

Comp683: Computational Biology

Lecture 7

Feb 3, 2025

Today → Single Cell Intro Day

- Automating Human Gating with Spade
 - What is a gate?
- Practical Considerations (normalization, batch effects)

SC Proteomics: Flow and Mass Cytometry

Flow → 18-20 proteins per cell at a rate of 10,000 cells per second!

Mass → 36-45 proteins at a rate of 1,000 cells per second

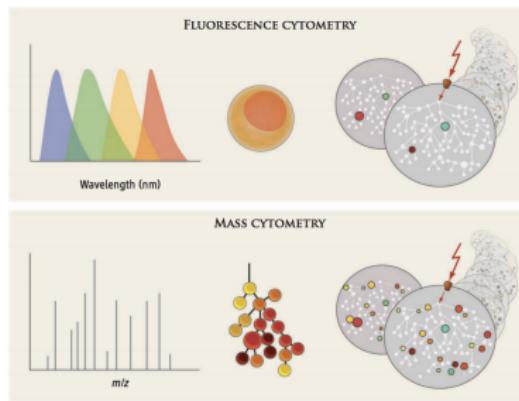


Figure: from Benoist and Hacohen. Science. 2011

I have my manually gated cell-populations, now what?

We look at particular immune cell-types to understand how the immune system is responding in particular circumstances (for example, pre-term birth).

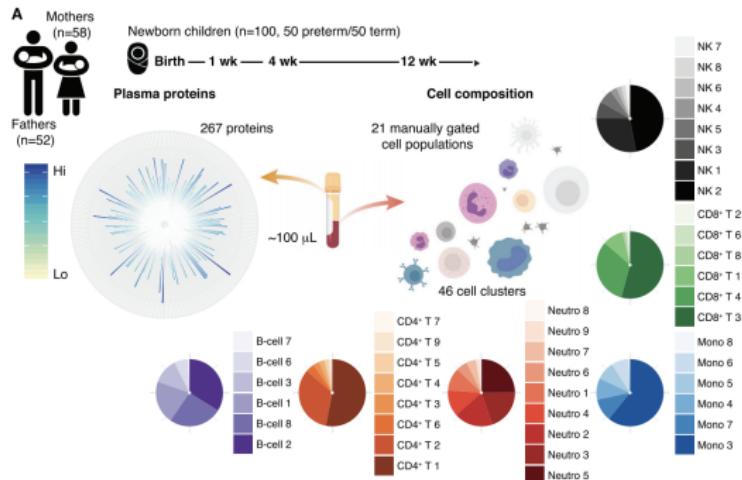


Figure: from Olin et al. Cell. 2018

What Properties of Cell-Populations do we Study?

You can count cells in each population (known as frequency, or f_q), or characterize signaling activity across multiple stimulations (e.g. experimental perturbations).

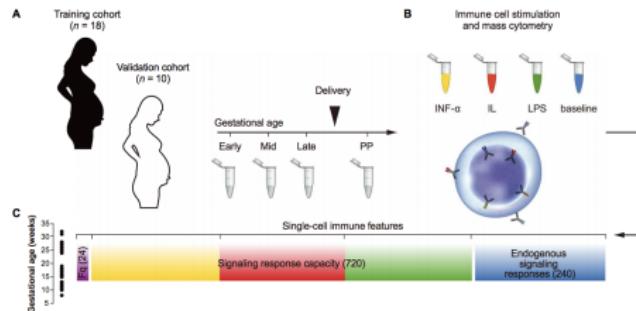


Figure: from Aghaeepour *et al.* Science Immunology. 2017

Extracting Features for Prediction Tasks

- Start with a matrix of **cells \times protein expression** for each patient sample
- Define populations through gating
- Compute function or frequency based features for each population

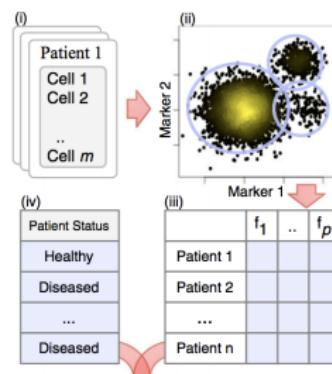


Figure: from Bruggner et al. PNAS. 2014

What Are Cell Frequencies?

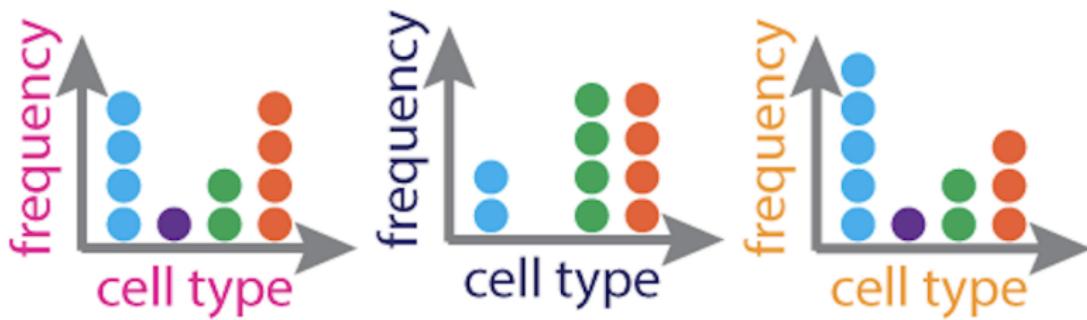


Figure: Simply count the number of cells in each profiled sample assigned to each specific type. This is a very simple hand engineered feature.

Practical Concerns. Converting data profiled with CyTOF into a matrix that can be used to understand a biological question.

clinical outcome classification

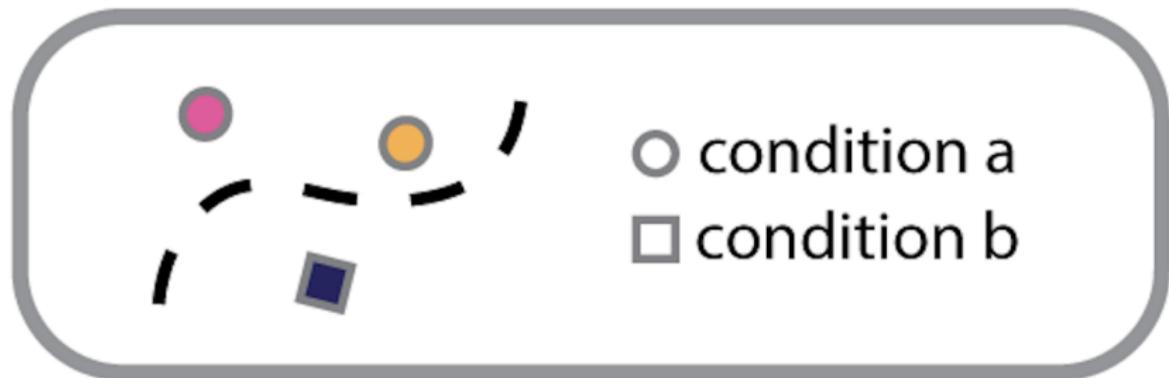


Figure: Each point is representing a different patient sample. How do we ultimately specify a set of features for each sample that can be used to classify them correctly?

FCS file

- The collection of cells for each sample are stored in an 'FCS' file. Cells are also called **events**.
- FCS stands for 'Flow Cytometry Standard' and contains information about the experiment, like human interpretable names for markers, channel names, information about any performed normalization, etc.
- You will need to process each individual sample file.

Dealing with These Data

You can find a lot of publicly available multisample flow and mass cytometry datasets on Flow Repository (<http://flowrepository.org/>)

File	Size	MD5sum	Type
Gates_PTLG021_IFNa_LPS_Control_1.fcs	27.9 MB	53e4367f87 ...	FCS 3.0
Gates_PTLG021_IFNa_LPS_Control_2.fcs	37.5 MB	7aab21b8cb ...	FCS 3.0
Gates_PTLG021_Unstim_Control_1.fcs	30.9 MB	b36fdfbf05 ...	FCS 3.0
Gates_PTLG021_Unstim_Control_2.fcs	26 MB	42676ee1b3 ...	FCS 3.0
Gates_PTLG025_IFNa_LPS_Control_1.fcs	26.7 MB	4cc5b6c07c ...	FCS 3.0
Gates_PTLG025_IFNa_LPS_Control_2.fcs	35.7 MB	f87c5e6356 ...	FCS 3.0
Gates_PTLG025_Unstim_Control_1.fcs	29.5 MB	1a3d2d9448 ...	FCS 3.0
Gates_PTLG025_Unstim_Control_2.fcs	28.9 MB	d8b3989dfb ...	FCS 3.0
Gates_PTLG026_IFNa_LPS_Control_1.fcs	32.6 MB	17ab8c9b98 ...	FCS 3.0
Gates_PTLG026_IFNa_LPS_Control_2.fcs	37.9 MB	b370371ed9 ...	FCS 3.0
Gates_PTLG026_Unstim_Control_1.fcs	36 MB	0f9c4dfd2d ...	FCS 3.0
Gates_PTLG026_Unstim_Control_2.fcs	39.8 MB	4687536e38 ...	FCS 3.0
Gates_PTLG027_IFNa_LPS_Control_1.fcs	26.8 MB	787f4a6366 ...	FCS 3.0
Gates_PTLG027_IFNa_LPS_Control_2.fcs	35.3 MB	3b2ab7e2a2 ...	FCS 3.0

Figure: One FCS file per sample.

Step 1: Separating Markers into Function vs Phenotypic vs Experimental

- There are 3 types of columns in an FCS file
- Phenotypic marker columns help us to characterize particular cell-populations (hint, usually starts with **CD**).
- Functional marker columns help us to quantify signaling or other function within a cell
- ‘Junk’ markers- help to keep track of which cells died, etc.
- After filtering out dead cells, we will only do analysis based on functional or phenotypic markers.

First Step of Manual Gating : Extract Live Cells

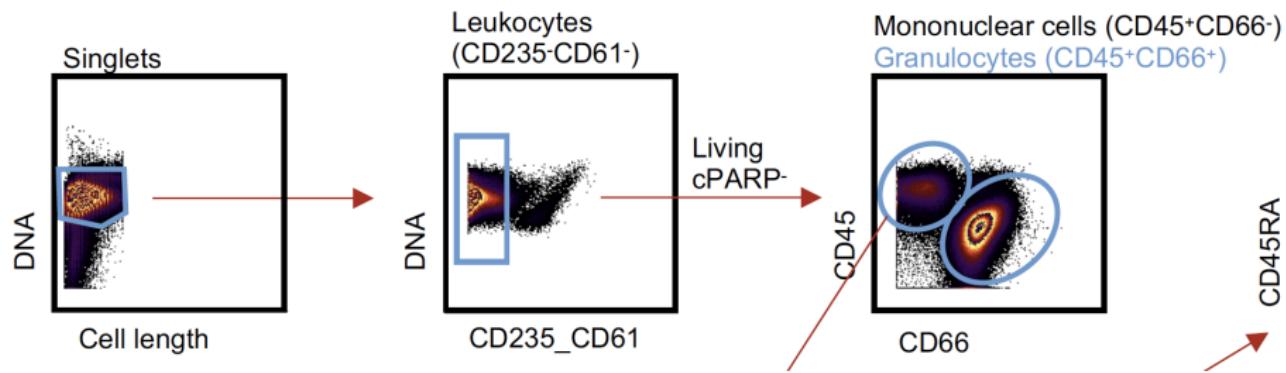


Figure: from Stanley *et al.* Nature Communications. 2020

Example Marker Names from an FCS File

```
>>> sample.pnn_labels  
['Time', 'Cell_length', 'DNA1', 'DNA2', 'CD45RA', 'CD133', 'CD19', 'CD22', 'CD11b', 'CD4', 'CD8', 'CD34', 'Flt3', 'CD20', 'CX3C', 'CD45', 'CD123', 'CD321', 'CD14', 'CD33', 'CD47', 'CD11c', 'CD7', 'CD15', 'CD16', 'CD44', 'CD38', 'CD13', 'CD3', 'CD61', 'CD64', 'HLA-DR', 'CD64', 'CD41', 'Viability', 'file_number', 'event_number', 'label', 'individual']
```

Figure: For example, DNA1 and DNA2 help us to find dead cells. Markers like 'CD19' help us to find specific cell-types. 'CD19' in particular characterized B-cells! Event number is a **junk marker example**, which is just counting the cells.

Step 2: Transform Marker Expressions

In the cell \times marker matrix, we are counting the number of detected ions or joins between protein and heavy-metal tagged antibody. We will use a transformation, to compress the upper end of the spectrum and enhance the lower end.

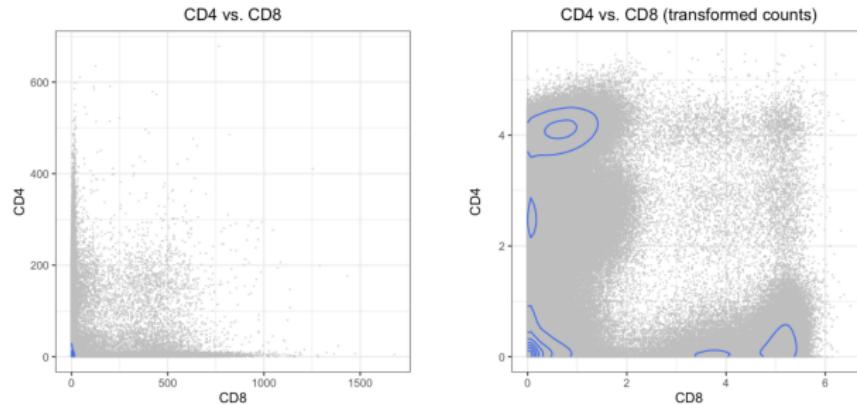


Figure: from https://biosurf.org/cytof_data_scientist.html

Arcsinh Transformation

In practice, especially with mass cytometry data, it is common practice to use an Arcsinh transformation, with co-factor aka scaling factor of 5. For count x , the transformed value $x' = \text{asinh}(\frac{1}{5}x)$

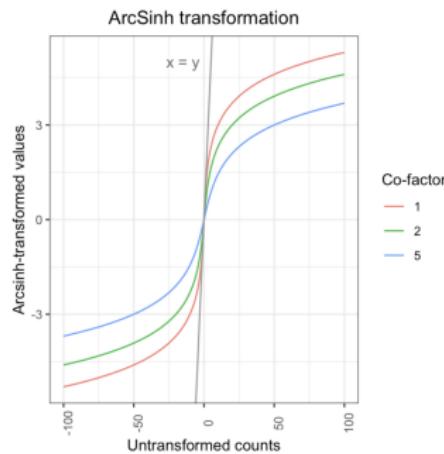


Figure: from https://biosurf.org/cytof_data_scientist.html

Effect of Normalization on Gating

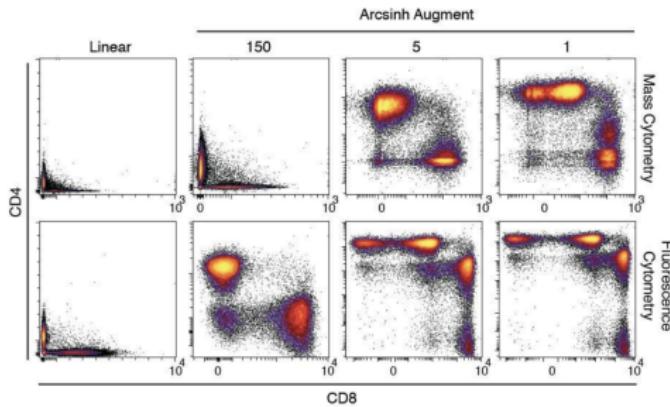


Figure: from Bendall *et al.* Science. 2011. The cofactor can cause sensitivity in the number of populations that emerge.

Tutorial

I have a tutorial for converting FCS files into matrices for both Python and R. https://github.com/stanleyn/fcs_tutorial.

Recap

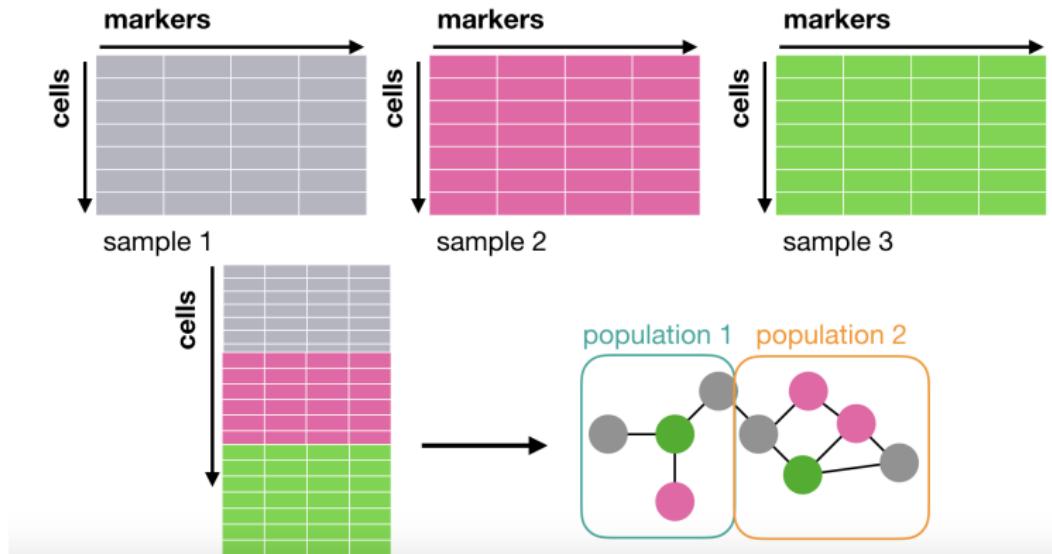


Figure: We are processing each FCS file, which corresponds to an individual sample. Ultimately cells are pooled across all samples.

To Automate Gating or to Not Automate Gating

Ask your collaborator to give you FCS files for each sample with only live cells included. If you ask them for gates, it will take them a lot of work. But you can make your own gates through unsupervised clustering!

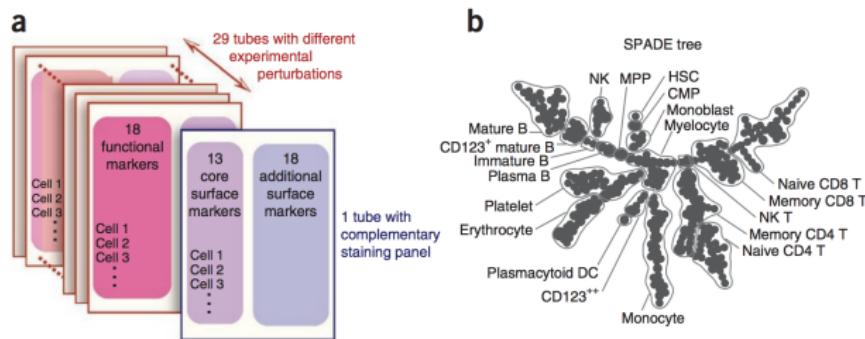


Figure: from Qiu *et al.* Nature Biotechnology. 2011. Do agglomerative hierarchical clustering based on the expression of measured markers.

Why Hierarchical Clustering?

It recapitulates our general understanding of cellular differentiation.

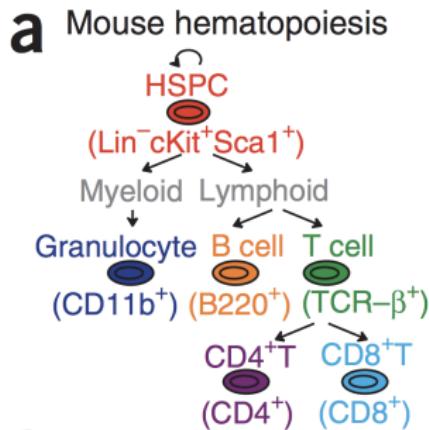


Figure: from Qiu *et al.* Nature Biotechnology. 2011. Cells can differentiate from Stem Cells to more specialized cell-types.

A Practical Consideration

- For N cells (and recall that N is large, like $>100K$), we need to compute an $N \times N$ distance matrix.
- If we have 100 samples, we will have around $100K \times 100$ total cells.
- We can't calculate all of those distances! So, right now, hierarchical clustering fails...

Question for You

Do you have any ideas about how you might reduce the size of this problem of the analysis of such a large number of cells across multiple samples?

Density-Dependent Downsampling

- Downsampling is a popular approach where a limited number of samples is samples for each FCS file.
- This is not ideal, because downsampling causes information loss.
- Spade does this in a density-dependent way, by sampling subsets of cells, and computing densities of their neighborhoods. Hence, Spade ensures that cells are represented across neighborhoods, especially in sparse neighborhoods.

Visualization in Spade : Cluster-Level

Construct a minimum spanning tree between clusters, based on the median expression of the markers.

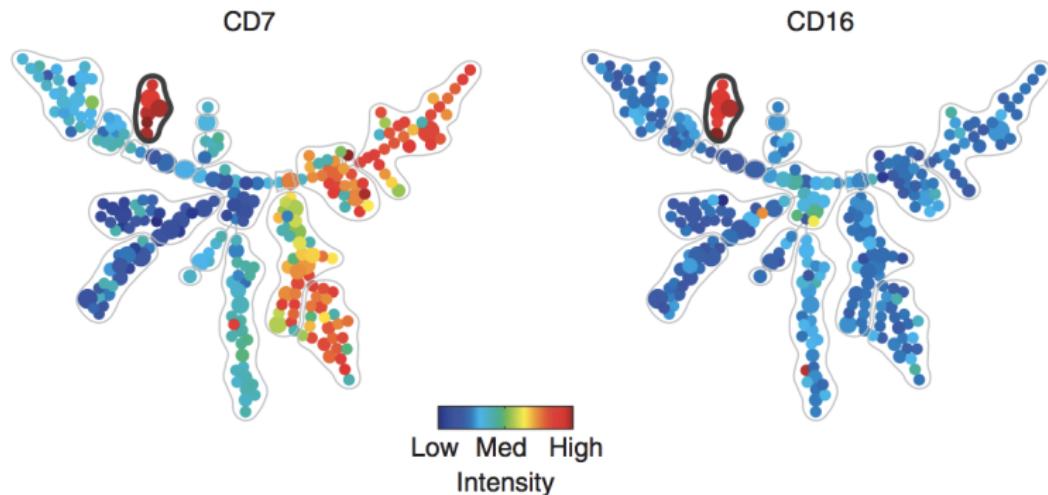


Figure: from Qiu *et al.* Nature Biotechnology. 2011.

Warning : Batch Effects

As with every biological assay, there is technical variation. For example, half the samples run on a machine in California, and the other half run on a machine in North Carolina. NC and CA samples might look very different from each other.

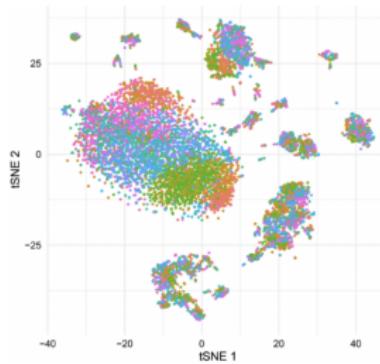


Figure: from Van Gassen *et al.* Cytometry A. 2019. Here cells are colored by batch.

Your job as a collaborator

- Batch effects are the most problematic when they correlate with the patient label that you are hoping to predict.
- If you could perfectly separate patients, it would be difficult to know if the success was because of a batch effect, or true biological signal.
- As a collaborator, you need to suggest for as much randomization as possible! E.g. have batches that have healthy and sick, for example

All of the Graph Knowledge Finally Applied....

Phenograph starts with a graph representation, sparsifies, and then clusters.

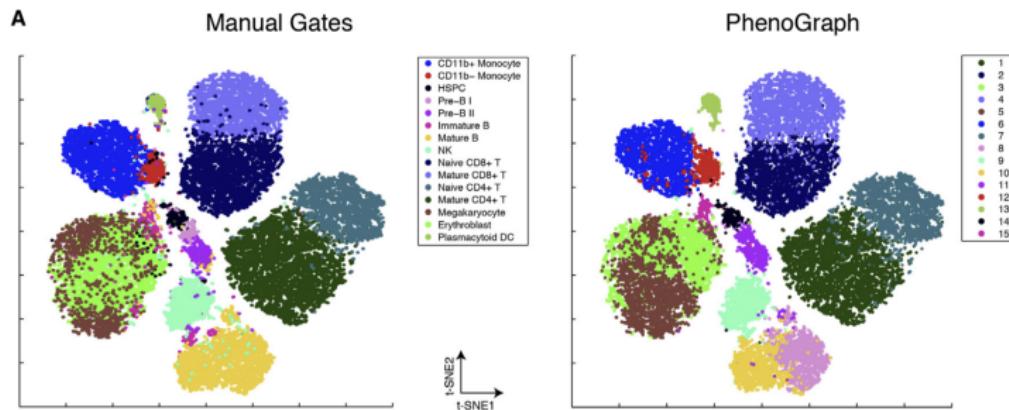


Figure: from Levine *et al.* Cell. 2015

A Direct Application of Louvain

- PhenoGraph is sold as being able to capture cell-populations of various sizes.
- A graph of cells is constructed for each samples
- Edges that exist are then turned into weighted edges, based on shared neighbors (a good contribution)
- The graph for each sample is partitioned with Louvain
- Clusters between samples are mapped with a metaclustering approach.

Communities Map to Cell-Populations

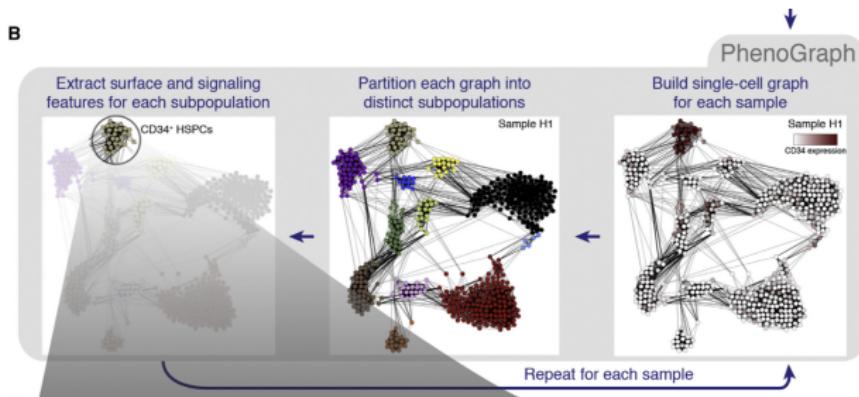


Figure: from Levine et al. Cell. 2015

Refining the k NN graph

Suppose an edge exists between nodes i and j . Then their edgeweighth, w_{ij} is defined as the Jaccard Score of shared neighbors as,

$$w_{ij} = \frac{|v(i) \cap v(j)|}{|v(i) \cup v(j)|} \quad (1)$$

Here, $v(i)$ is the set of neighbors of node i . $|\cdot|$ is cardinality (number of neighbors)

Evaluating Similarity to Manual Gates

It's a bit reassuring to know that the performance (in terms of F-measure) is stable, regardless of the choice of k .¹

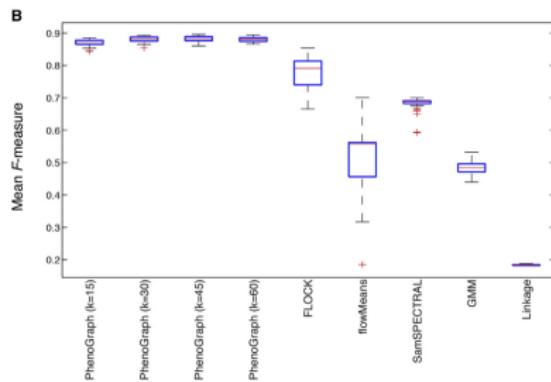


Figure: from Levine *et al.* Cell. 2015

¹discussion of F measure <https://www.nature.com/articles/nmeth.2365.pdf>

Healthy vs AML with Cell Frequencies

Calculate the proportion of each sample's cells assigned to a particular metacluster. As you can see, there are differences in frequency between healthy (first few rows) and AML.

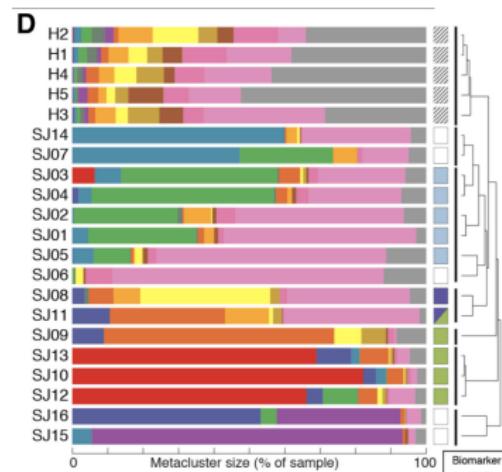


Figure: from Levine *et al.* Cell. 2015

You are ready for your first CyTOF dataset

If the data have signal, cell frequencies are a pretty powerful biologically interpretable feature that can allow us to classify patient samples.

To recap,

- Define per sample clusters
- Define metaclusters representing all samples
- Map cells from individual cells to metaclusters
- Compute frequency features
- You have now build a feature matrix that can be used for classification!

Example of a Powerful Frequency Feature

Frequency differences can be quite prominent in clinical settings. In this example, we are studying differences between patients who received steroid treatment after surgery, versus not.

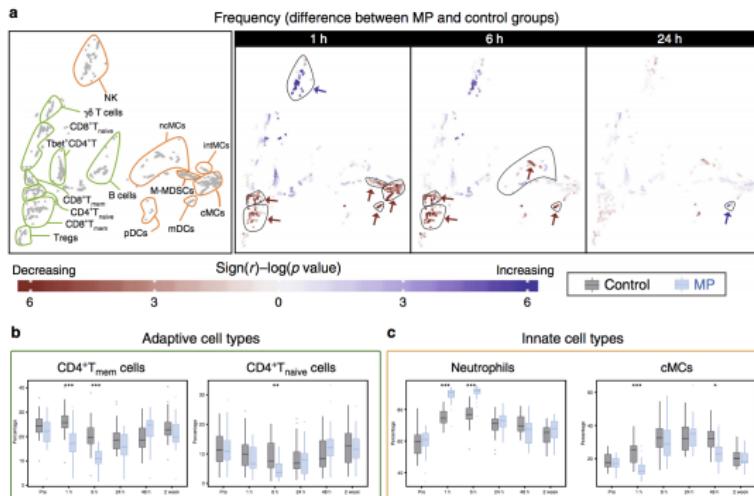


Figure: from Nature Communications. 2019.