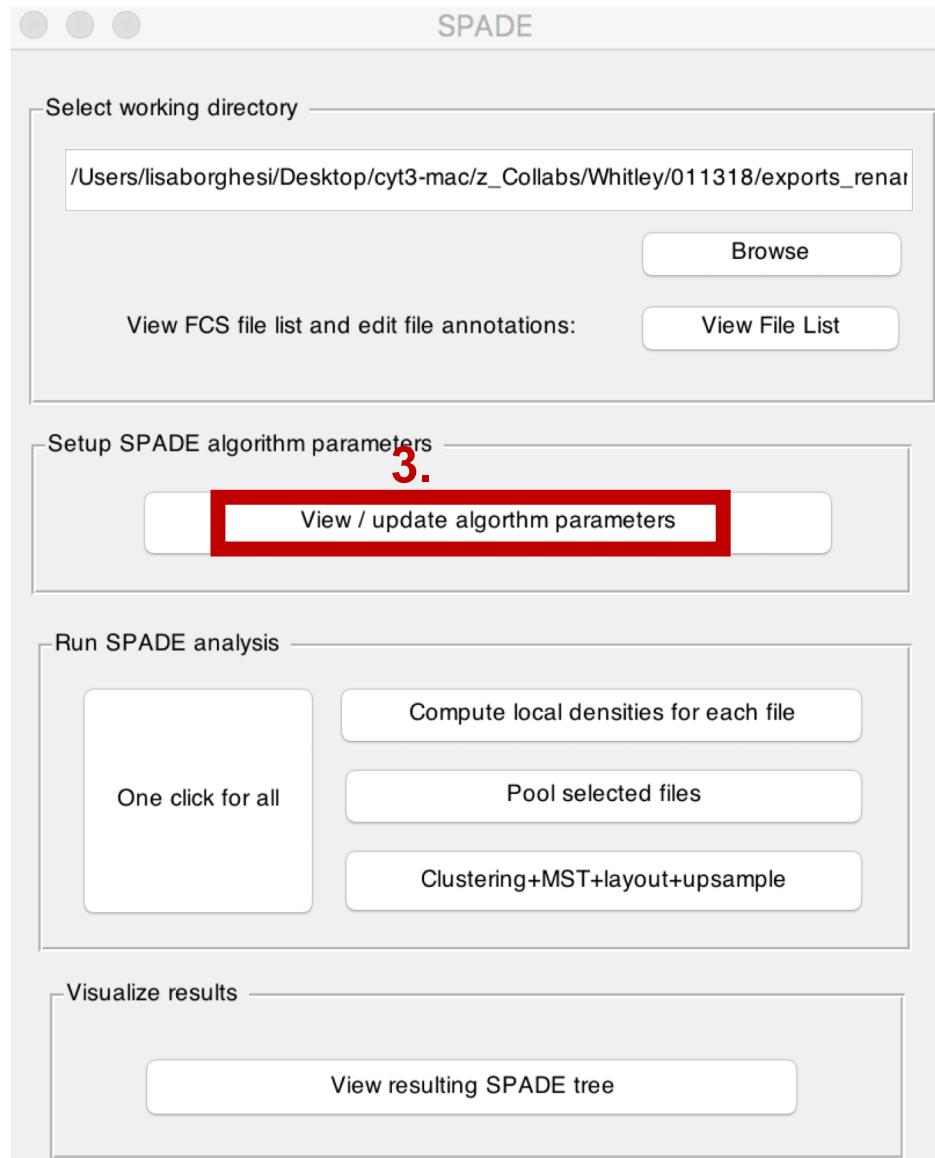
Import gated populations into SPADE

1. Click “Browse” and navigate to folder that contains gated fcs files
2. Select the folder and click “Close”

SPADE

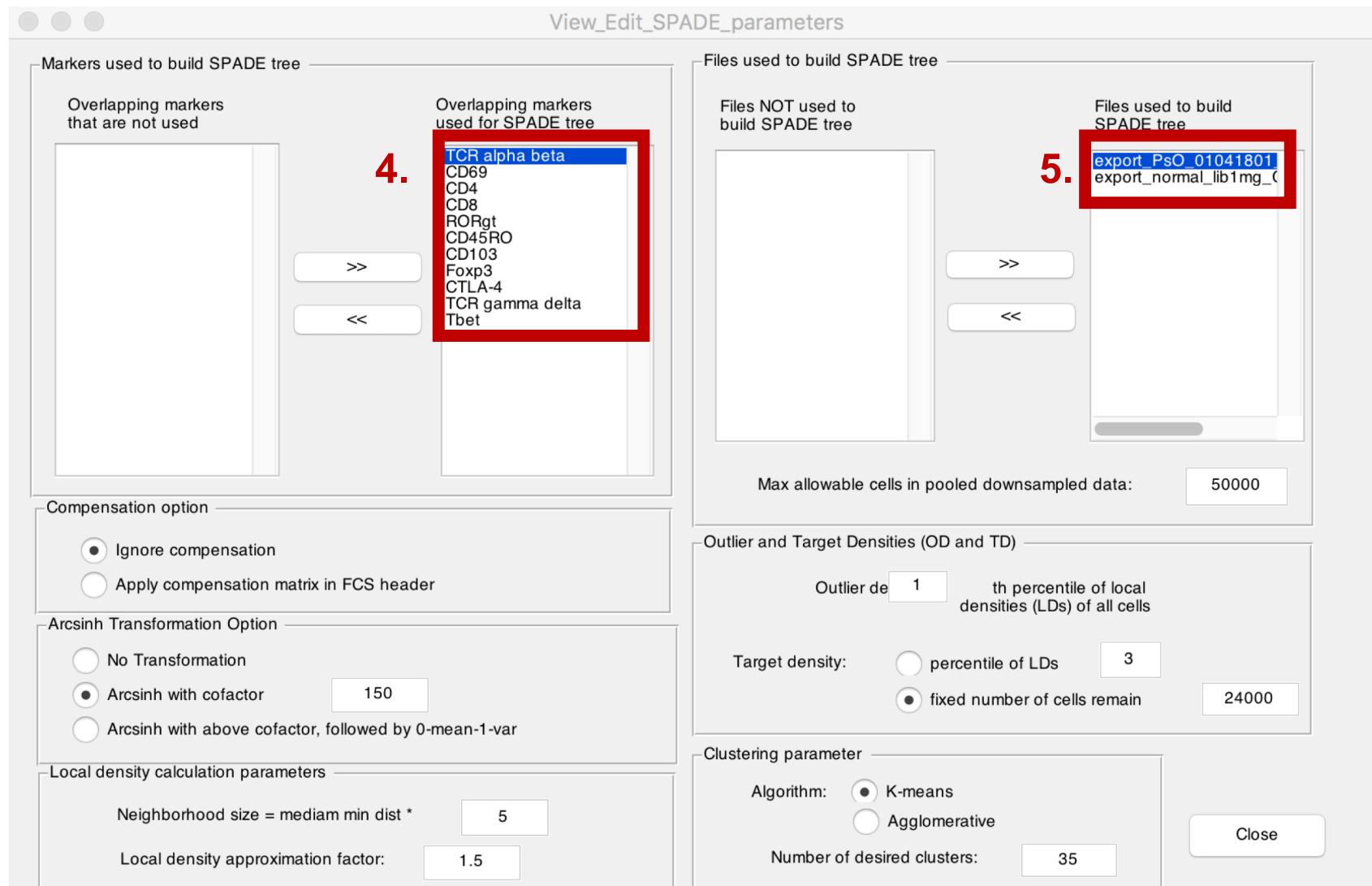


Setup SPADE parameters



3. Click “View/update algorithm parameters”

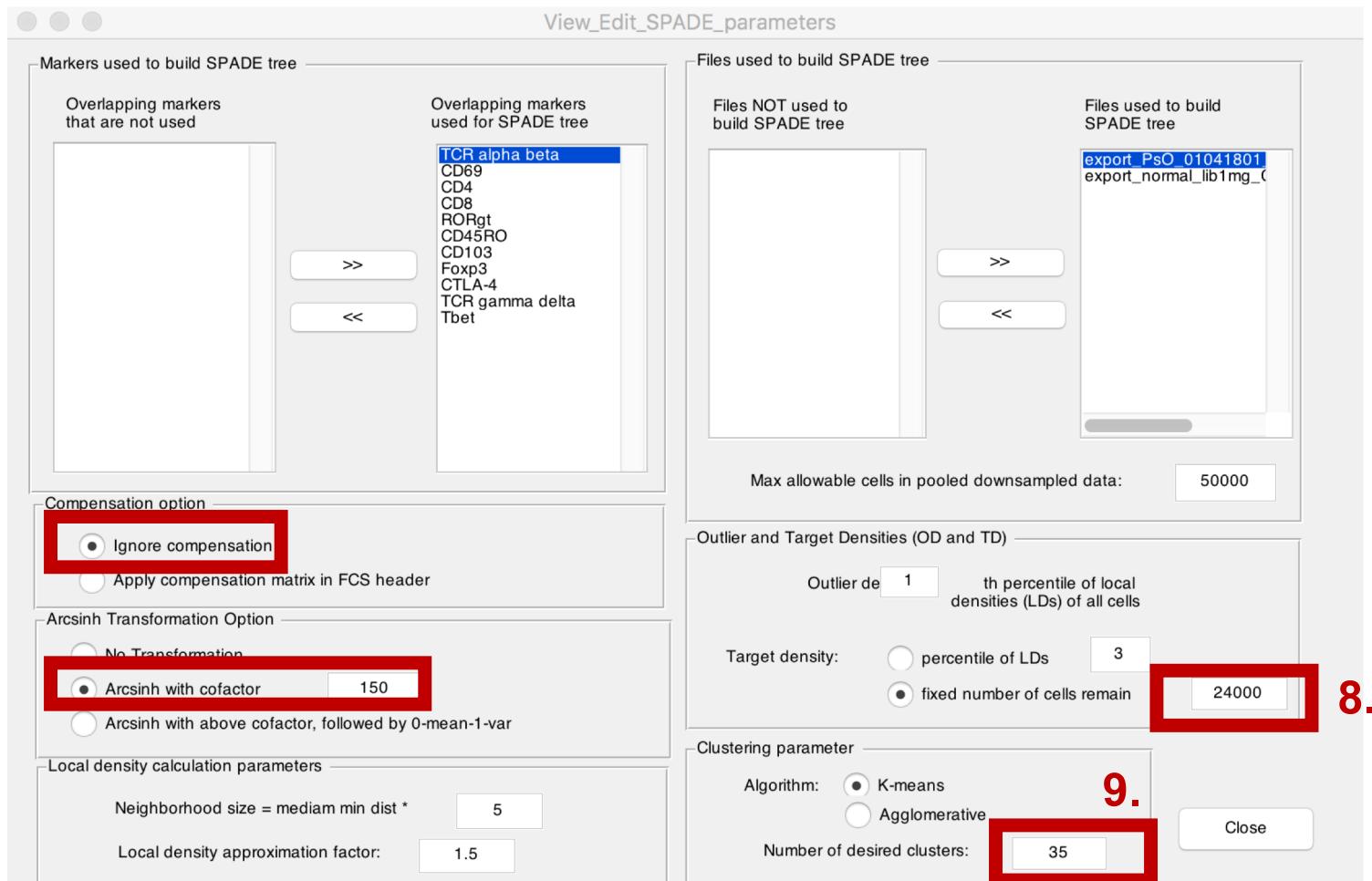
Setup SPADE parameters



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

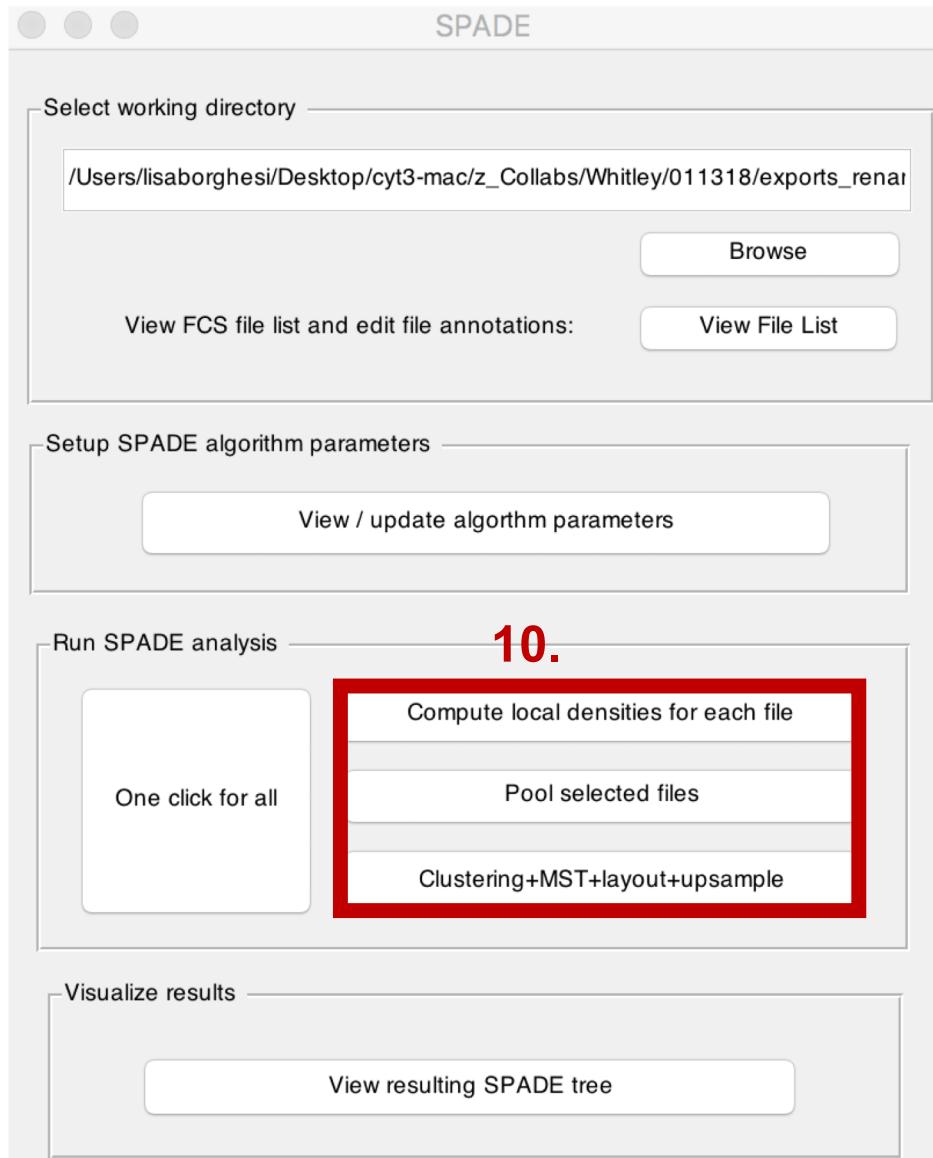
5. Select files to use for building SPADE tree (ditto)

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

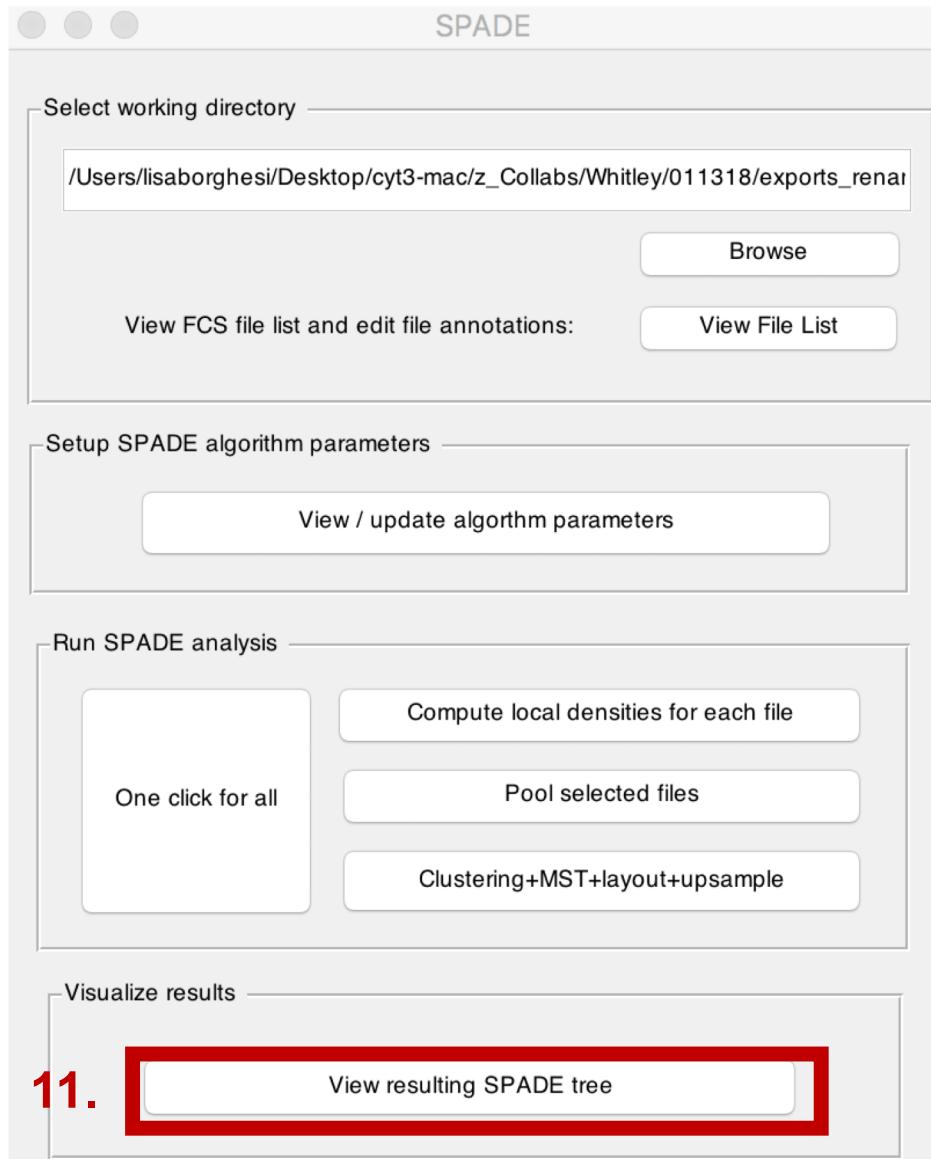
Run SPADE analysis



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

show tree in new window Arch Layout Spring RadioExpand

Show node/cluster index
Edit SPADE tree layout

Selected nodes: add to annotation

Scale span of selected nodes:

Rotate select nodes:

Change node size:

SPADE tree annotation

Auto Suggest Annotation View Annotations Remove

No show Show all Show selected

Annotation bubble size: 5

overlay information by coloring nodes

Select a marker: POOLED Ref files for ratio

TCR alpha beta TCRab

TCR alpha beta
CD69
CD4
CD8
ROR γ t
CD45RO
CD103
Foxp3
CTLA-4
TCR gamma delta
Tbet
Fileld
CellFreq

TCR alpha beta
POOLED
export_normal_lfbm9_CD3_dump

add to ref remove from ref

Color definition: expr (selected), ratio, cell freq

Color scheme: JET (selected), half JET, Gray scale

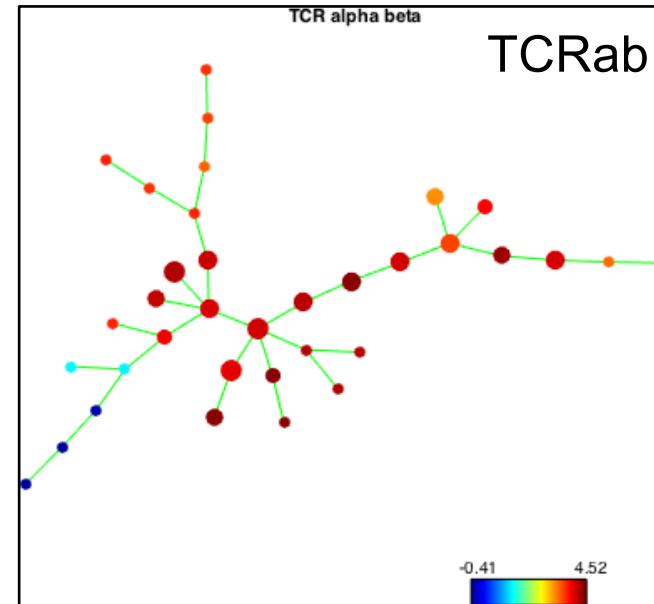
Export results to PS, GML, TXT, FCS, network

Tree colored by markers Tree colored by CellFreq

Tree to GML & TXT Node/Annot expr & CellFreq to TXT

Clustering result to FCS EarthMoverDist b/w samples Annot to cytobank

→ 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

Color options

overlay information by coloring nodes

Select a marker:

- TCR alpha beta
- CD69
- CD4
- CD8
- ROR γ t
- CD45RO
- CD103
- Foxp3
- CTLA-4
- TCR gamma delta
- Tbet
- FileInd
- CellFreq

Select a file

Ref files for ratio

export_PsO_01041801_CD3_dump.l
export_normal.lib1mg_CD3_dump.l

Select comparison file

Select reference file

add to ref remove from ref

Color definition

- expr
- ratio
- cell freq

Color scheme

- JET
- half JET
- Gray scale

Export results to PS, GML, TXT, FCS formats

Tree colored by markers Tree colored by CellFreq

Tree to GML & TXT Node/Annot expr & CellFreq to TXT

Clustering result to FCS EarthMoverDist b/w samples Annot to cytobank

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

Color options

overlay information by coloring nodes

Select a marker:

- TCR alpha beta
- CD69
- CD4
- CD8
- RORgt
- CD45RO
- CD103
- Foxp3
- CTLA-4
- TCR gamma delta
- Tbet
- FileInd
- CellFreq

Select a file

POOLED
export_PsO_01041801_CD3_dump.i
export_normal_lib1mg_CD3_dump.i

Ref files for ratio

export_normal_lib1mg_CD3_dump.i

Color definition

- expr
- ratio
- cell freq

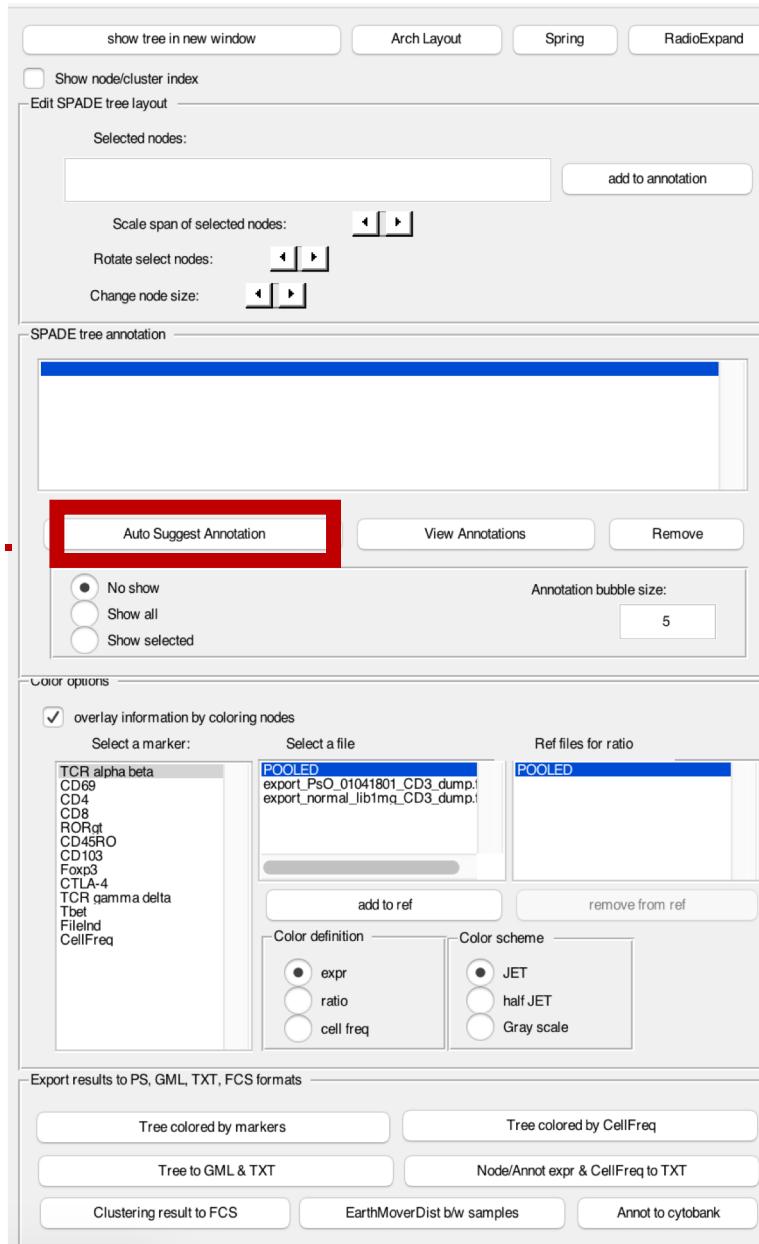
Color scheme

- JET
- half JET
- Gray scale

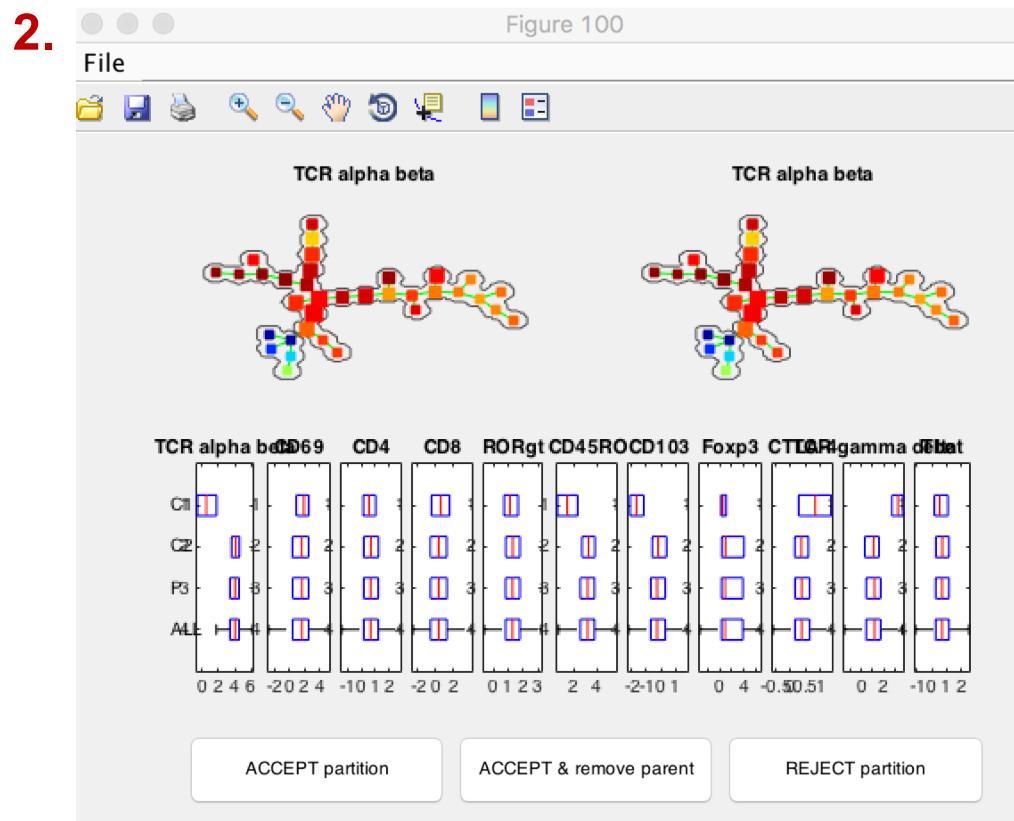
Export results to PS, GML, TXT, FCS formats

Export
SPADE trees
or files

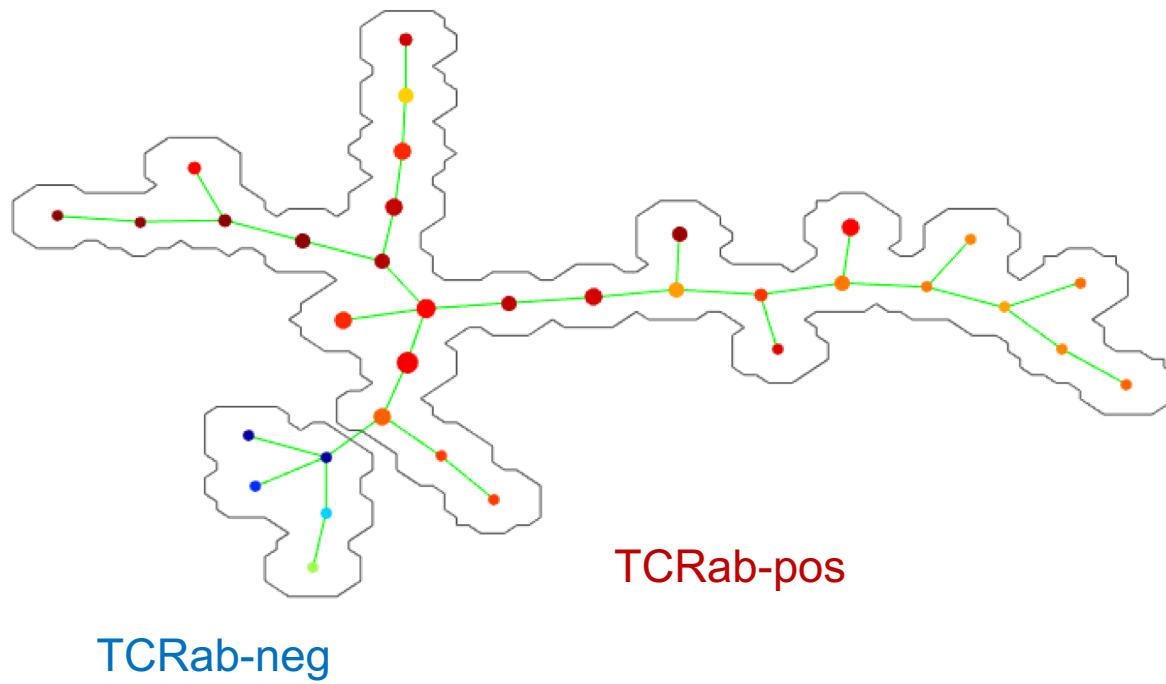
Annotate SPADE tree (unbiased)



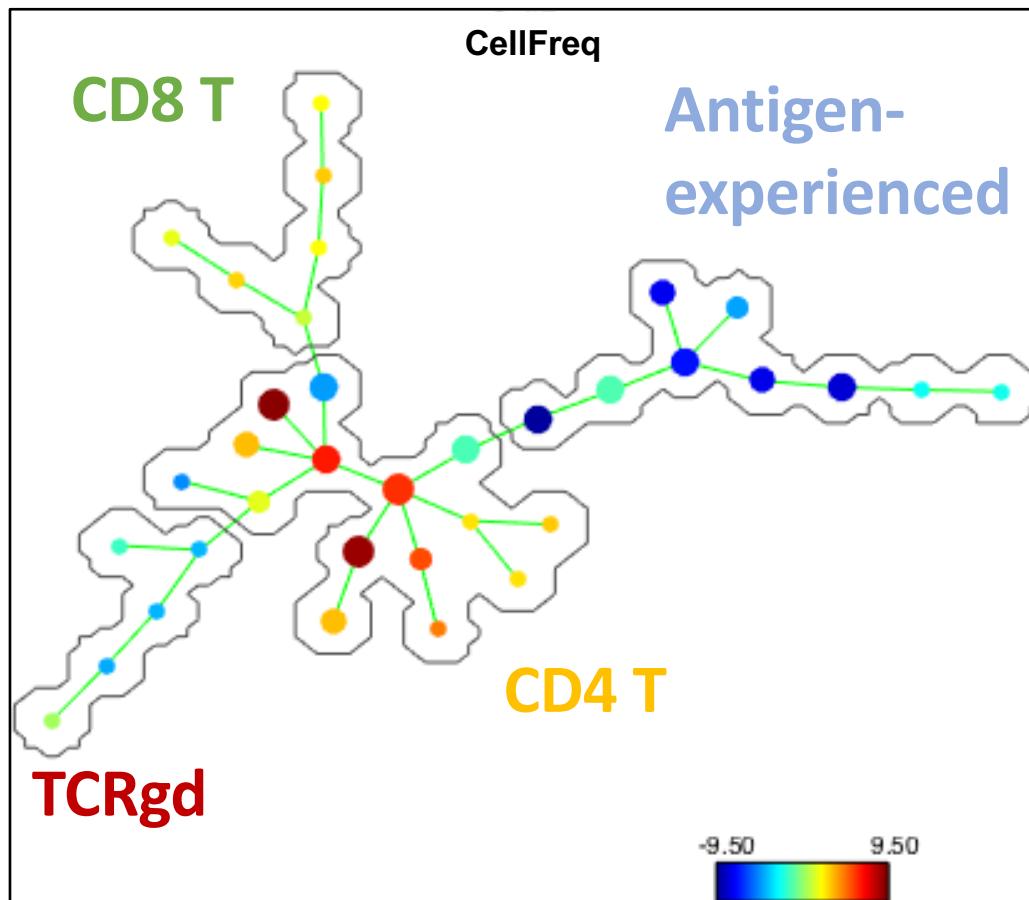
1. Select “Auto Suggestion Annotation”
2. In popup window, accept/reject proffered partition



Annotations (unbiased)



Changes in population frequency PsO_Stelara relative to Normal



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**