

Comp790-166: Computational Biology

Lecture 13

February 23, 2022

Good Morning Question

Who can tell us about how to define the edges and the node signals in Meld?

Today

- Finish up last results discussion from MELD
- Differential abundance discussion with Cydar
- Start differential abundance analysis with Milo

Intermission for Announcements

- Homework 1 is due by 11:59pm eastern time on **Friday** February 25
- Reminder : Extra office hours Thursday 1-2
- Project proposal template is now online https://github.com/natalies-teaching/Comp790-166-CompBio-Spring2022/blob/main/Project_Proposal/Project_Proposal.pdf
- Sign up a time to present your project on March 7 or March 9, <https://docs.google.com/spreadsheets/d/1fX52jKWDWbJ01iB6D7FHv1DNQ53LPs0S8yNCUnUbSwQ/edit?usp=sharing>

Review : MELD Optimization Problem

To find an appropriate \mathbf{z} , an optimization problem can be defined as,

$$\mathbf{y} = \underset{\mathbf{z}}{\operatorname{argmin}} \underbrace{\|\mathbf{x} - \mathbf{z}\|_2^2}_{\mathbf{a}} + \underbrace{\beta \mathbf{z}^T \mathcal{L} \mathbf{z}}_{\mathbf{b}} \quad (1)$$

Meld Results

Computing the EES cleans up some of the noise and helps to better identify prototypical cells in each experimental condition.

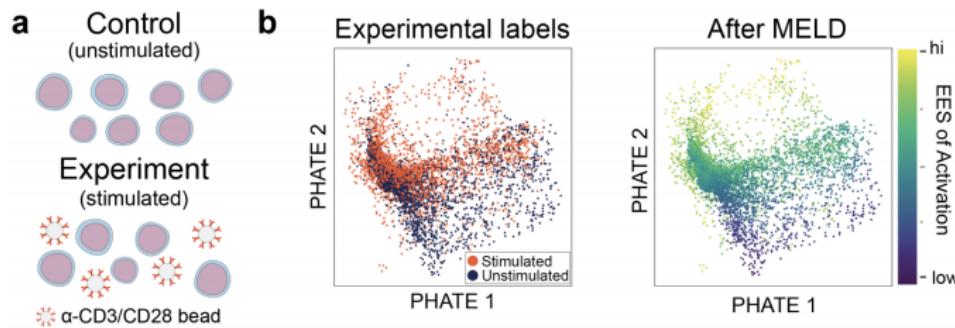


Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021.

Gene Expression Profiles Based on RES and EES

You can look at the gene expression profiles of cells with similar EES scores.

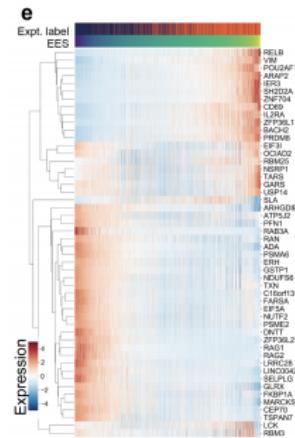


Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021.

Zooming in on High and Low Frequency Regions

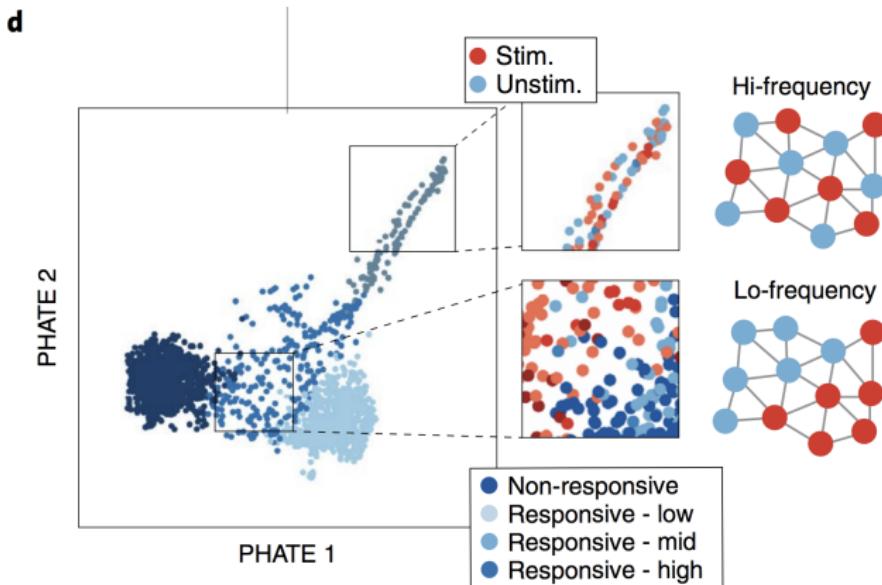


Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021.

A General Question: What's Different Between Clinical Groups

In this example, the abundances of particular cell-types are being compared between patients who have varying mortality likelihoods from COVID.

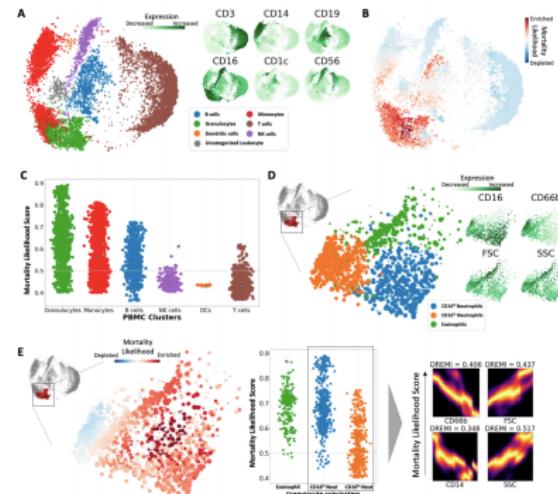


Figure: from Kuchroo et al. 2020. <https://www.biorxiv.org/content/10.1101/2020.11.15.383661v1.abstract>

Problem Formally Stated

Given two patient phenotypes, which cell-populations are statistically, significantly different between groups in terms of **frequency, function**, or 'state'?

- We are exploring this question in a statistical way, rather than through building a classifier or a model. Therefore, we need to look out for multiple testing problems!
- In contrast to Meld, we are no longer looking for prototypical cell examples associated with each condition. Instead, we are testing overlapping subsets of cells for significance.

Welcome Cydar (Nature Methods 2017).

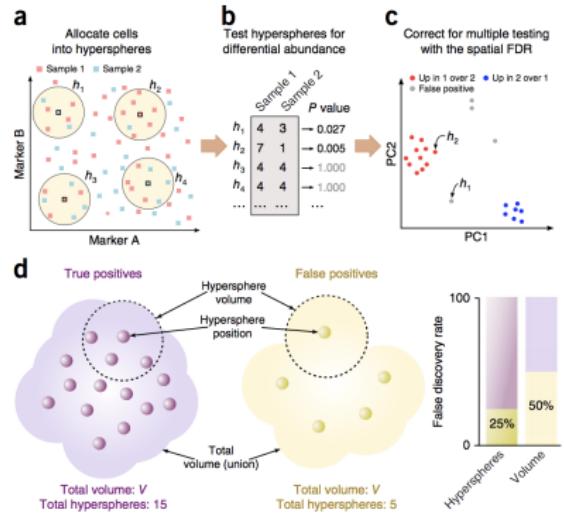


Figure: from Lun *et al.* Nature Methods. 2017.

Clustering vs Other Approaches

- The authors claim they can just cluster cells and then study differential abundance (DA) in each cluster
- They rightfully suggest that the quality of downstream interpretation can be affected by noisy cells, clustering parameters, etc.
- To deal with this, they develop a 'hypersphere' based approach

Assigning Cells to Hyperspheres

Hyperspheres are designed to be centered around existing individual cells.
In particular, around every 10th cell.

- Define the radius of each hypersphere, r , as $r = 0.5\sqrt{M}$. M is the number of markers or parameters measured for each cell
- Any cell is assigned to the hypersphere if their distance to the hypersphere center is within $r = 0.5\sqrt{M}$
- Note that this definition imposes overlap between hyperspheres, or having cells assigned to more than one hypersphere.

Hyperspheres Illustrated

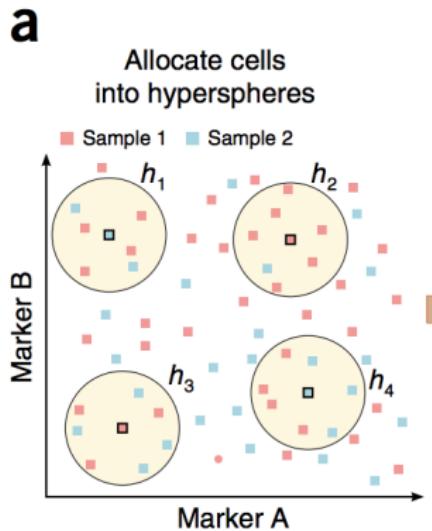


Figure: from Lun *et al.* Nature Methods. 2017.

Testing Differences Between Groups

- First, for each hypersphere, and for each sample, you are going to calculate the proportion of sample's cells assigned to the hypersphere. Again, cells will be counted twice because they can belong to multiple hyperspheres.
- Then you can apply some statistical test (like Wilcoxon Rank Sum Test) to test if the proportion of cells assigned to each hypersphere are the same
- The Null hypothesis is that the mean proportion of cells belonging to each hypersphere should be the same between groups (e.g. treatment and control)

	Sample 1	Sample 2	P value
h_1	4 3		→ 0.027
h_2	7 1		→ 0.005
h_3	4 4		→ 1.000
h_4	4 4		→ 1.000
...

Figure: from Lun et al. Nature Methods. 2017.

Example Null Hypothesis for tReg Hypersphere

H_0 : The number of tRegs is the same between healthy and sick people

H_1 : The number of tRegs between healthy and sick people is not the same

The caveat here is we're doing many, many of such tests so significance can arise by chance!

Intuition Behind Spatial FDR Idea

The spatial FDR can be interpreted as the proportion of the total volume (rather than the sum of individual hypersphere volumes) that is occupied by false positively differentially abundant hyperspheres.

- Hypersphere density differs across the high-dimensional space. So, we will soon see that each hypersphere is weighted by the reciprocal of its density of neighboring hyperspheres.

Spatial FDR

False discoveries are when the null hypothesis (that the abundance is the same between groups) is *falsely* rejected. Cydar computes a spatial FDR, which considers the proportion of the total volume of differentially abundant hyperspheres that are occupied by false-positive hyperspheres.

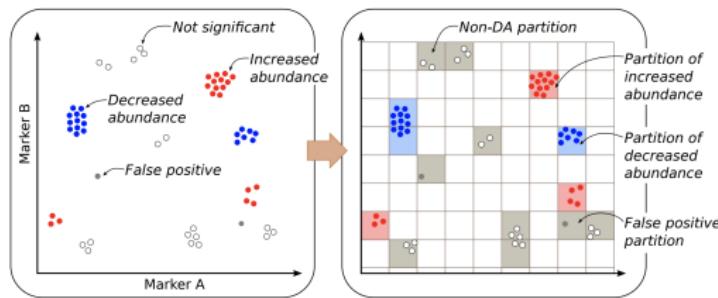


Figure: from Lun *et al.* Nature Methods. 2017.

Spatial FDR, Continued

Each circle is representing a hypersphere colored by increase in abundance (red), decrease (blue), no change (white), or false positive (gray). On the right shows a partition of the space, which is ultimately labeled depending on the hyperspheres comprising it. Partition can be labeled as false positive.

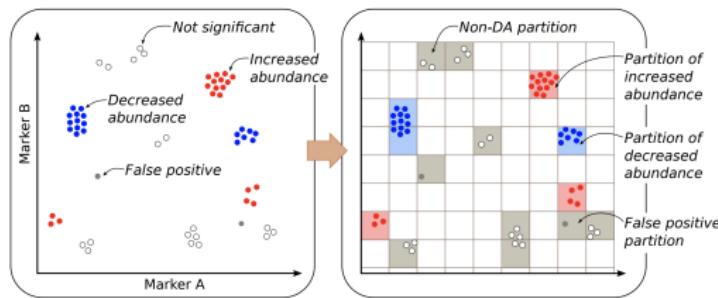


Figure: from Lun *et al.* Nature Methods. 2017.

Weighted Benjamini-Hochberg

- Assume that for n hyperspheres that there are n ordered p -values from the statistical test with $p_1 \leq p_2 \leq \dots p_n$.
- Imagine a partition of your M dimensional space. For a particular hypersphere, its local density will represent how representative it is of that partition.
- For hypersphere, I , define w_I as the weight, which is inversely related to the density of hypersphere I . Local density is defined by the distance of each hypersphere to its 50th nearest neighbor.

Weighted BH, Coontinued

Assuming ordered pvalues $p_1 < p_2 < \dots < p_n$, then the weighted BH method will reject any null hypothesis where the p -value is less than the following. Here α is some threshold at which you would like to control your FDR.

$$\max_i \left\{ p_{(i)} : p_{(i)} \leq \alpha \frac{\sum_{l=1}^i w_{(l)}}{\sum_{l=1}^n w_{(l)}} \right\}$$

Cydar Applied in Practice

They applied Cydar to an MEF dataset (mouse embryonic fibroblast). Samples were collected at 13 timepoints between day 0 and day 20. The goal was to detect subpopulations that change over time.

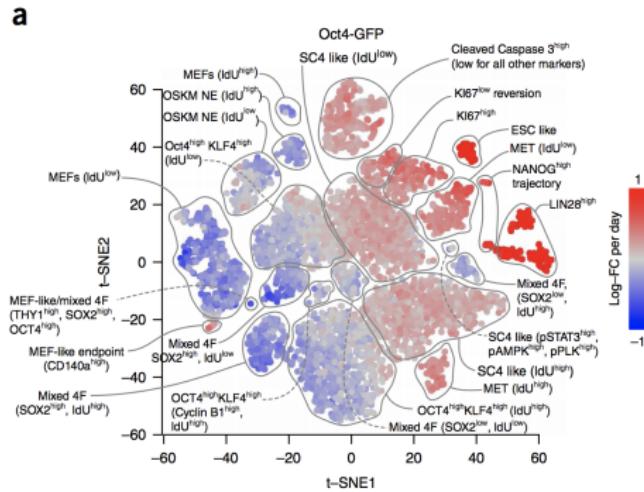


Figure: from Lun et al. Nature Methods. 2017. Each plotted point represents the median position of differentially abundant hyperspheres at an FDR of 5%

A Practical Point about Annotation

Getting a colored tSNE like this is just the beginning. You then need to do the following to describe your cell-populations, or to annotate them by hand.

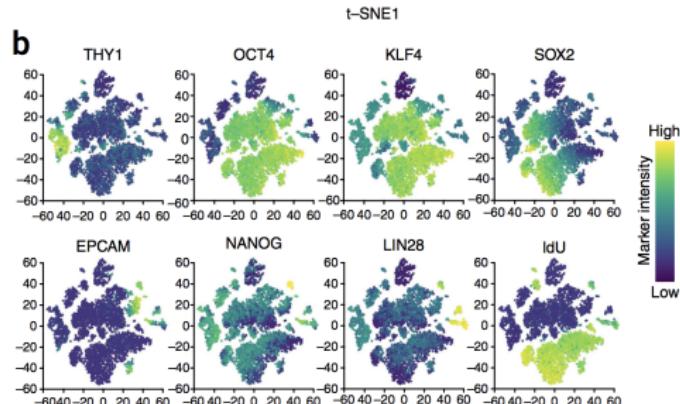


Figure: from Lun et al. Nature Methods. 2017. Color cells by the expression of individual markers.

There has been some work automating this.

Welcome GateFinder (Aghaeepour *et al.* Bioinformatics. 2018). The goal of GateFinder is to tell you the combinations of markers that characterize your cell-population of interest.

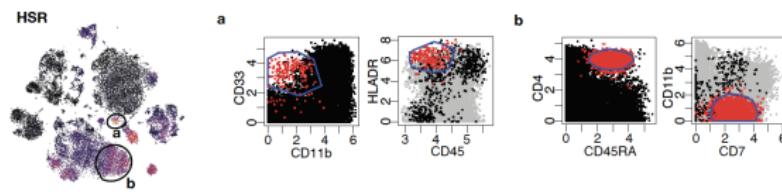


Figure: Nature Communications. 2020. We applied GateFinder to figure out what types of cells our prioritized cells were based on the combinations of markers that were expressed.

Thoughts...

- Why not just define clusters, test clusters, and do something simple like dividing the p -value threshold for significance by the number of tests? (aka Bonferroni)
- My guess it that that is completely driven by visualization. They want a way to visualize individual cells, not cluster centers.
- All of this hypersphere business seems a bit expensive and time consuming if you could just do k -means → test → correct

Example of Cluster-Based Testing

We have done something simpler. We calculated a score for each cell based on a linear combination of similarity (in marker space) to each cluster and that cluster's score.

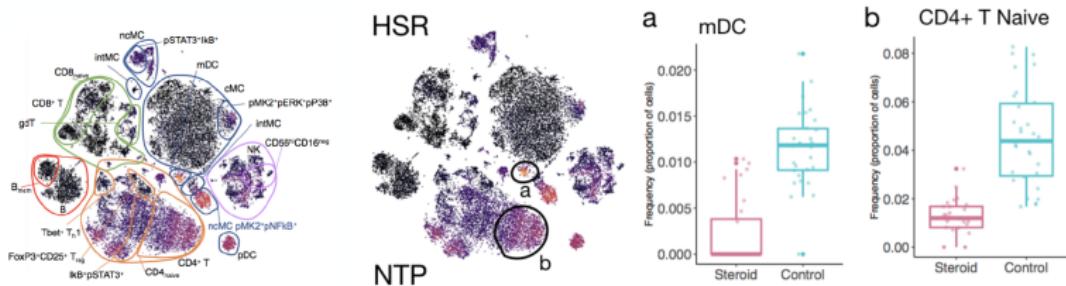
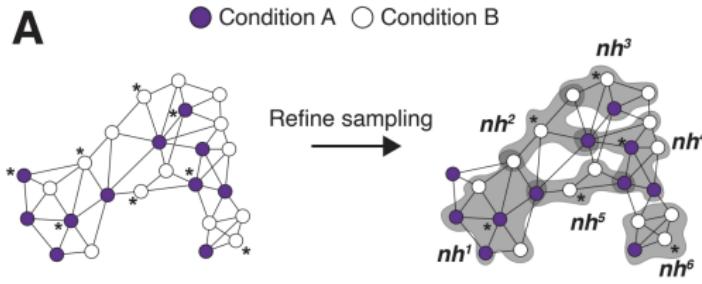


Figure: from Stanley *et al.* Nature Communications. 2020.

Milo

Milo is a graph-based version of Cydar, just recently on BioArXiv.

<https://www.biorxiv.org/content/10.1101/2020.11.23.393769v1>.

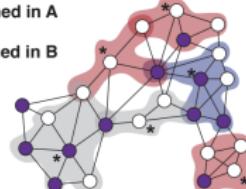


Assign cells to neighbourhoods

	Condition A	Condition B
nh ¹	4 . .	3 . .
nh ²	1 . .	3 . .
nh ³	1 . .	3 . .
nh ⁴	4 . .	2 . .
nh ⁵	2 . .	2 . .
nh ⁶	1 . .	3 . .
.	.	.
nh ⁱ	C _i . .	.

Enriched in A
Enriched in B

$$C_i \sim NB(\mu_i, \phi_i)$$



Test neighbourhoods for differential abundance

General Overview of Milo

- Build k -NN graph of cells
- Define a representative set of nodes to serve as the ‘center’ of neighborhoods across the graph
- Define the neighborhood of a node, j , as the collection of cells that are connected to node j by an edge.
- Count cells in each neighborhood. You end up with a matrix of samples \times counts of cells across neighborhoods.
- Test for differential abundance in neighborhoods Spatial FDR again to control for the proportion of neighborhoods that are false-positive.

How Does Milo Do?

MCC (Matthews Correlation Coefficient) is a performance metric that measures performance from integrating multiple performance metrics.

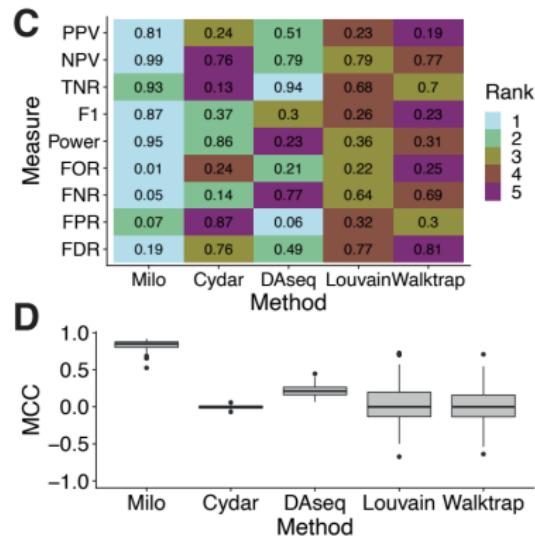


Figure: from Dann *et al.* 2020. BioArXiv

Resulting Visualization of Milo

Milo visualizes the graph between the subsets of selected nodes that were used to form neighborhoods.

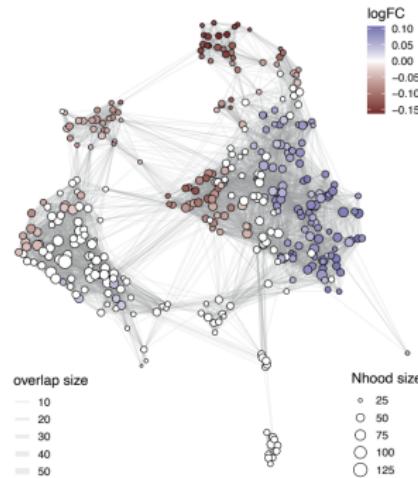


Figure: from Dann *et al.* 2020. BioArXiv. Here the data are single thymic epithelial cells sampled from mice from age 1 to 52 weeks.

Connecting to Ground Truth Labels of the Cells

Each cell has an ‘age’ associated with it. We can see that cells belonging to neighborhoods that had an increased abundance of cells with age are colored blue.

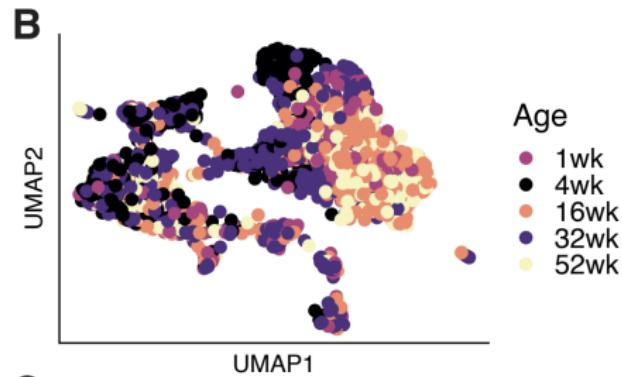


Figure: from Dann *et al.* 2020. BioArXiv. Cells are colored by the age of the mouse that they came from.

Thoughts on Milo + Comparison with Meld

- **Neighborhoods Initialized Randomly.** It seems that we can really do better than choosing nodes at random to serve as the centers of the neighborhoods.
- This problem of choosing seeds on a graph is actually hard. How do you choose seeds that are sufficiently equidistant from other seeds.
(For example, this would be easier on a grid)