Introduction to high-parameter analysis

For mass cytometry

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Let's get started

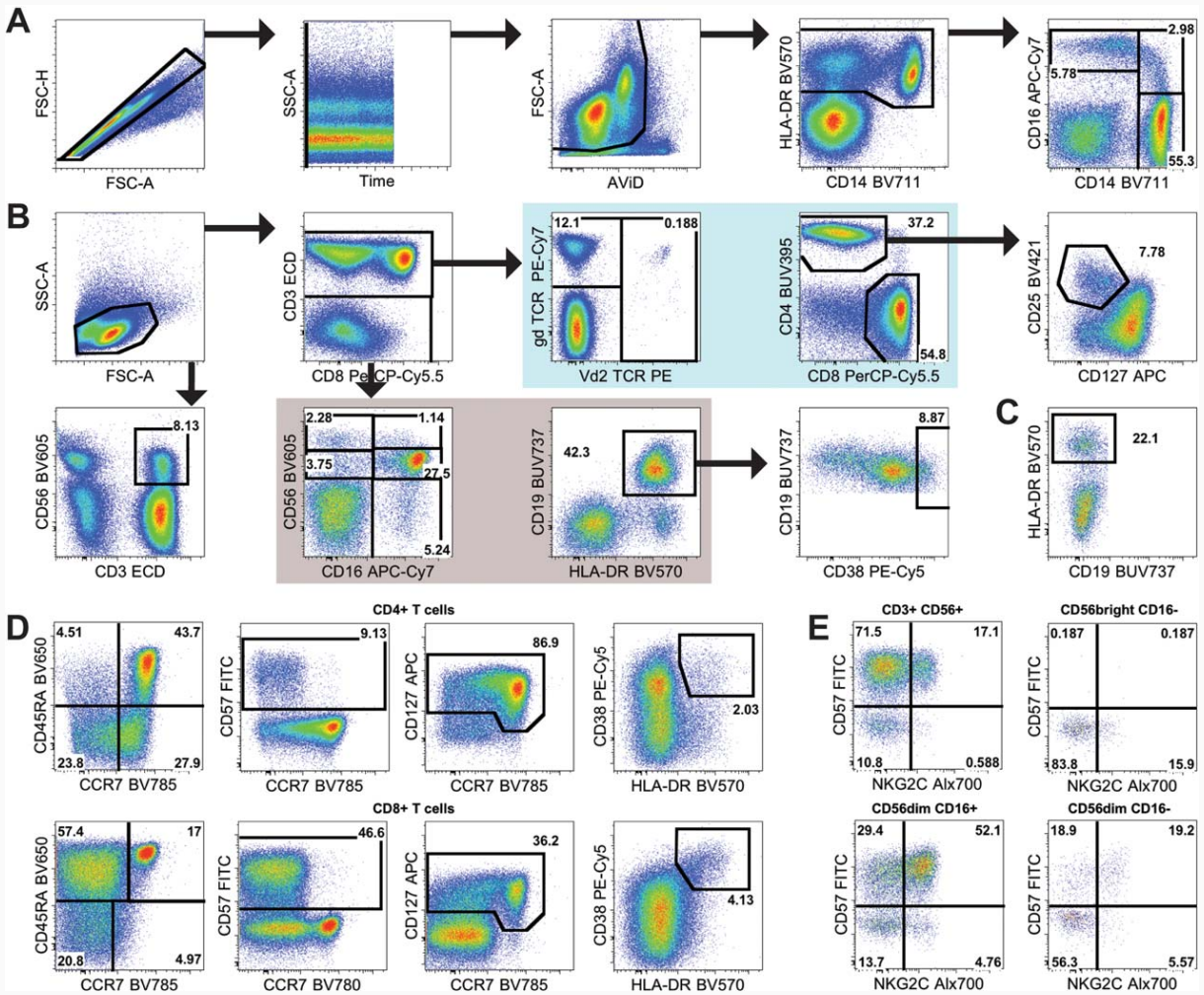
What are the goals of our analysis?

- Identify all cell types present in the sample
- Compare population frequencies between samples
- Compare antigen expression between samples
- Track cell development pathways
- Characterise functional state of populations

What challenges are presented by mass cytometry data?

- Larger number of parameters than conventional cytometry
- Visualizing the patterns in the data becomes difficult
- Manual gating is difficult to impossible
  - p(p-1)/2 number of bivariate combinations (435 combinations for 30 paramaters)
  - subjective
  - very likely to miss populations

What challenges are presented by mass cytometry data?



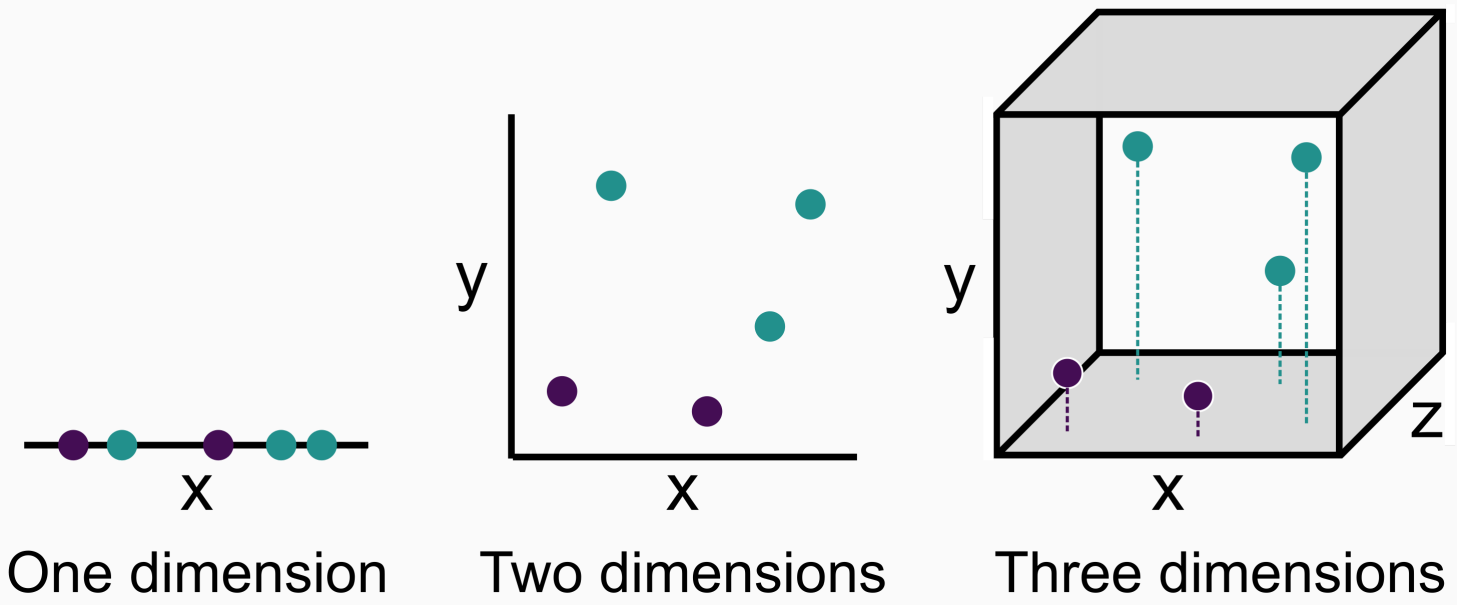
Moncunill et al., 2014

How do we solve these challenges?

We turn to computational, **machine learning** methods to gate populations for us.

But these techniques can perform poorly when the number of parameters is high. This is due to a phenomenon known as **the curse of dimensionality**.

The curse of dimensionality

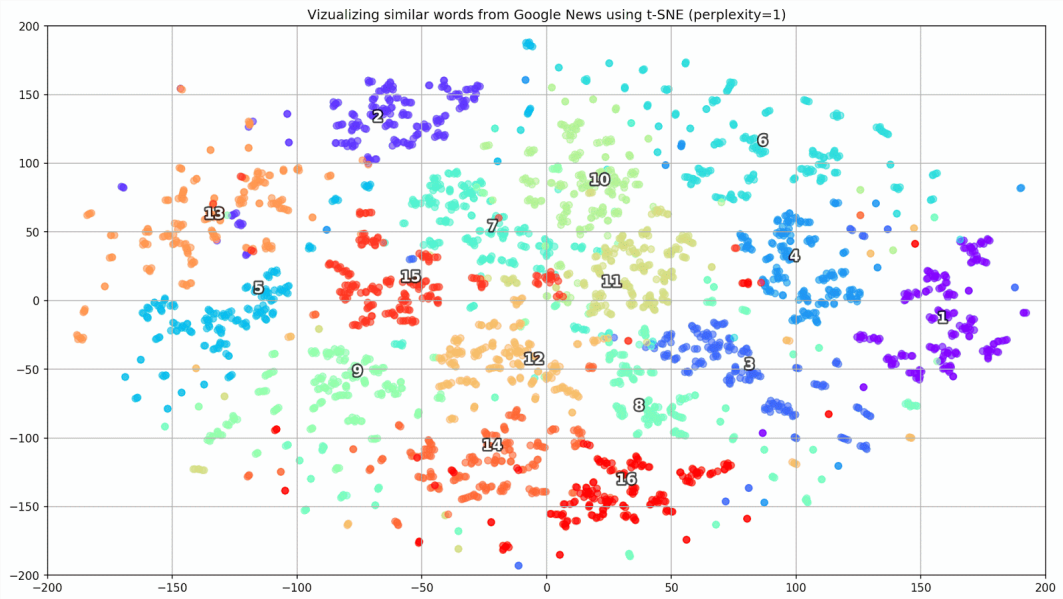


- Algorithms that rely on calculating the **distance** between points, suffer in high-dimensional space
- In high dimensions most of the "volume" of the **feature space** is empty, or **sparse**
- Algorithms are more likely to start learning from noise in the data, rather than signal
- Distance also starts to lose its meaning

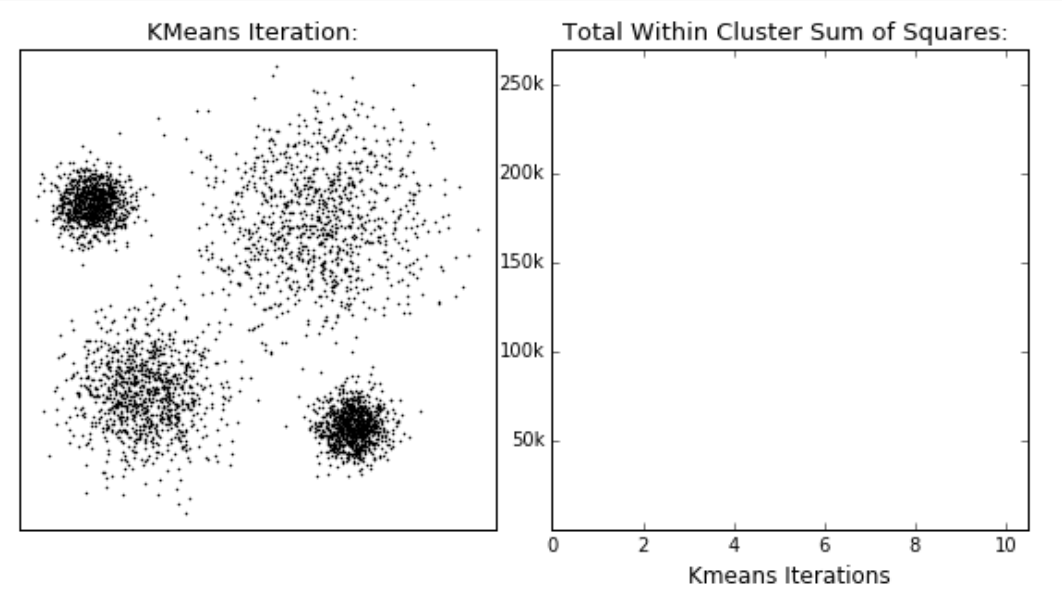
How do we solve these challenges?

Unsupervised machine learning!

Dimension reduction



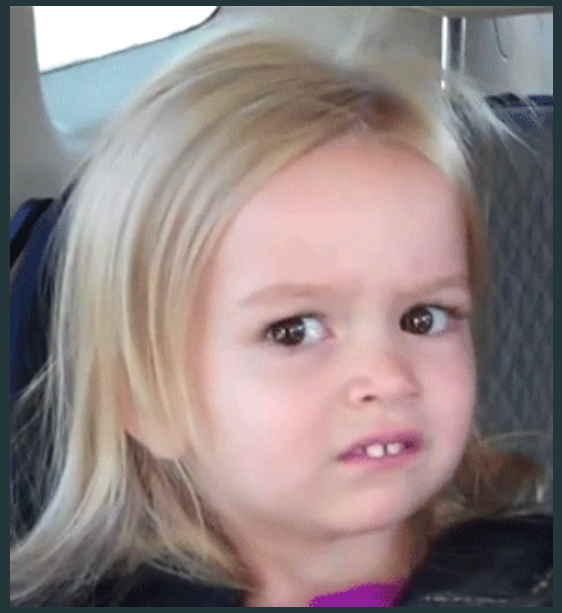
Clustering



What is dimension reduction?

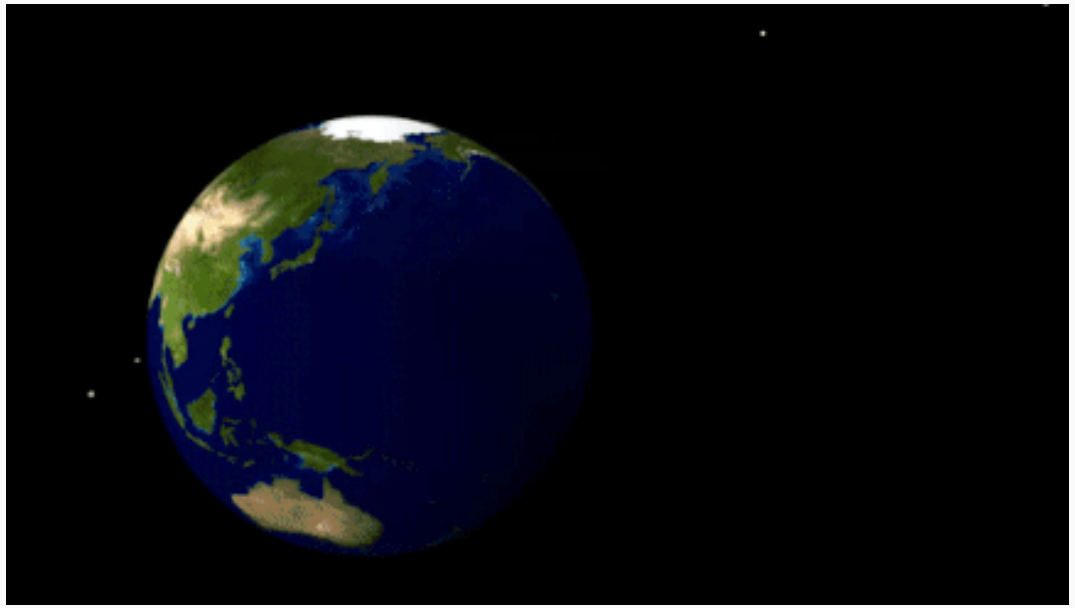
Dimension reduction algorithms aim to convert a large number of dimensions into a smaller number of dimensions, while preserving as much of the original, high-dimensional information as possible.

What is dimension reduction?

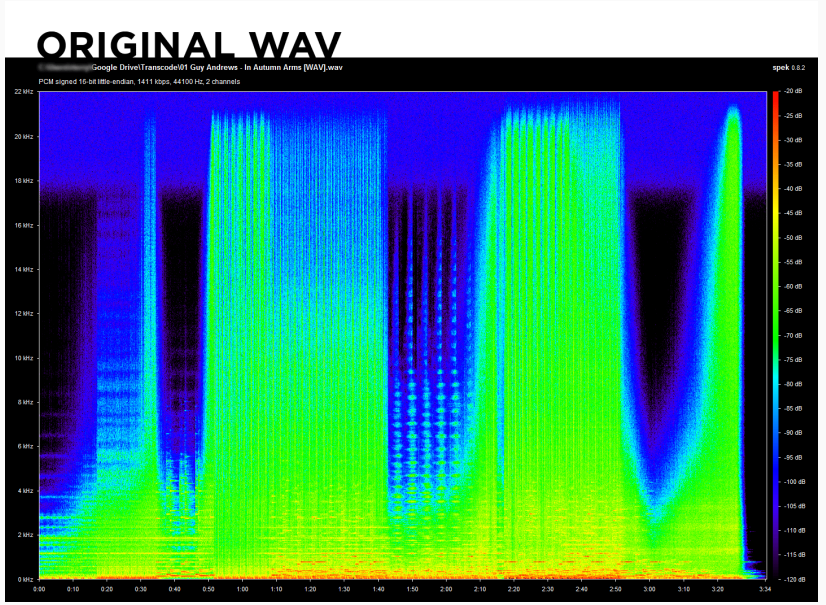


What is dimension reduction?

Mercator projection



Audio compression



Common dimension reduction algorithms

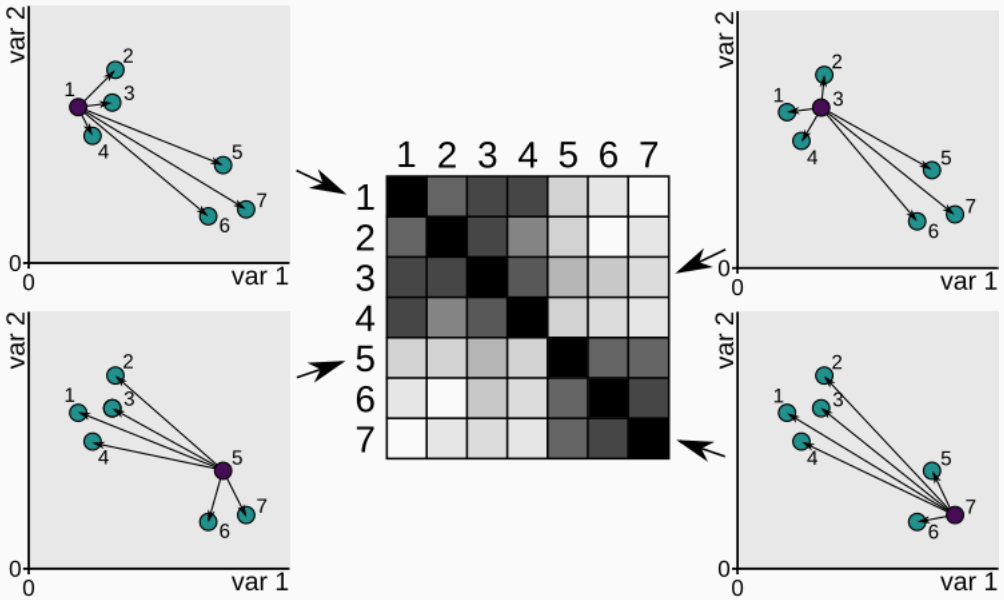
- **Principal components analysis**
  - Linear dimension reduction
  - The first few PCs explain most of the variation in the data, the rest can be discarded
  - Usually performs poorly for flow cytometry data
- **t-distributed stochastic neighbor embedding (t-SNE)**
  - Non-linear dimension reduction
  - Computationally expensive
  - Works well for flow cytometry data
- **Uniform manifold approximation and projection (UMAP)**
  - Non-linear dimension reduction
  - Not as computationally expensive
  - Works well for flow cytometry data

A brand new algorithm called PHATE was released in December: (https://www.biorxiv.org/content/10.1101/120378v1)

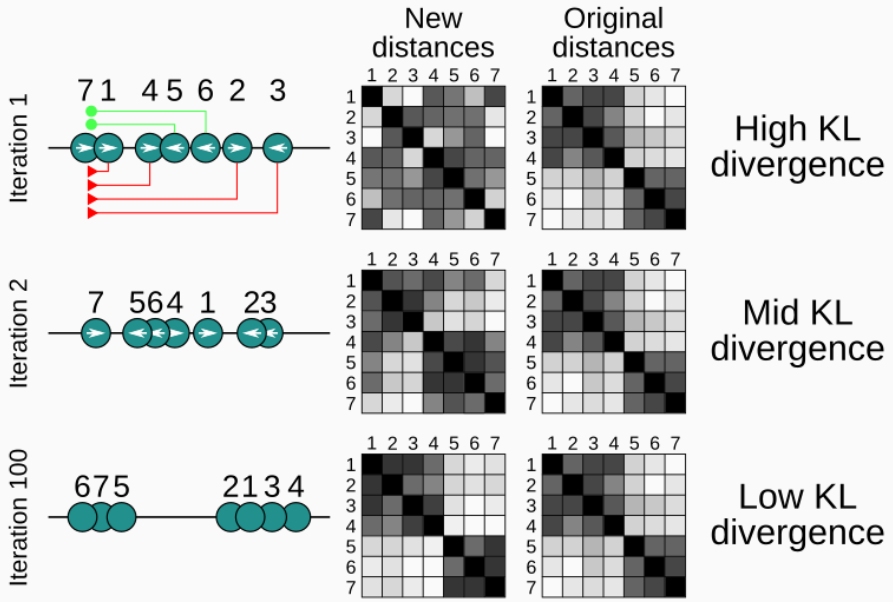
t-SNE

How does t-SNE work?

Calculate distances between each event, and every other event



Randomly distribute the events in a new, two-dimensional space. Iteratively move events closer together that were close by originally

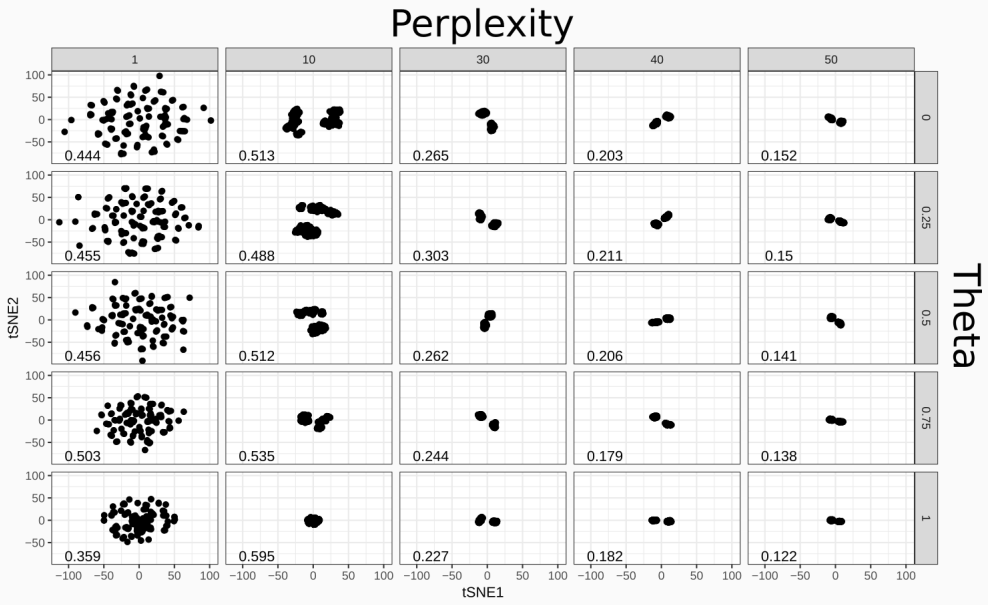


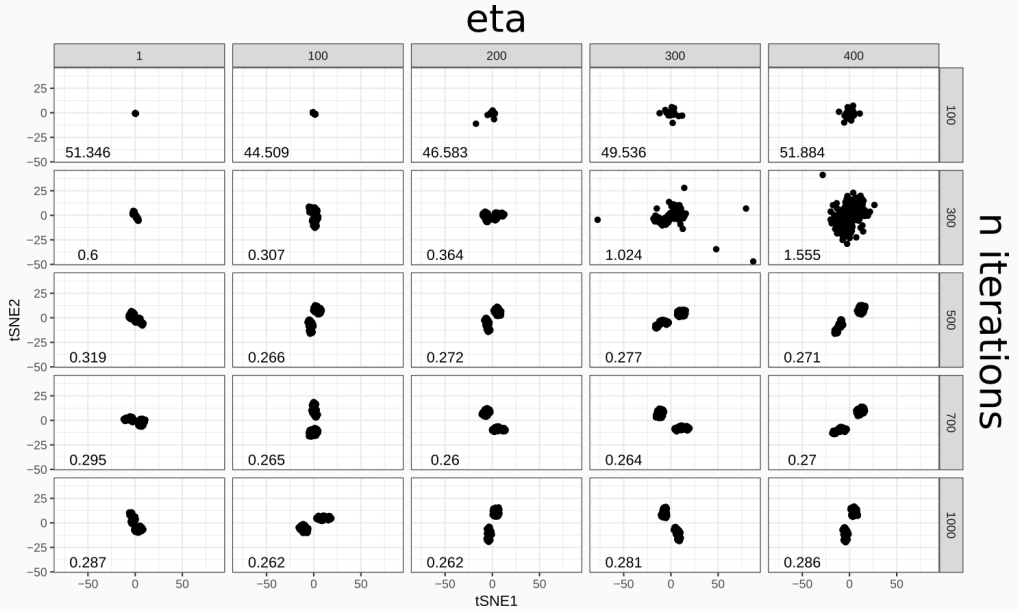
What does a t-SNE look like for mass cytometry data?

Hyperparameters of t-SNE

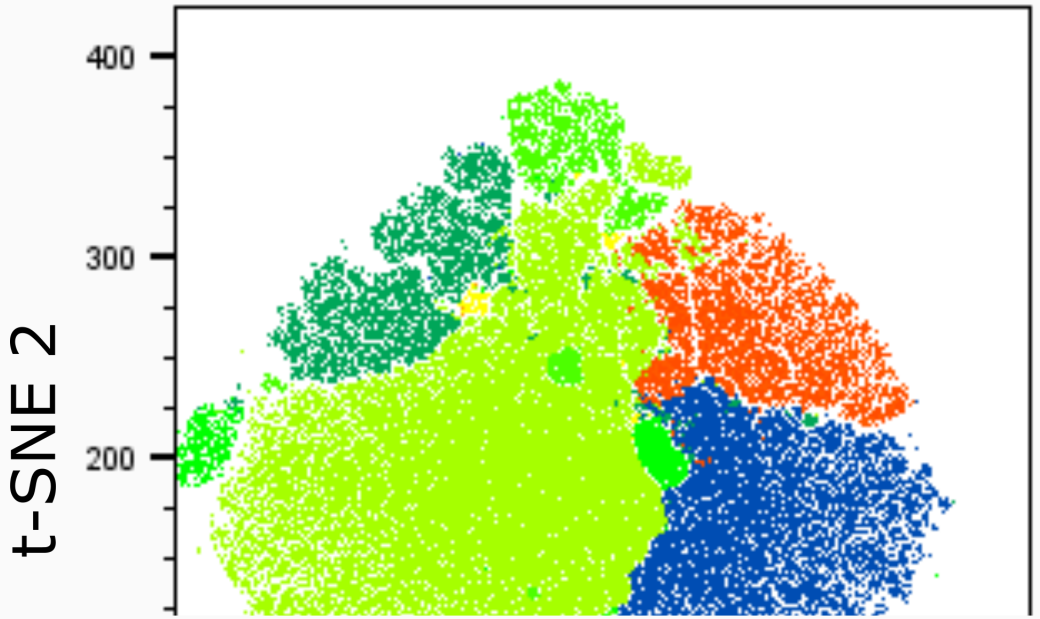
The t-SNE algorithm has **hyperparameters** that control what the final embedding looks like:

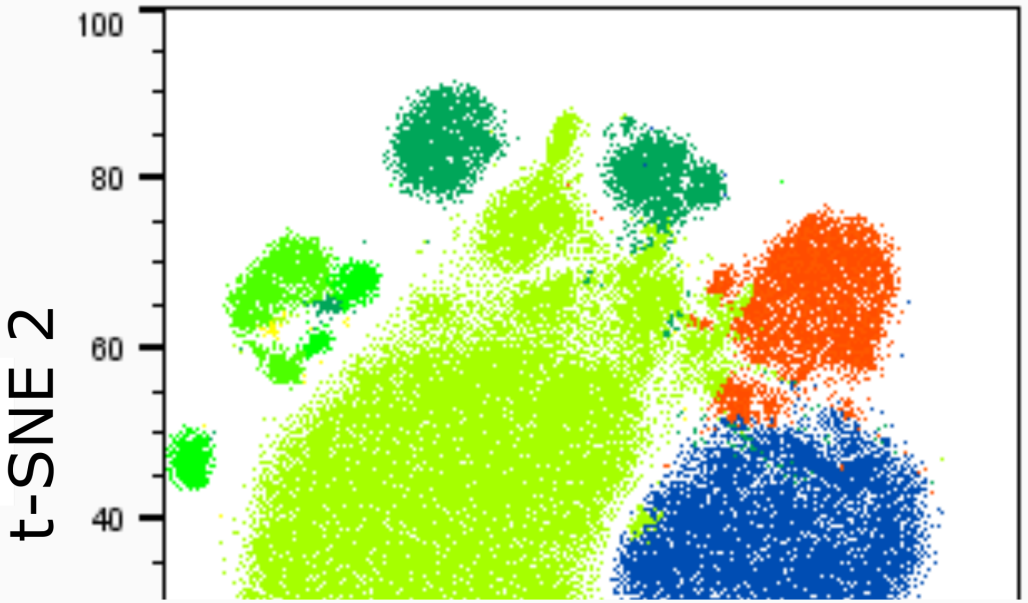
- perplexity (emphasis on global vs local structure)
- theta (0 -> 1 increases speed but decreases accuracy)
- eta (learning rate, default value usually fine)
- n iterations / epochs (must be high enough to converge)





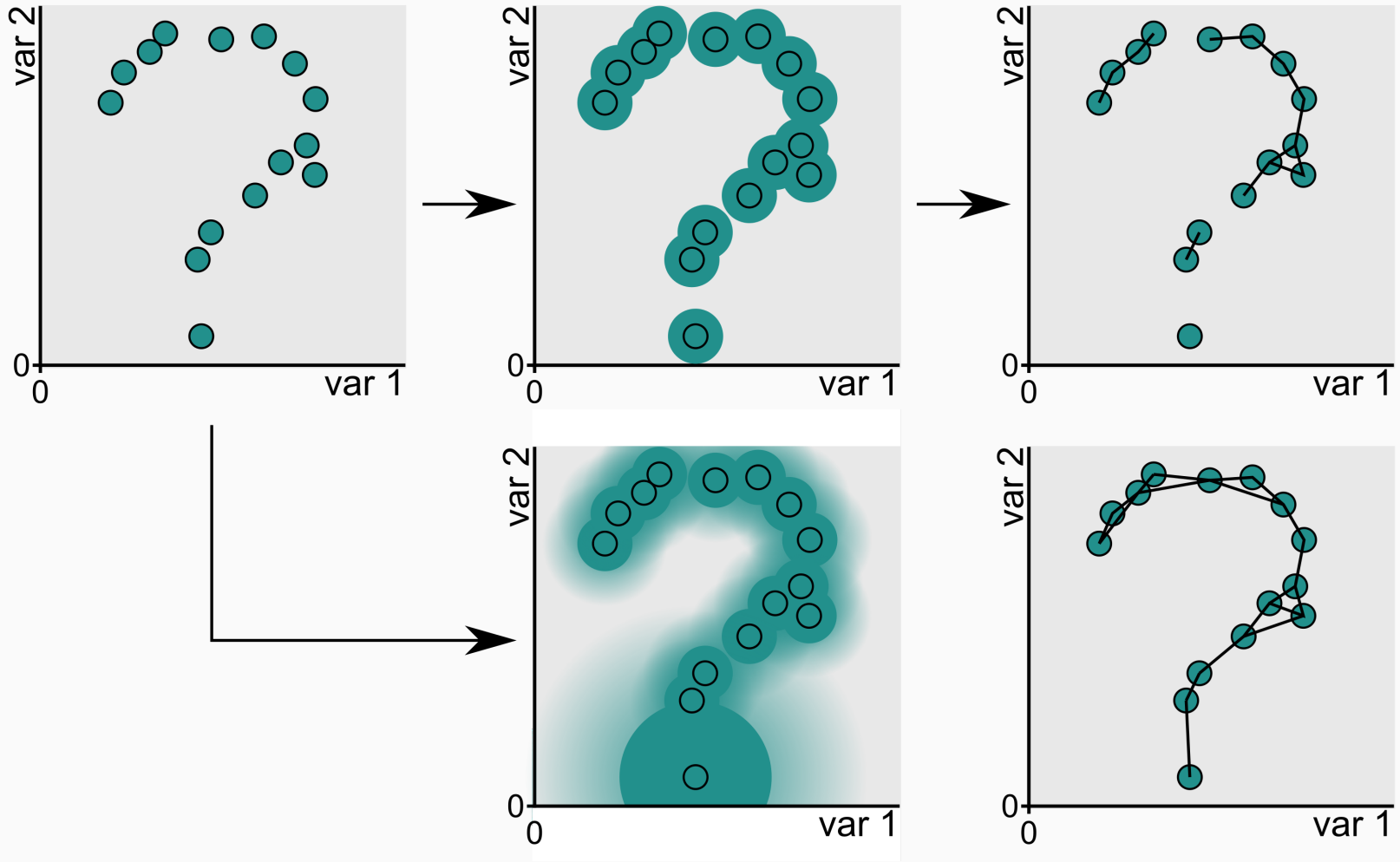
Hyperparameters of t-SNE



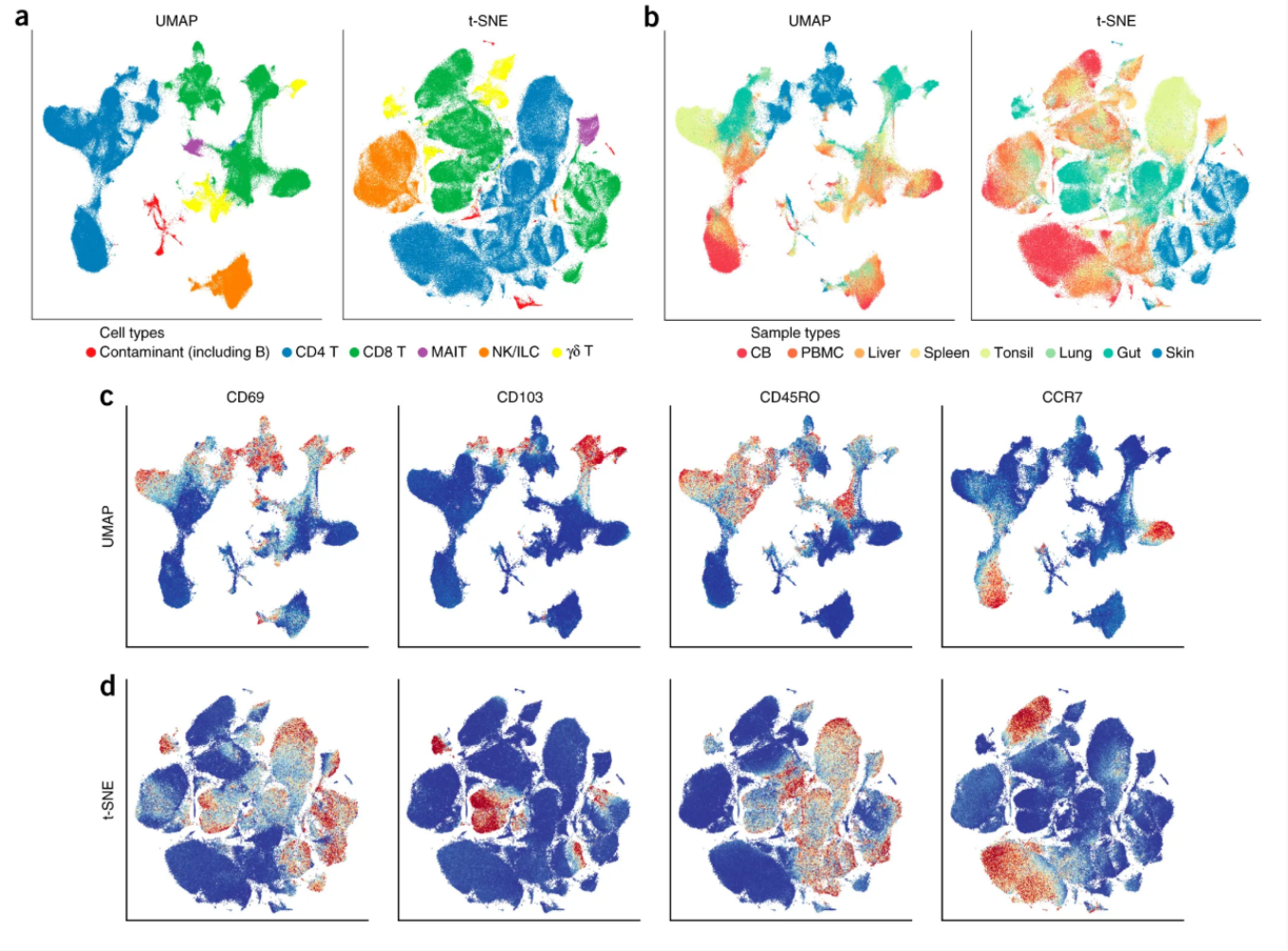


UMAP

How does UMAP work?



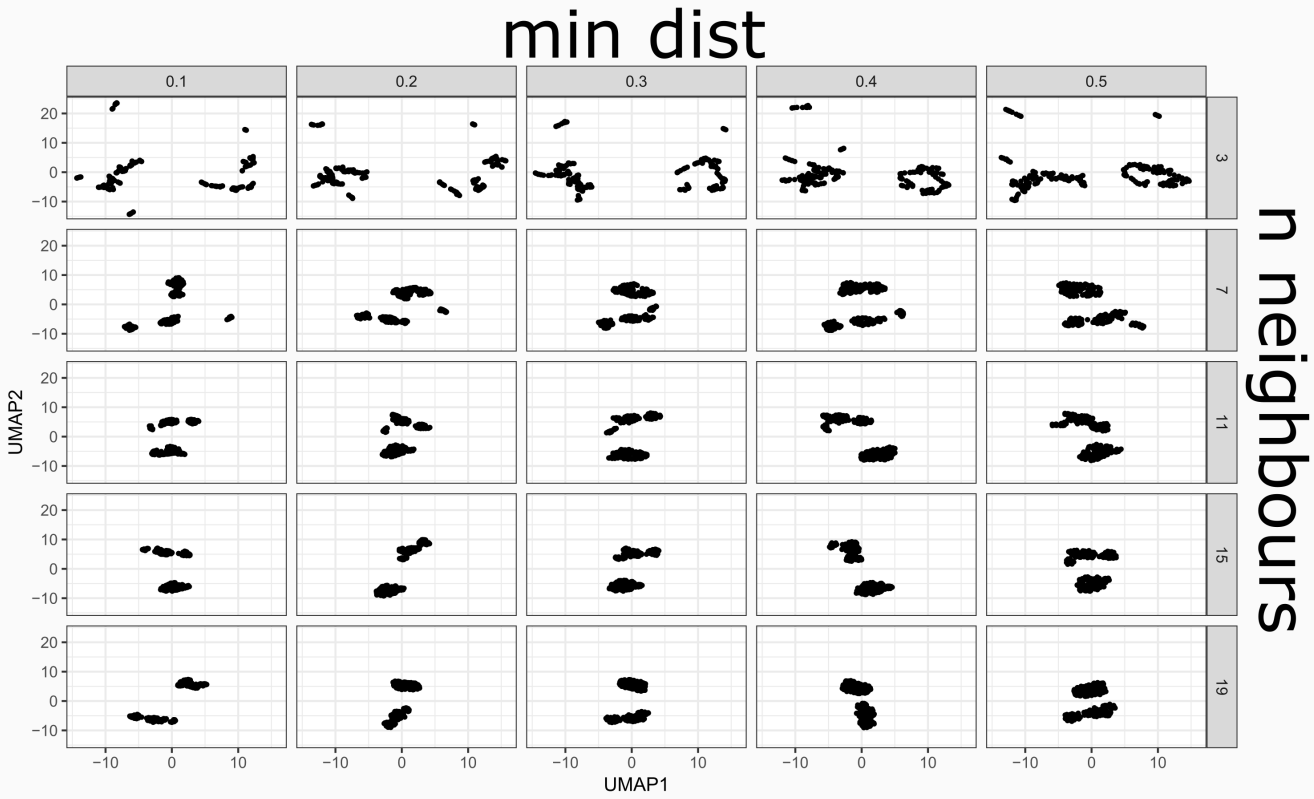
What does a UMAP look like for mass cytometry data?



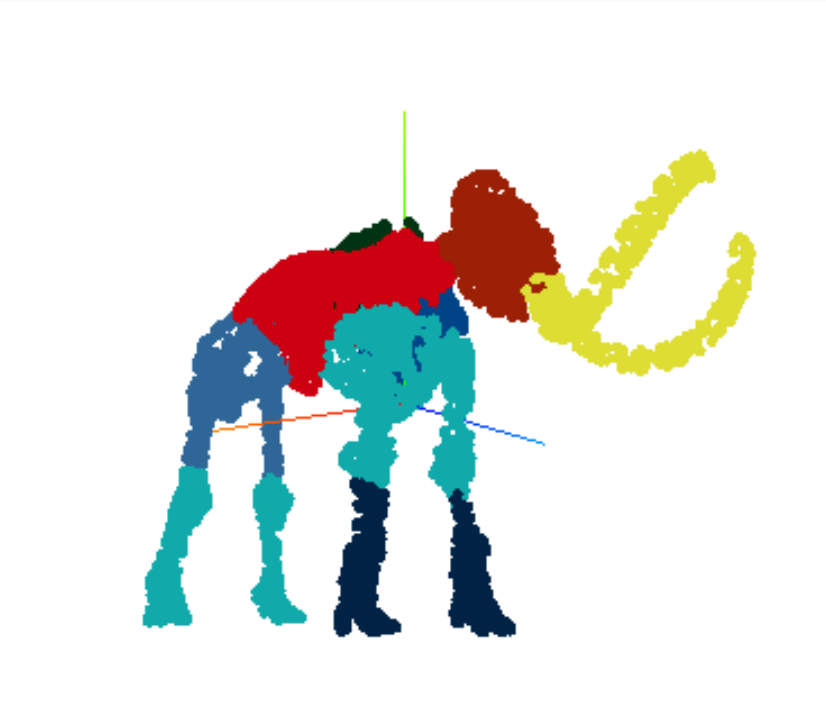
Hyperparameters of UMAP

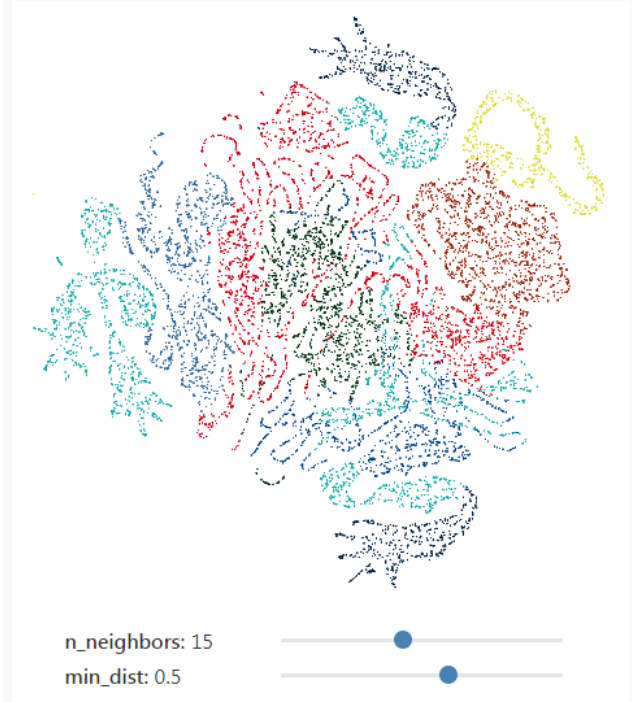
The UMAP algorithm also has hyperparameters that control what the final embedding looks like:

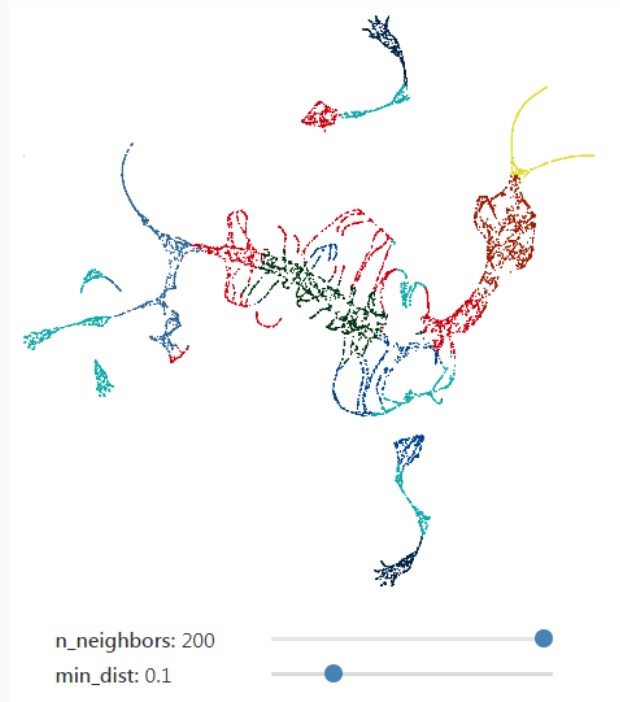
- n neighbours (emphasis on global vs local structure)
- min distance (clumpy vs spread out points)
- n iterations / epochs (must be high enough to converge)



Hyperparameters of t-SNE

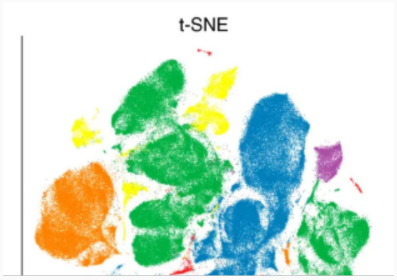






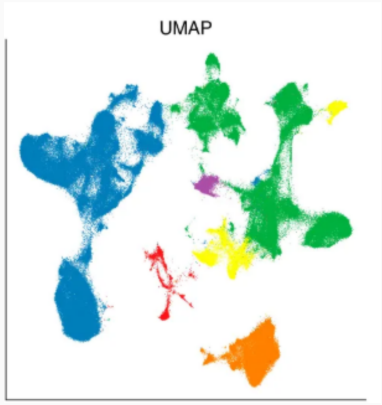
t-SNE vs. UMAP

t-SNE



- Preserves local structure but not global structure
- Computationally expensive
- Cannot map new data onto embedding
- Gives a different embedding each run
- Islands of points tend to be more globular

UMAP



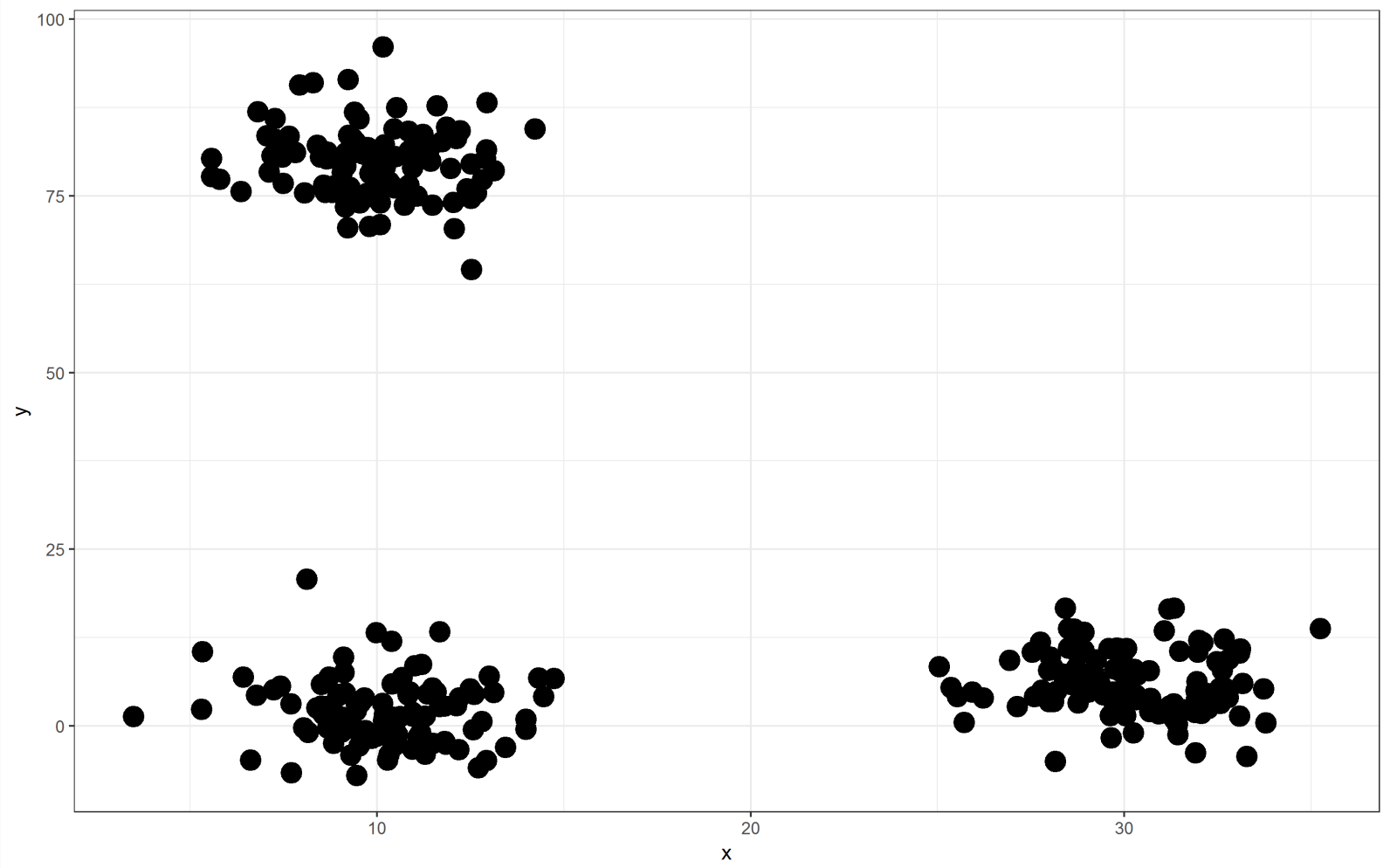
- Preserves local and *some* global structure
- Computationally less expensive
- New data can be mapped onto existing embedding
- Gives the same embedding each run
- Islands of points tend to follow a continuum

What is clustering?

Clustering algorithms aim to partition the dataset into discrete **clusters** (populations). A cluster is a set of data points that are more similar to each other, than data points in other clusters.

What is clustering?

What is clustering?



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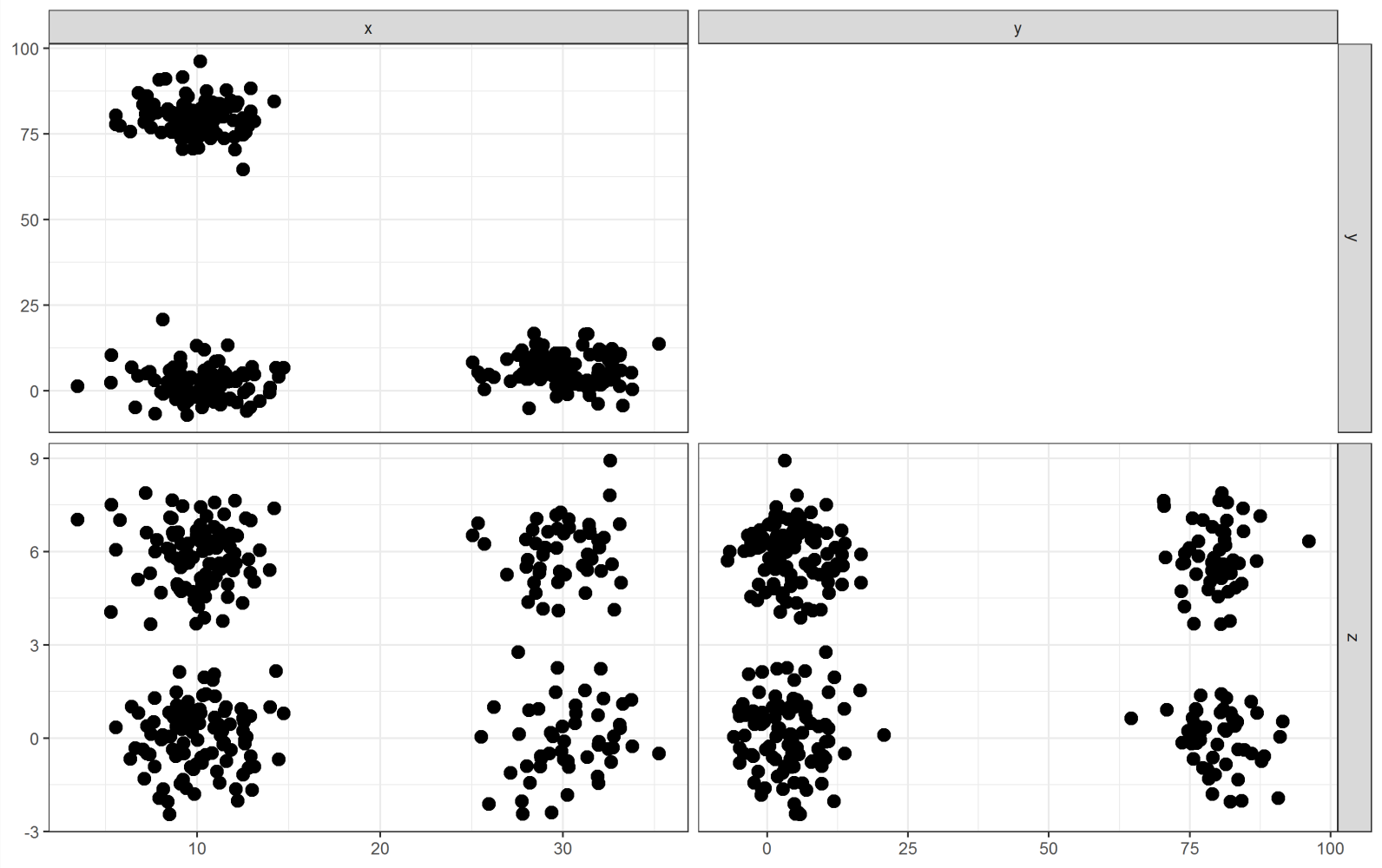

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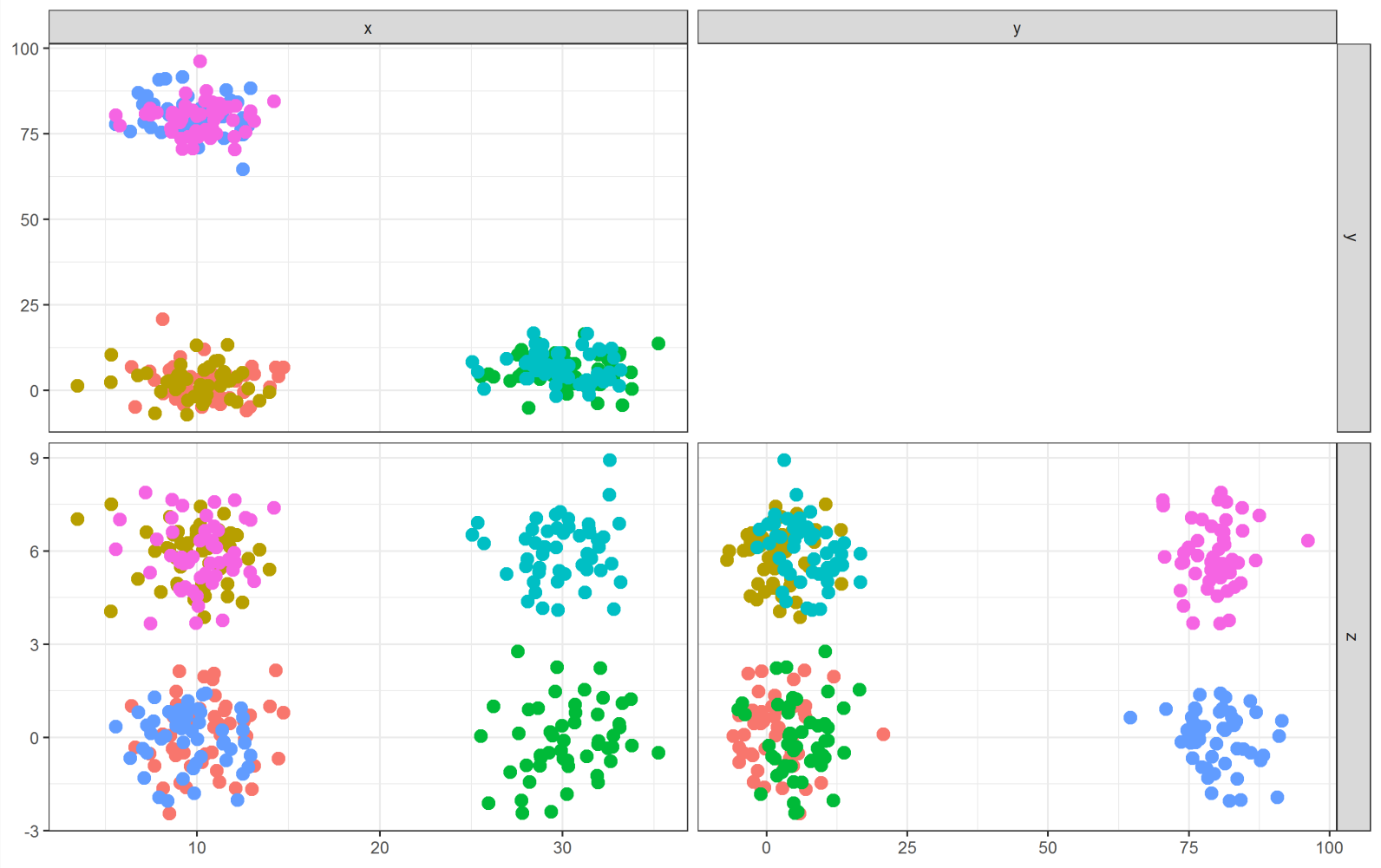

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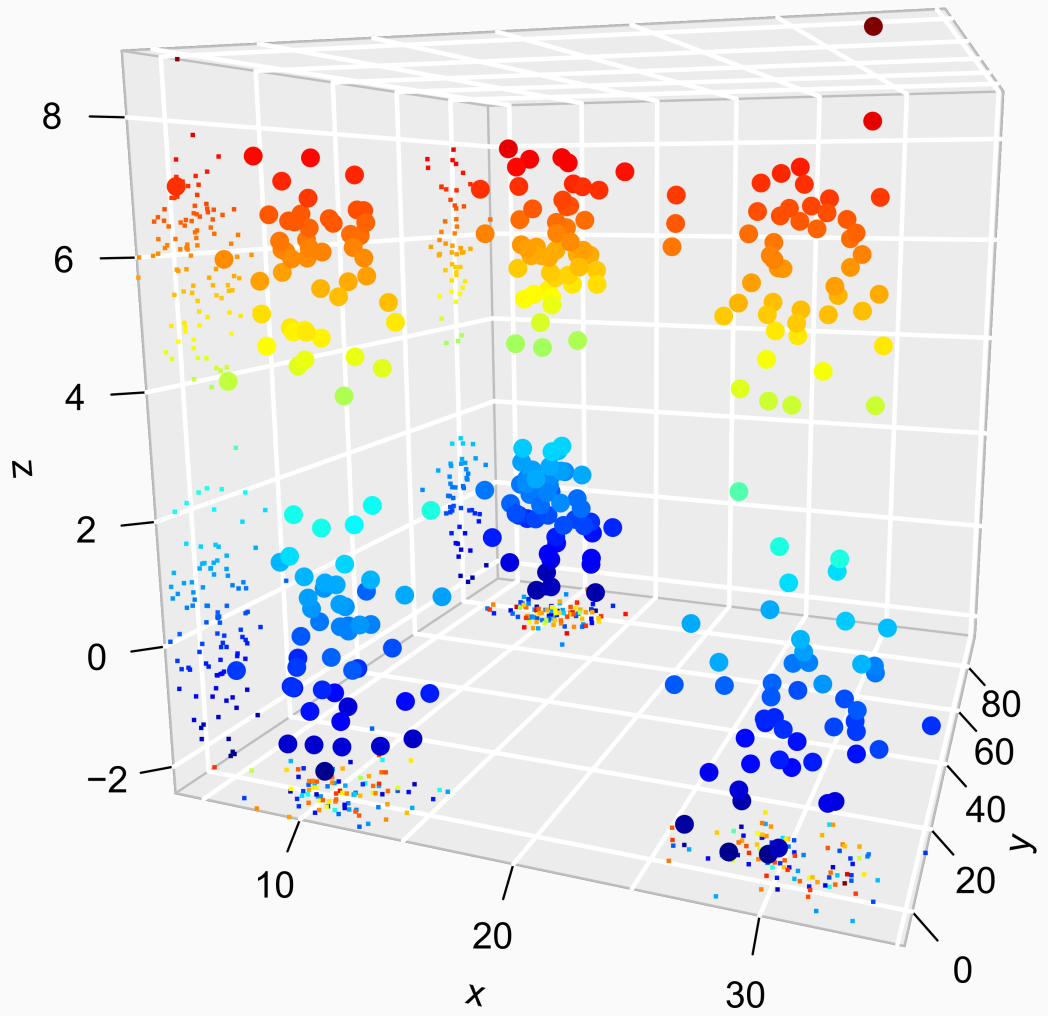


Common clustering algorithms

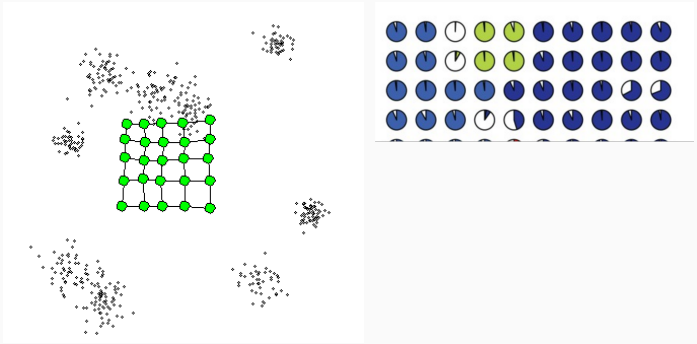
- **k-means/medians** (not great for cytometry)
- **Hierarchical clustering** (not great for cytometry)
- **flowMeans and flowPeaks** (outdated)
- **SPADE** (probably superceded by newer algorithms)
- **flowSOM** (state of the art)
- **Phenograph** (state of the art)
- **flowGrid** (very new, state of the art)

flowGrid was released in April 2019: (https://bmcsystbiol.biomedcentral.com/articles/10.1186/s12918-019-0690-2#article-info)

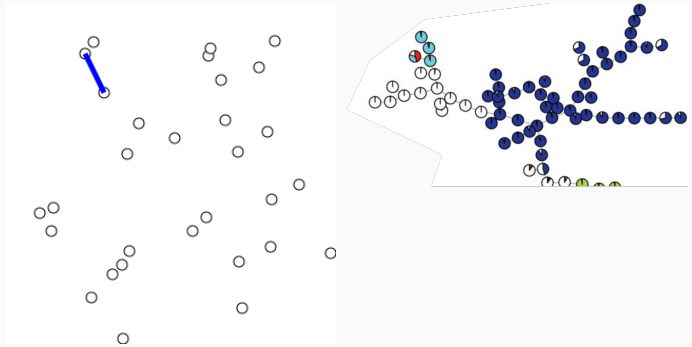
flowSOM

How does flowSOM work?

1 - Reduce the number of dimensions using a **self-organizing map (SOM)**

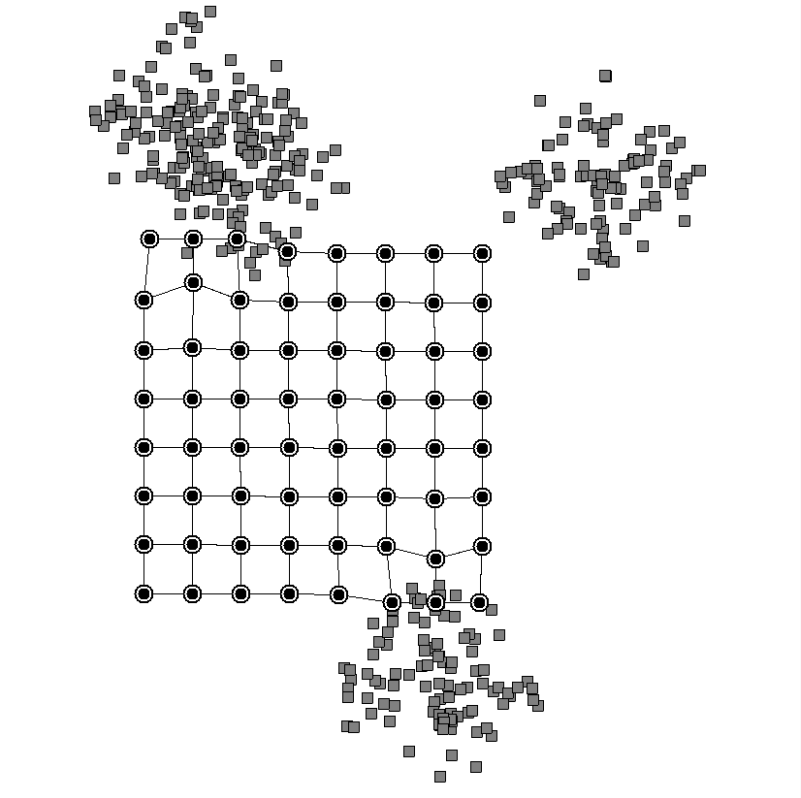


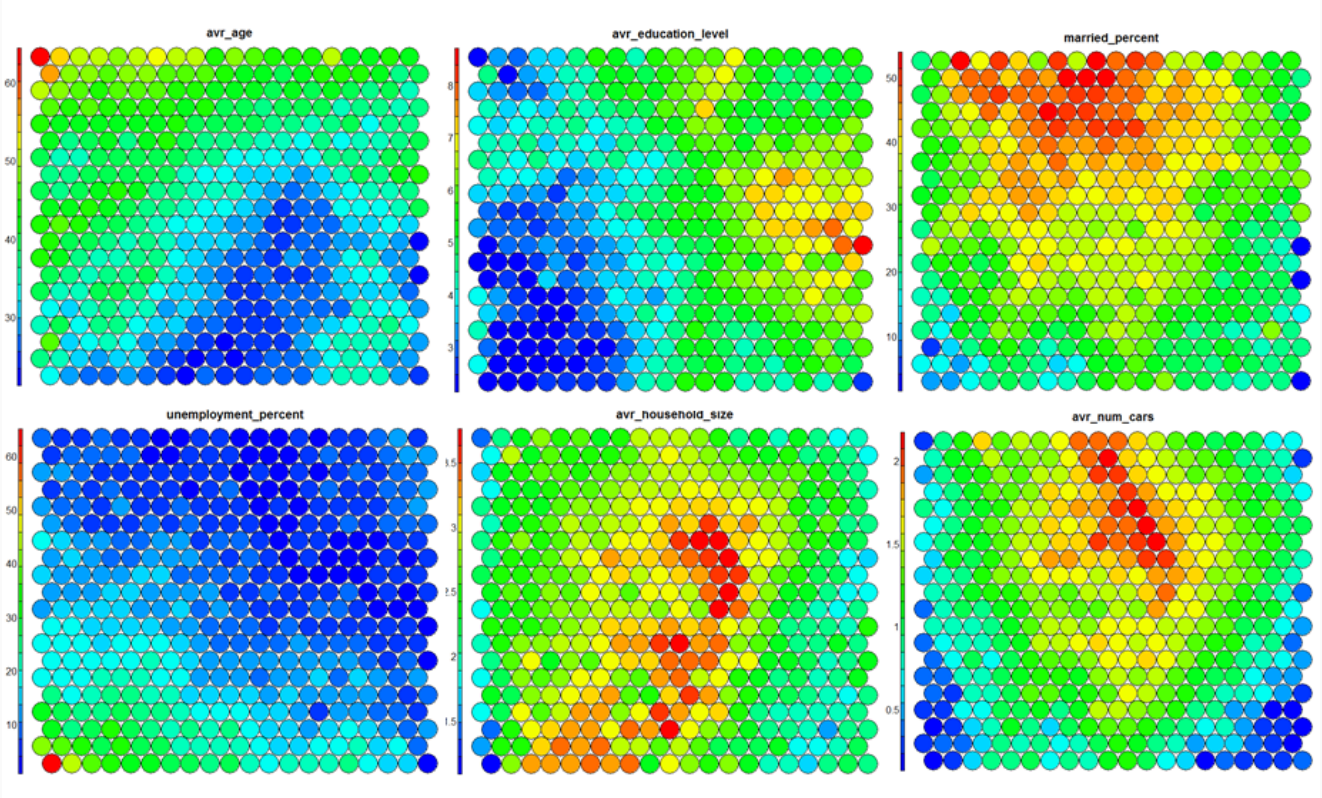
2 - Arrange the SOM into a **minimal spanning tree** for visualisation



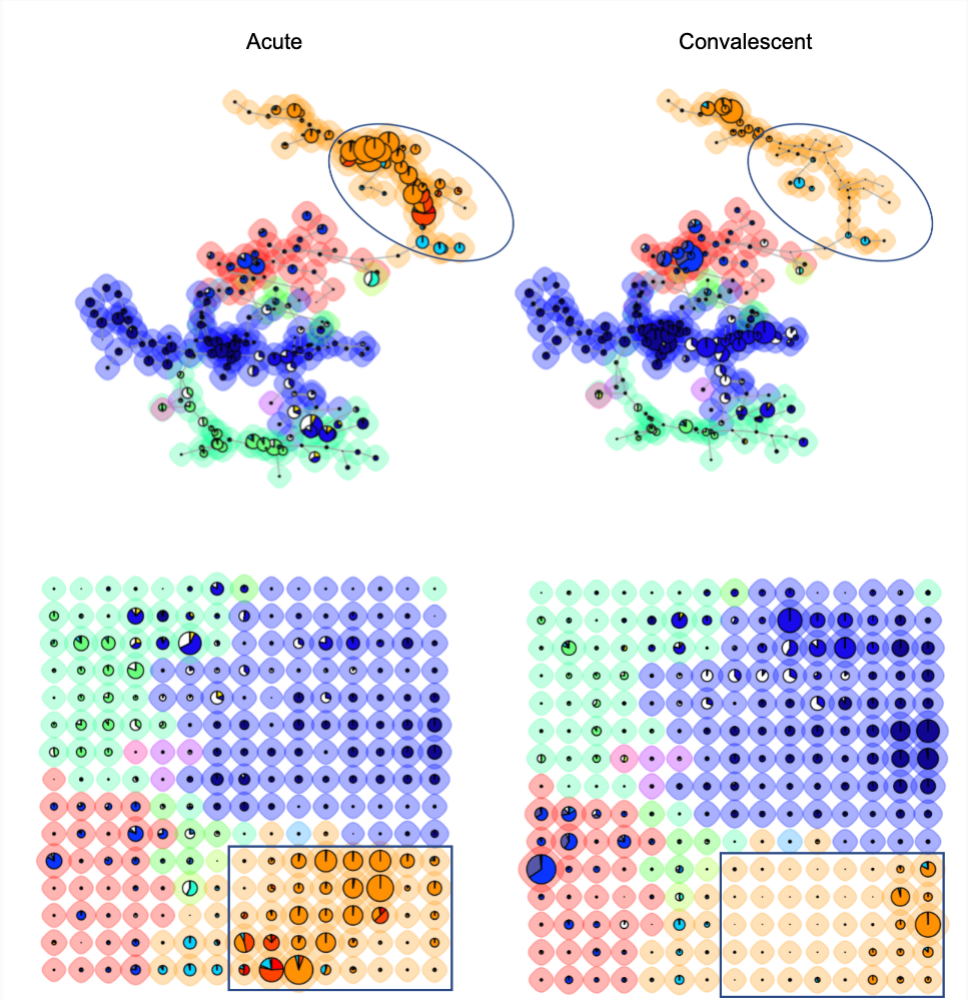
3 - Find clusters of nodes in the SOM, and place the events in the cluster of their node

Creating the self-organizing map (SOM)



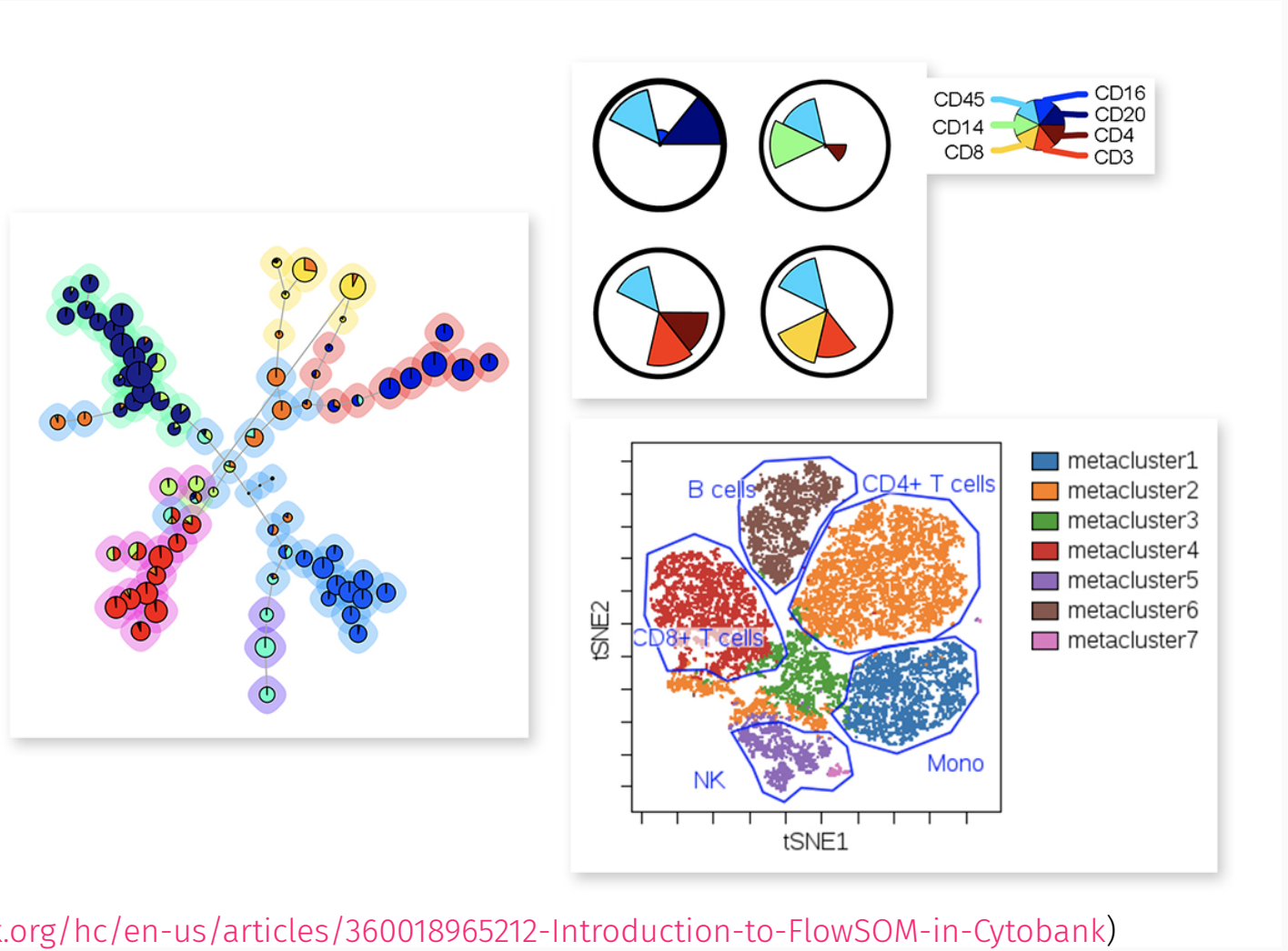


Arrange the SOM into a minimal spanning tree



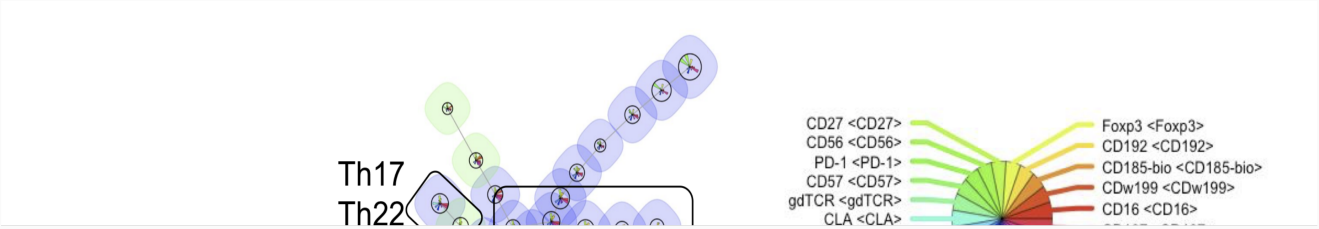
(https://blog.cytobank.org/2019/03/15/beginners-guide-to-flowsom-profiling-the-innate-immune-response-to-viral-infection/)

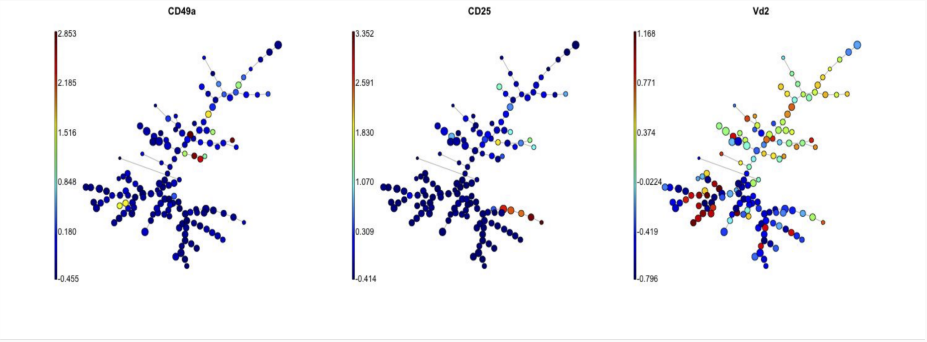
Cluster the nodes



(https://support.cytobank.org/hc/en-us/articles/360018965212-Introduction-to-FlowSOM-in-Cytobank)

What does flowSOM look like for mass cytometry data?

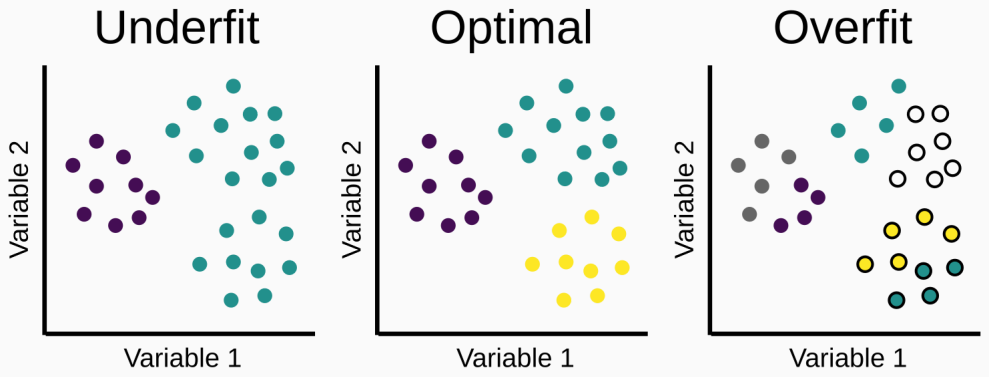




(https://github.com/hammerlab/t-cell-data/issues/29)

Choosing the number of clusters

- Identifying the number of clusters in a dataset is an **ill-posed problem**
- Some clustering algorithms need us to state how many clusters to find. Some identify clusters automatically, but may disagree with each other
- Deciding on the the number of clusters in a dataset can be hard and there may not even be one correct answer
- While clustering is a form of **unsupervised** machine learning, ALWAYS validate your clusters manually



Choosing the number of clusters with flowSOM

1 - Use *a priori* knowledge about the number of clusters

2 - Manually try a range of "sensible" numbers of clusters

3 - Allow flowSOM (in R) to choose for you

Whichever method you choose, you MUST evaluate your clustering model

Validating a clustering model

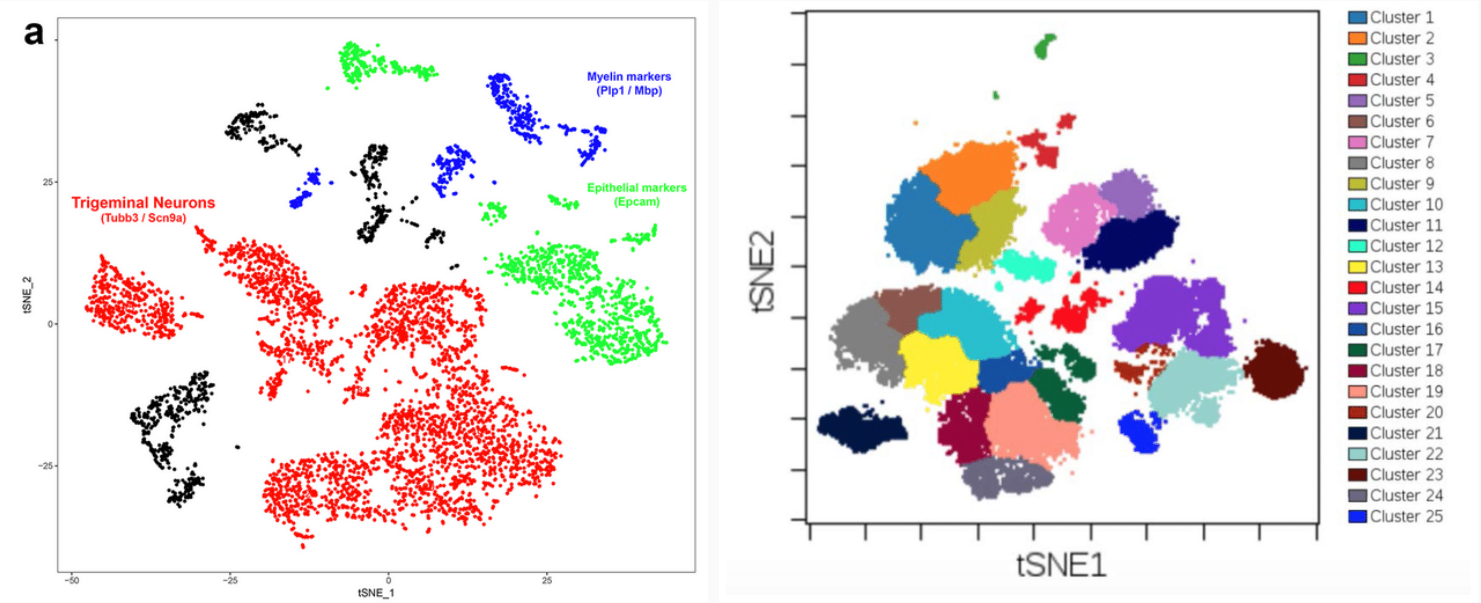
1 - Map cluster labels onto a lower-dimensional representation

2 - Map cluster labels onto bivariate plots

3 - Plot expression data for each cluster

Validating a cluster model

1 - Map cluster labels onto a lower-dimensional representation

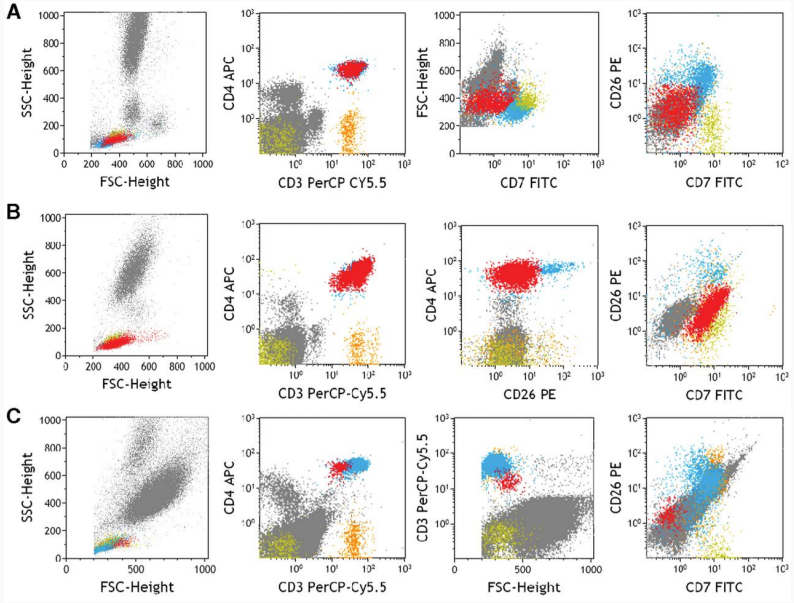


Pro: simple method to evaluate clusters in a single bivariate plot

Con: relies on the lower-dimensional embedding being a faithful representation of the data

Validating a cluster model

2 - Map cluster labels onto bivariate plots

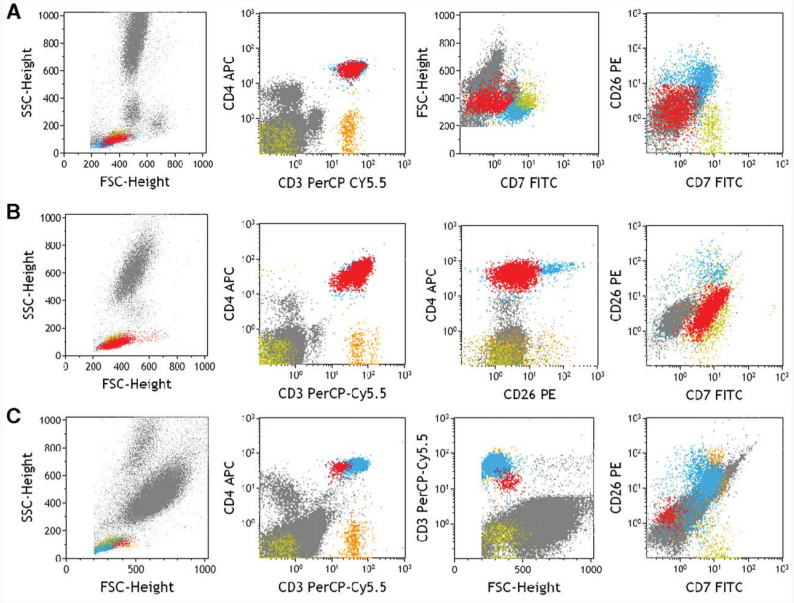


Pro: Allows us to incorporate our expert knowlege of biology

Con: Time consuming, impossible to interrogate all combinations

Validating a cluster model

3 - Plot expression data for each cluster



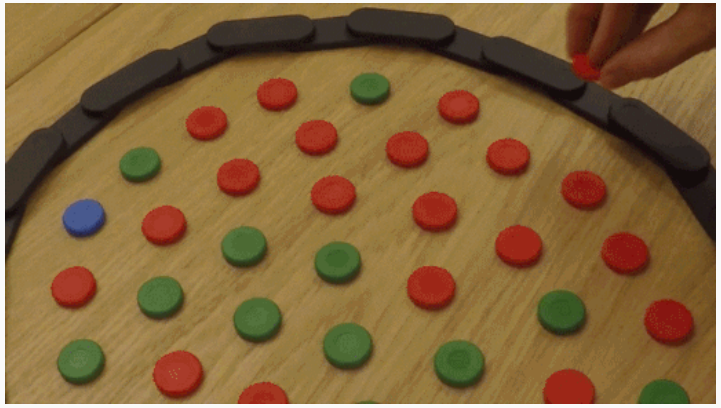
NEED TO REPLACE THIS IMAGE WITH ONE FROM PHIL **Pro:** Allows us to incorporate our expert knowlege of biology

Con: Time consuming, impossible to interrogate all combinations

What if the clustering model doesn't fit well?

If the model **under-clusters**, increase the number of clusters, duh.

If the model **over-clusters**, decrease the number of clusters, OR manually merge clusters you believe represent the same cell type.



Practical tips for dimension reduction and clustering

Practical tips

1 - Clean and transform[1] your data first!

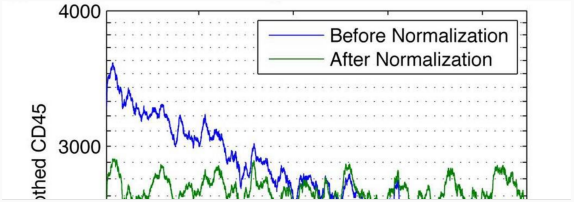
2 - Merge .fcs files together, analyse, then split apart later

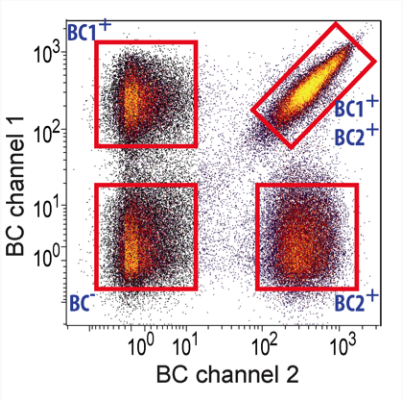
3 - Downsample if necessary

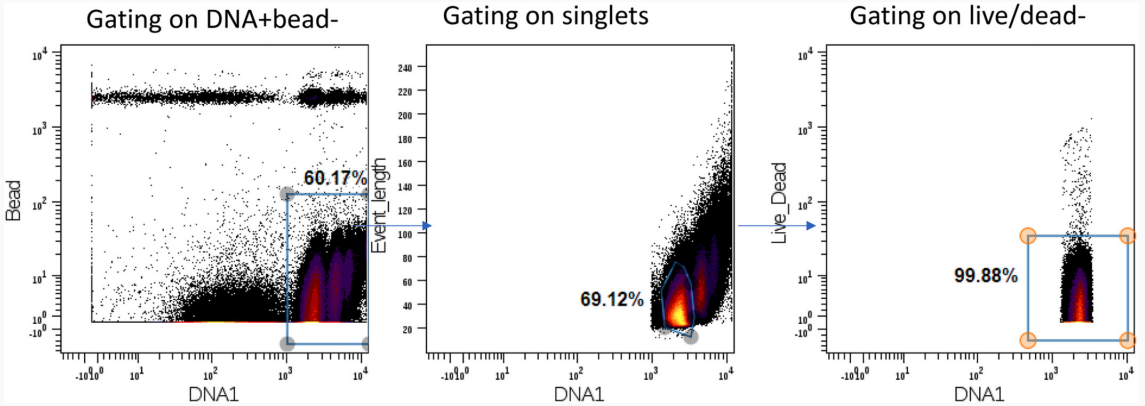
1 - or let FlowSOM do this for you (it uses logicle)

1 - Clean and transform your data

Bead normalization, de-barcoding, bead exclusion, doublet exclusion, dead cell exclusion

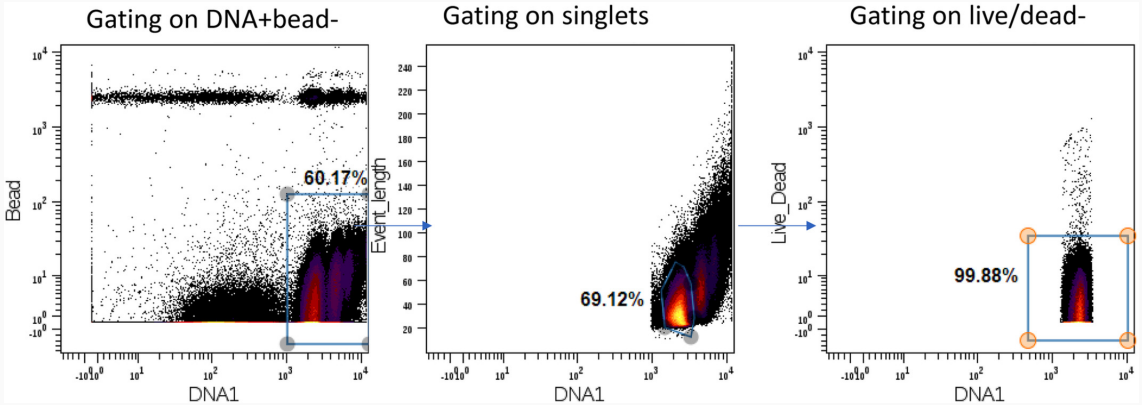






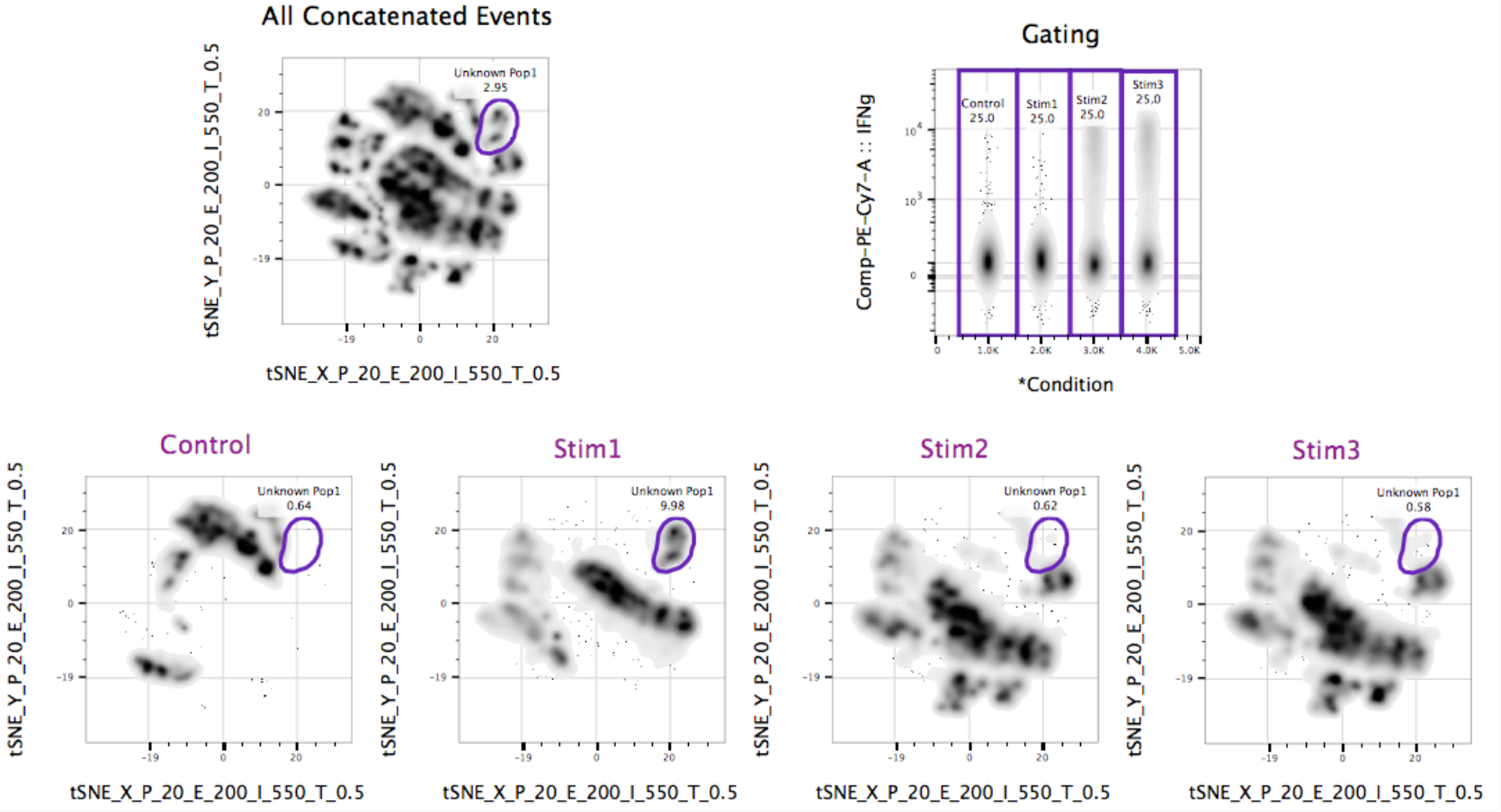
Check out the cytofclean R package: (https://github.com/JimboMahoney/cytofclean)

1 - Clean and transform your data



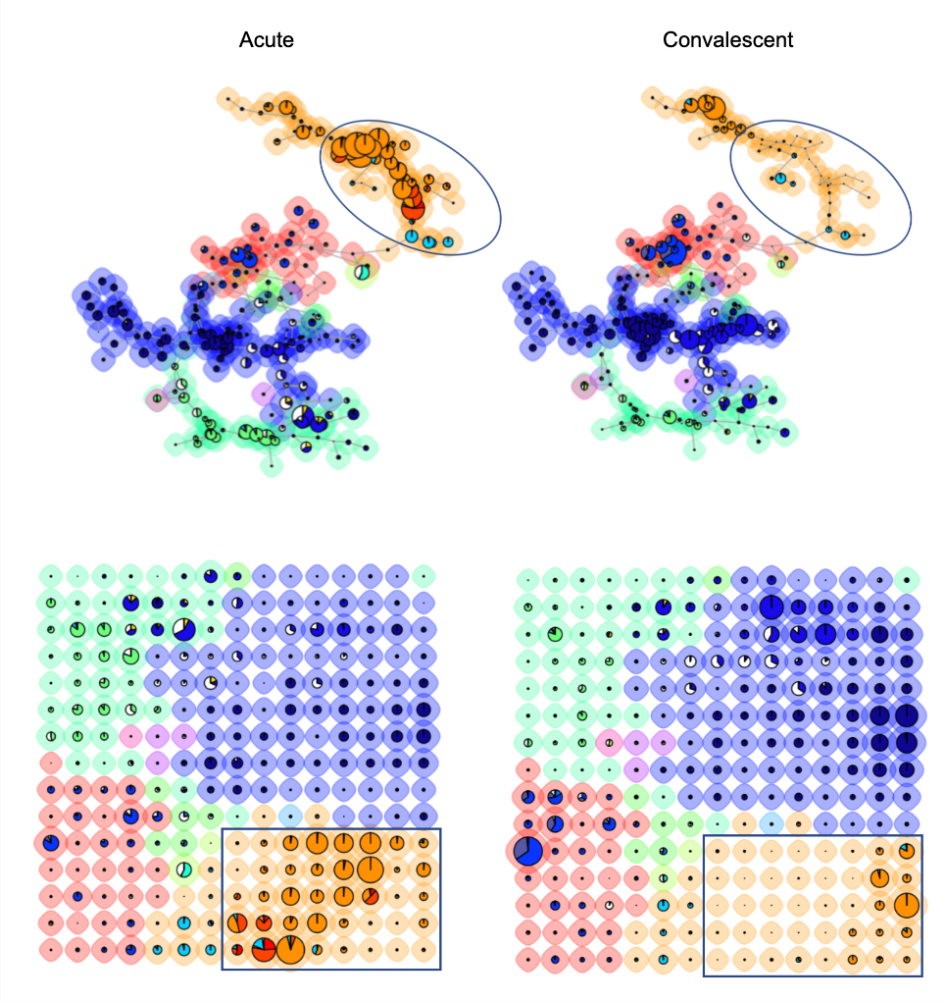
GET IMAGE FROM DINA SHOWING ARCSIN VS LOG TRANSFORMATION

2 - Merge .fcs files together

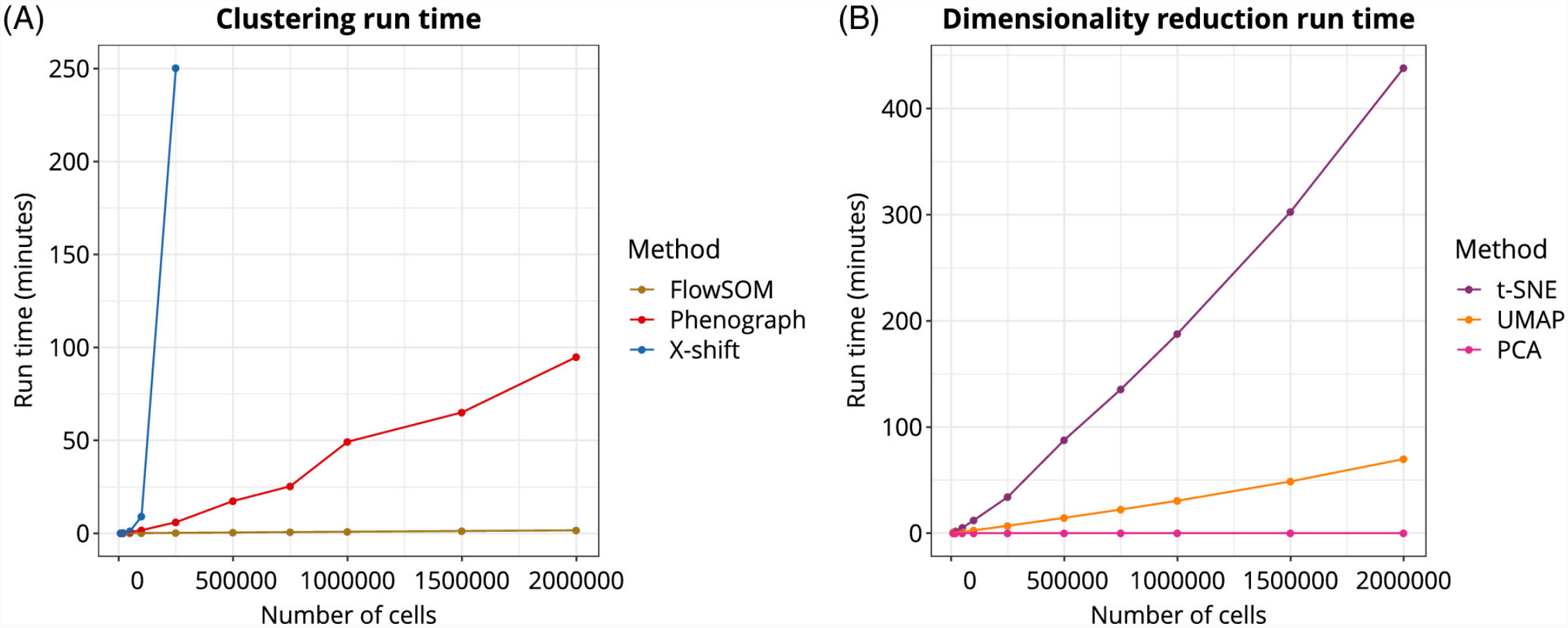


(https://www.flowjo.com/learn/flowjo-university/flowjo/tutorial/31)

2 - Merge .fcs files together



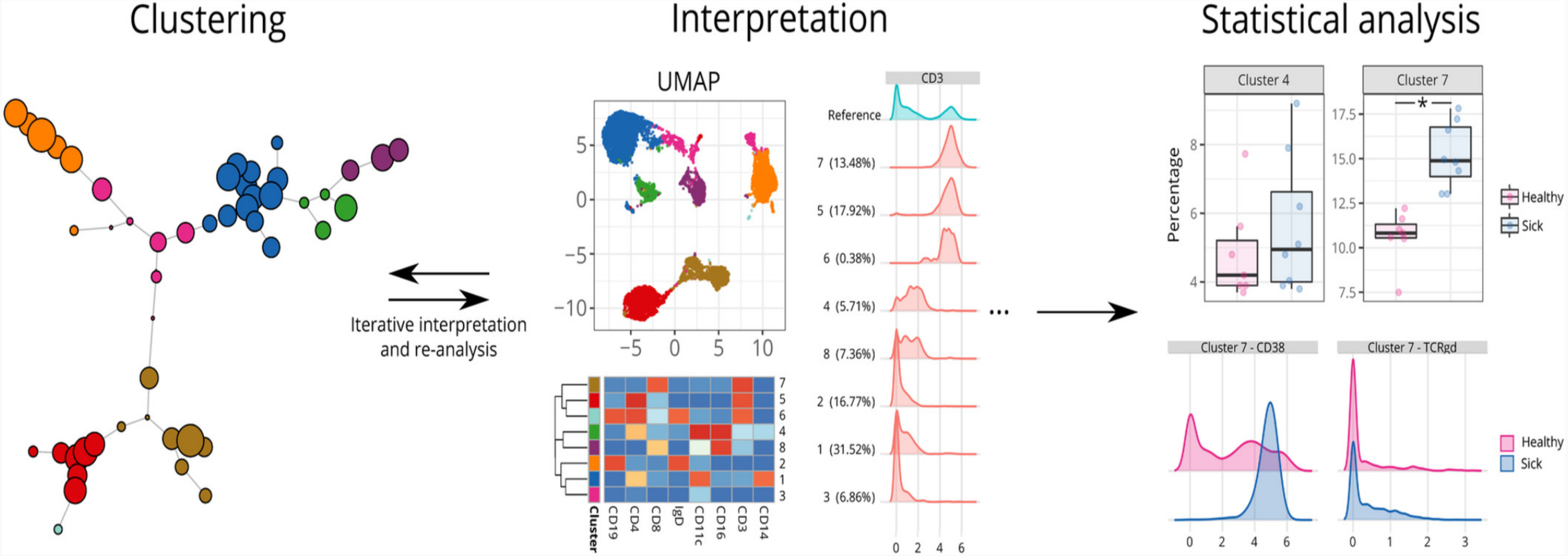
3 - Downsample if necessary



Pedersen & Olsen, 2019

The big picture

Overview of high-dimensional analysis



Pedersen & Olsen, 2019

Thanks for your attention!