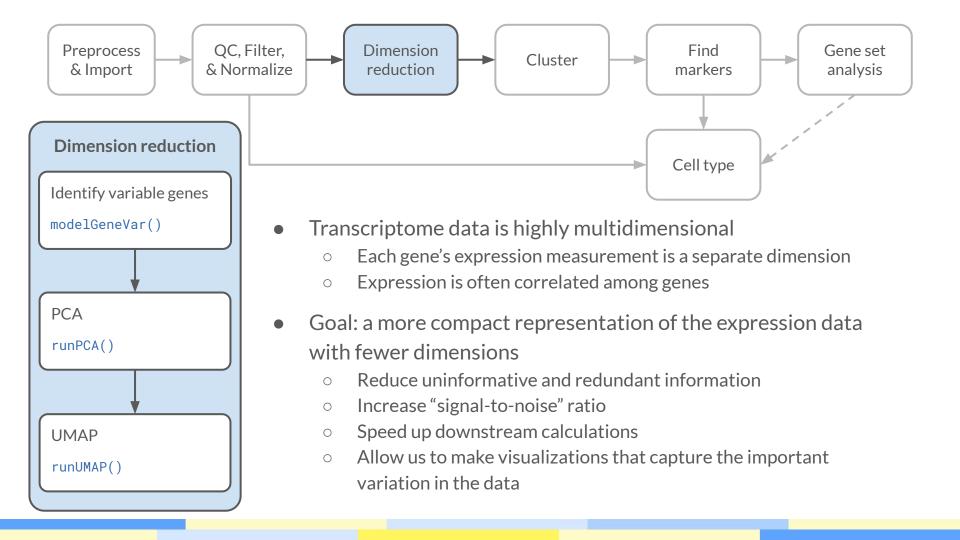
Dimensionality Reduction and Clustering of Single-cell Data

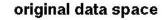
The Data Lab

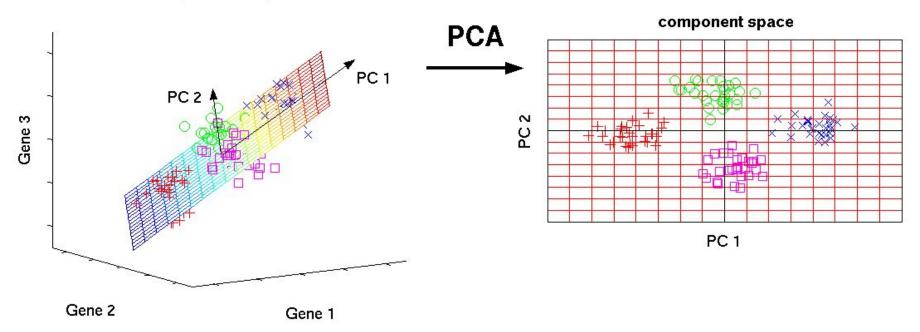


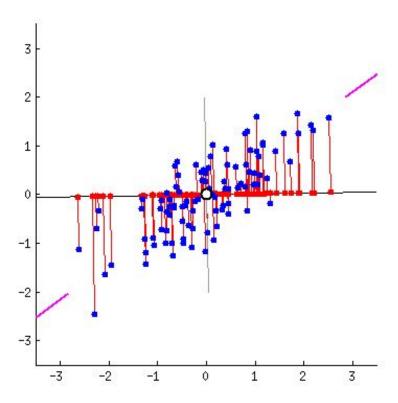
Dimensionality Reduction Methods

- Feature selection
 - Select the most (biologically) variable genes
- Principal Components Analysis
 - linear transformation of input data
 - usually to tens of dimensions
 - removes much of the noise; retains most of the signal
 - useful as input to many downstream analyses (clustering, etc.)
- UMAP and/or tSNE
 - o reduce down to 2 or 3 dimensions
 - transformation is highly non-linear
 - much slower than PCA

Principal Components Analysis (PCA)







https://builtin.com/data-science/step-step-explanation-principal-component-analysis

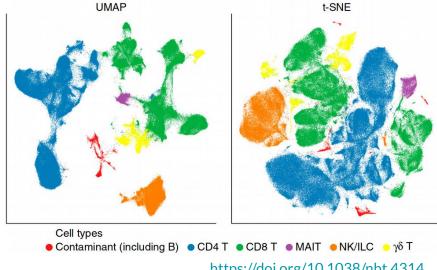
Assumptions/Limitations of PCA

- PCA is a linear transformation of the input data
 - Fast!
 - Reversible if we keep all dimensions
 - Usually we don't keep everything... removing higher dimensions reduces effects of noise
- Assumes ~ normal distributions for error
 - For scRNA-seq count data, this can be approximated with log-scale normalization
- Sensitive to outliers

UMAP and tSNE

Machine learning methods for dimensionality reduction

Details are beyond the scope of this course, but the basic steps are these:



https://doi.org/10.1038/nbt.4314

- Calculate the similarity between pairs of data points
- Find a representation in low dimensionality space (mapping) that recapitulates the similarity matrix
 - How? Start with a mapping then progressively update it by how well the distances in the low dimension space match the original distances

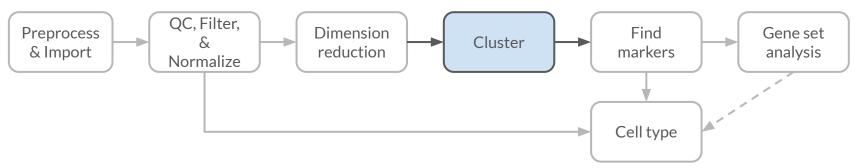
A nice visualization/playground for tSNE: https://distill.pub/2016/misread-tsne/

Assumptions/Limitations of UMAP & tSNE

- No assumptions about shape of data
 - Performs better when structures may not have "normal" distributions
- Tends to produce more visually distinct clustering
 - Nice for visualization, but be careful!
 - Distances between points may be misleading
 - Similar challenge to squashing a globe onto a flat map... but more extreme!
- Non-reversible (can't infer original data from mapping)
 - Don't use the resulting coordinates for analysis!
- Can be slow
 - Common to use PCA first for partial dimension reduction, then UMAP/tSNE on that
 - UMAP is (usually) faster

To the notebooks, Batman!

Clustering Cells



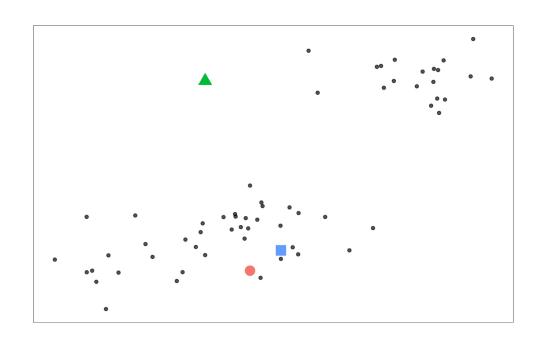
Dimensionality reduction often results in visible "clusters", but how do we define those? *Many methods!*

- hierarchical clustering
 - Join closest points/groups recursively
- k-means clustering
 - Pick a number k, then find the "best" way to divide cells into that many groups
 - Assumes clusters are "spherical"
- graph-based clustering
 - Connect cells to other cells with similar expression, then divide up the graph into clusters

Step 1: Pick k random centers

Step 2: Assign points to clusters by which center is closest

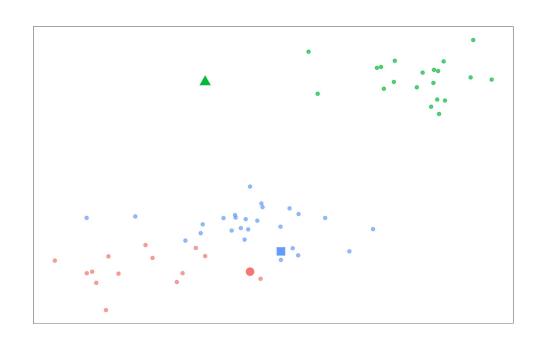
Step 3: Find new centers as the mean locations of all points in a cluster



Step 1: Pick k random centers

Step 2: Assign points to clusters by which center is closest

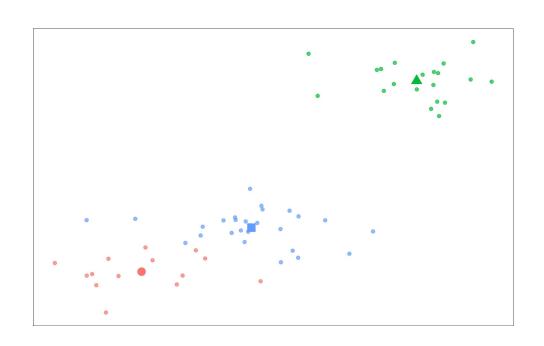
Step 3: Find new centers as the mean locations of all points in a cluster



Step 1: Pick k random centers

Step 2: Assign points to clusters by which center is closest

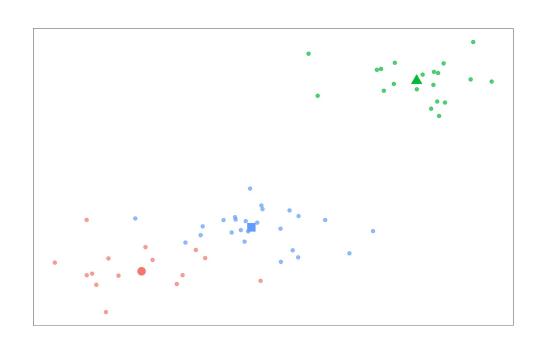
Step 3: Find new centers as the mean locations of all points in a cluster



Step 1: Pick k random centers

Step 2: Assign points to clusters by which center is closest

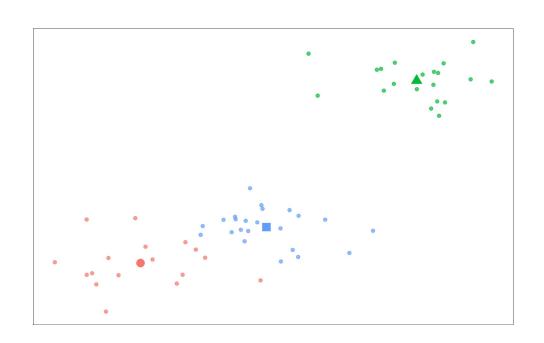
Step 3: Find new centers as the mean locations of all points in a cluster



Step 1: Pick k random centers

Step 2: Assign points to clusters by which center is closest

Step 3: Find new centers as the mean locations of all points in a cluster



Graph-based Clustering

Step 1: Calculate similarity matrix among points .

Step 2: Build a weighted network graph connecting points to their neighbors

Step 3: Divide network graph into "neighborhoods" based on connection patterns

Many options at each step! The algorithms can determine how many clusters to assign.

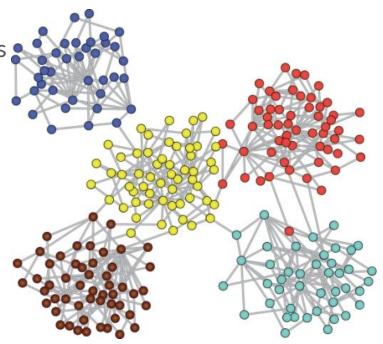
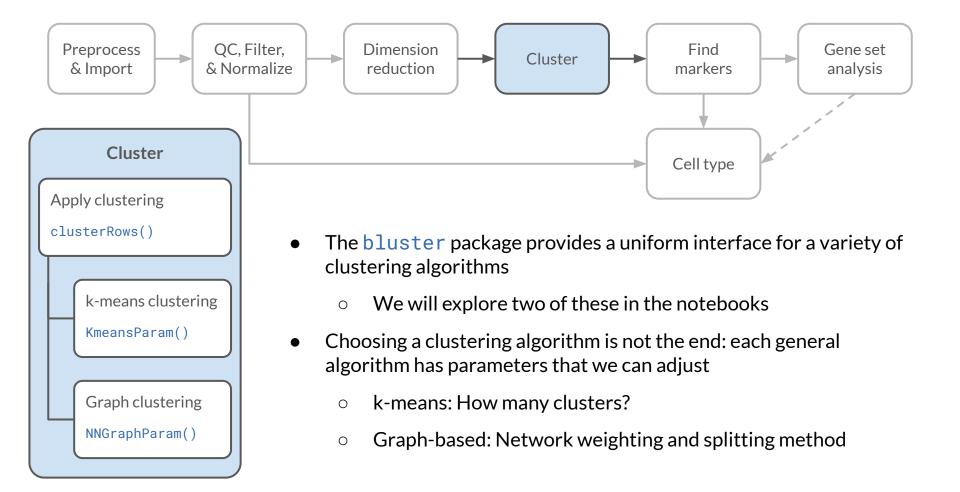


Image from:

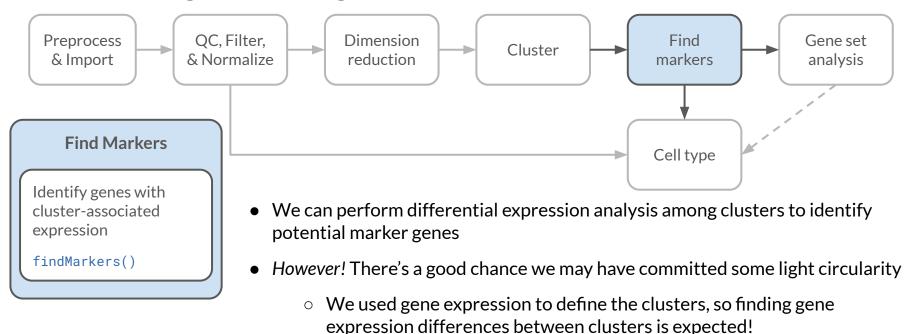
https://github.com/benedekrozemberczki/awesome-community-detection



What do the clusters represent?

- Groups of cells with distinct gene expression patterns
- What does that mean?
 - maybe cell types?
 - o sometimes cell states?
 - o perhaps perturbations?
- Interpretation will vary based on the sample you are using!
 - do not expect a simple mapping of clusters to cell types
- Clustering is usually somewhat stochastic
 - o parameter choice and random seeds will affect clusters
 - use caution when interpreting clustering results!

Identifying marker genes



 Don't rely too much on the specific statistics we calculate (for more, see the OSCA section on p values)