Comp790-166: Computational Biology

Lecture 10

February 25, 2021

Announcements

- Thank you for your homework. Feedback within two weeks....
- We will have a reading summary (see my email). You can choose any paper assigned this week or next.
- Project proposal template is online, `https://github.com/stanleyn/Comp790-166-Comp-Bio/tree/main/Projects`

Discussion about Project Proposals

- **Abstract:** Sell your idea in 3-5 sentences. This is really good practice for figuring out what the story is with a project. Convince us why we should care.
- **Formal Problem Statement:** This should be a 1 to 2 sentence summary of what your problem is. Easier said than done.....
- **Contributions:** Think about a list of contributions and then put this into formal writing.
- **Intended Experiments:** Realistically you can aim for 1 to 2 experiments (more if you want!)
- **Implementation:** What is the product that you will give to the scientific community? (e.g. well-documented open source software)

Preliminary Results

It's great if you have preliminary results. If not simple outline a timeline (for example: data cleaning by X data, implementation of baseline methods by Y date)

Today

- Differential cell-population analysis
  - Cydar
  - DiffCyt
  - Milo
- Contrastive PCA

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.

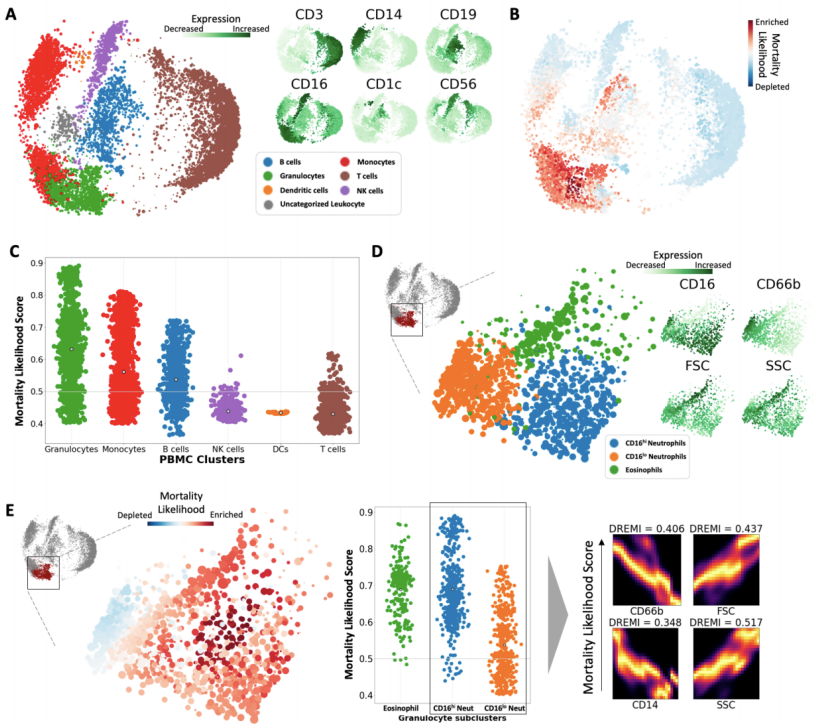


Figure: from Kuchroo *et al.* 2020. `https://www.biorxiv.org/content/10.1101/2020.11.15.383661v1.abstract`

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).

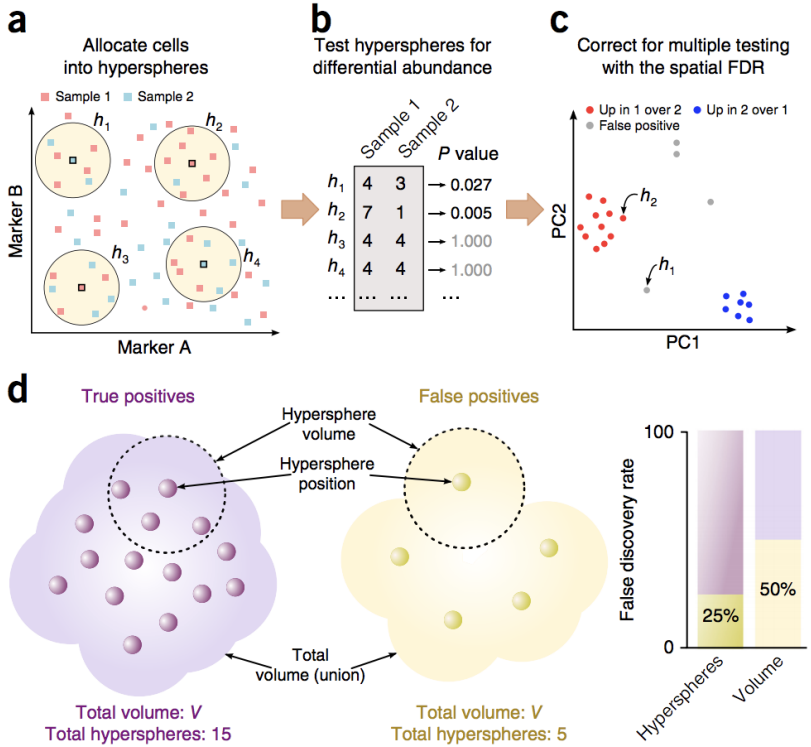


Figure: from Lun *et al.* Nature Methods. 2017.

Clustering vs Other Approaches

- The authors claim they can just cluster cells and then study differential abundance (DA) in each cluster
- The 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

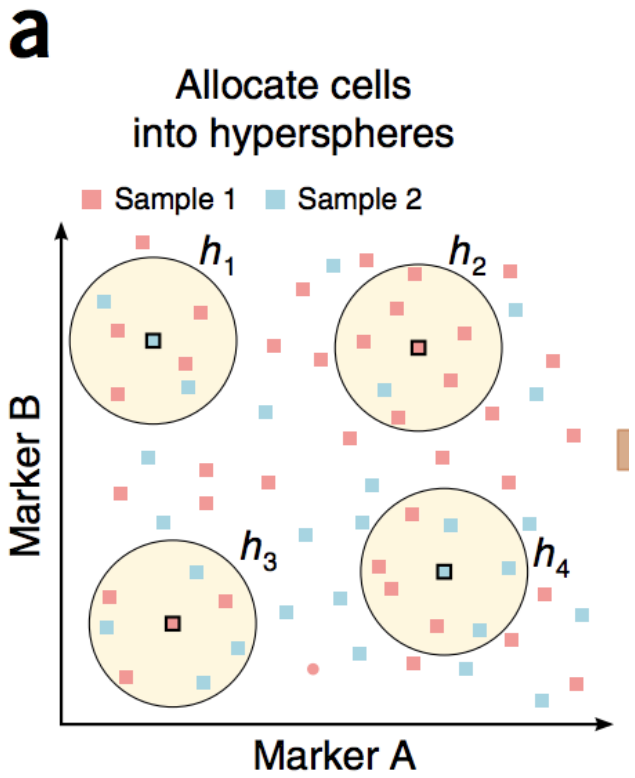
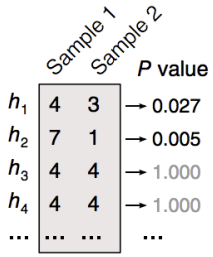


Figure: from Lun *et al.* Nature Methods. 2017.

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)



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 were are 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 recipricol of its density or 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.

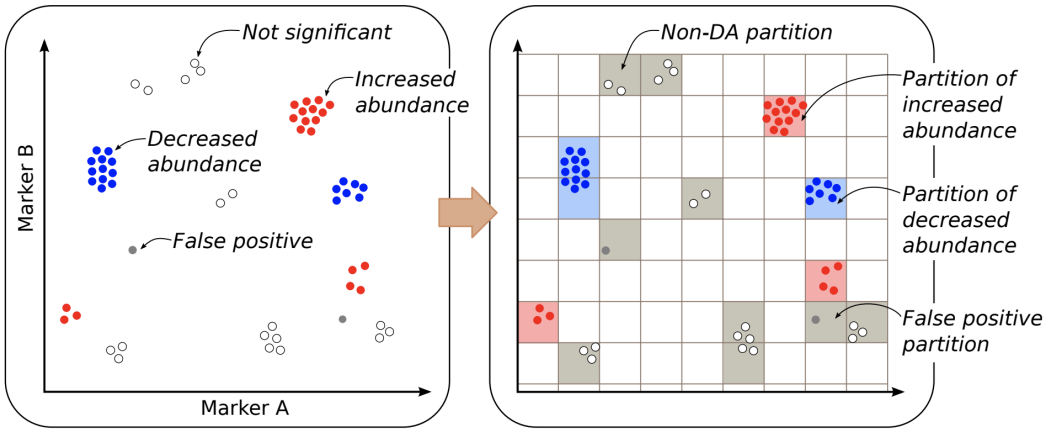


Figure: from Lun *et al.* Nature Methods. 2017.

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, where a hypersphere within a given partition will be chosen as representative

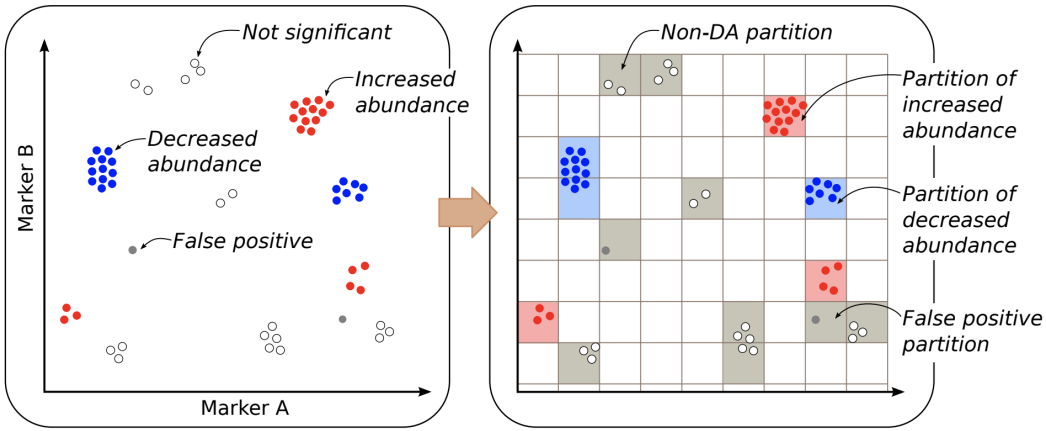


Figure: from Lun *et al.* Nature Methods. 2017.

Weighted Benjamini-Hochberg

- Assume that for n hyperspheres that there are n ordered p-values from the statistical test with $p_1 \leq p_2 \leq \ldots p_n$.
- 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, l, define w_l as the weight, which is inversely related to the density of hypersphere l. If there are few hyperspheres within a local region, there is a high probability that a particular hypersphere will be selected as representative of that partition.

Weighted BH, Coontinued

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 subppulations that change over time.

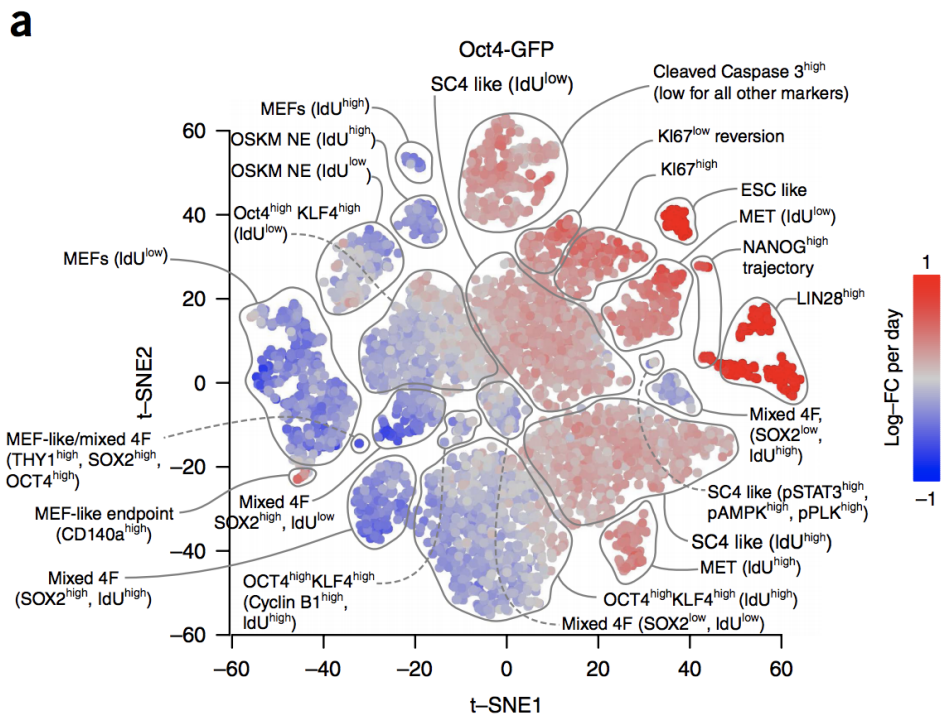


Figure: from Lun *et al.* Nature Methods. 2017. Each plotted point represents the median position of differentially abundant hyperspheres at an FDR of 5%

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.

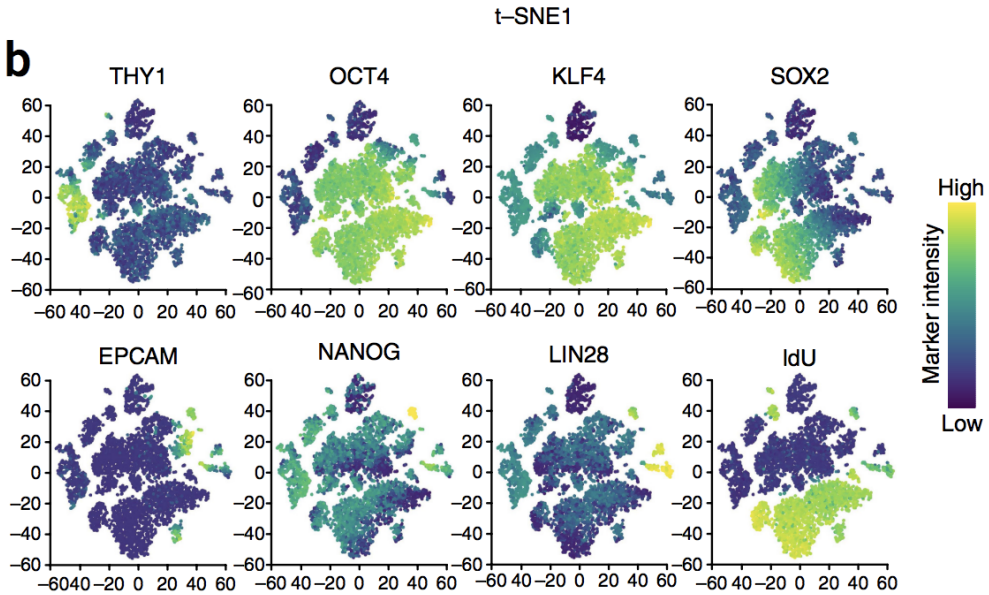


Figure: from Lun *et al.* Nature Methods. 2017. Color cells by the expression of individual markers.

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.

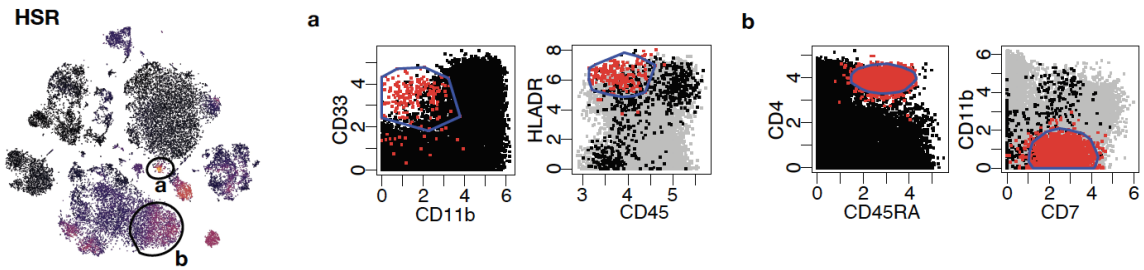


Figure: from Stanley *et al.* Nature Communications. 2020. We applied GateFinder to figure out what types of cells our prioritized cells were based on the combinations of markers that were expressed.

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 Bonferonni)
- 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 $\rightarrow$ test $\rightarrow$ 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.

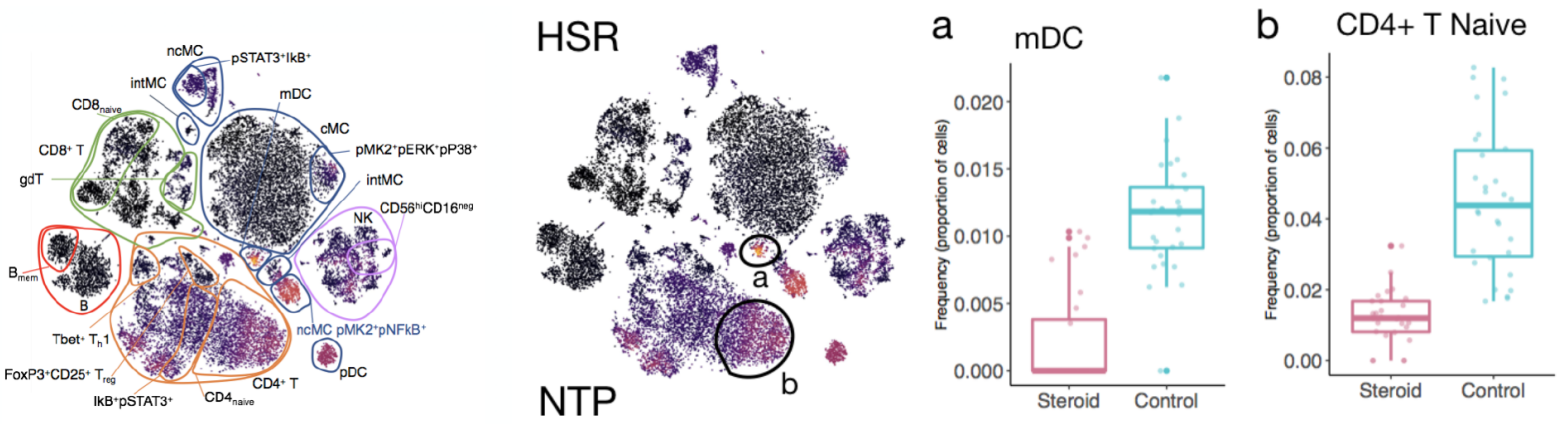
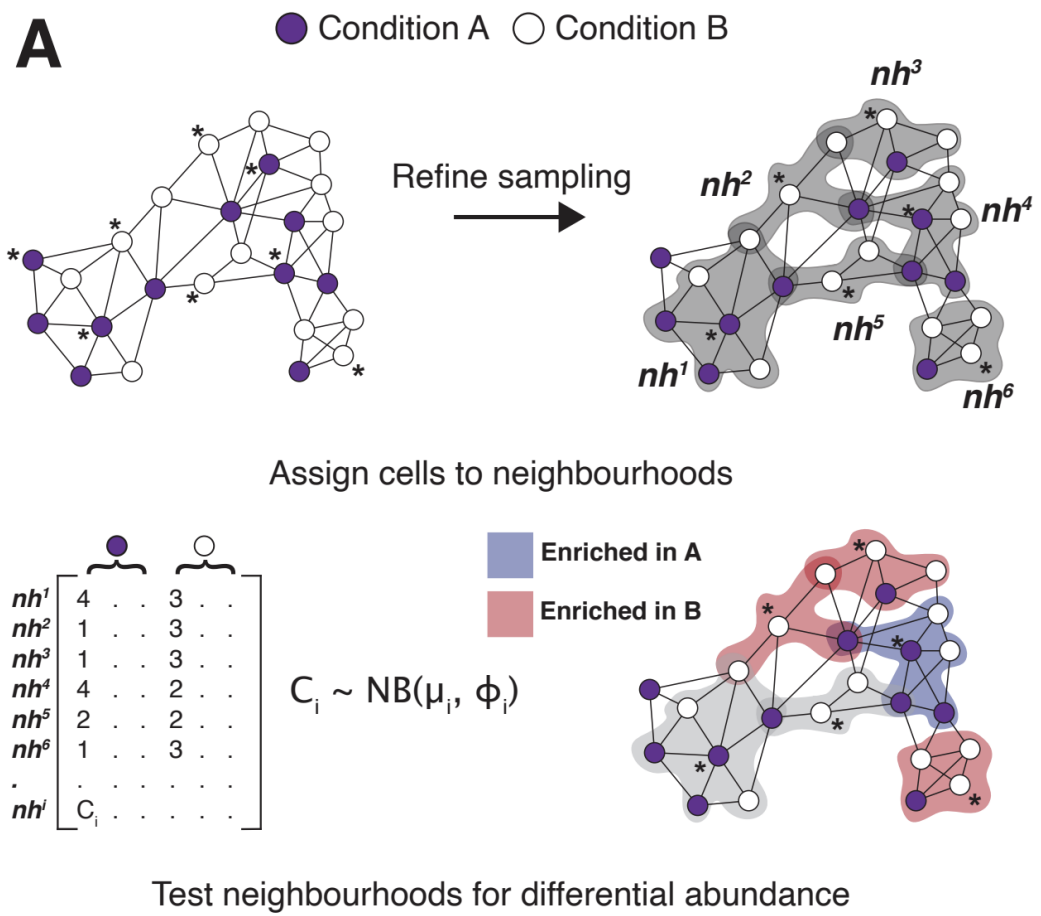


Figure: from Stanley *et al.* Nature Communications. 2020.

Milo

Milo is a graph-based version of Cydar, just recently on BioArXiv. `https://www.biorxiv.org/content/10.1101/2020.11.23.393769v1`.



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.

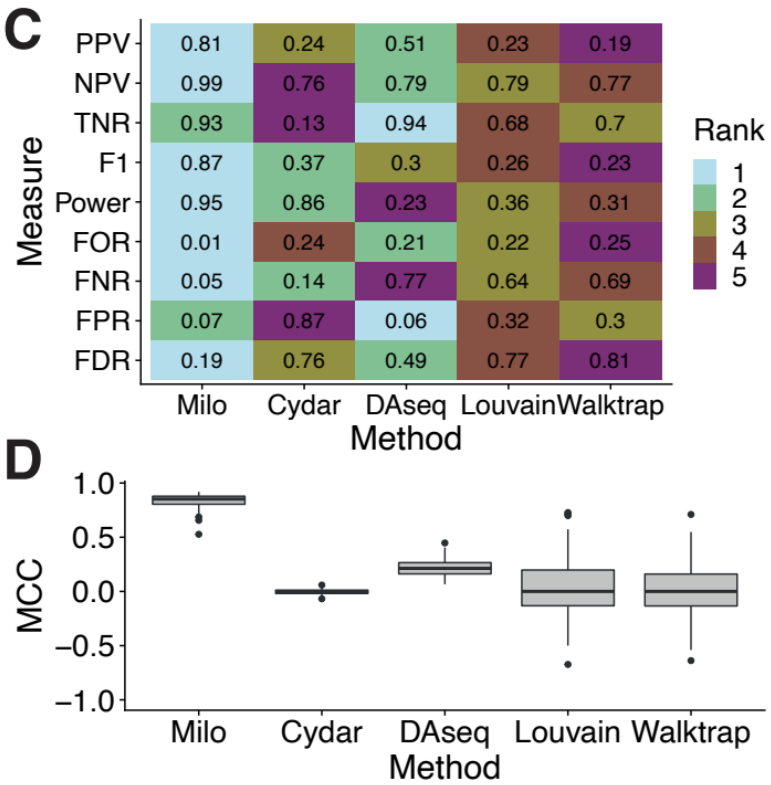


Figure: from Dann *et al.* 2020. BioArXiv

Resulting Visualization of Milo

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

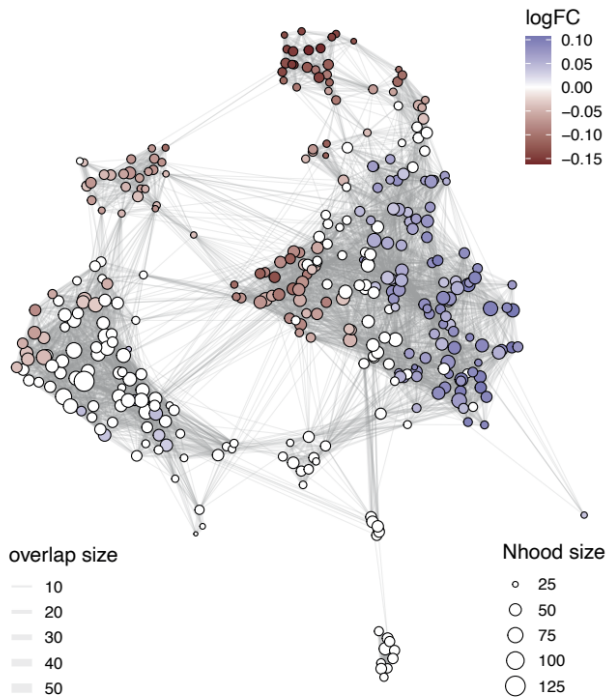


Figure: from Dann *et al.* 2020. BioArXiv. Here the data are single thymic epithelial cells sampled from mice from age 1 to 52 weeks.

Connecting to Ground Truth Labels of the Cells

Each cell as 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.

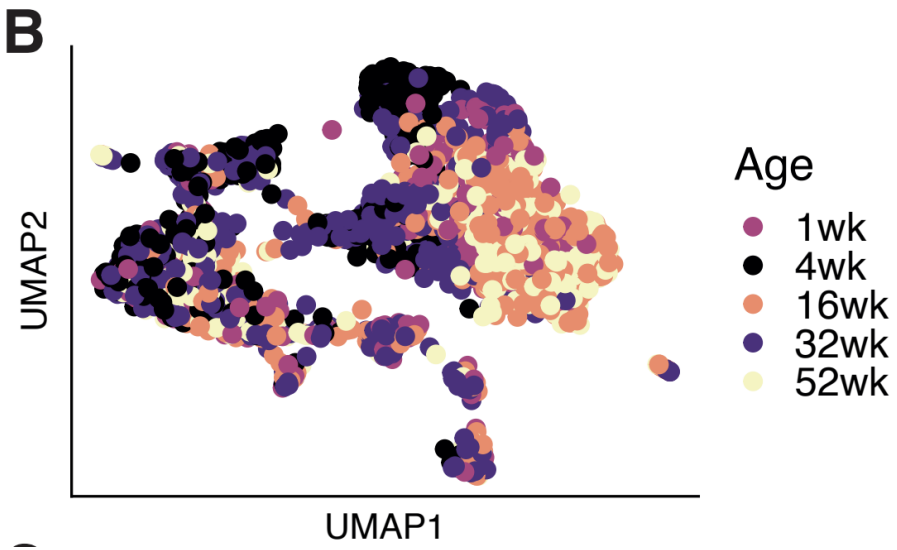


Figure: from Dann *et al.* 2020. BioArXiv. Cells are colored by the age of the mouse that they came from.

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)

Meld vs DA Style Approach

- **Meld:** Collect the set of prototypical cells from each condition (according to Meld Score). Examine differences in prototypical cells from each condition.
- **DA Approach:** Coarse grain cells according to clusters, hyperspheres, neighborhoods, whatever. Do multiple tests and correct.
- Which do you all think is better? Or how about a hybrid of the two?

What if we thinking about a set of control samples as a *background* that we can compare samples from our experiments to?

Example Question and Application

- Consider high-dimensional gene expression measurements collected from people from all over the world.
- Suppose these patient samples also correspond to healthy and cancer patients.
- If the question is to find gene expression patterns associated with cancer subtypes, PCA on our samples may mostly reflect demographic variation between patients, rather than biological variation related to cancer subtypes.

Intro to Contrastive PCA

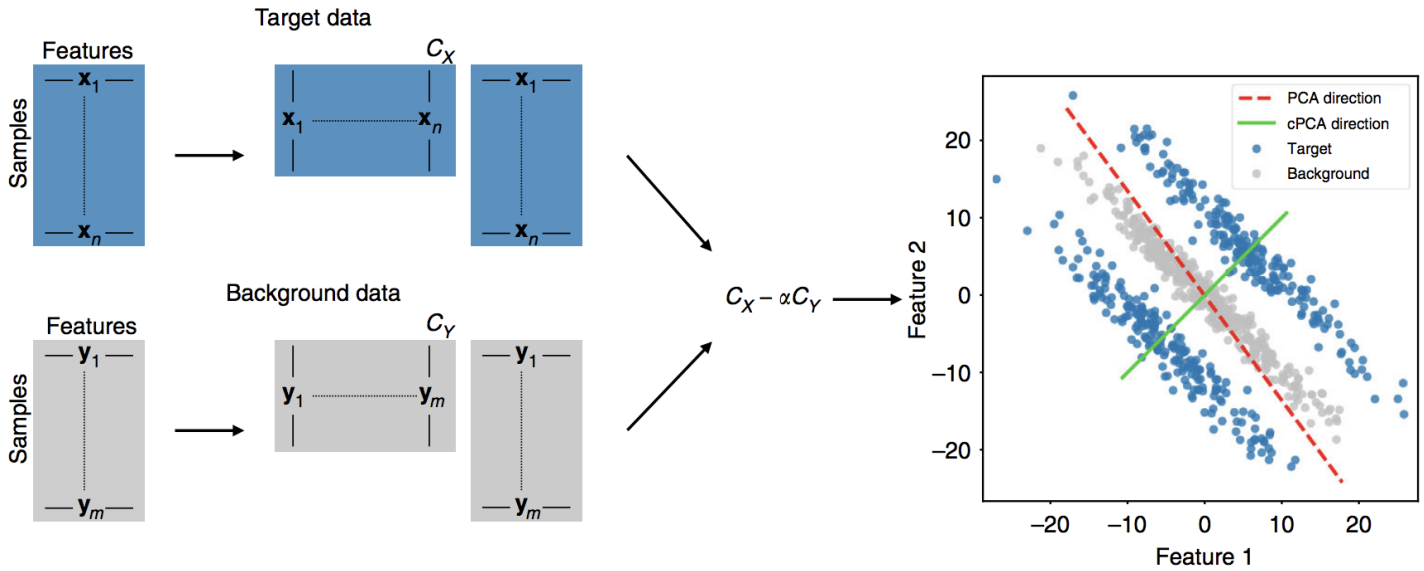


Figure: from Abid *et al.* Nature Communications. 2018. When projecting the data, the goal is to find the target direction that has the highest variance in the target data in comparison to the background data.

Thinking About Background Data

- Given two groups of datapoints (e.g. patient measurements), you can imagine there is variance common to both datasets and variance characteristic of each one.
- For example, thinking about a control group and a disease group, both have population-level variation, but the disease group has particular disease subtypes.
- As another example, consider time series data when you want to decouple variation from a particular timepoint from variation across the entire time series.
- Choice of basckground dataset is important here and should ideally contain ‘structure‘ that we would like to remove form the target data.

Motivating Biological Examples

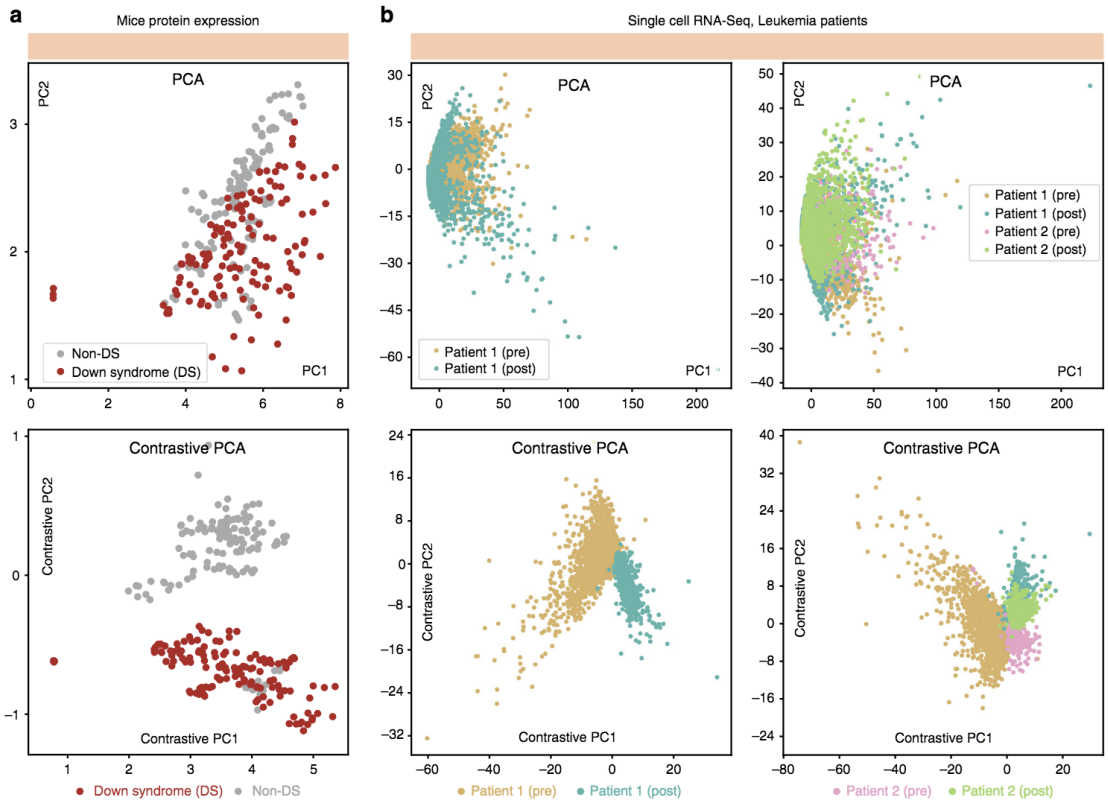


Figure: from Abid *et al.* Nature Communications. 2018. (Left) : Protein expression in Down Syndrome vs Non Down Syndrome Mice. Single cell data pre and post transplant.

cPCA Problem Setup

- Assuming we start with d-dimensional target data $\{\mathbf{x}_i \in \mathbb{R}^d\}$ background data $\{\mathbf{y}_i \in \mathbb{R}^d\}$

For some direction vector, $\mathbf{v} \in \mathbb{R}^d_{\text{unit}}$ the variance it accounts for in the target and background data can be expressed as,

$$\text{Target data variance} : \lambda_X(\mathbf{v}) \stackrel{\text{def}}{=} \mathbf{v}^T \mathbf{C}_X \mathbf{v}$$

$$\text{Background data variance} : \lambda_Y(\mathbf{v}) \stackrel{\text{def}}{=} \mathbf{v}^T \mathbf{C}_Y \mathbf{v}$$

What is happening here and what does this remind you of?

Given a contrast parameter $\alpha \geq 0$ that quantifies the trade-off between having high target variance and low background variance, cPCA computes the contrastive direction $\mathbf{v}^*$ by optimizing

$$\mathbf{v}^* = \text{argmax}_{\mathbf{v} \in \mathbb{R}^d_{\text{unit}}} \; \lambda_X(\mathbf{v}) - \alpha \lambda_Y(\mathbf{v})$$

This problem can be rewritten as

$$\mathbf{v}^* = \text{argmax}_{\mathbf{v} \in \mathbb{R}^d_{unit}} \; \mathbf{v}^T \left(C_X - \alpha C_Y \right) \mathbf{v}$$

cPCA is Quite Simple!

Algorithm 1 cPCA for a Given α

Inputs: target data $\{\mathbf{x}_i\}_{i=1}^n$; background data $\{\mathbf{y}_i\}_{i=1}^m$; contrast parameter α; the number of components k.

Centering the data $\{\mathbf{x}_i\}_{i=1}^n$, $\{\mathbf{y}_i\}_{i=1}^m$.
Calculate the empirical covariance matrices:

$$C_X = \frac{1}{n}\sum_{i=1}^{n} \mathbf{x}_i\mathbf{x}_i^T, C_Y = \frac{1}{m}\sum_{i=1}^{m} \mathbf{y}_i\mathbf{y}_i^T.$$

Perform eigenvalue decomposition on

$$C = (C_X - \alpha C_Y).$$

Compute the the subspace $V \in \mathbb{R}^k$ spanned by the top k eigenvectors of C.
Return: the subspace V.

Figure: Just do eigendecomposition on **C** and consider the eigenvectors corresponding to the top k eigenvalues of C.

Effect of Varying α

For $\alpha = 0$, cPCA will create directions that maximize the target variance. For higher α, directions with smaller background variance become more important.

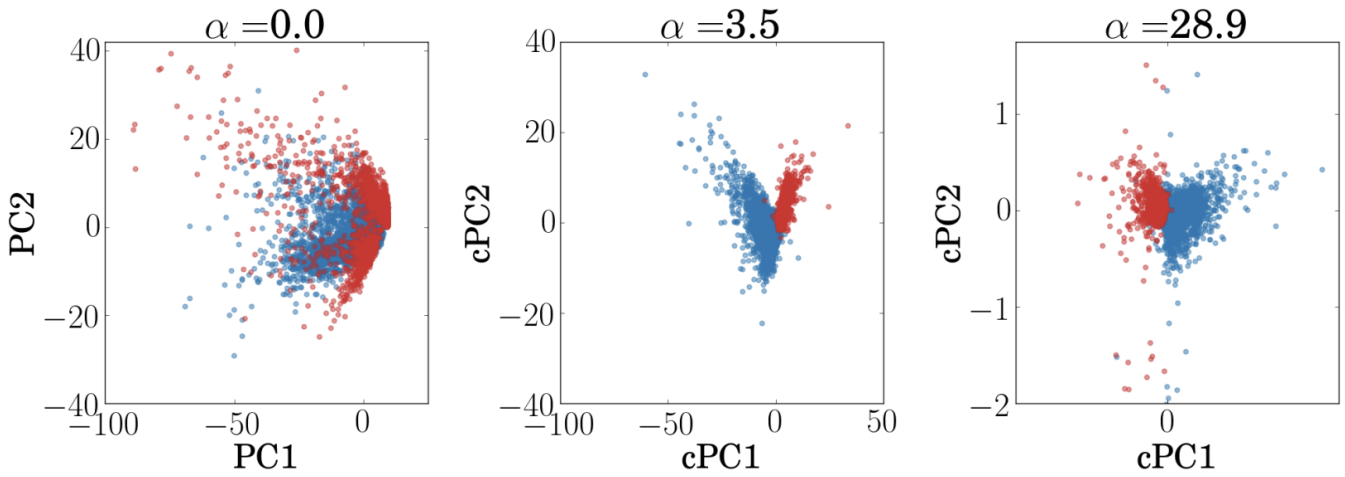


Figure: from Abid *et al.* Nature Communications. 2018. This dataset is visualizing cells from two different samples.

Recap

- DA analysis with Cydar and Milo
- Thinking about Meld vs DA style analyses
- Contrastive PCA

Next time,

- Batch Effects
- Integrating Multiple Single Cell Datasets