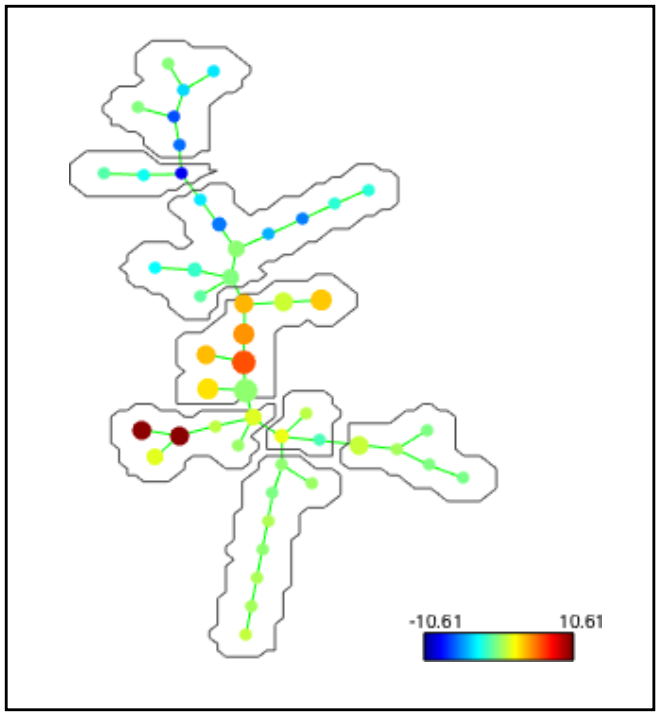
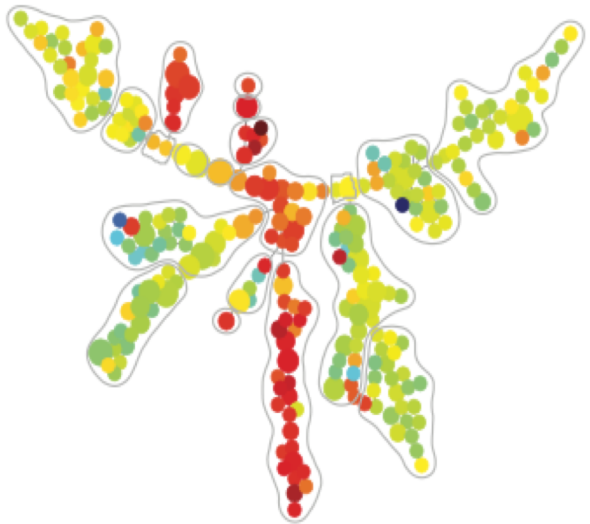
SPADE Step-By-Step



Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

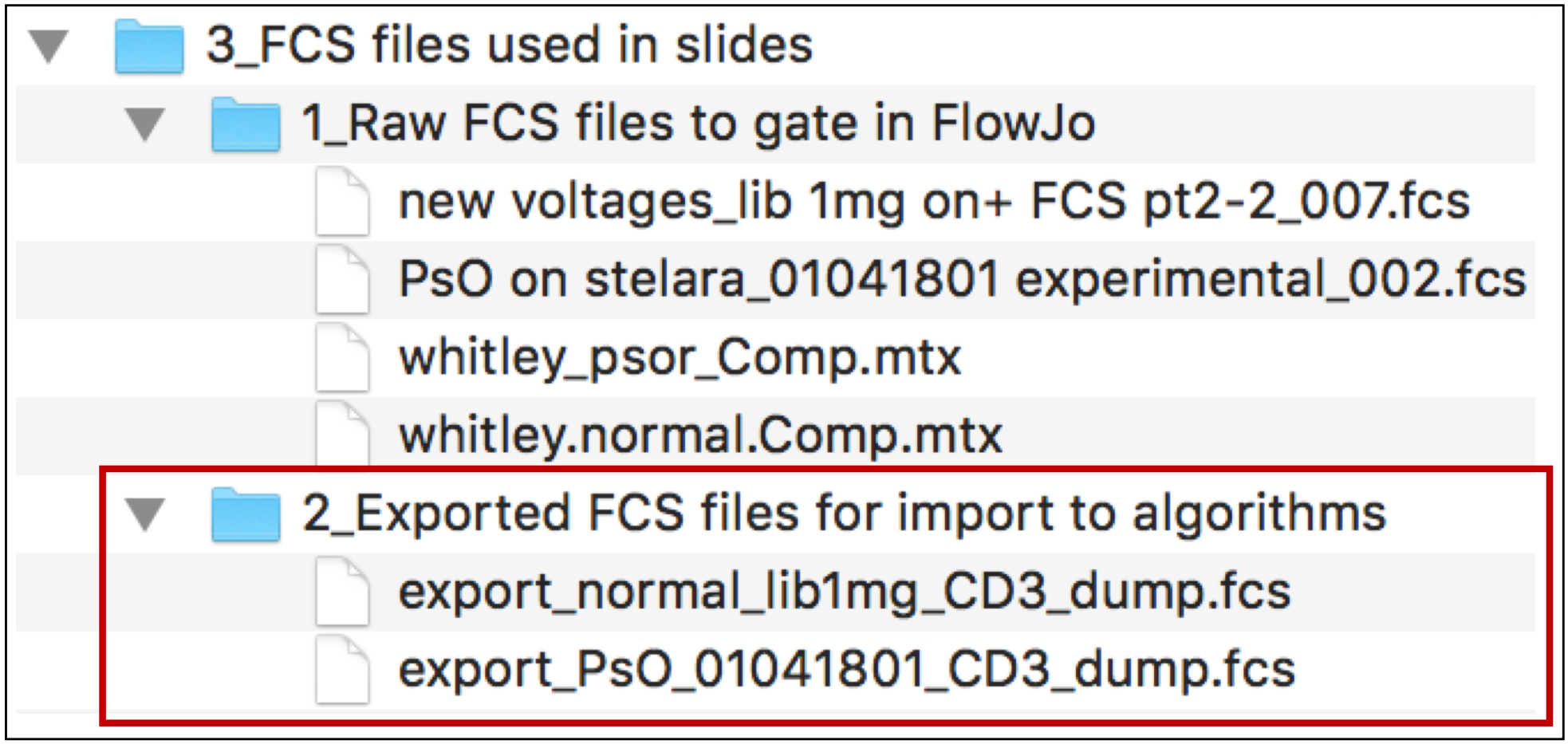
SPADE developer provides great instructions including screenshots

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.

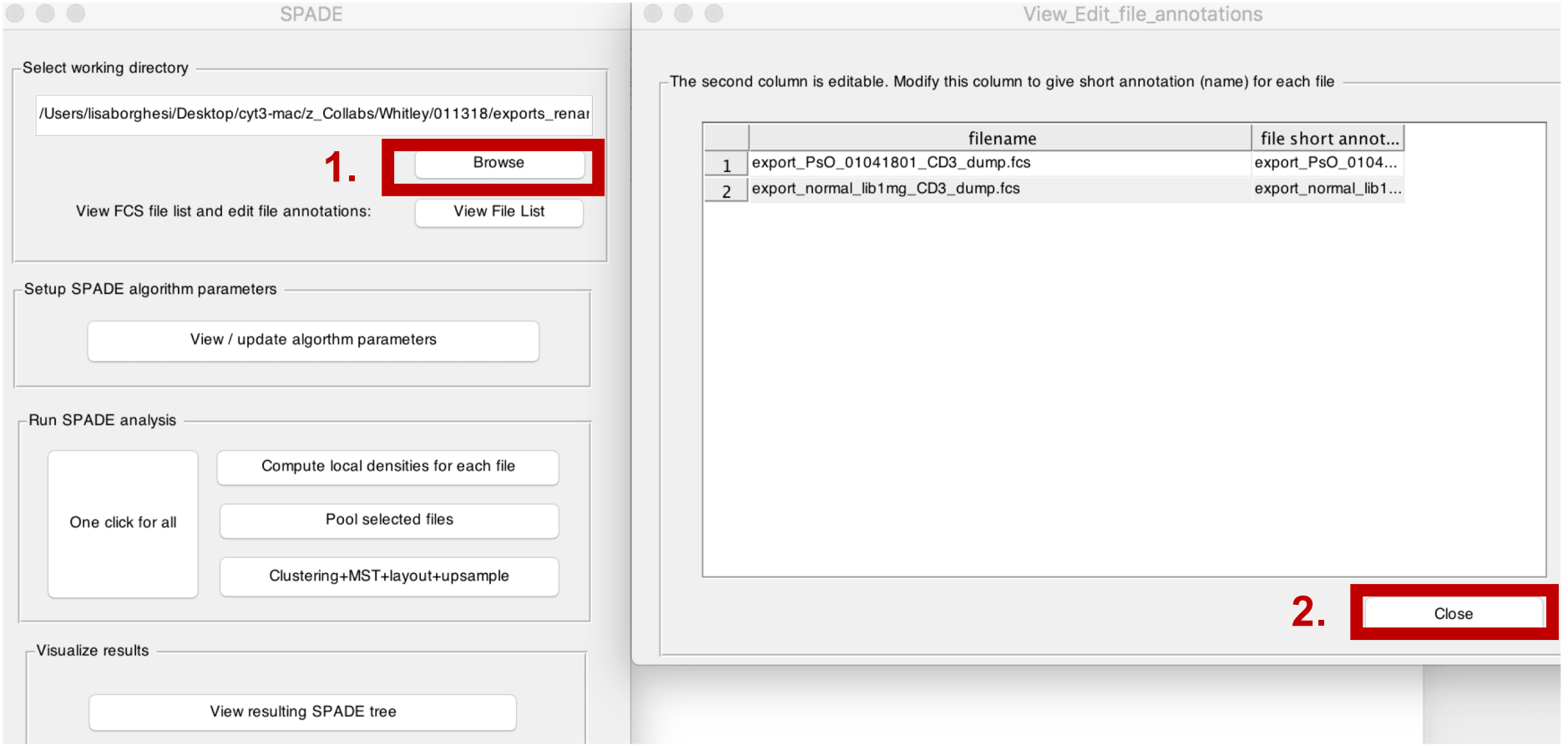


Import gated populations into SPADE

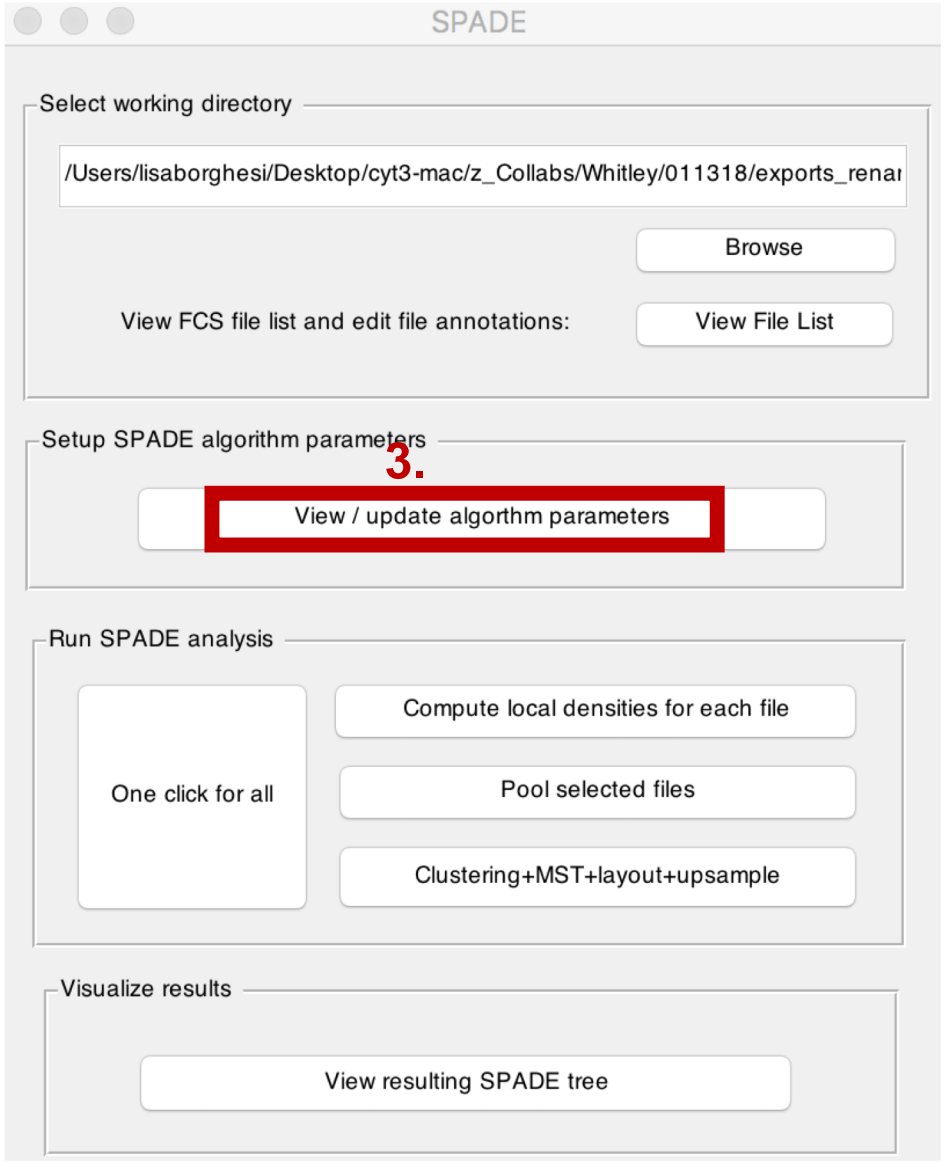
1. Click “Browse” and navigate to folder that contains gated fcs files

2. Select the folder and click “Close”

SPADE

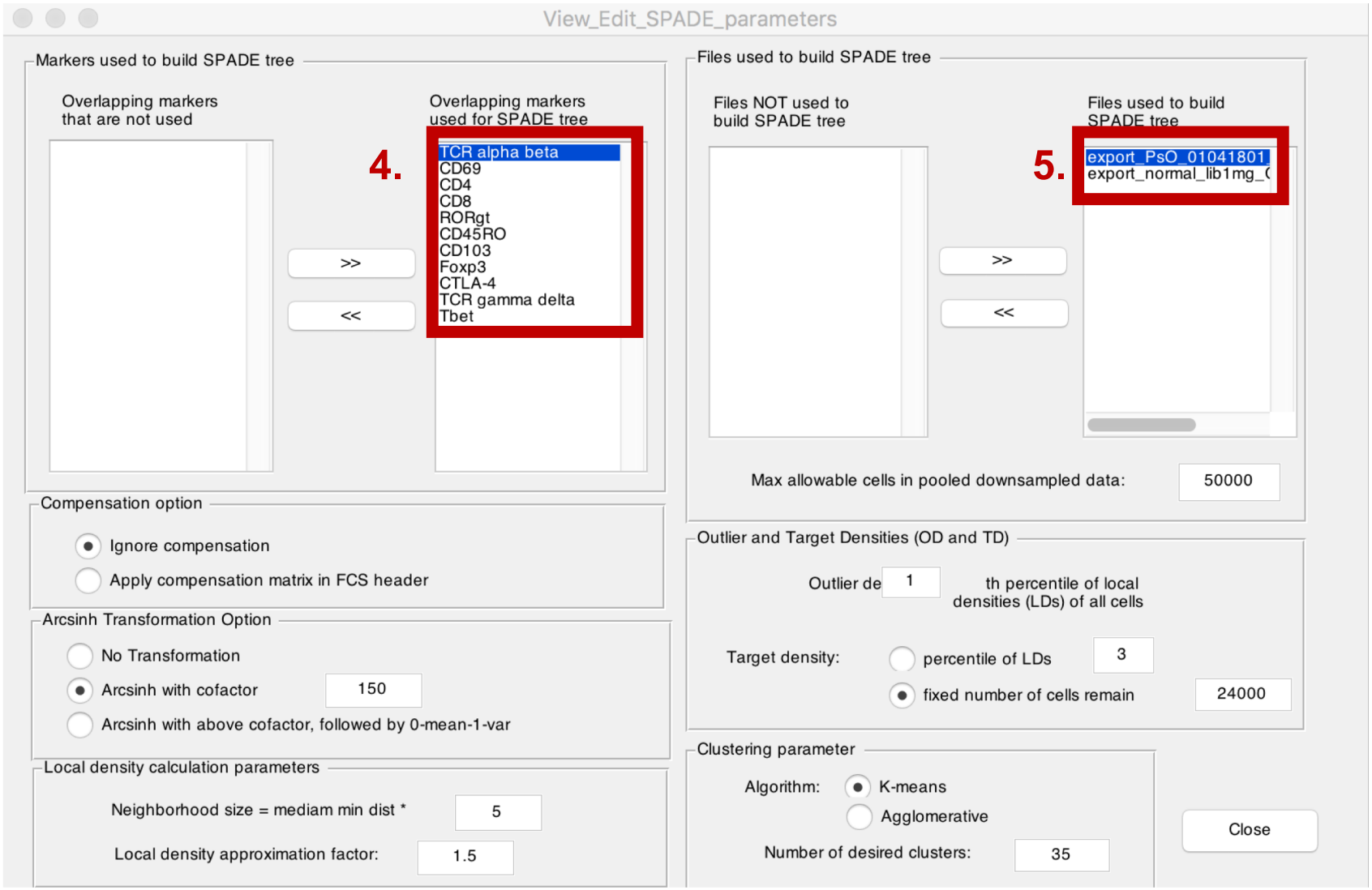


Setup SPADE parameters



3. Click “View/update algorithm parameters”

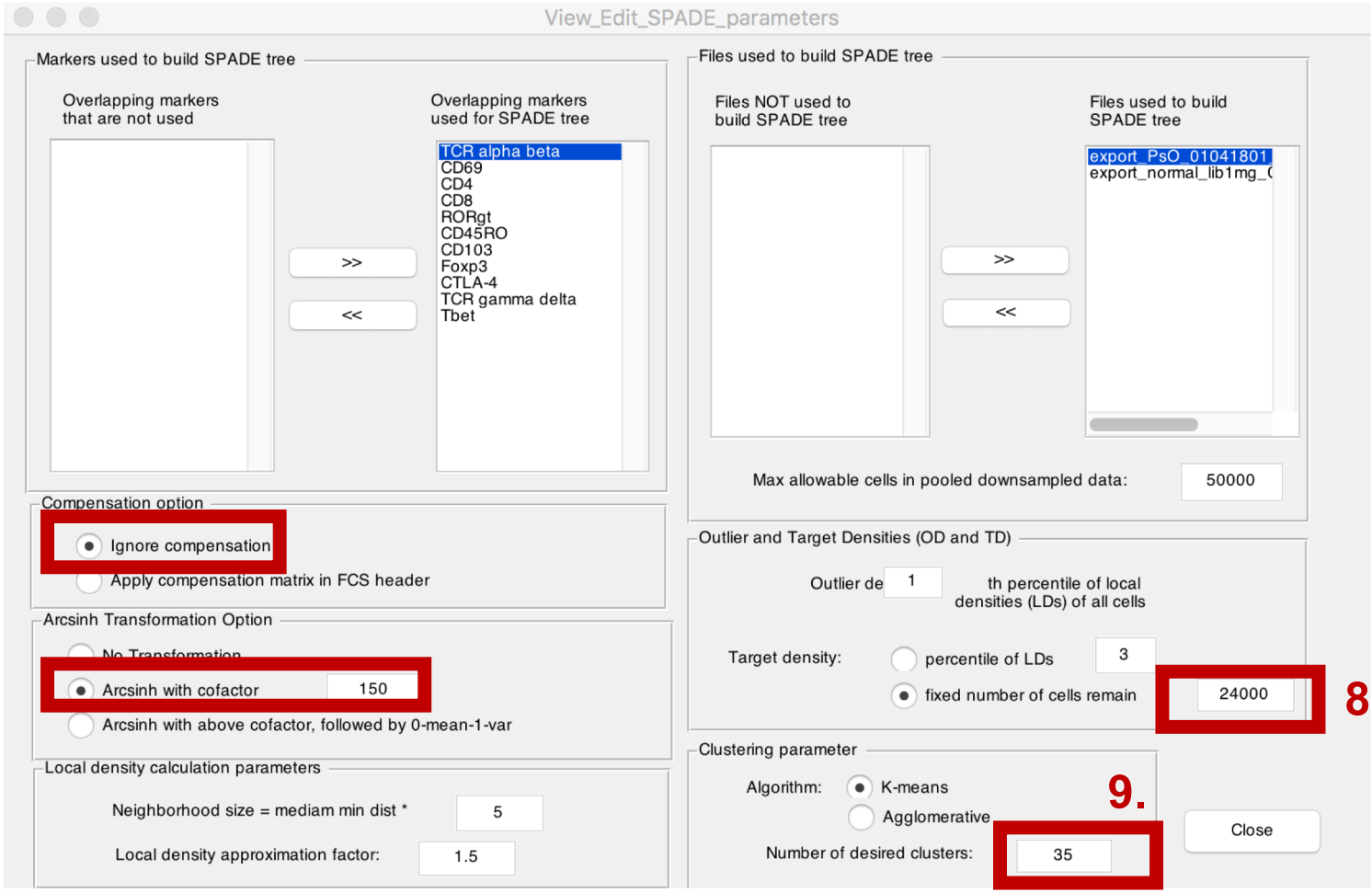
Setup SPADE parameters



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

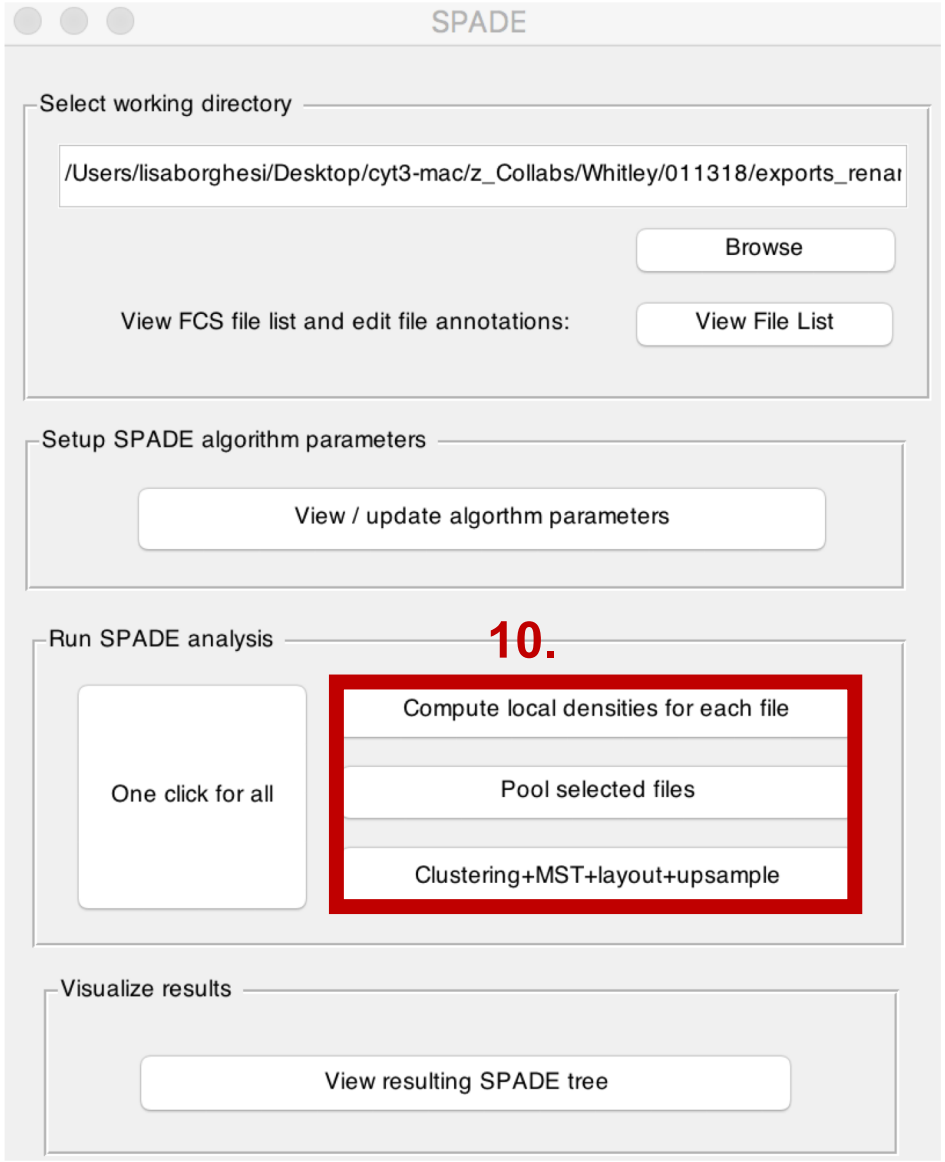
5. Select files to use for building SPADE tree (ditto)

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



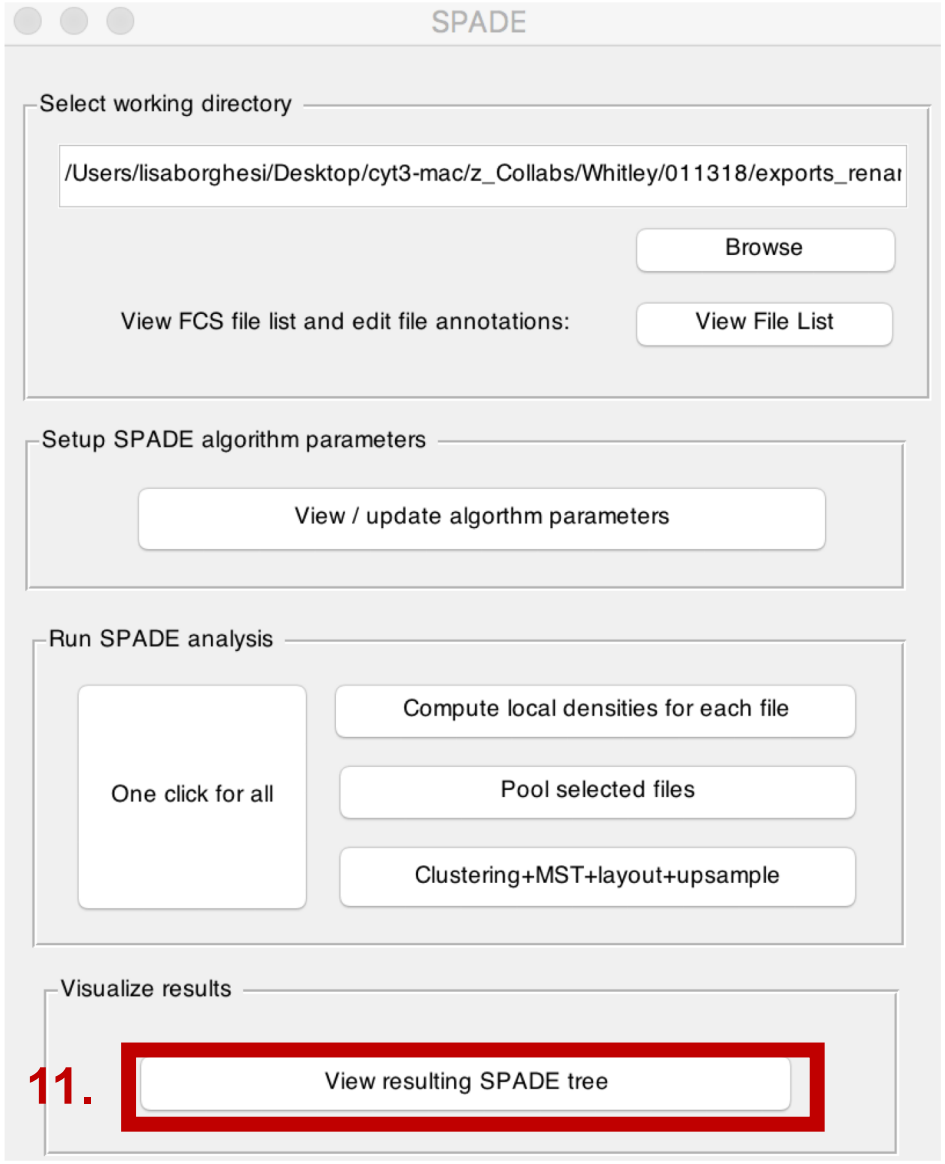
10. Today we'll sequentially walk through each step in the algorithm.

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

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

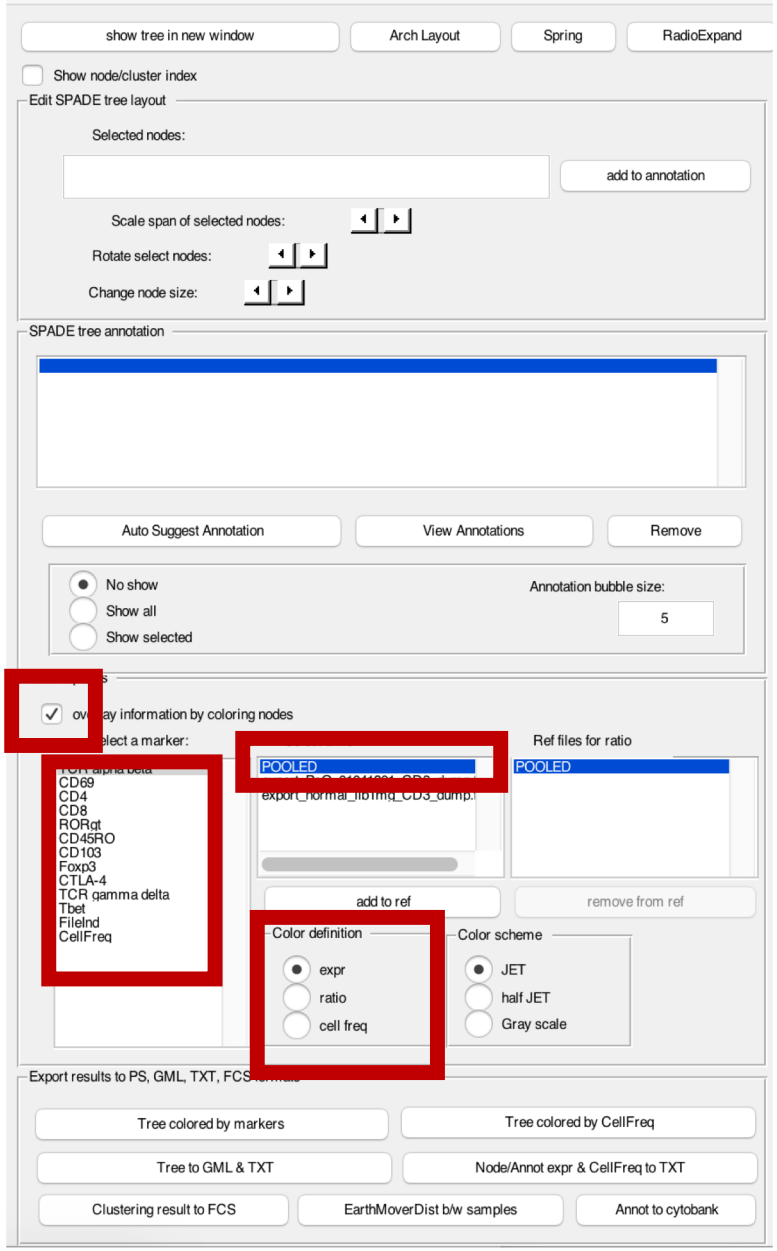
c) Clustering
→ Fast. Feedback is "Done"

Run SPADE analysis

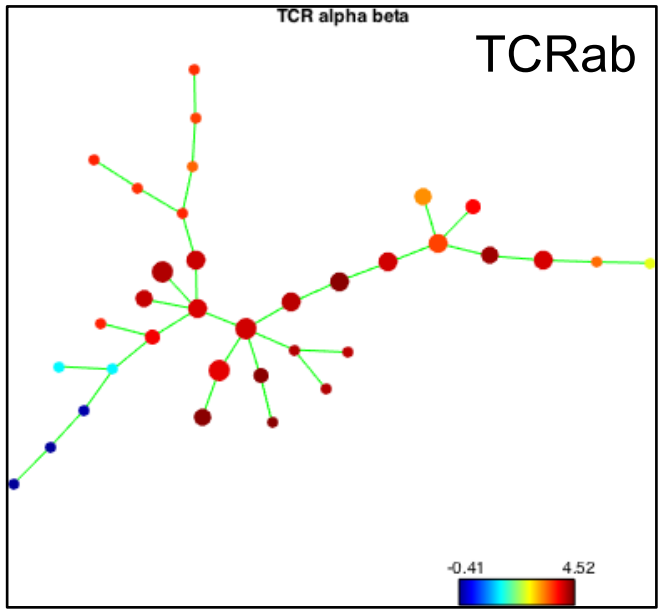


11. View resulting SPADE tree

Color tree by marker

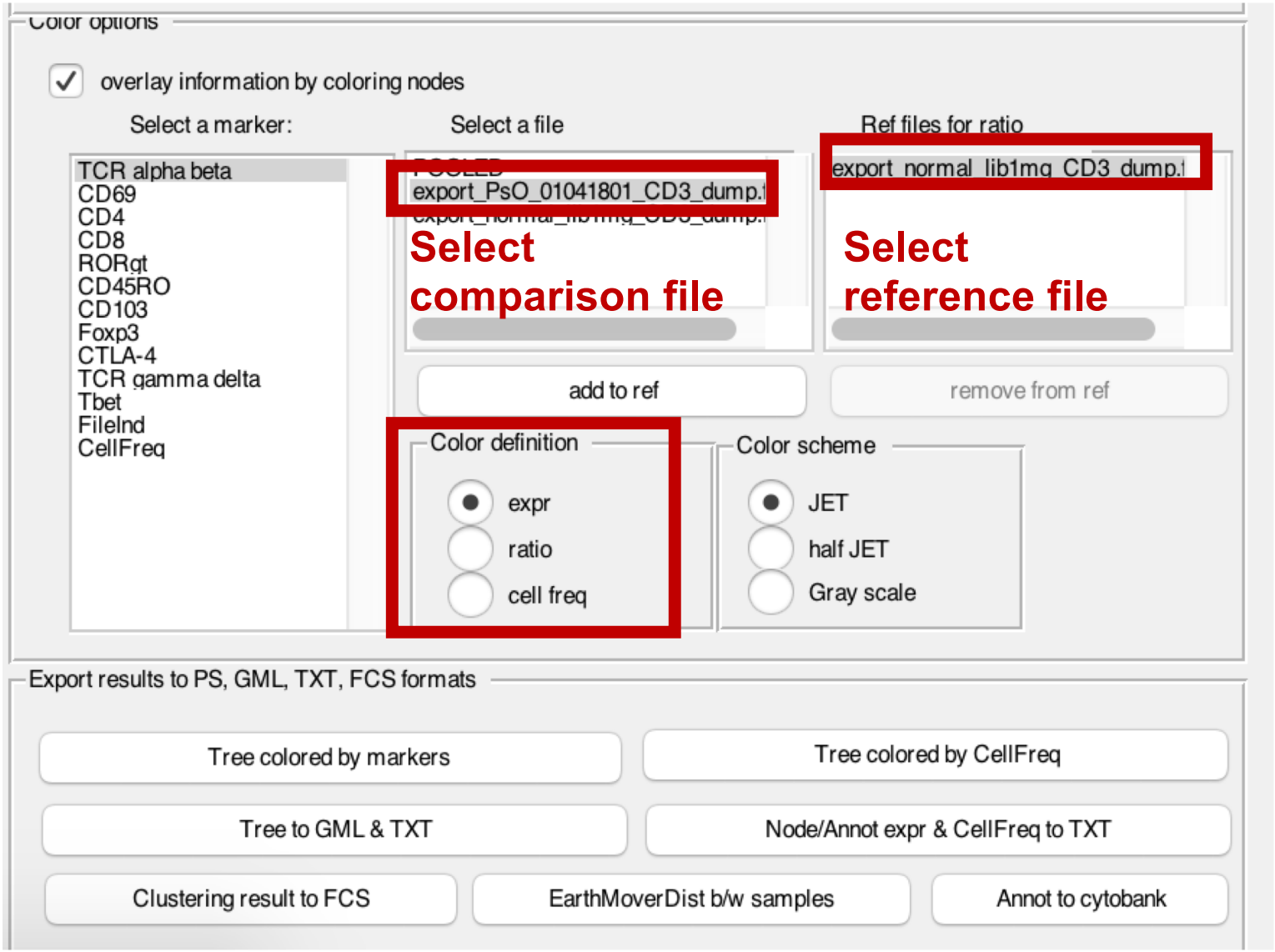


→ Circle size proportional to pop frequency



- 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



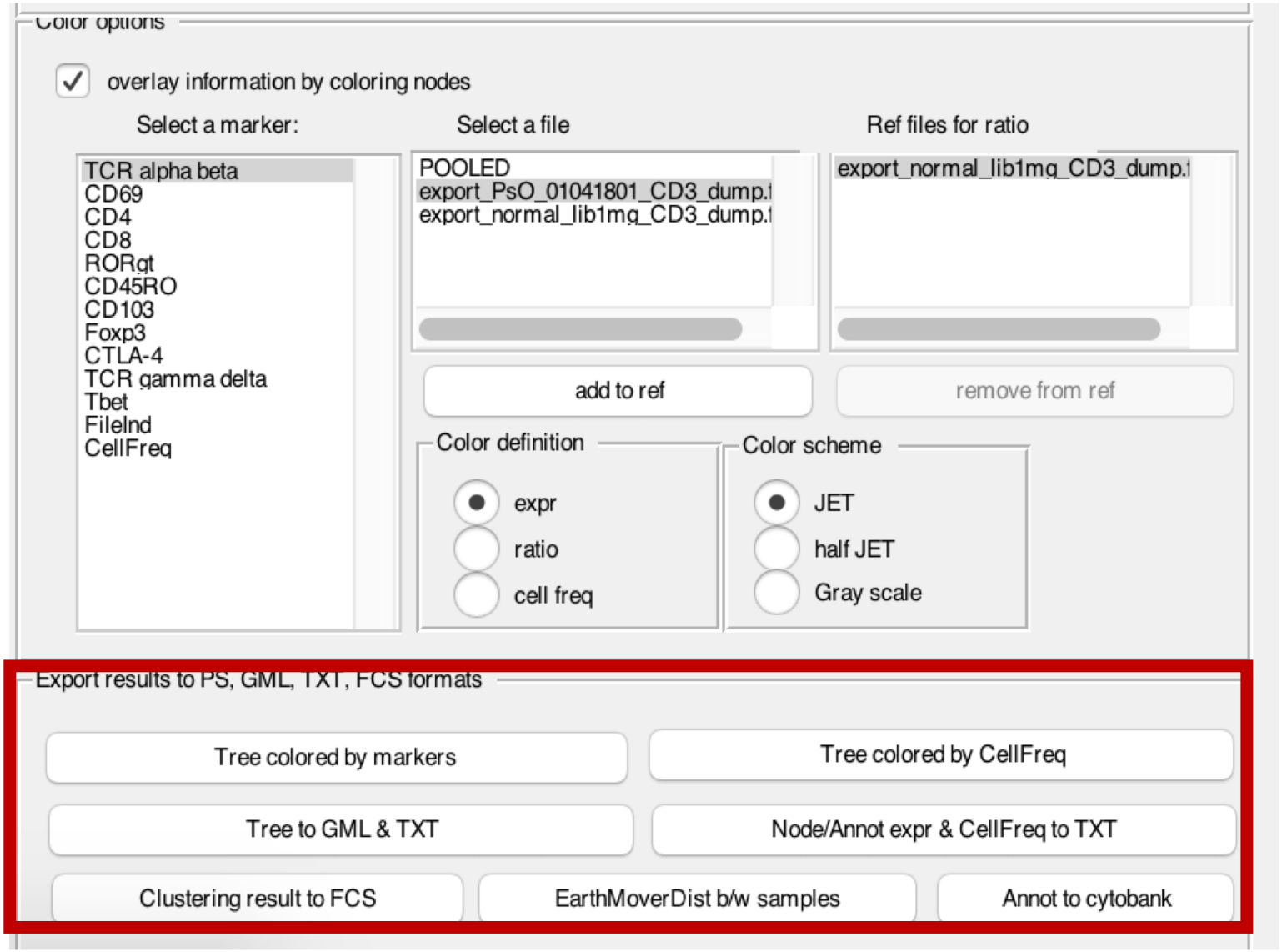
Or compare samples:

Expr = median fluor intensity of cells in that cluster

Ratio = the difference b/w two samples

Cell freq = freq. of cells in cluster

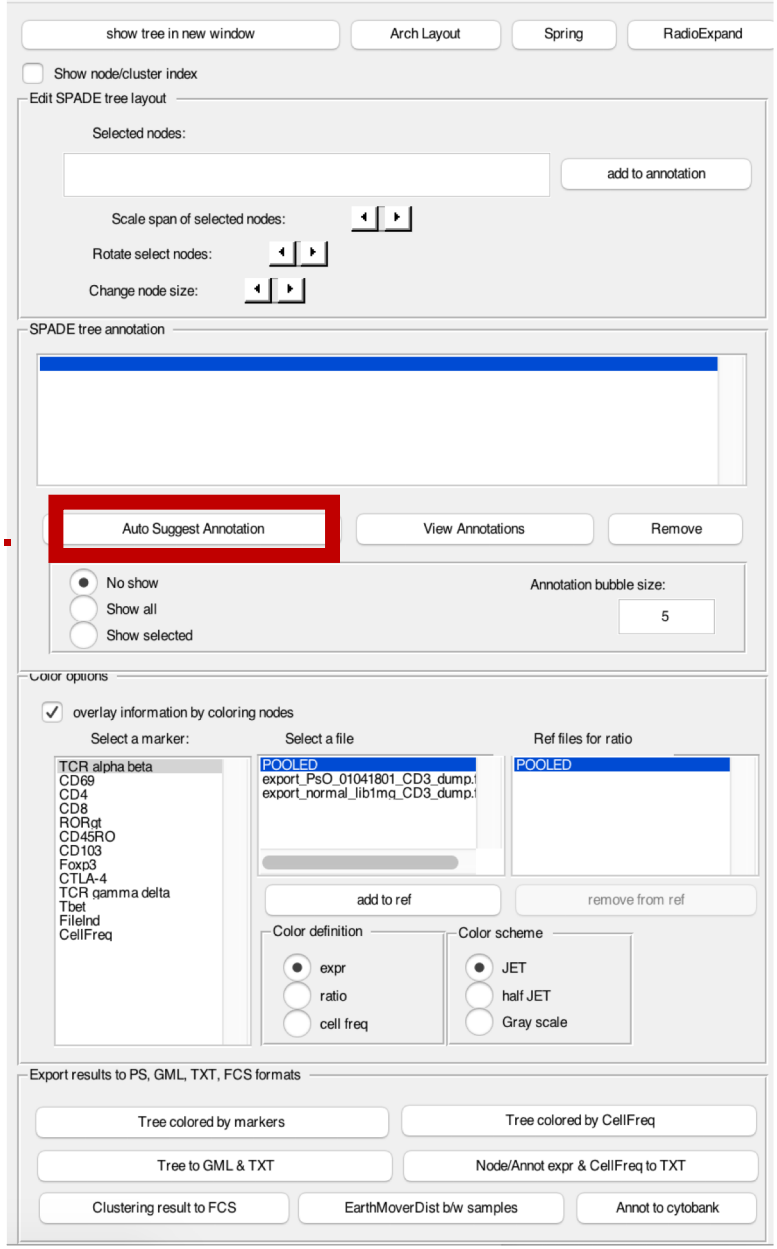
Compare samples



Export SPADE trees or files

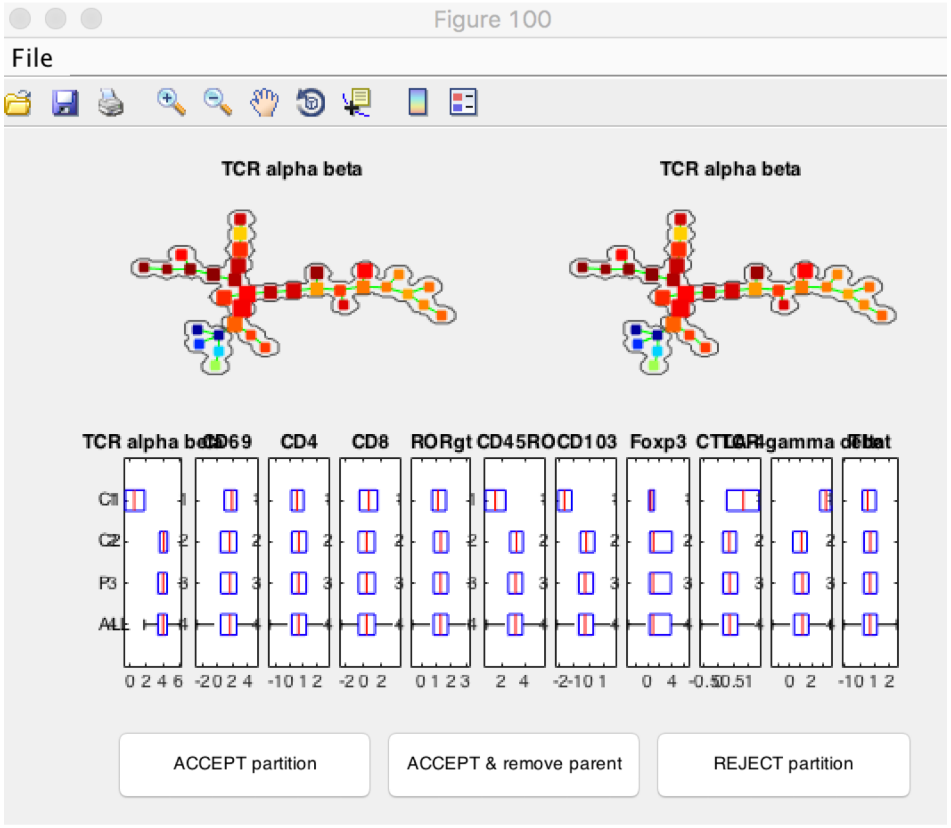
Annotate SPADE tree (unbiased)

1.

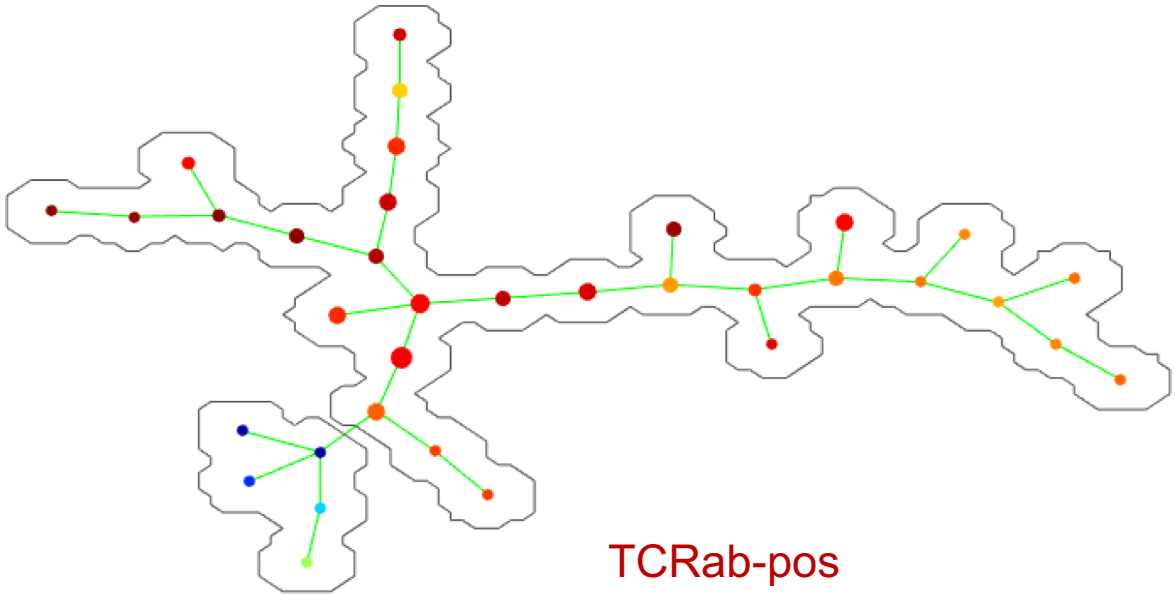


1. Select “Auto Suggestion Annotation”
2. In popup window, accept/reject proffered partition

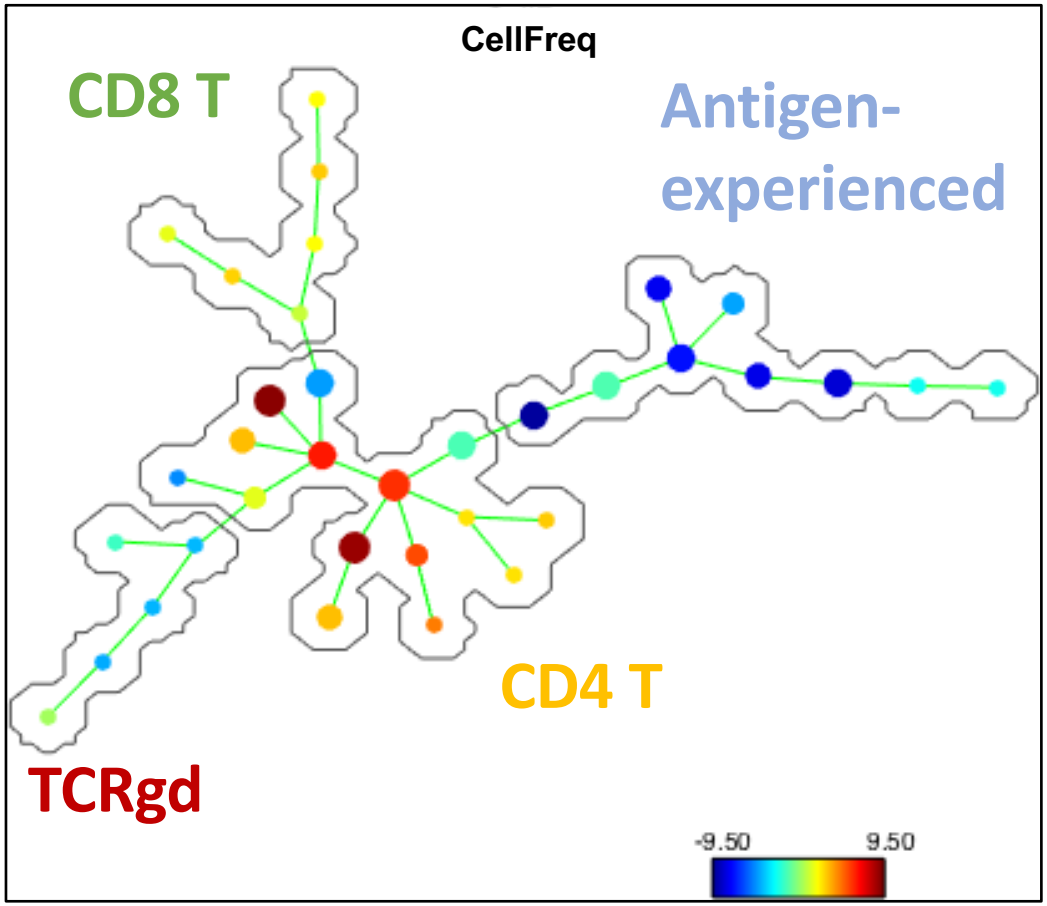
2.



Annotations (unbiased)



Changes in population frequency PsO_Stelara relative to Normal



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Reviews

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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.
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Algorithms

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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**
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