scRNAseq clustering tools

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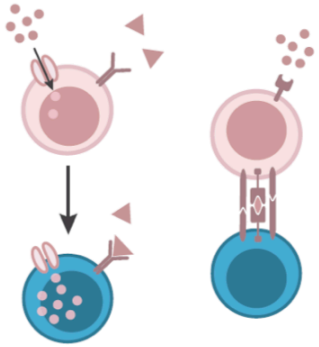
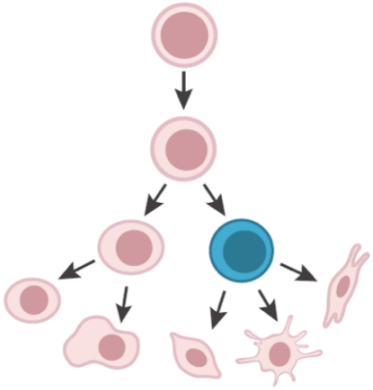
Cell identity

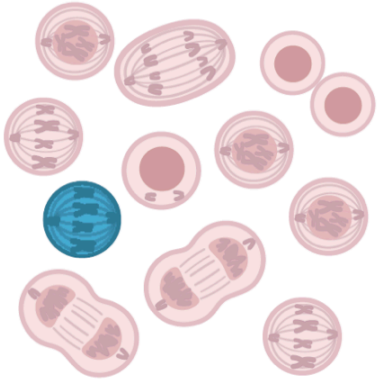
Environmental stimuli



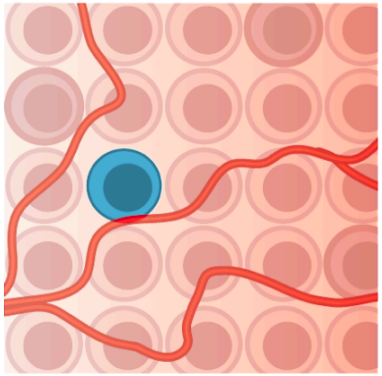
Cell development



Cell cycle



Spatial context



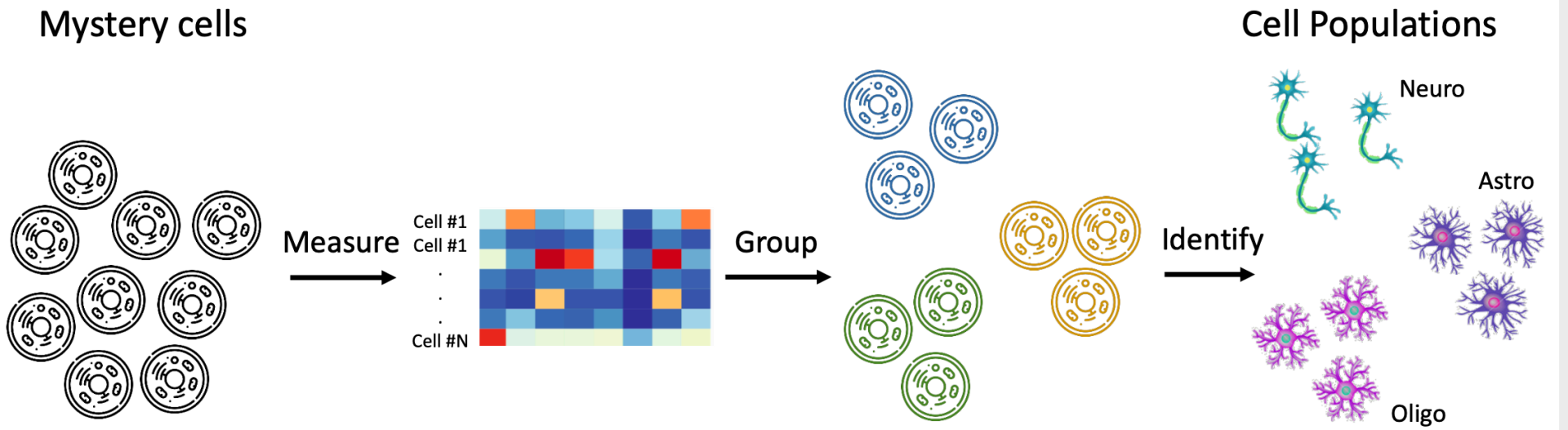
What is a cell type?

- A cell that performs a specific function?
- A cell that performs a specific function at a specific location/tissue?
- Not clear where to draw the line between cell types and **subpopulations** within a cell type.
- Also important to distinguish between **cell type** and **cell state**.
  - A cell state may be infected/non infected
  - Metabolically active/inactive
  - Cell cycle stages
  - Apoptotic

Outline

- Basic clustering theory
- Graph theory introduction
- Examples of different tools for clustering single cell data
- Celltype prediction

How can we identify populations?



Considerations for clustering

- Hypotheses:
  - What is a cell type? What cell types are in my tissue?
  - What is the number of clusters *k*?
- Choices:
  - Gene set selection
  - Similarity measure
  - Algorithm and hyper parameters of that algorithm.
- Different choice leads to different results. Validate, interpret and repeat steps.

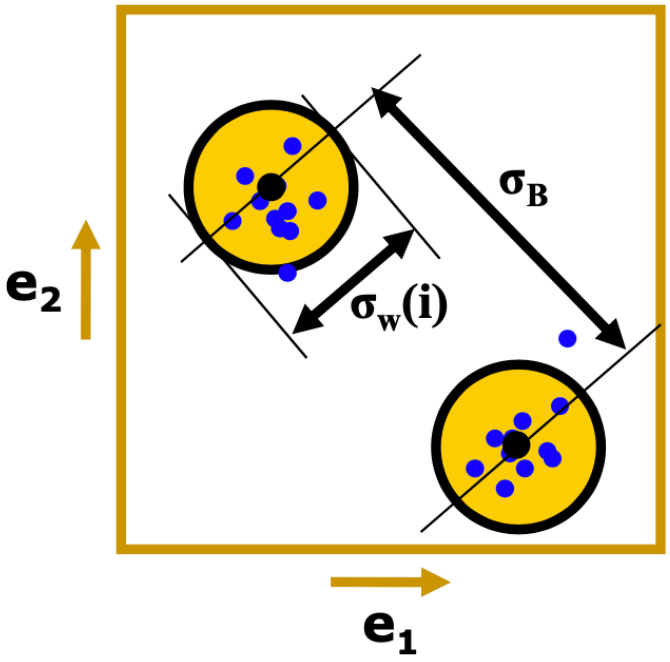
What is clustering?

- “The process of organizing objects into groups whose members are similar in some way”
- Typical methods are:
  - Hierarchical clustering
  - K-means clustering
  - Density based clustering
  - Graph based clustering

The main idea

- Structure when:
  1) Samples within cluster resemble each other *(within variance, σW(i))*
  2) Clusters deviate from each other

  (between variance, σB)



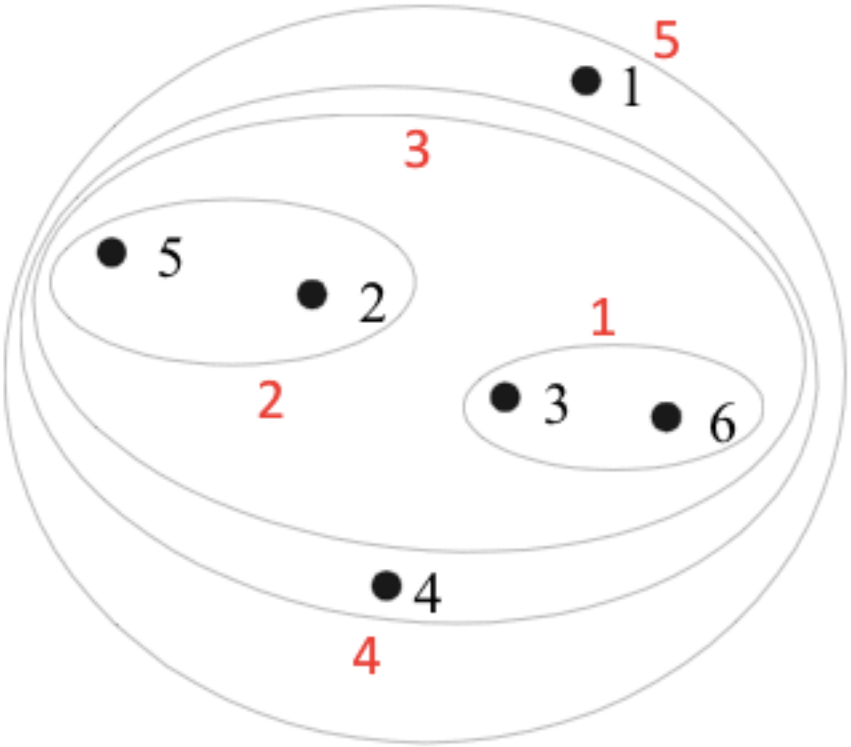
Group samples such that:

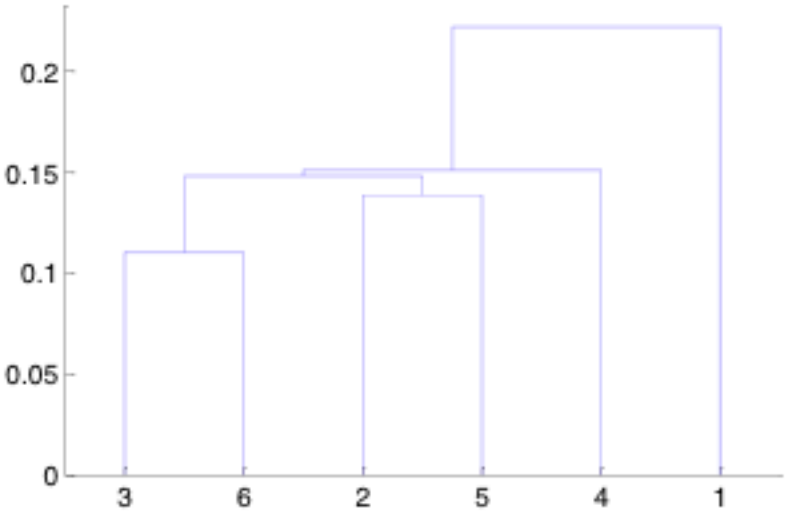
$$\min\left(\frac{\sum_{\forall clusters}\sigma_W(i)}{\sigma_B}\right) \rightarrow \sigma_W\text{: small \&}\ \sigma_B\text{: large}$$

Hierarchical clustering

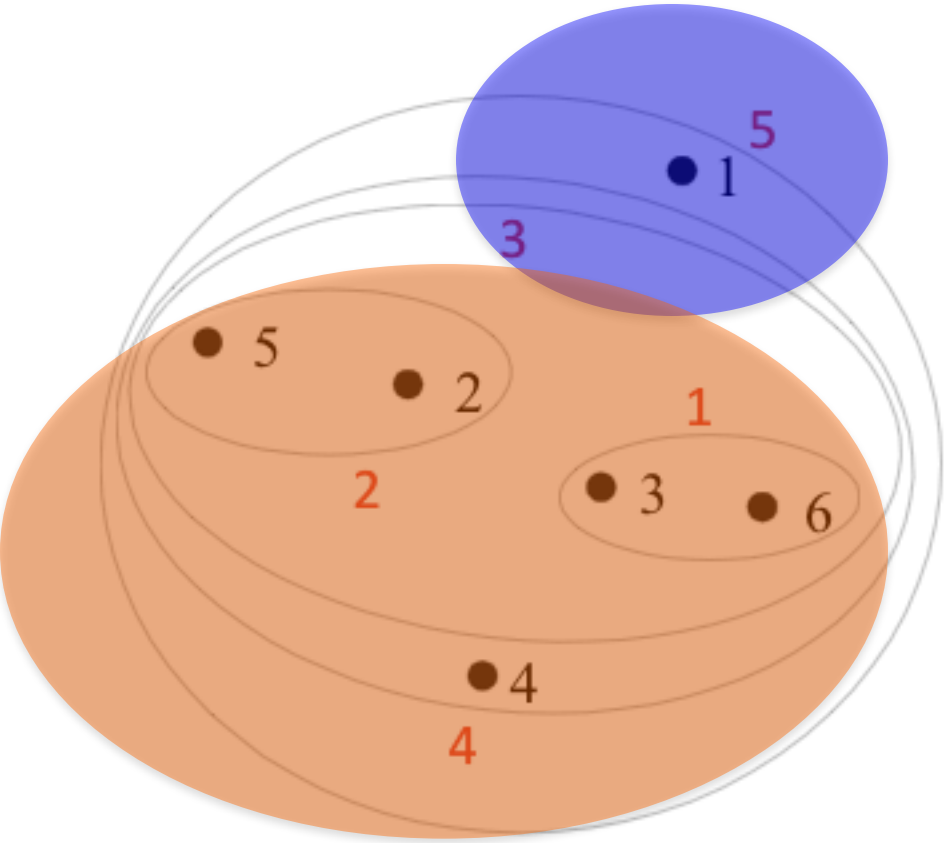
- Builds on **distances** between data points
- **Agglomerative** – starts with all data points as individual clusters and joins the most similar ones in a bottom-up approach
- **Divisive** – starts with all data points in one large cluster and splits it into 2 at each step. A top-down approach
- Final product is a **dendrogram** representing the decisions at each merge/division of clusters

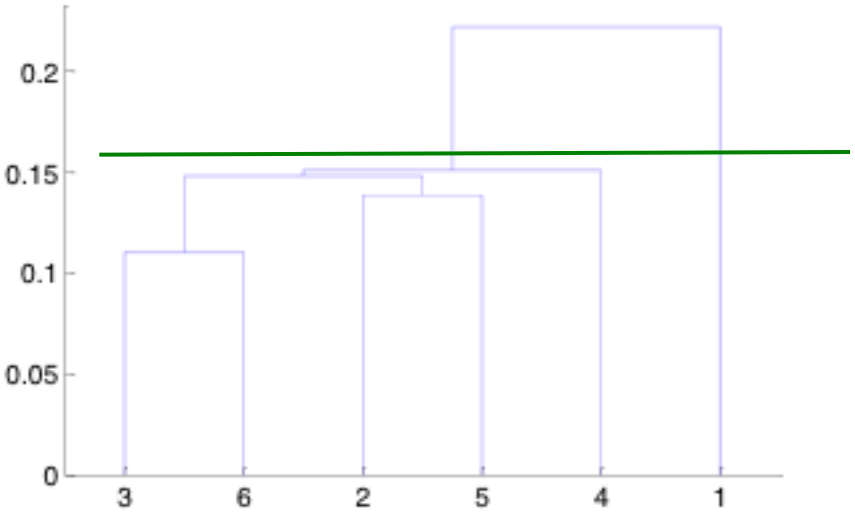
Hierarchical clustering





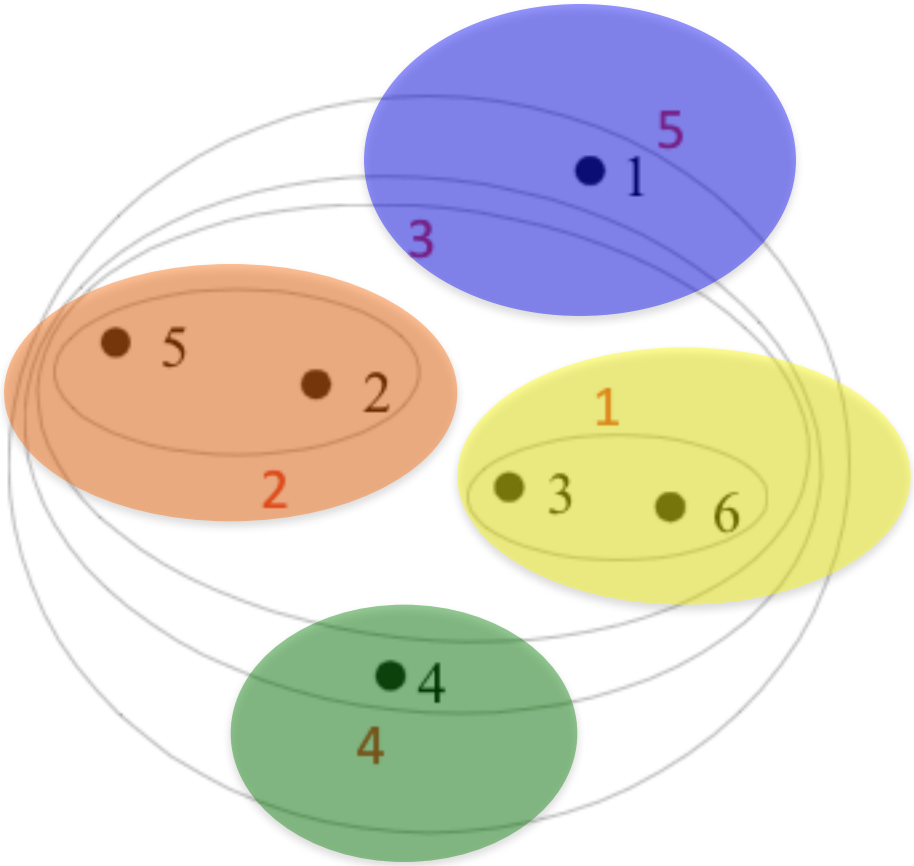
Hierarchical clustering

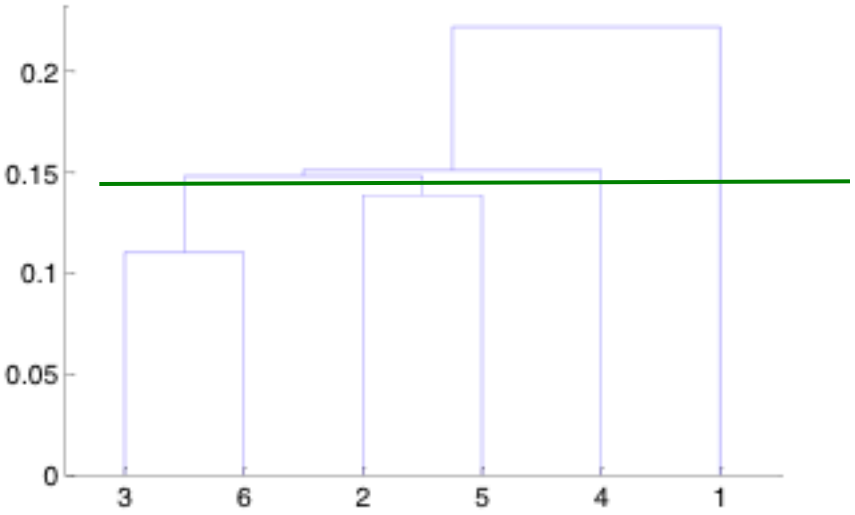




Clusters are obtained by cutting the tree at a desired level

Hierarchical clustering

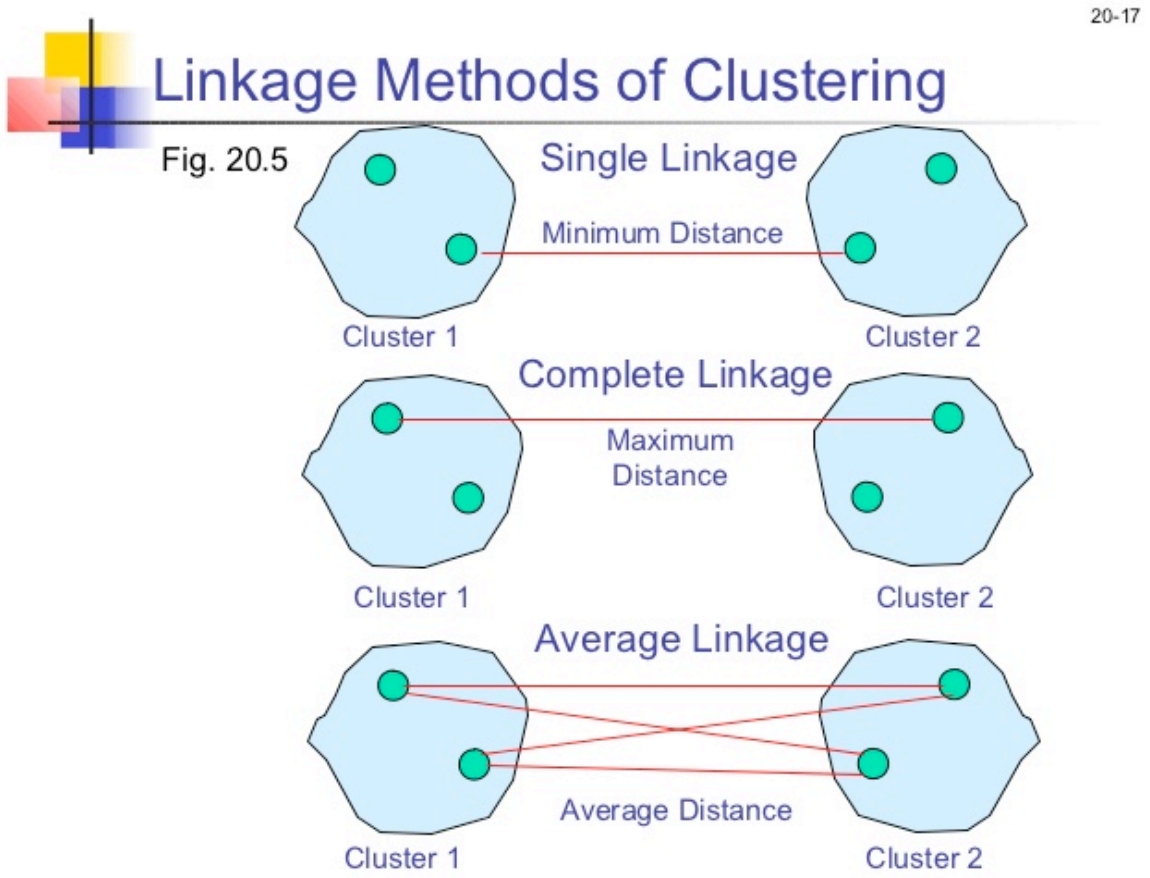




Clusters are obtained by cutting the tree at a desired level

Linkage criteria

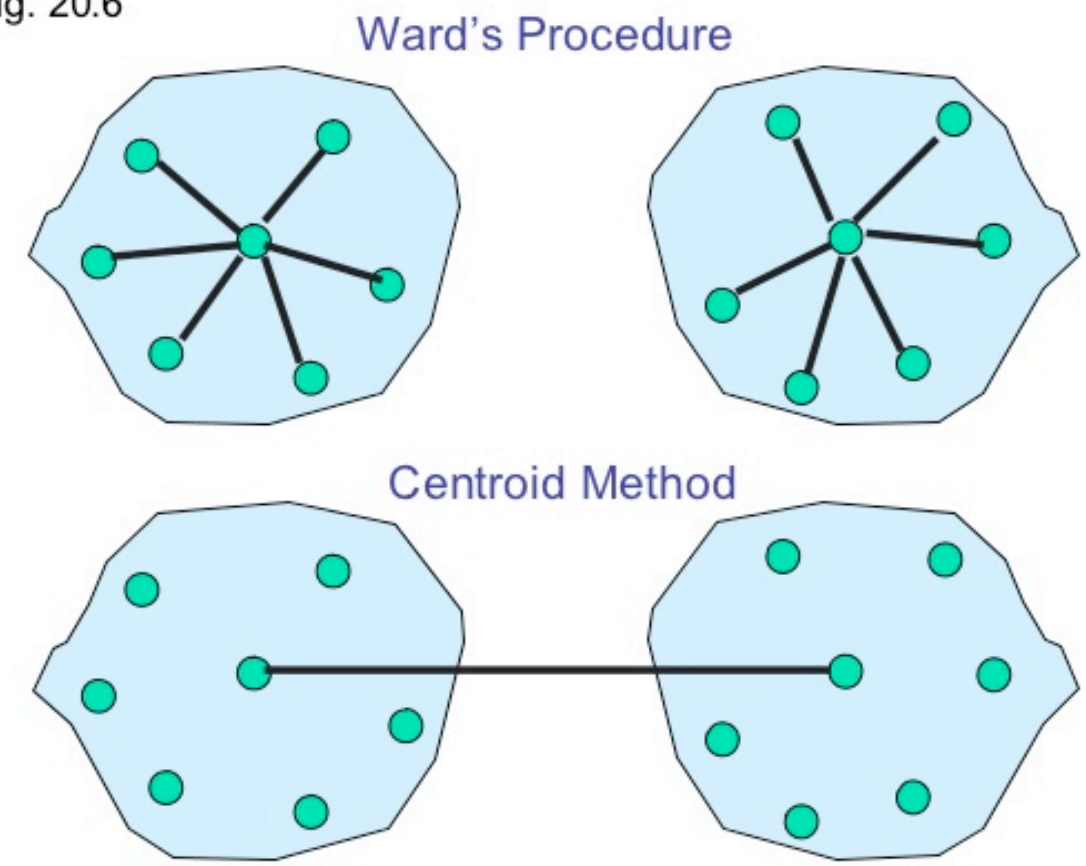
- Calculation of similarities between 2 clusters (or a cluster and a data point)



http://www.slideshare.net/uzairjavedsiddiqui/malhotra20

Other Agglomerative Clustering Methods

Fig. 20.6

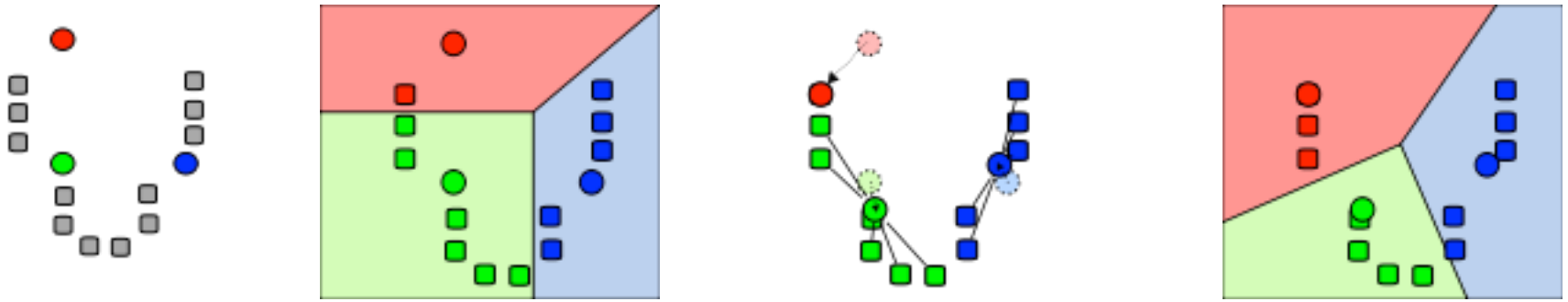


- Ward (minimum variance method). Similarity of two clusters is based on the increase in squared error when two clusters are merged.

K-means clustering

1. Starts with random selection of cluster centers (centroids)
2. Then assigns each data points to the nearest cluster
3. Recalculates the centroids for the new cluster definitions
4. Repeats steps 2-3 until no more changes occur.

Can use same distance measures as in hclust.



https://en.wikipedia.org/wiki/K-means_clustering

Network/graph clustering

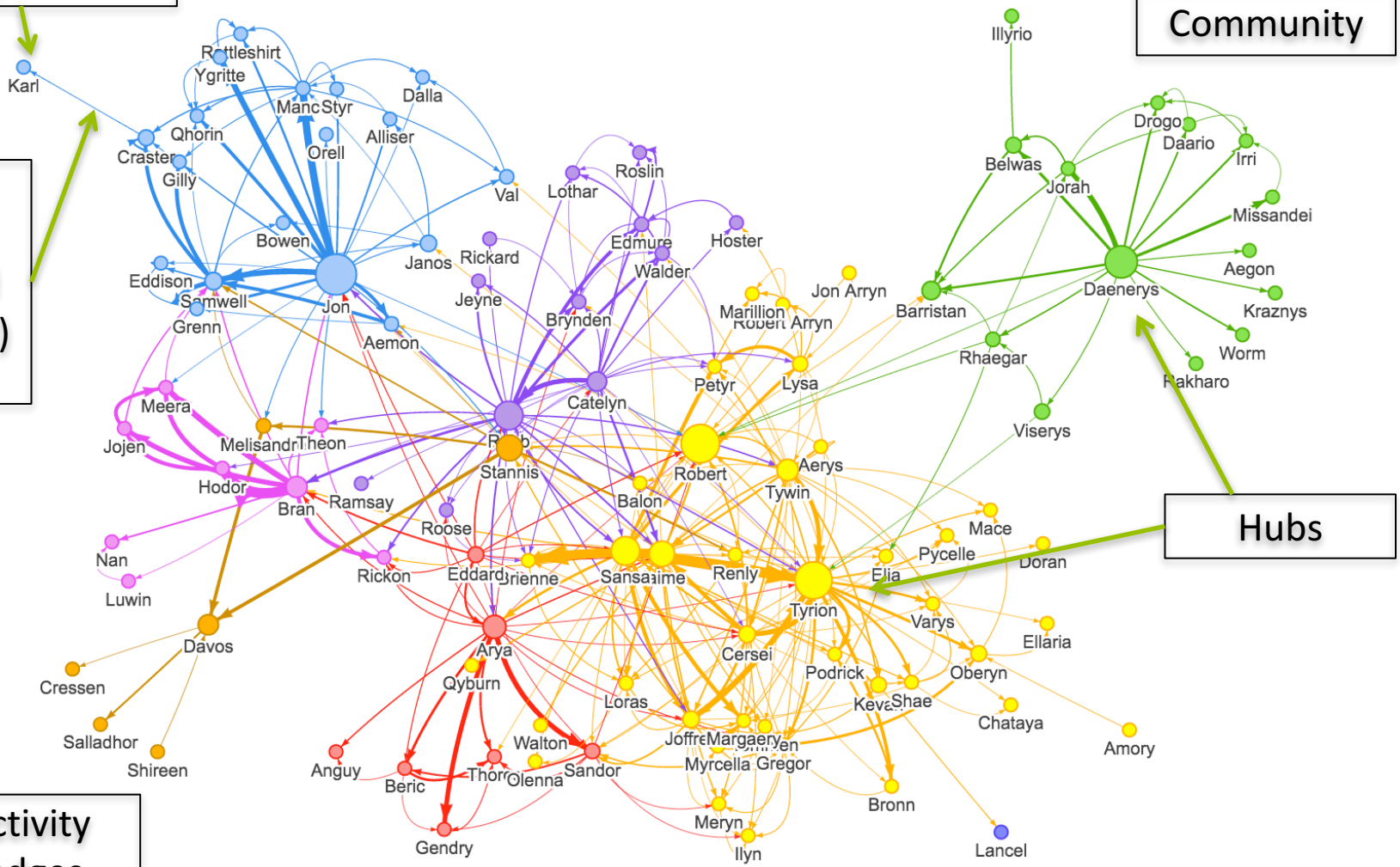
Node/Vertice

Edge – (weighted & directed)

Community

Hubs

Connectivity - # of edges



(http://www.lyonwj.com/2016/06/26/graph-of-thrones-neo4j-social-network-analysis/)

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Types of graphs

- The ***k*-Nearest Neighbor** (***k*NN**) graph is a graph in which two vertices *p* and *q* are connected by an edge, if the distance between *p* and *q* is among the *k*-th smallest distances from *p* to other objects from *P*.
- The **Shared Nearest Neighbor** (**SNN**) graph has weights that defines proximity, or similarity between two edges in terms of the number of neighbors (i.e., directly connected vertices) they have in common.

SNN graph

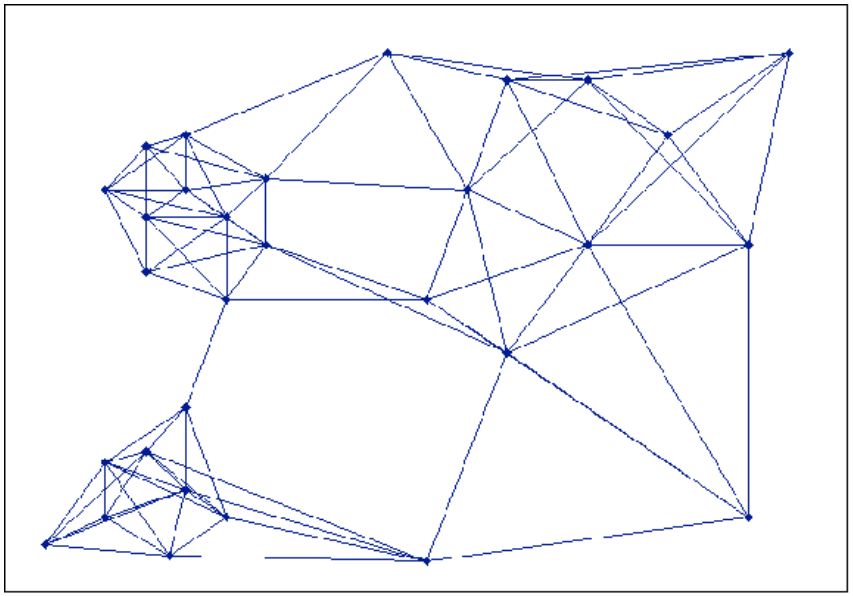


Figure 2. Near Neighbor Graph

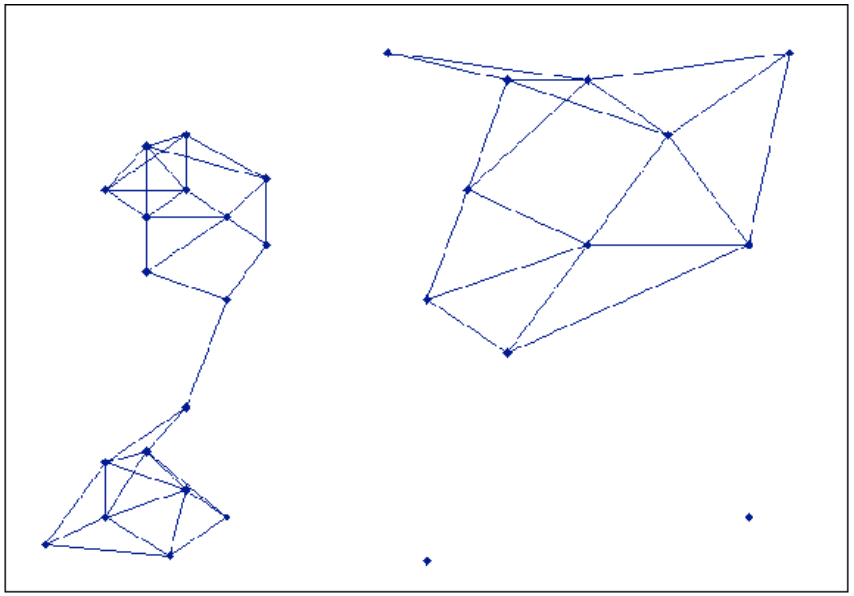
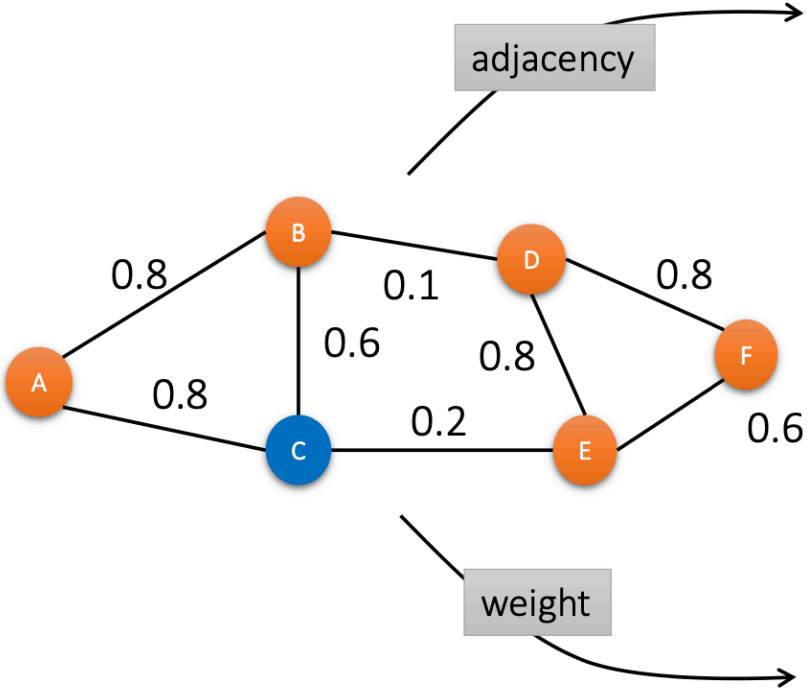


Figure 3. Unweighted Shared Near Neighbor Graph

(Ertöz et al. Semantic scholar, 2002)

Graphs, adjacency and weight matrices

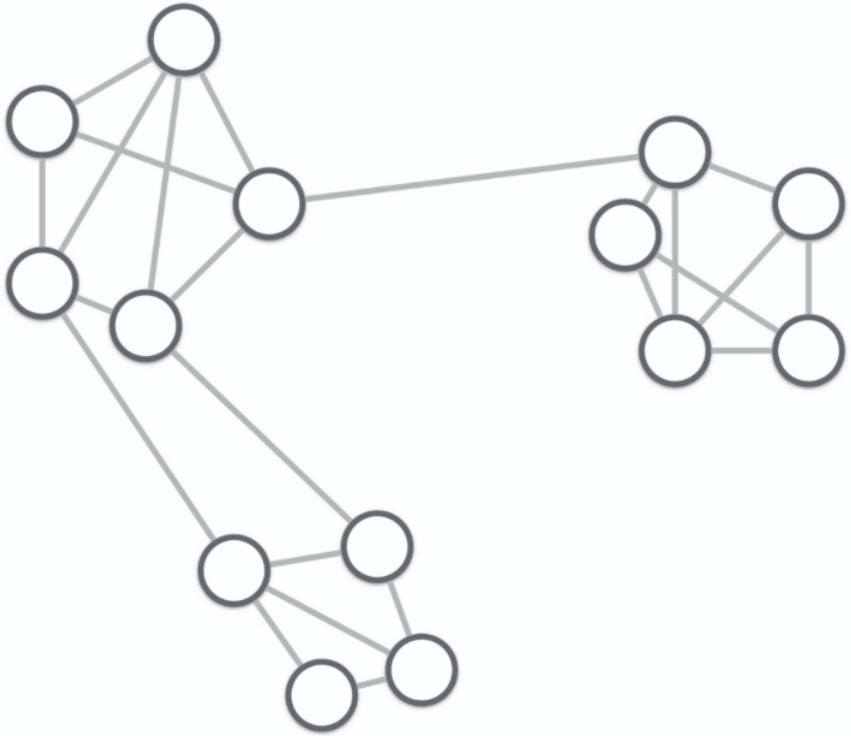


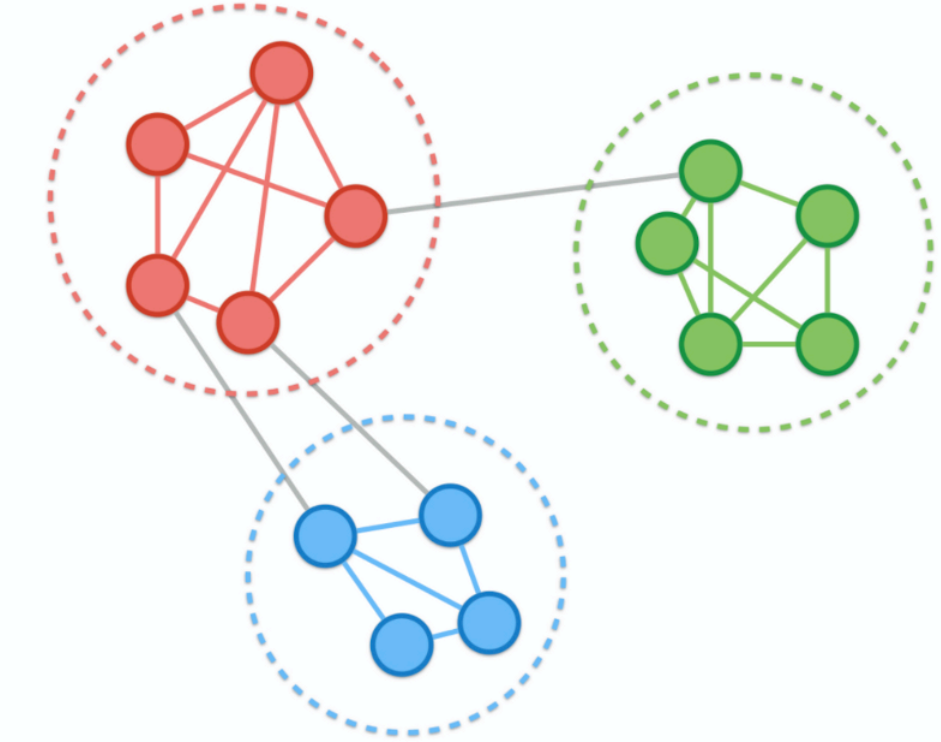
$$A = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 \end{pmatrix} \end{array}$$

$$W = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 0.8 & 0.8 & 0 & 0 & 0 \\ 0.8 & 0 & 0.6 & 0.1 & 0 & 0 \\ 0.8 & 0.6 & 0 & 0 & 0.2 & 0 \\ 0 & 0.1 & 0 & 0 & 0.8 & 0.8 \\ 0 & 0 & 0.2 & 0.8 & 0 & 0.6 \\ 0 & 0 & 0 & 0.8 & 0.6 & 0 \end{pmatrix} \end{array}$$

Community detection

Communities, or clusters, are usually groups of vertices having higher probability of being connected to each other than to members of other groups.



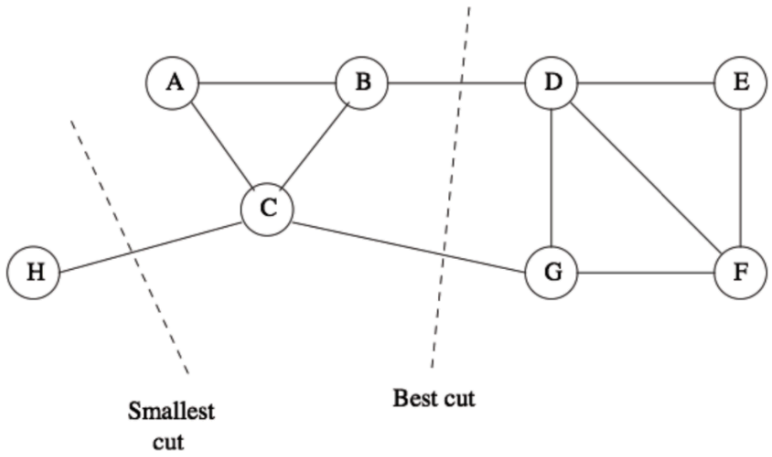


Community detection

- Main objective is to find a group (community) of vertices with more edges **inside** the group than edges linking vertices of the group with the rest of the graph.

Graph cuts

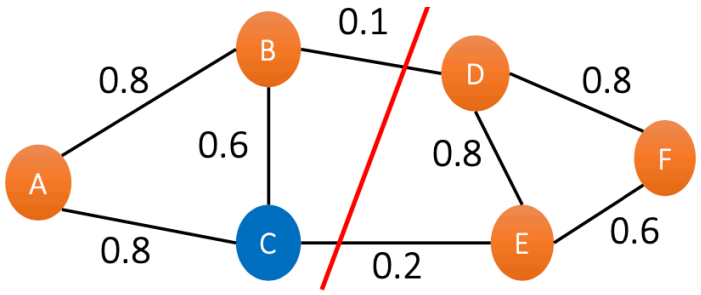
- Graph cut partitions a graph into subgraphs
- Cut cost is the sum of weights of the edges.
- Clustering by graph cuts: find the smallest cut that bi-partitions the graph
- The smallest cut is not always the best cut – may give many small disjoint cluster



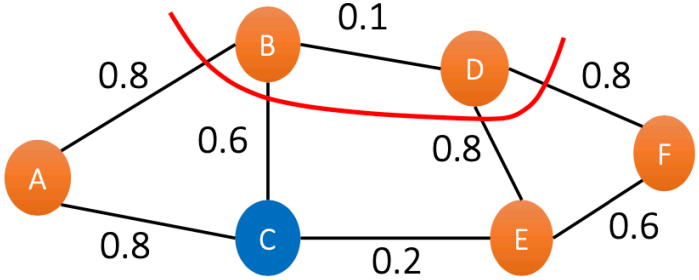
Normalized cut

- Normalized cut computes the cut cost as a fraction of the total edge connections to all the nodes in the graph.

- cut(S,T) = 0.1 + 0.2 = 0.3
- vol(S)= 0.3 + 0.6 + 0.8 + 0.8 = 2.5
- vol(T)= 0.3 + 0.8 + 0.8 + 0.6 = 2.5
- Ncut(S,T) = 0.3/2.5 + 0.3/2.5 = 0.24



- cut(S,T) = 0.8 + 0.6 + 0.8 + 0.8 = 3.0
- vol(S) = 3.0 + 0.1 = 3.1
- vol(T) = 3.0 + 0.8 + 0.2 + 0.6 = 4.6
- Ncut(S,T) = 3.0/3.1 + 3.0/4.6 = 1.62



Normalized cut

- Searching for the best normalized cut is NP-hard
- We need a heuristic method to solve the problem:
  - Spectral clustering
  - Louvain
  - Markov clustering
  - Leiden
  - …

For single cell data

- Can start with distances based on correlation, euklidean distances in PCA space etc. Same as for hclust/k-means.
- Buld a KNN graph with cells as vertices.
  - Find **k** nearest neighbors to each cell.
  - The size of **k** will strongly influence the network structure.
- Can reduce network based on shared neighbors.
- Find clusters with community detection method.
- Graphs can also be used for trajectory analysis

How to work with networks

- Igraph package – implemented for both R, python and Ruby
- Has most commonly used layout optimization methods and community detection methods implemented.
- Simple R example at:

https://jef.works/blog/2017/09/13/graph-based-community-detection-for-clustering-analysis/

- Tutorial to igraph at:

http://kateto.net/networks-r-igraph

Distance between cells

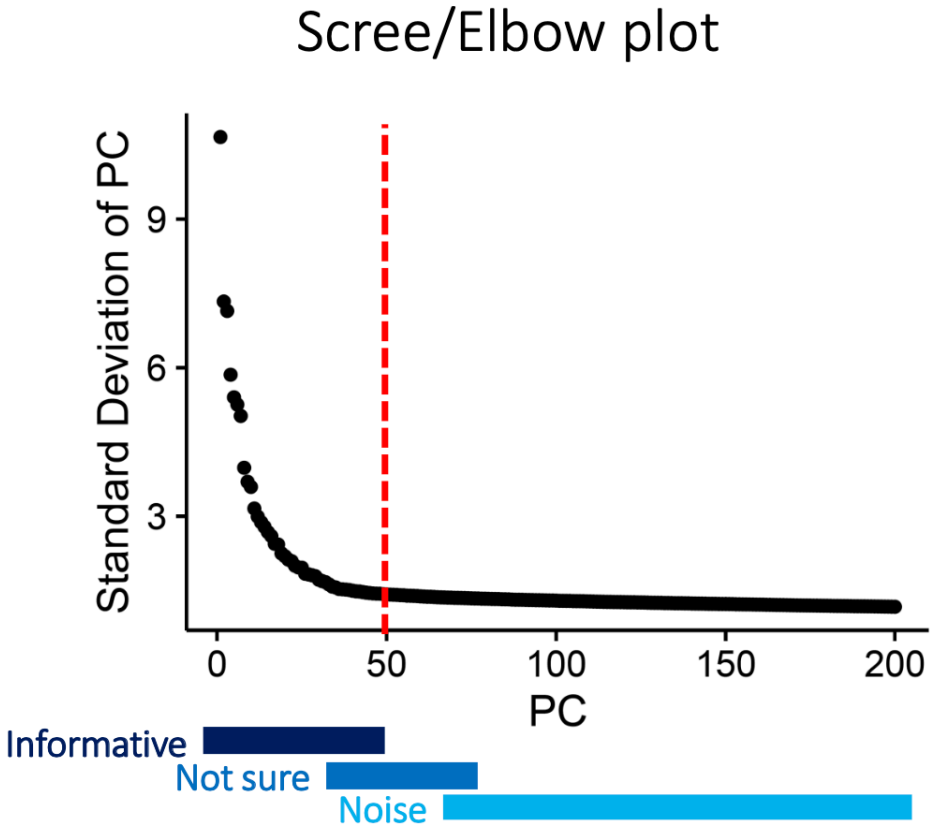
- All clustering methods need to define distances between cells. Things to consider are:
  - What gene set should be included?
    - Commonly used: Highly variable genes
  - What space to calculate distance in?
    - Commonly done in PCA space
    - Can also be full space, tSNE, UMAP etc.
  - How many dimensions to include?
  - What distance measure?

Different distance measures

- Most commonly used in scRNA-seq:
  - Euclidean distance
  - Inverted pairwise correlations (1-correlation)
- Other common methods are:
  - Manhattan distance
  - Mahalanobis distance
  - Maximum distance

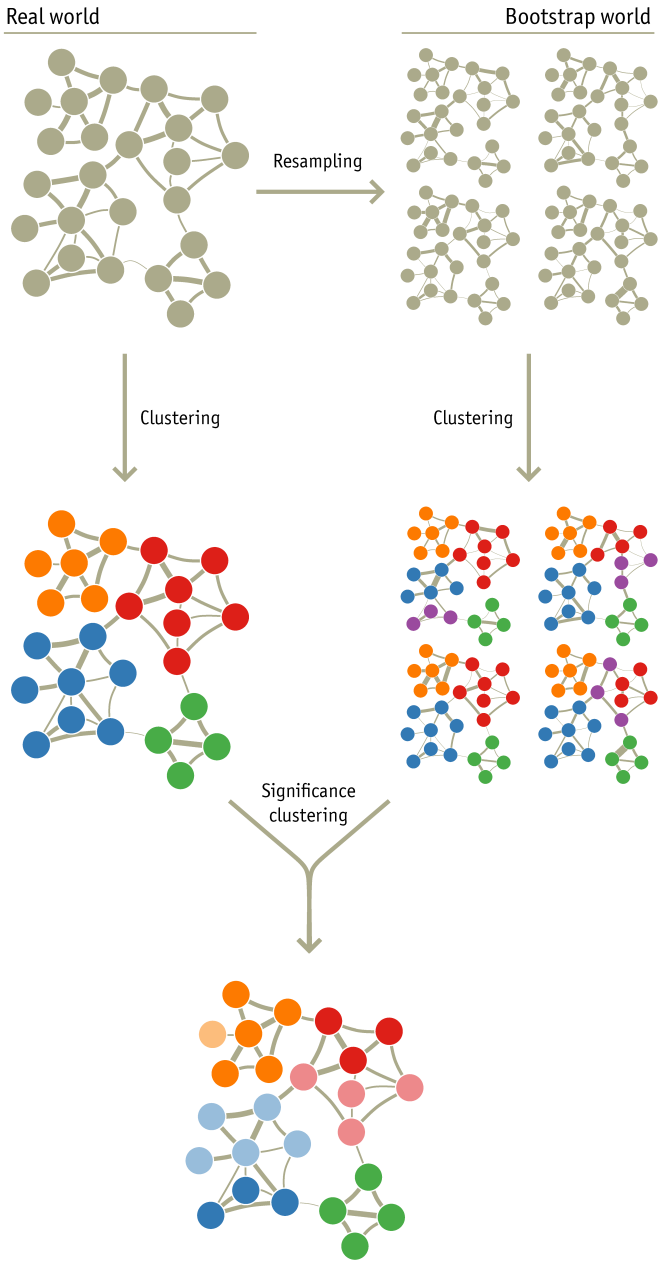
Selection of principal components

- To overcome the extensive technical noise in scRNA-seq data, it is common to cluster cells based on their PCA scores
- Each PC represents a 'metagene' that (linearly) combines information across a correlated gene set
- Depending on the heterogeneity of your data more/less PCs should be selected.



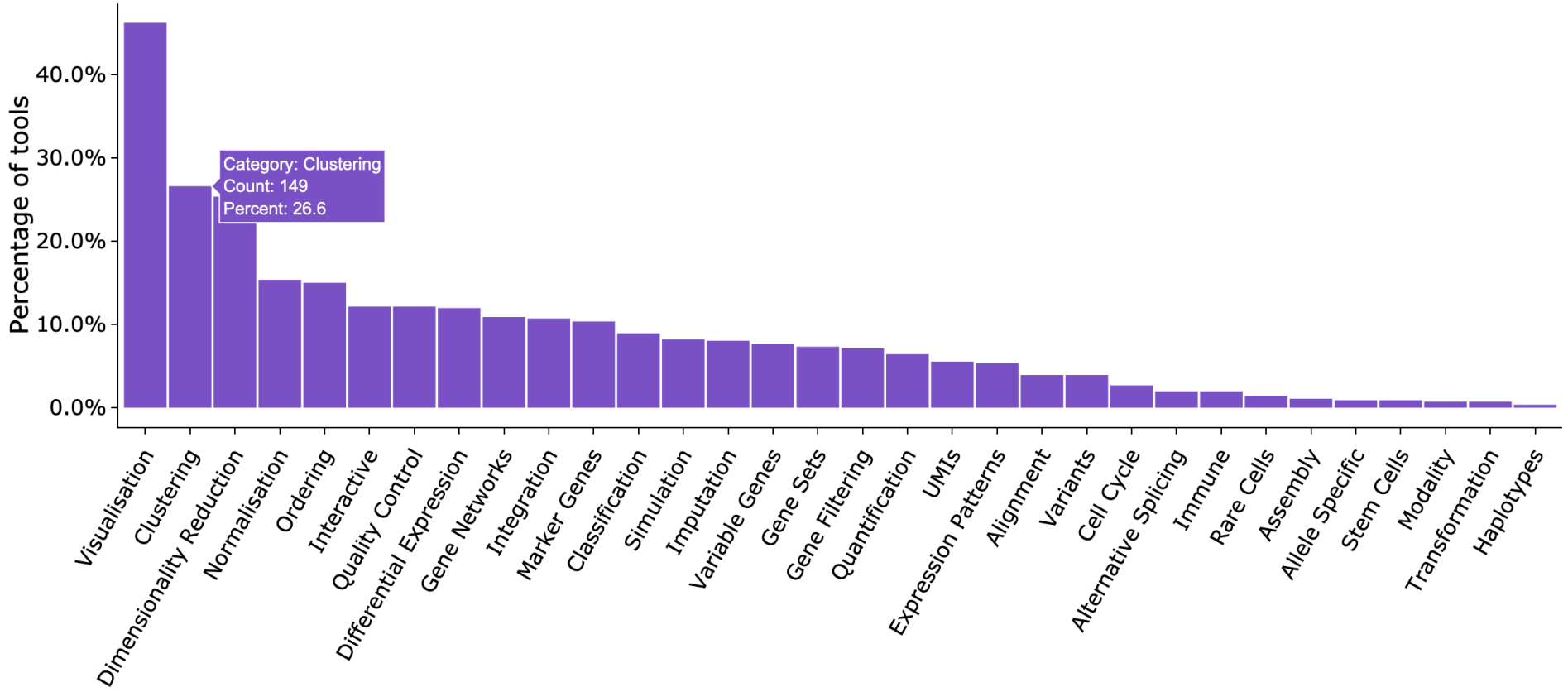
Bootstrapping

- How confident can you be that the clusters you see are real?
- You can always take a random set of cells from the same cell type and manage to split them into clusters.
- Most scRNAseq packages do not include any bootstrapping. Scran has function **bootstrapCluster.**



(Rosvall et al. *Plos One* 2010)

Many tools for clustring scRNAseq data



https://www.scrna-tools.org/

Main pipelines

- Scater + Scran – EBI groups, Marioni, Lun, McCarthy
- Seurat – Satija lab
- Monocle – Trapnell lab
- Pagoda – Kharchenko lab

Seurat

- Developed for drop-seq analysis – compatible with 10X output files. But works also for other types of data.
- Contains function for
  - Data normalization
  - Detection of variable genes
  - Regression of batch effects and other confounders
  - Prediction of cell cycle score
  - JackStraw to detect significant principal components
  - tSNE and other dimensionality reduction techniques
  - Clustering based on SNN graphs
  - Many different methods for Differential expression

Seurat - FindClusters

- First construct a KNN (k-nearest neighbor) graph based on the euclidean distance in PCA space.
- Prune the edge weights between any two cells based on the shared overlap in their local neighborhoods (Jaccard distance).
- To cluster the cells, modularity optimization techniques to iteratively group cells together.
  - Louvain
  - Louvain with multilevel refinement
  - Leiden
  - SLM

Seurat

- Also contains functions for:
  - Spatial reconstruction of single cell data using *in situ* references (Zebrafish embryos)
  - Integrated analysis across platforms
  - Analysis of multimodal datasets (e.g. RNA + protein)

SCRAN – Single Cell RNA ANalisys

- Uses SingleCellExperiment class – same as in Scater package
- Cyclone method for predicting cell cycle phase.
- Basics deconvolution strategy for size factors.
- Detection of variable genes by deconvolution of technical and biological variance.
- MNNCorrect/fastMNN for batch correction
- Also contains method for SNN graphs and community detection.

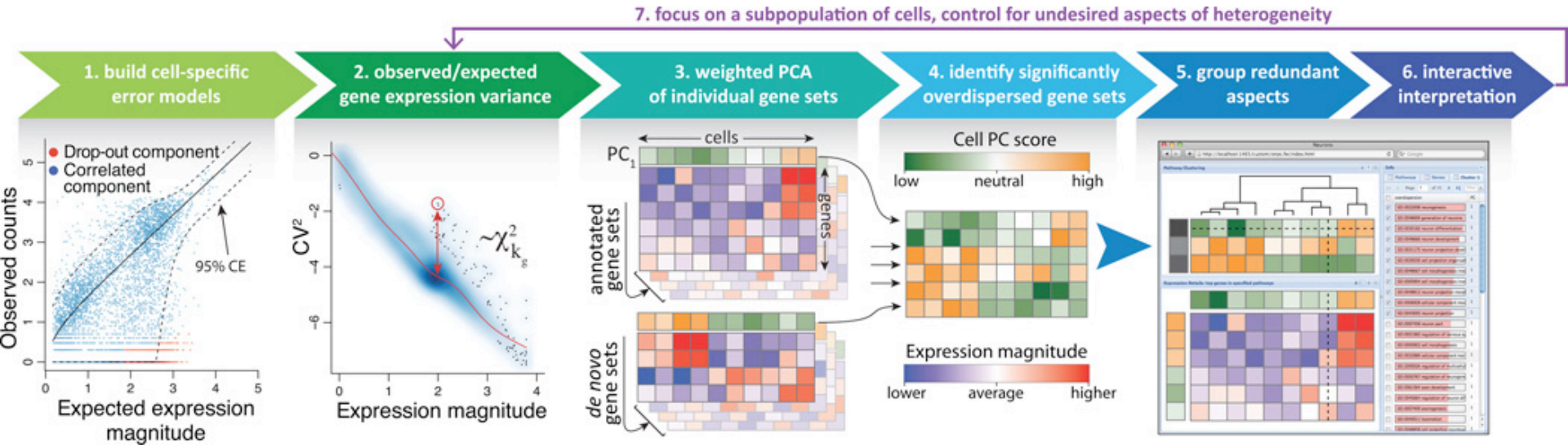
Shared nearest neighbor (SNN)-Cliq

- Similarity matrix using Euclidean distance (can use other distances)
- List the *k*-nearest-neighbors (KNN)
- Edge between cells if at least one shared neighbor
- Weights based on ranking of the neighbors
- Graph partition by finding cliques
- Identify clusters in the SNN graph by iteratively combining significantly overlapping subgraphs
- Implemented in Matlab and Python

(Xu et al *Bioinformatics* 2015)

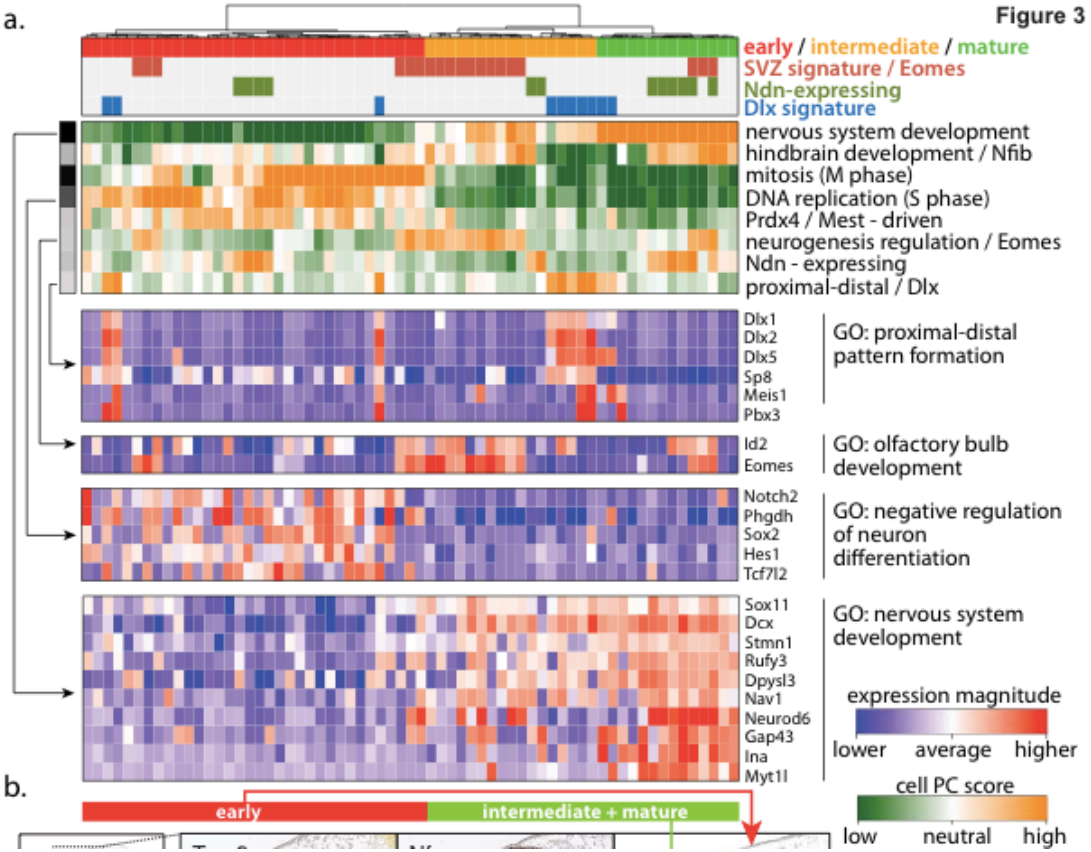
Pagoda – Pathway And Geneset OverDispersion Analysis

Implemented in the SCDE package



(Fan et al. *Nature Methods* 2016)

Pagoda – Pathway And Geneset OverDispersion Analysis



- Helps with biological interpretation of data
- Important to have good and relevant gene sets
- High memory consumption when running Pagoda
- Also has methods for removing batch effect, detected genes, cell cycle etc

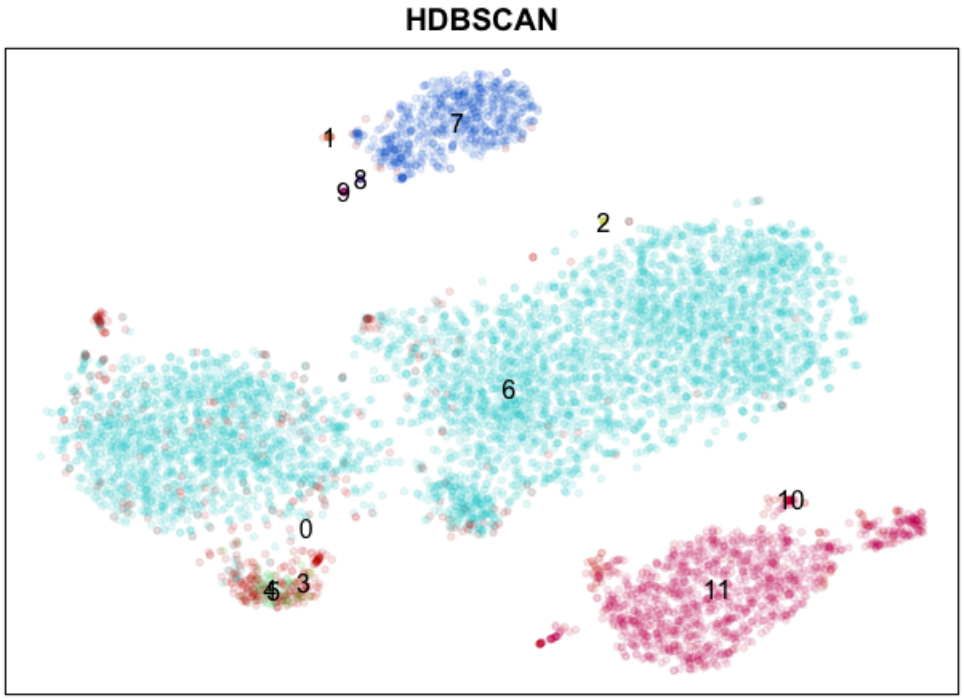
(Fan et al. *Nature Methods* 2016)

Pagoda2

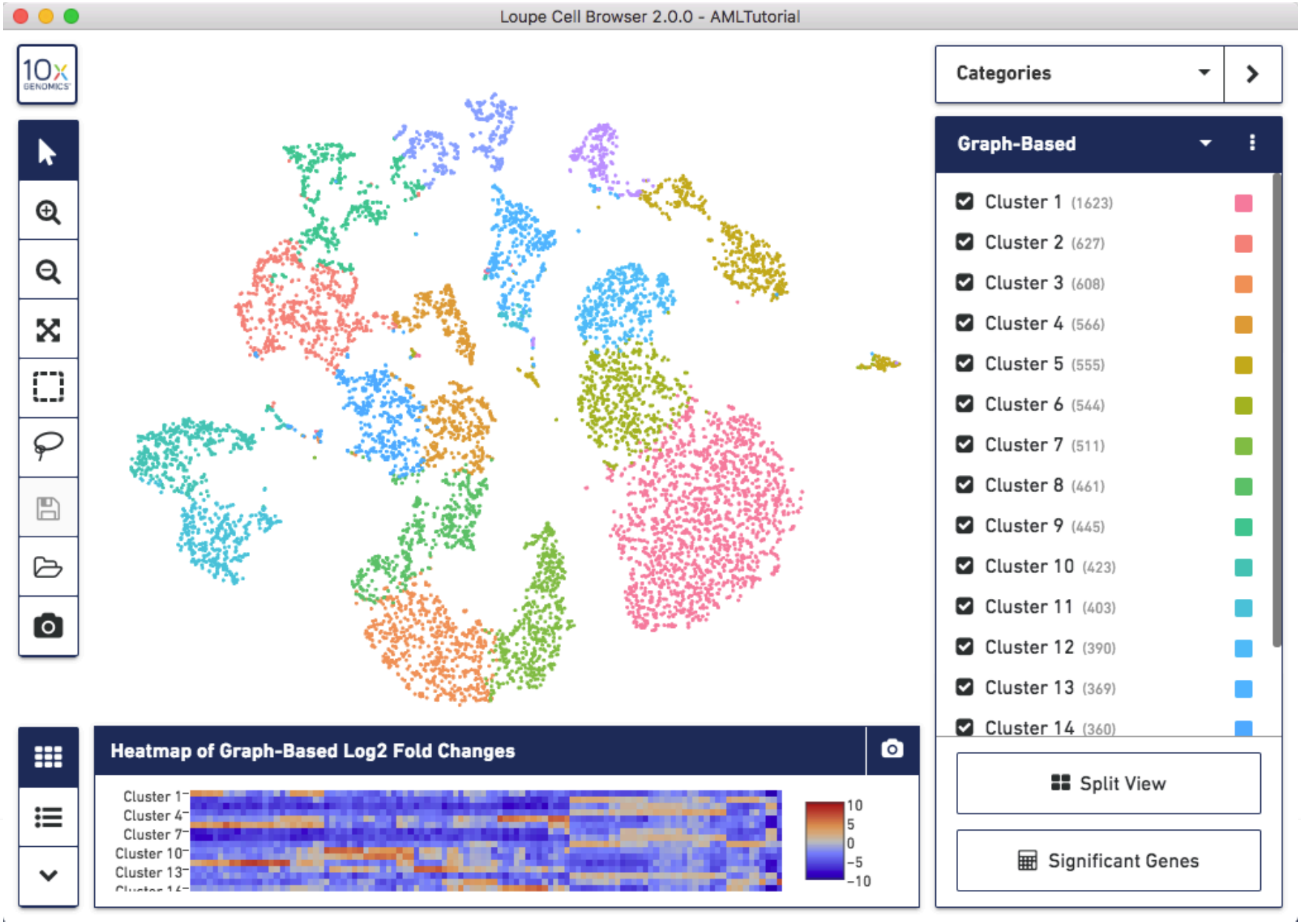
- Similar error modelling
- Now include KNN graph clustering
- largeViz for dimensionality reduction
- Can visualize gene sets.
- https://github.com/hms-dbmi/pagoda2

HDBSCAN

- Hierarchical DBSCAN – density based clustering on tSNE



Loupe – Cell Browser, from 10X Genomics



Which clustering method is best?

- Depends on the input data
- Consistency between several methods gives confidence that the clustering is robust
- The clustering method that is most consistent – best bootstrap values is not always best
- In a simple case where you have clearly distinct celltypes, simple hierarchical clustering based on euclidean or correlation distances will work fine.

Comparison of clustering methods

F1000Research

F1000Research 2018, 7:1141 Last updated: 11 SEP 2018

Check for updates

RESEARCH ARTICLE

REVISED A systematic performance evaluation of clustering methods for single-cell RNA-seq data [version 2; referees: 2 approved]

Angelo Duò[1,2], Mark D. Robinson[1,2], Charlotte Soneson[1,2]

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[2]SIB Swiss Institute of Bioinformatics, Zurich, 8057, Switzerland

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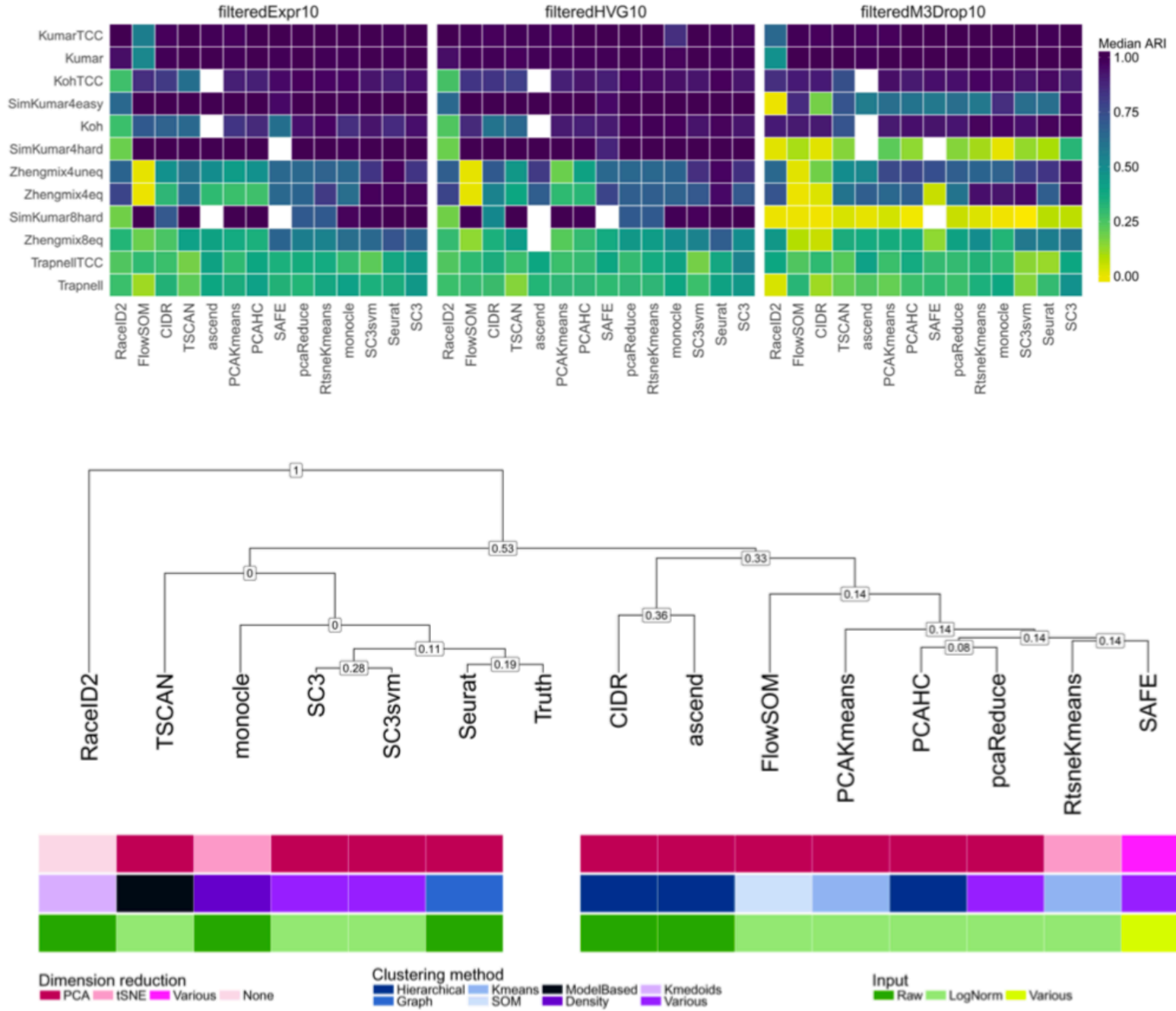
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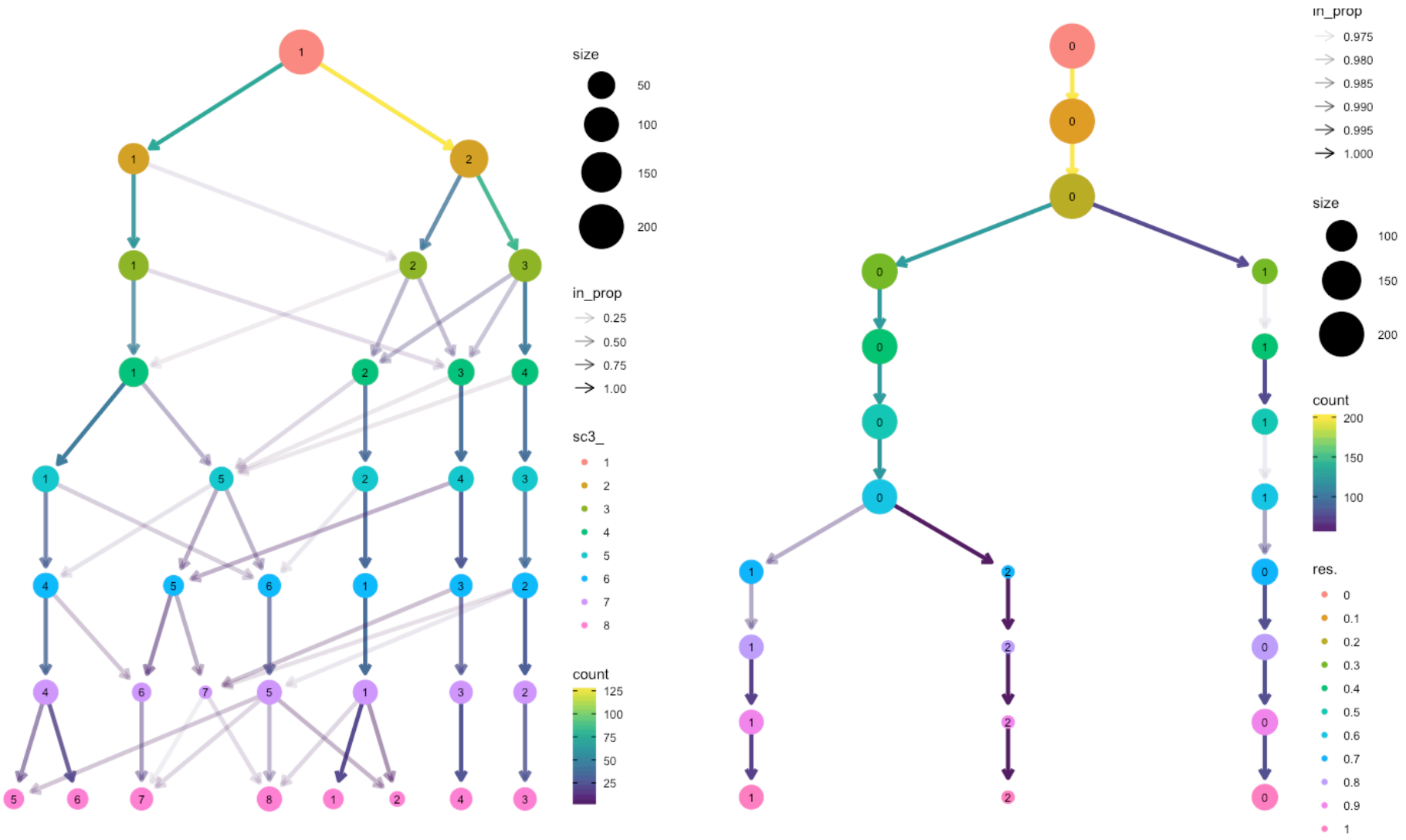
KumarTCC
Kumar
KohTCC
SimKumar4easy
Koh
SimKumar4hard
Zhengmix4uneq
Zhengmix4eq
SimKumar8hard
Zhengmix8eq
TrapnellTCC
Trapnell
RaceID2
FlowSOM
CIDR
TSCAN
ascend
PCAKmeans
PCAHC
SAFE
pcaReduce
RtsneKmeans
monocle
SC3svm
Seurat
SC3
Median ARI
1.00
0.75
0.50
0.25
0.00
1
0.53
0.33
0
0
0.14
0.36
0.14
0.11
0.14
0.14
0.28
0.19
0.08
RaceID2
TSCAN
monocle
SC3
SC3svm
Seurat
Truth
CIDR
ascend
FlowSOM
PCAKmeans
PCAHC
pcaReduce
RtsneKmeans
SAFE
Dimension reduction
PCA
tSNE
Various
None
Clustering method
Hierarchical
Kmeans
ModelBased
Kmedoids
Graph
SOM
Density
Various
Input
Raw
LogNorm
Various

How many clusters do you really have?

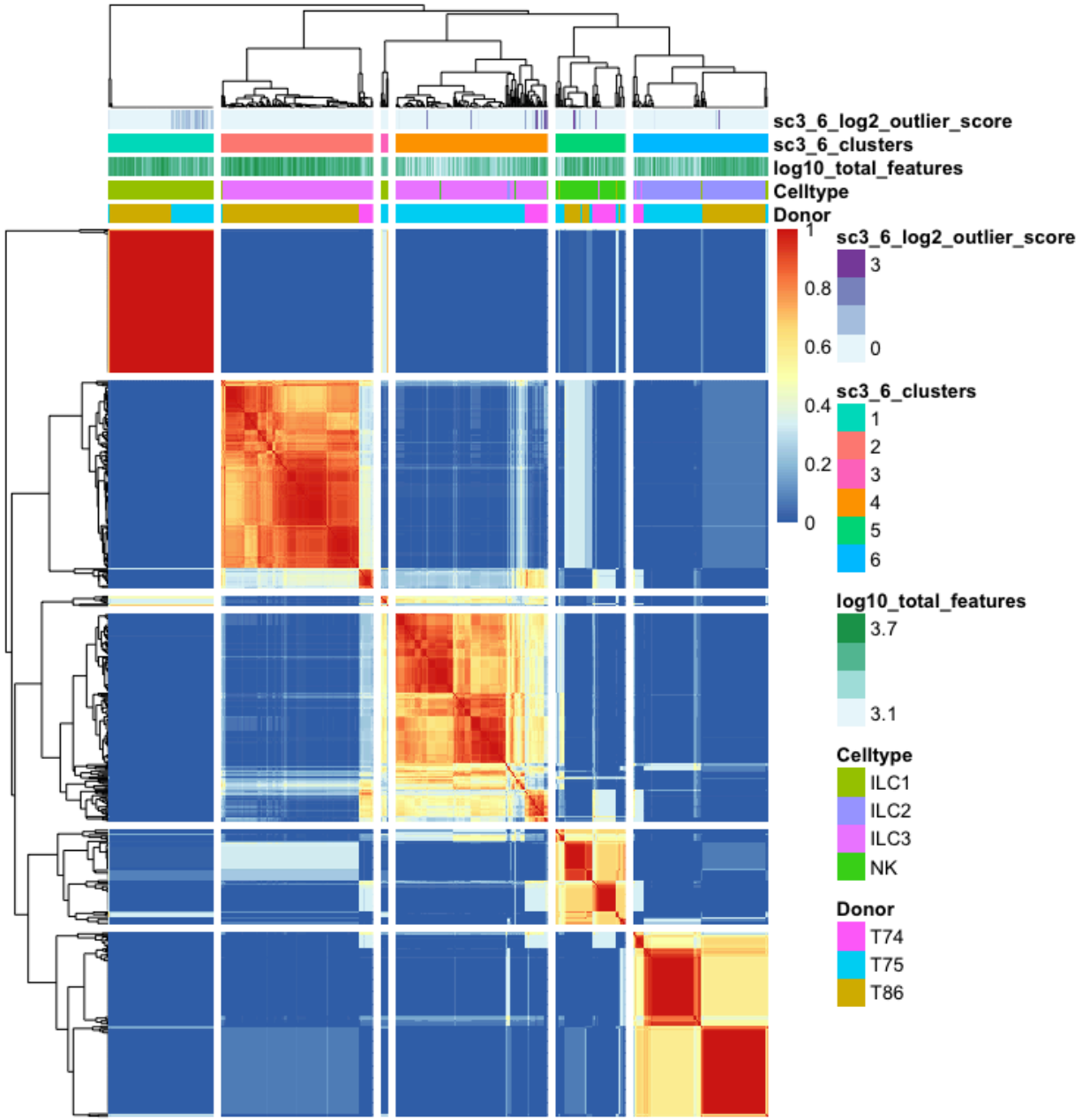
- It is hard to know when to stop clustering – you can always split the cells more times.
- Can use:
  - Do you get any/many significant DE genes from the next split?
  - Some tools have automated predictions for number of clusters – may not always be biologically relevant
- Always check back to QC-data – is what your splitting mainly related to batches, qc-measures (especially detected genes)

Clustree – R package

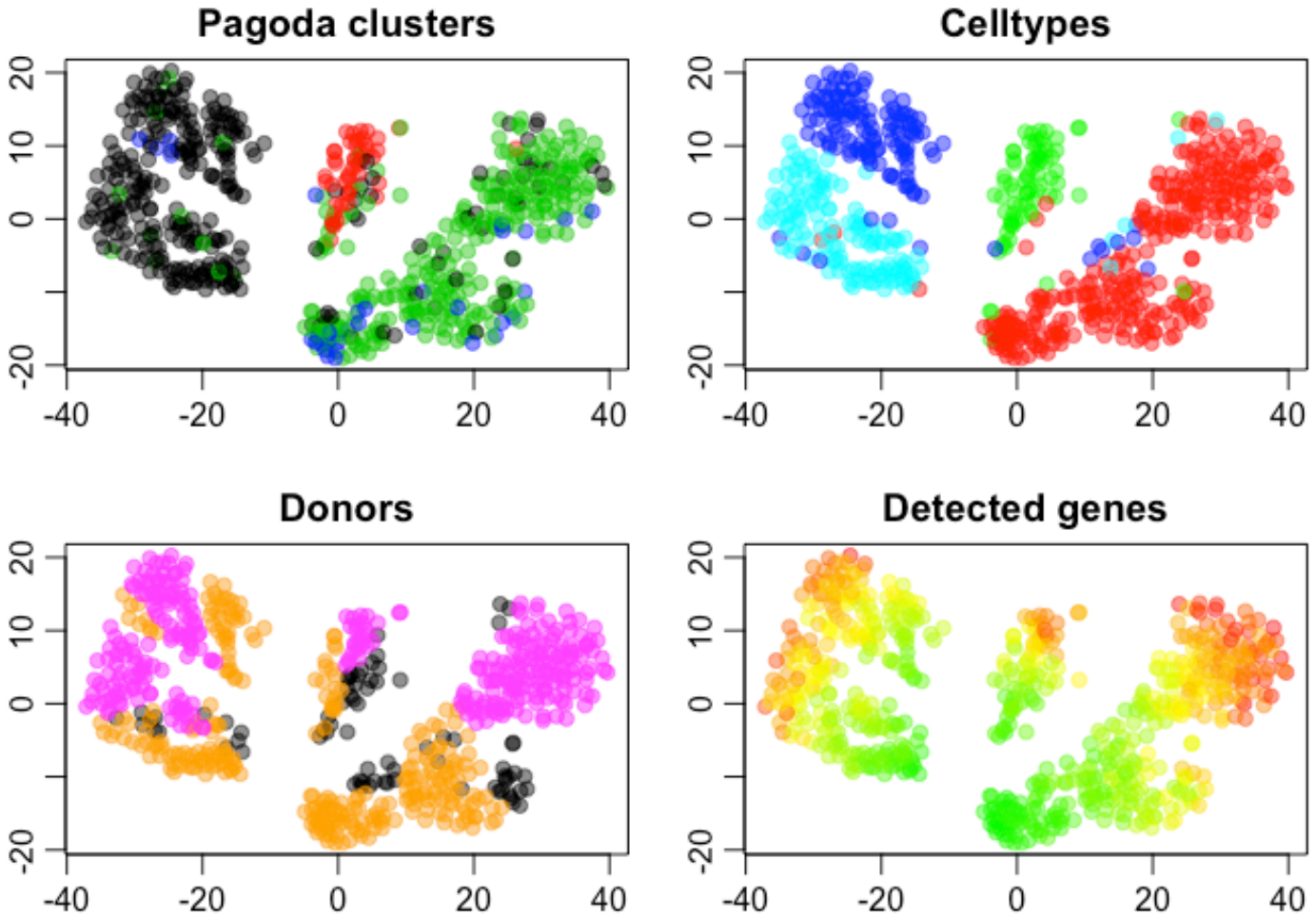


https://cran.r-project.org/web/packages/clustree/vignettes/clustree.html

Check QC data



Check QC data



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Subclustering

- Most of the variation in a heterogeneous data set will be between broad celltypes.
- By selecting one celltype and rerunning HVG-selection and PCA – most of the variation will be differences between subtypes.

From clusters to celltypes

- Using lists of DE genes and prior knowledge of the biology
- Using lists of DE genes and comparing to other scRNAseq data or sorted cell populations.
- Infer labels from other scRNAseq dataset(s) from the same tissue
  - Correlation between clusters
  - Different data integration methods
  - Programs for inferring celltypes

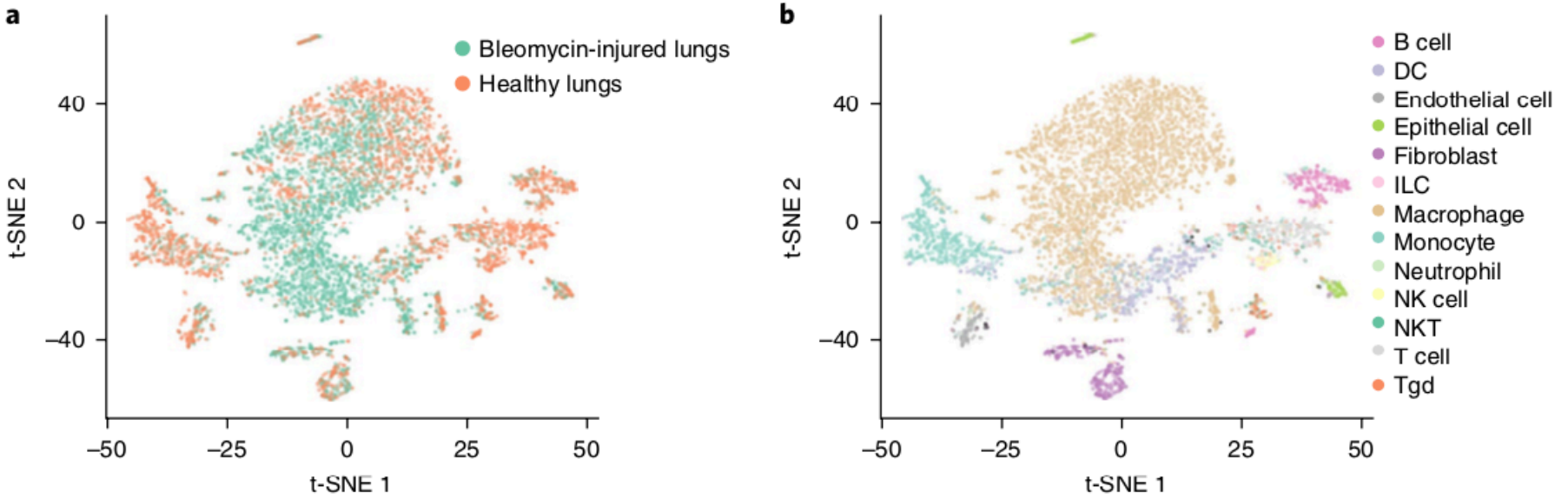
Databases with celltype gene signatures

- PanglaoDB - panglaodb.se
  - Human: 208 samples, 56 tissues, 0.7 M cells
  - Mouse: 798 samples, 147 tissues, 3.3 M cells
  - paper under review

- CellMarker – http://biocc.hrbmu.edu.cn/CellMarker/
  - Human: 13,605 cell markers of 467cell types in 158 tissues
  - Mouse: 9,148 cell makers of 389 cell types in 81 tissues
  - Zhang et al. NAR 2018

singleR

- Annotation of scRNAseq by reference bulk transcriptomes
- Reference from ImmGen, Encode and Blueprint Epigenomics.
- Webportal you can upload your data to.



(Aran et al. Nature Immunol. 2019)

- scPred – Hernandez et al bioRxiv 2018
  - unbiased feature selection from a reduced-dimension space, and machine-learning classification
  - Support vector machine or other models.

- Moana – Wagner & Yanai bioRxiv 2018
  - Hierarchical machine learning framework – classification of celltypes at different levels.
  - PBMC classifier, Pancreas cell type classifier.

- CaSTLe – Lieberman et al PLOS One 2018
  - XGBoost classification model trained on one dataset and predicted onto another dataset

Conclusions

- Clearly distinct celltypes will give similar results regardless of method
- Subclustering within celltypes may require careful selection of variable genes, dim reduction etc.
- Consistent results from different methods and agreement with tSNE/UMAP layout is always best!
- Use your biological knowledge to evaluate the results – but try to be unbiased!

Resources

- Good course at: https://hemberg-lab.github.io/scRNA.seq.course/
- Many of the packages have very thorough tutorials on their websites
- Repo with scRNA-seq tools: https://github.com/seandavi/awesome-single-cell
- Single cell assay objects for many datasets: https://hemberg-lab.github.io/scRNA.seq.datasets/
- Conquer datasets - salmon pipeline to many different datasets: http://imlspenticton.uzh.ch:3838/conquer/
- EBI Single cell expression atlas: https://www.ebi.ac.uk/gxa/sc