Single Cell RNA-seq Clustering

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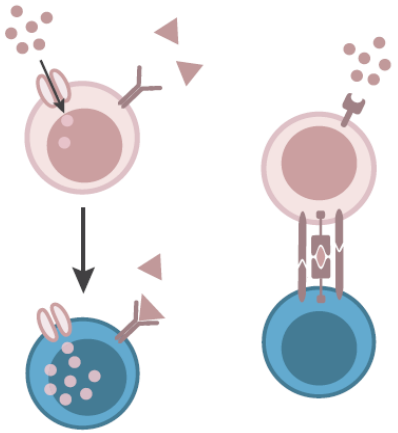
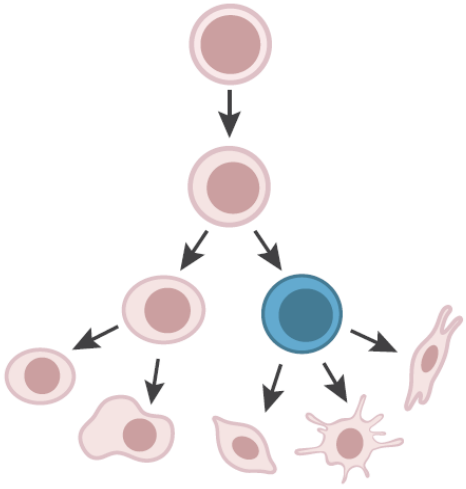
Cell Identity determined by diverse factors

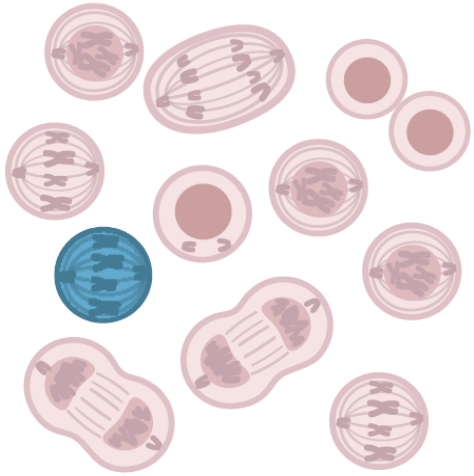
Environmental stimuli



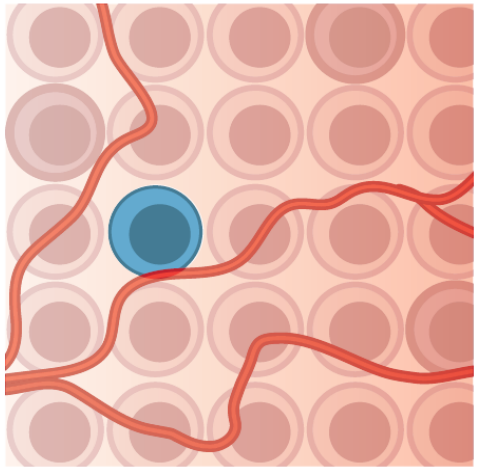
Cell development



Cell cycle

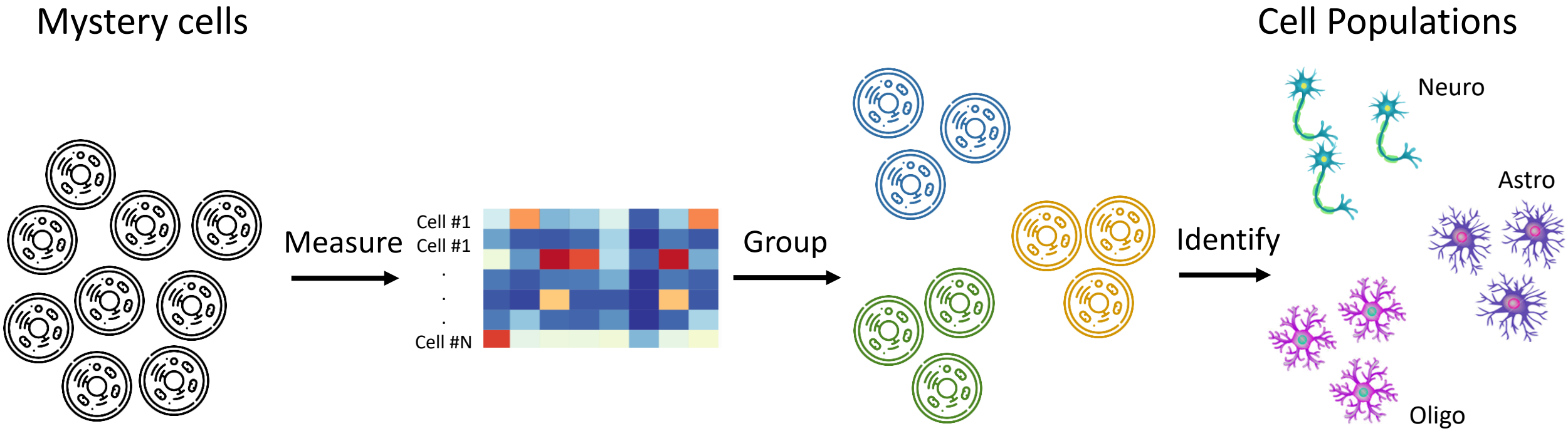


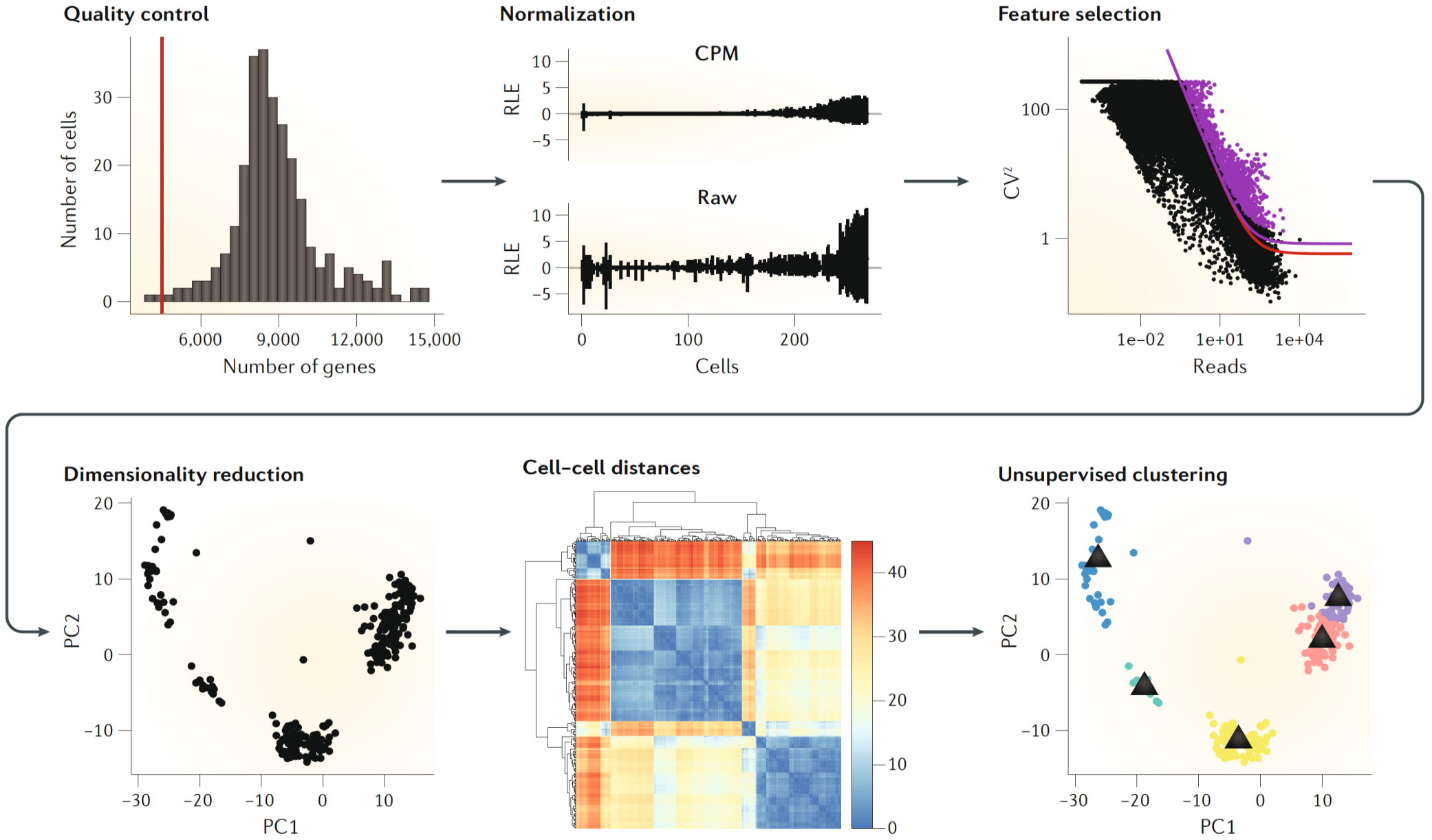
Spatial context



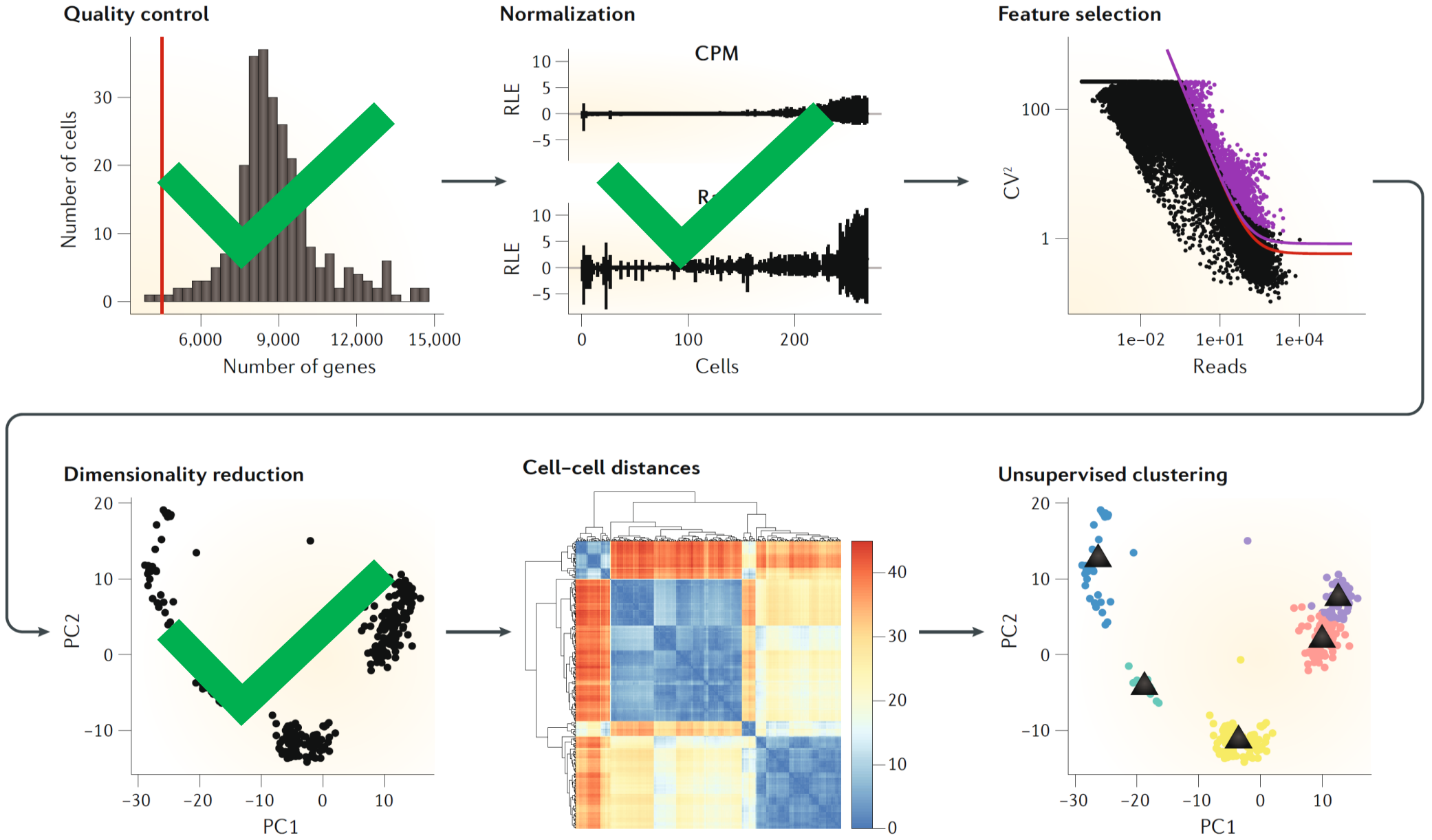
Wagner et al. (https://doi.org/10.1038/nbt.3711)

How can we *automatically* identify cell populations?





Kiselev et al. (https://doi.org/10.1038/s41576-018-0088-9)



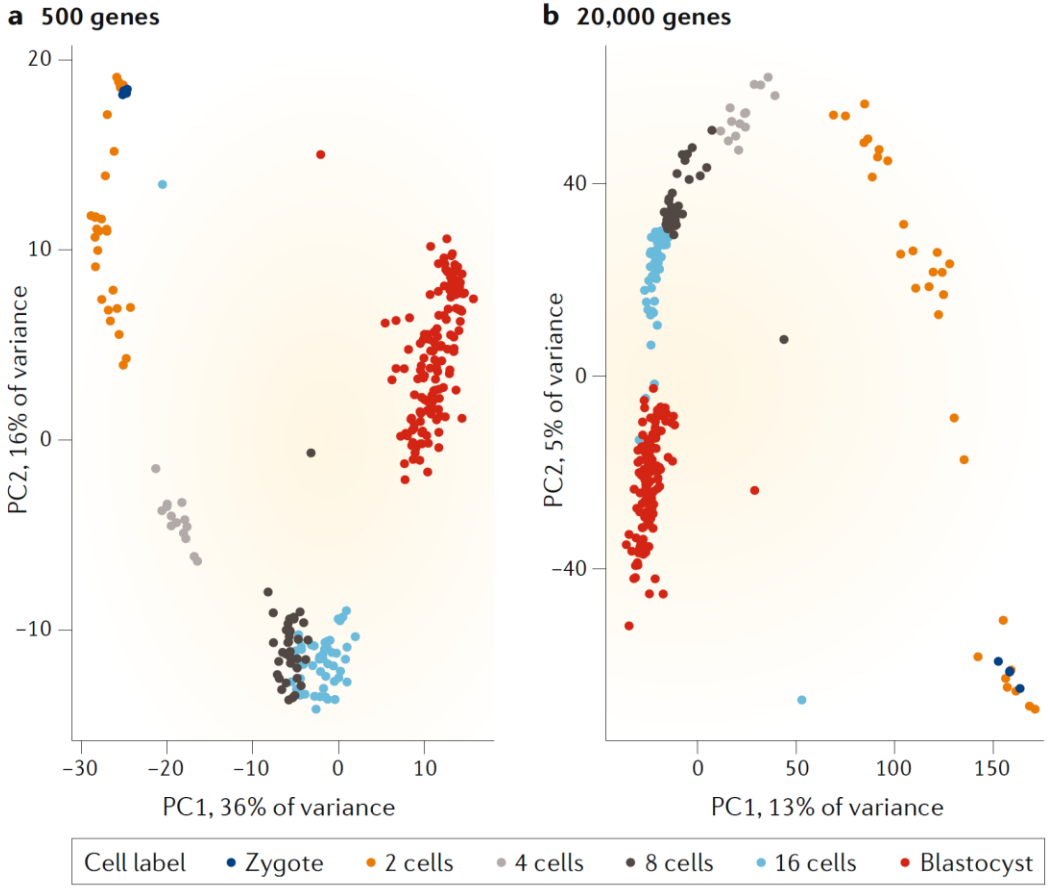
Kiselev et al. (https://doi.org/10.1038/s41576-018-0088-9)

Outline

- **Feature selection**
- Introduction to clustering
  - Hierarchical clustering
  - *k*-Means clustering
  - Graph-based clustering
- Validation
- scRNA-seq clustering
  - Single Cell Consensus Clustering (SC3)
  - Seurat

Feature selection

- Curse of dimensionality
  - More features (genes) -> noise dominates distances between samples (cells), effectively all cells get ‘same’ distance
- Remove genes which only exhibit technical noise
  - Increase the signal:noise ratio
  - Reduce the computational complexity



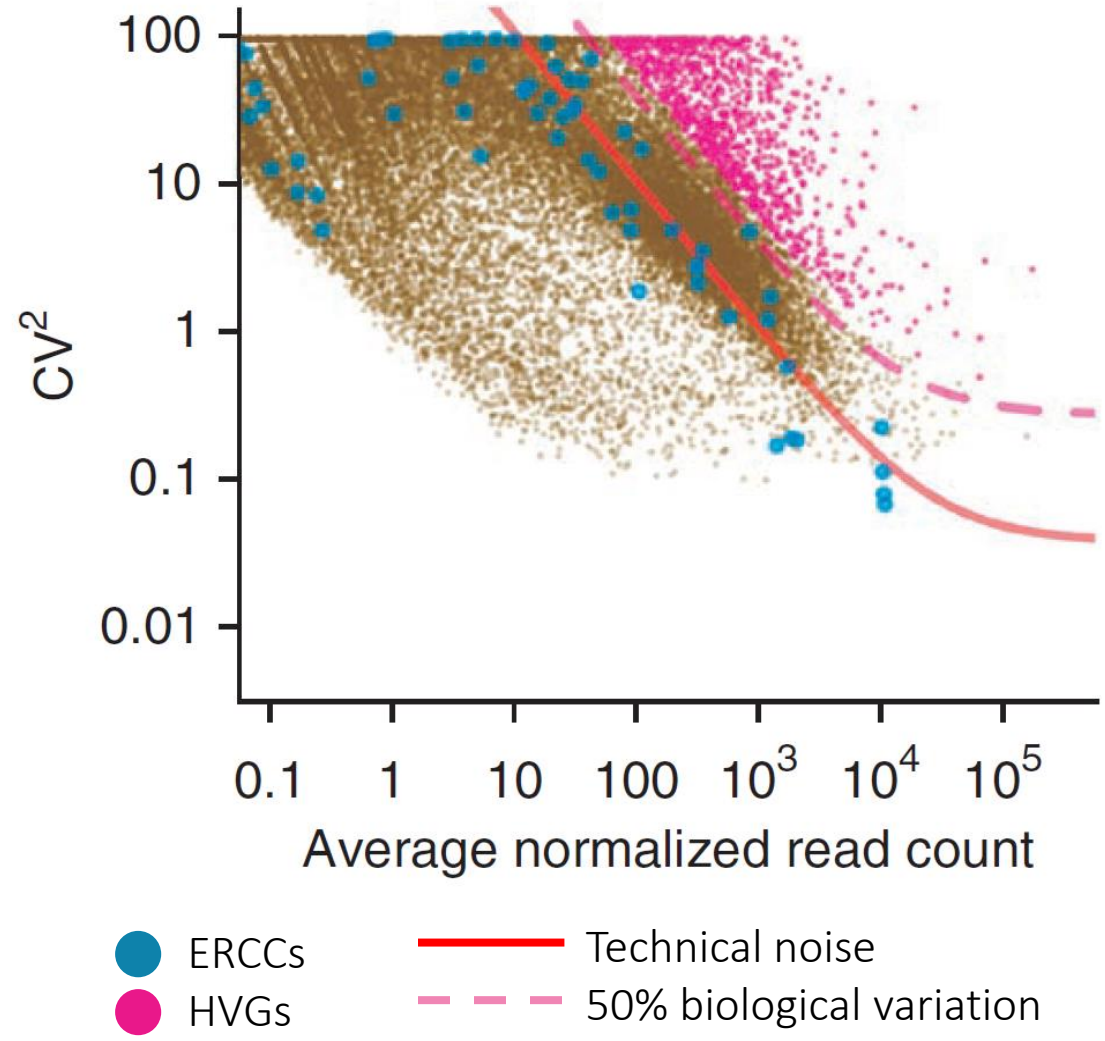
Kiselev et al. (https://doi.org/10.1038/s41576-018-0088-9)

Feature selection

Highly Variable Genes (HVG)

- $CV = \frac{var}{mean} = \frac{\sigma}{\mu}$
- Fit a gamma generalized linear model to spike ins (ERCCs)
- No ERCCs?

  Estimate technical noise based on all genes



Brennecke et al. (https://doi.org/10.1038/nmeth.2645)

Feature selection

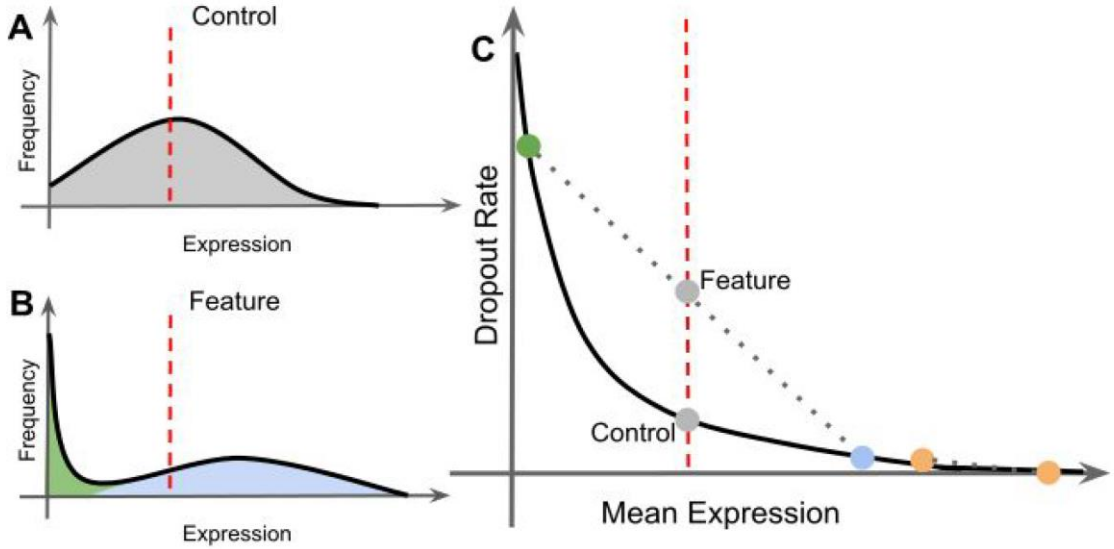
M3Drop: Dropout-based feature selection

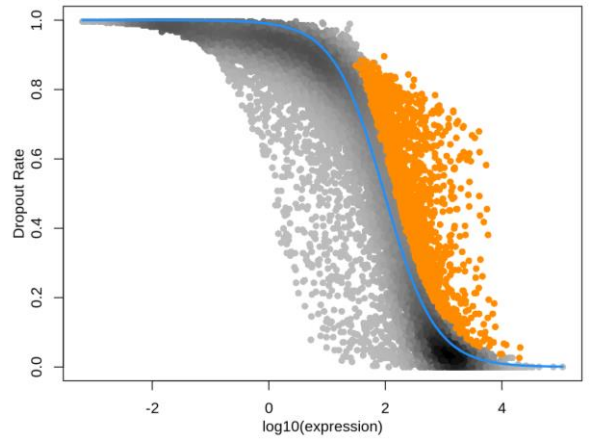
- Reverse transcription is an enzyme reaction thus can be modelled using the Michaelis-Menten equation:

$$P_{dropout} = 1 - \frac{S}{K_M + S}$$

S: average expression

K_M: Michaelis-Menten constant





Brennecke et al. (https://doi.org/10.1038/nmeth.2645)

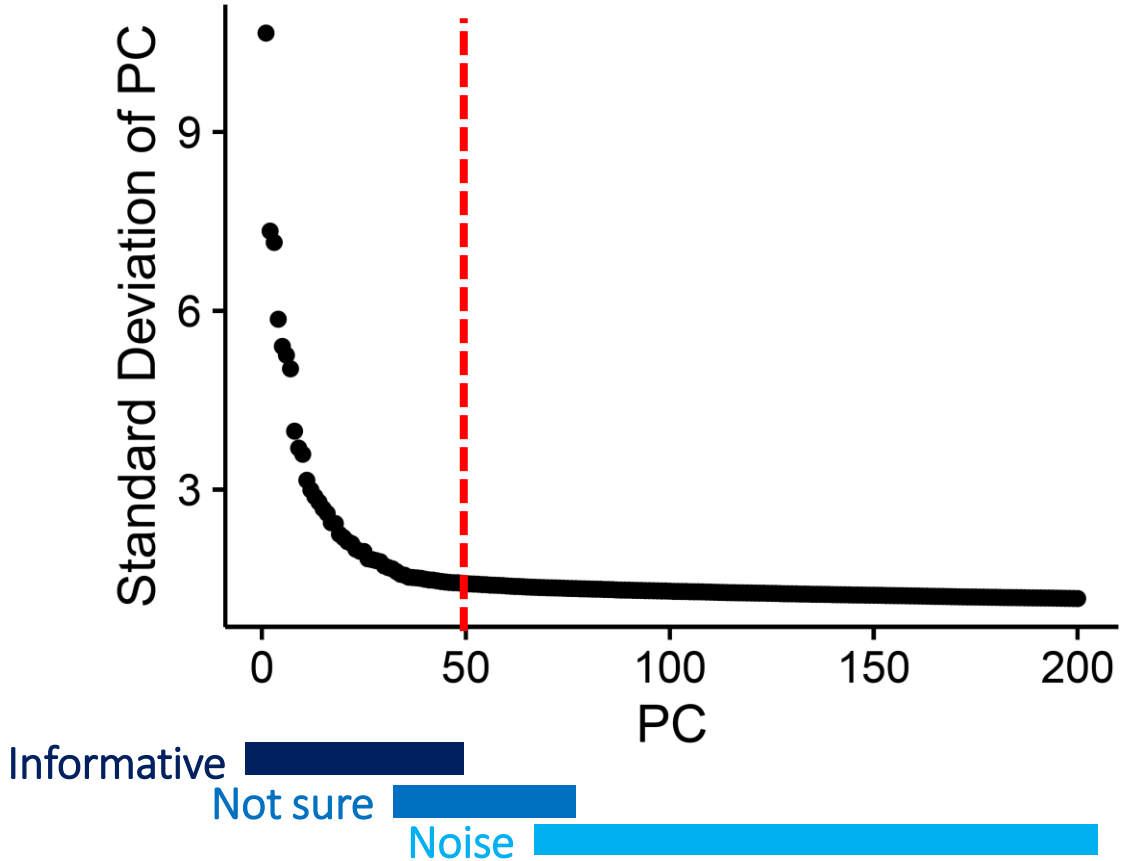
Feature selection

Selecting 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

Scree/Elbow plot



Outline

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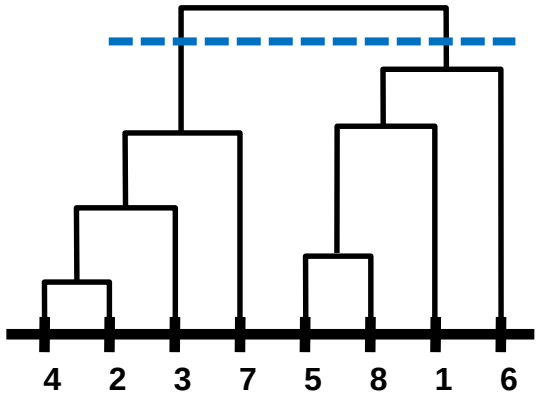
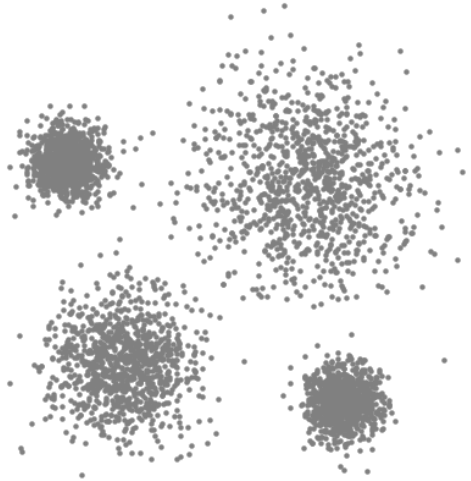
  - Seurat
- Validation

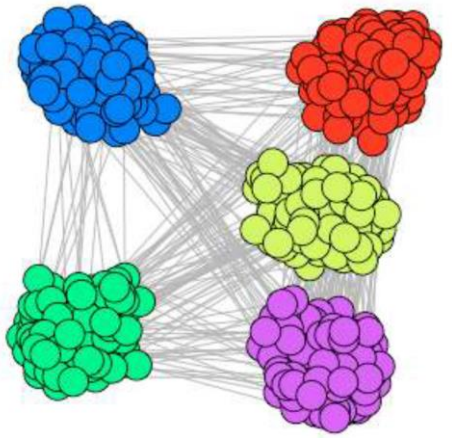
Many clustering approaches



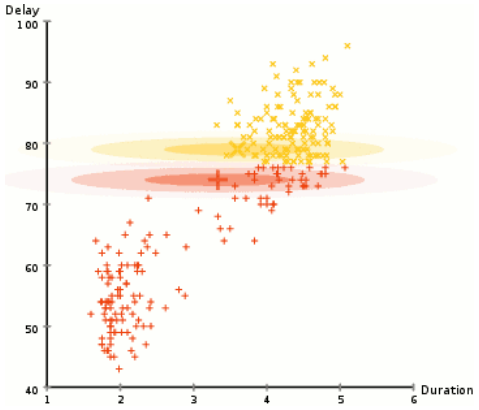
Hierarchical Clustering



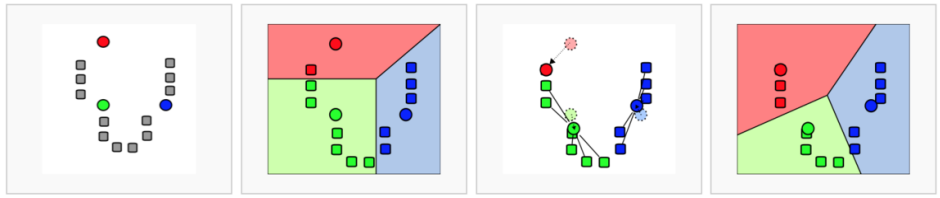
Mean shift clustering



Graph-based clustering

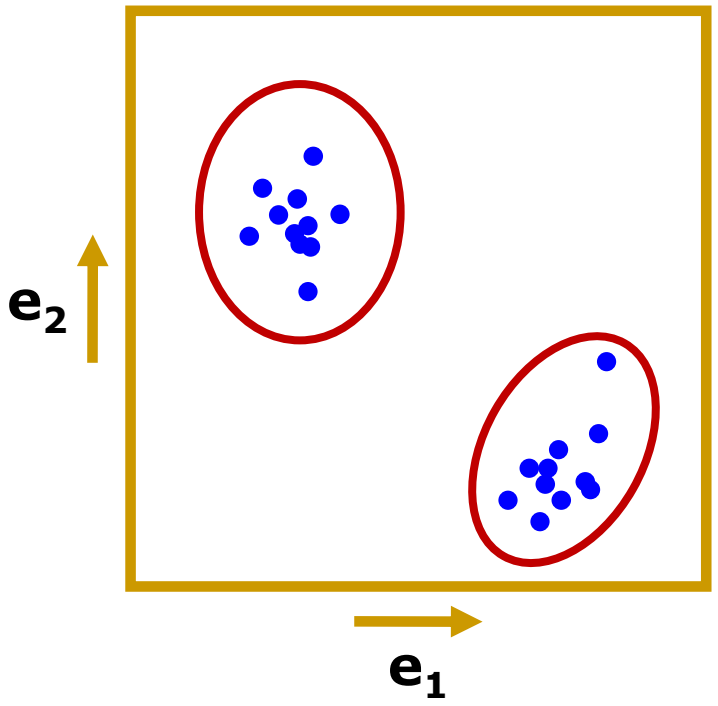


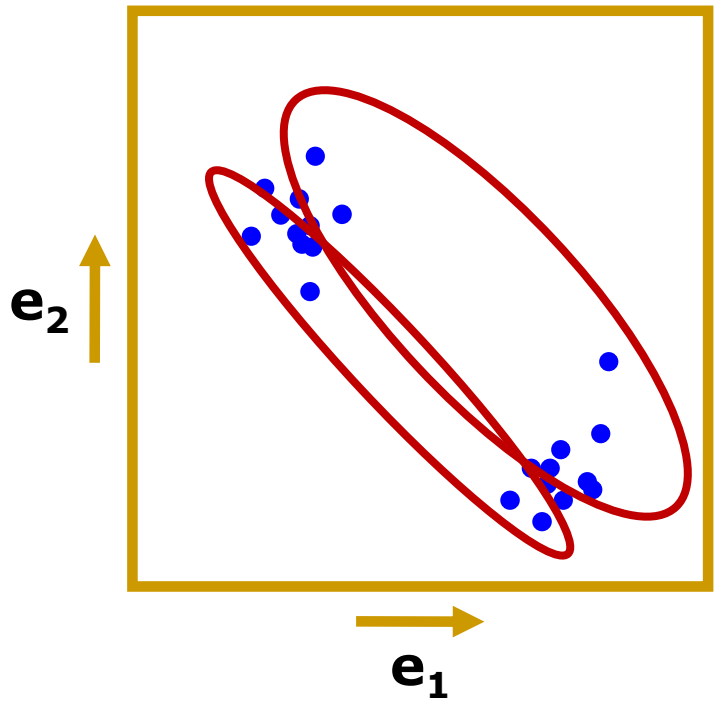
Gaussian mixture modeling



k-means clustering

Clustering

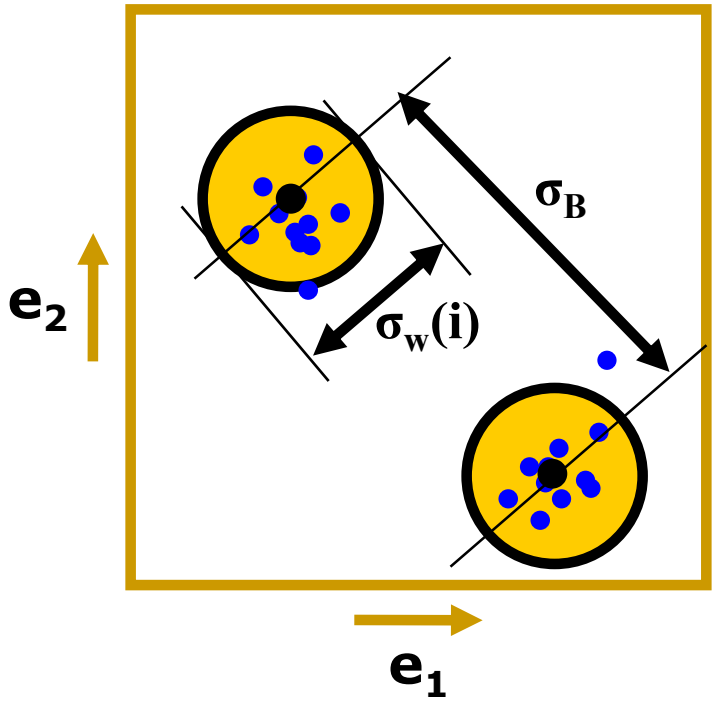




Clustering

- Structure when:

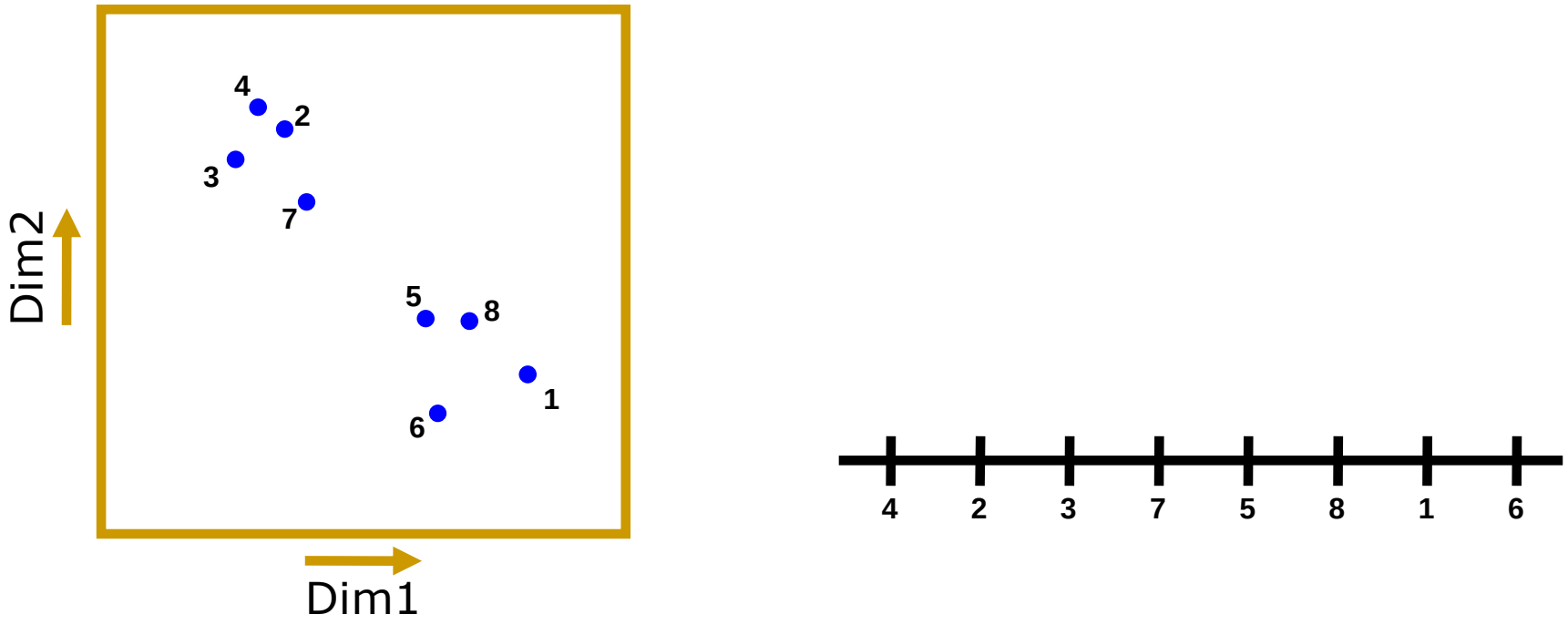
1) Samples within cluster resemble each other *(within variance, $\sigma_W(i)$)*
2) Clusters deviate from each other *(between variance, σ_B)*



- Group samples such that:

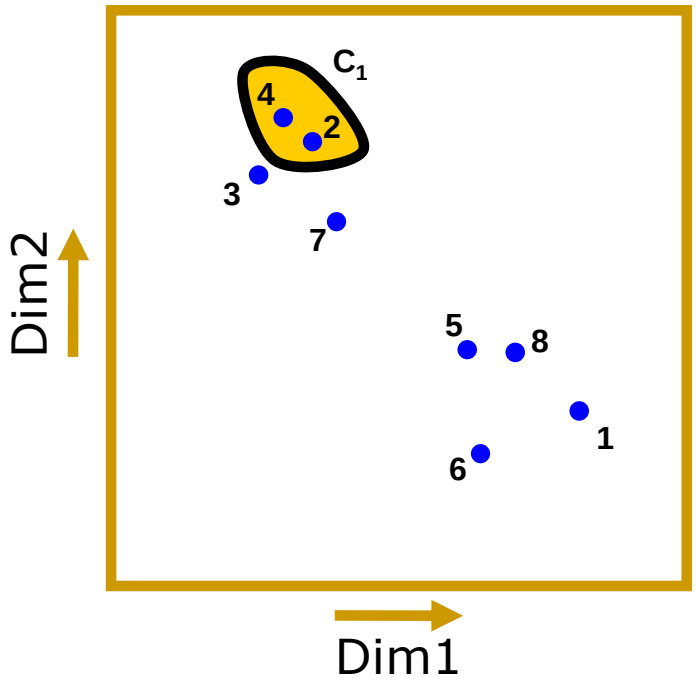
$$\min\left(\frac{\sum_{\forall clusters}\sigma_W(i)}{\sigma_B}\right) \rightarrow \begin{matrix}\sigma_W\text{: small \&} \\ \sigma_B\text{ : large}\end{matrix}$$

Hierarchical clustering

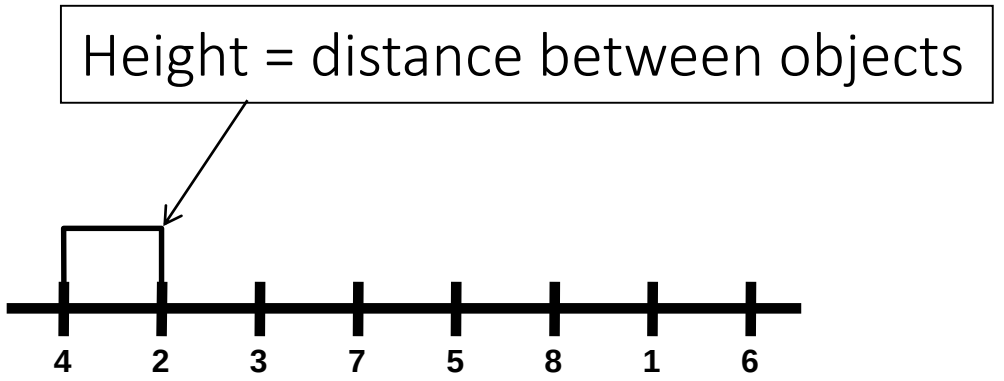


Find most similar objects (genes) and group them

Hierarchical clustering

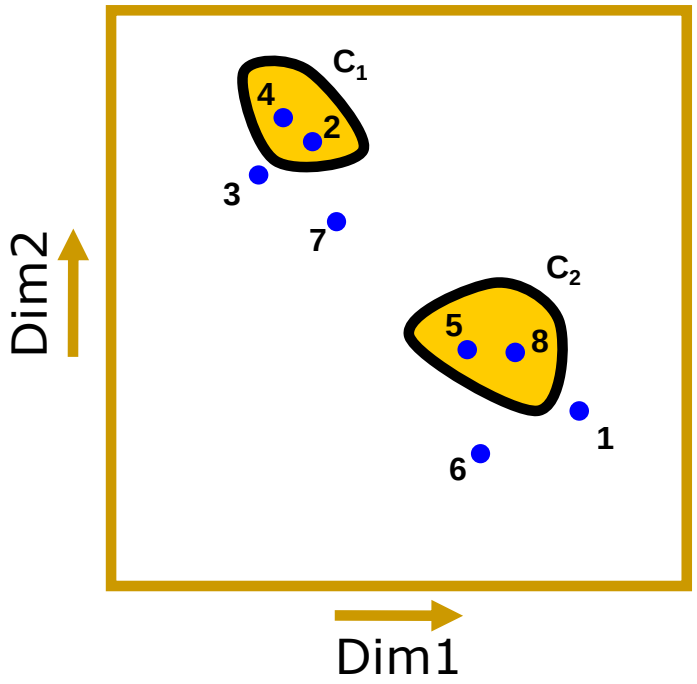


dendrogram

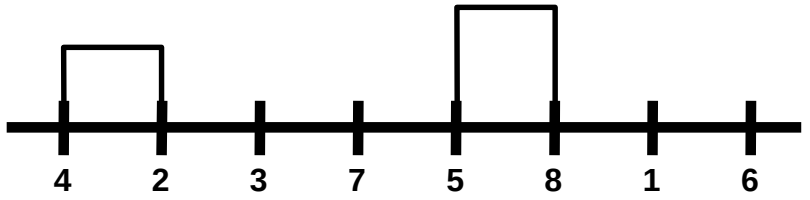


These are: objects 4 and 2
Again, find most similar objects (genes or clusters) and group them

Hierarchical clustering



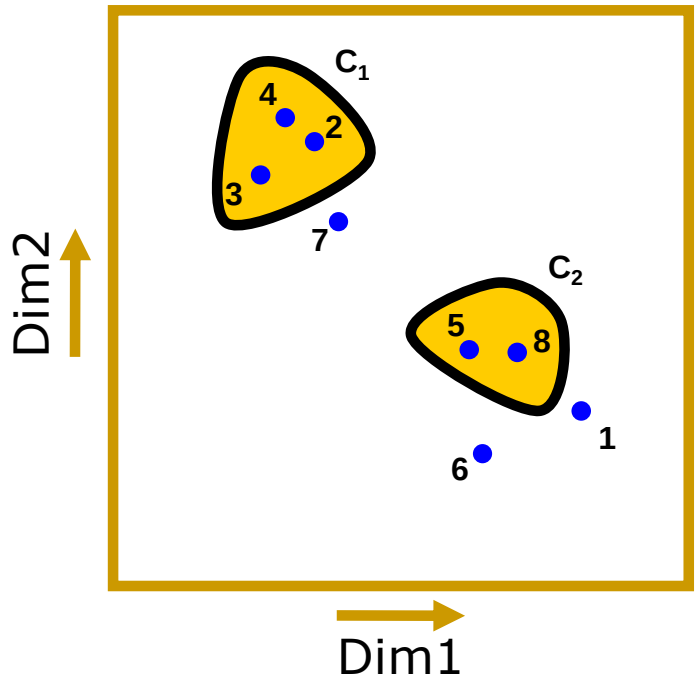
dendrogram



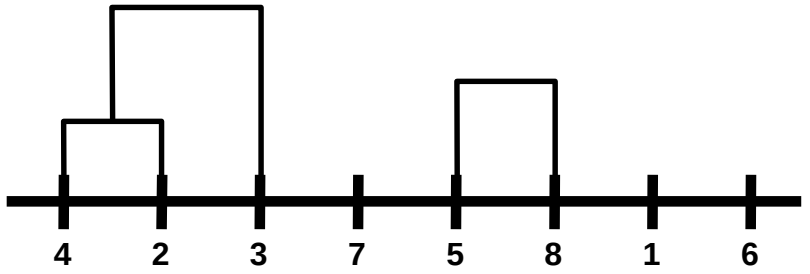
These are: objects 5 and 8

Repeat finding most similar objects (genes or clusters) and grouping them

Hierarchical clustering

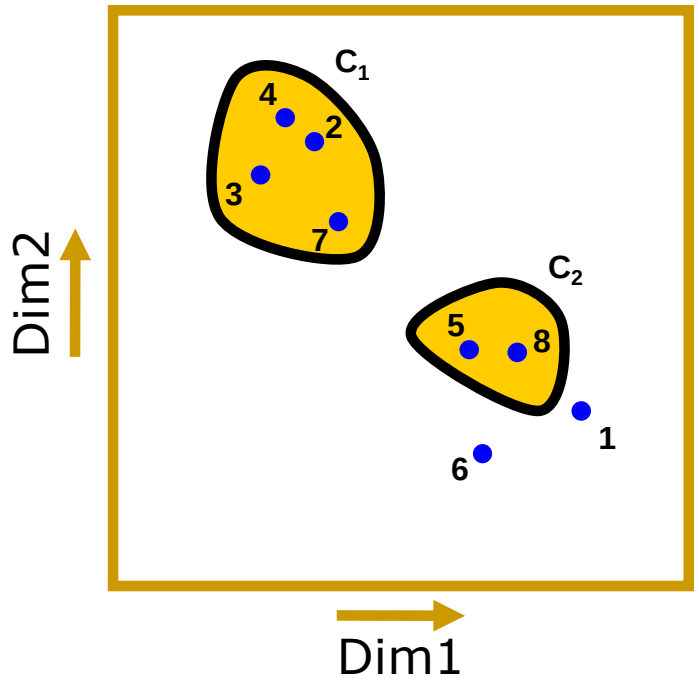


dendrogram

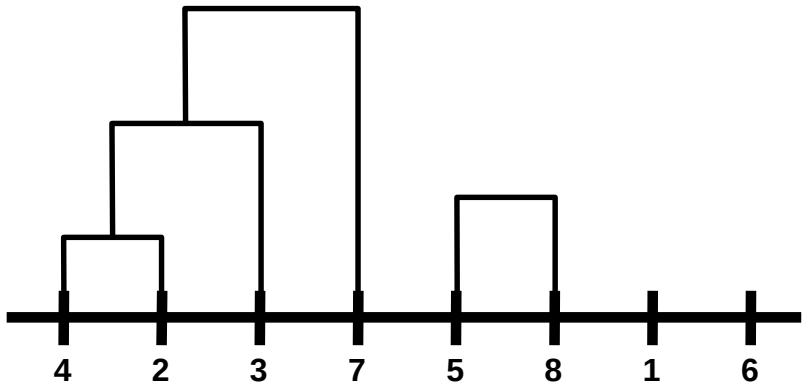


Join object 3 and cluster 1
Repeat process

Hierarchical clustering

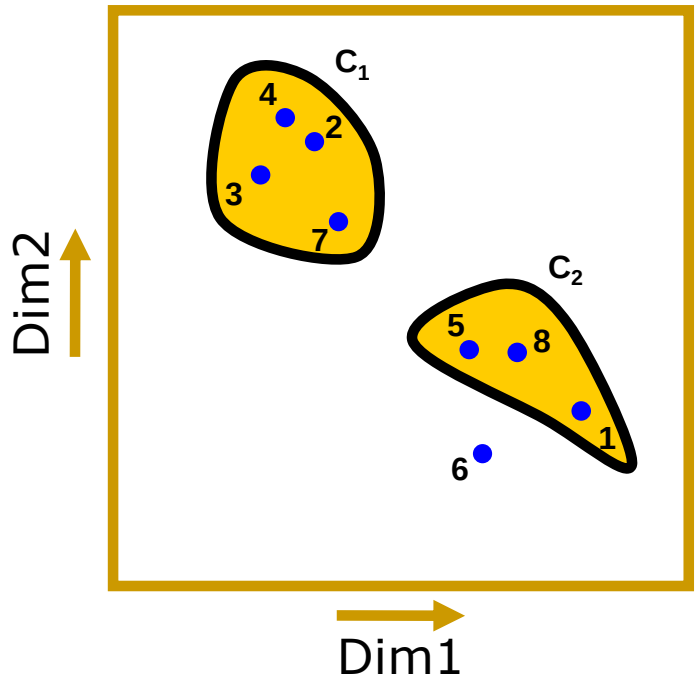


dendrogram

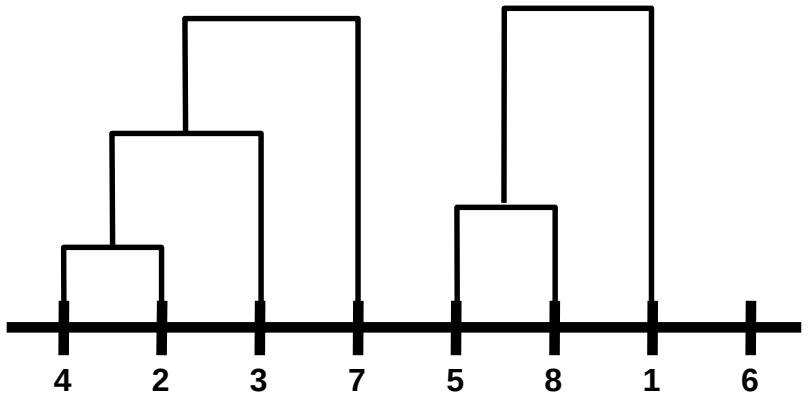


Join [object 7 and cluster 1] -> [cluster 1]
Repeat process

Hierarchical clustering

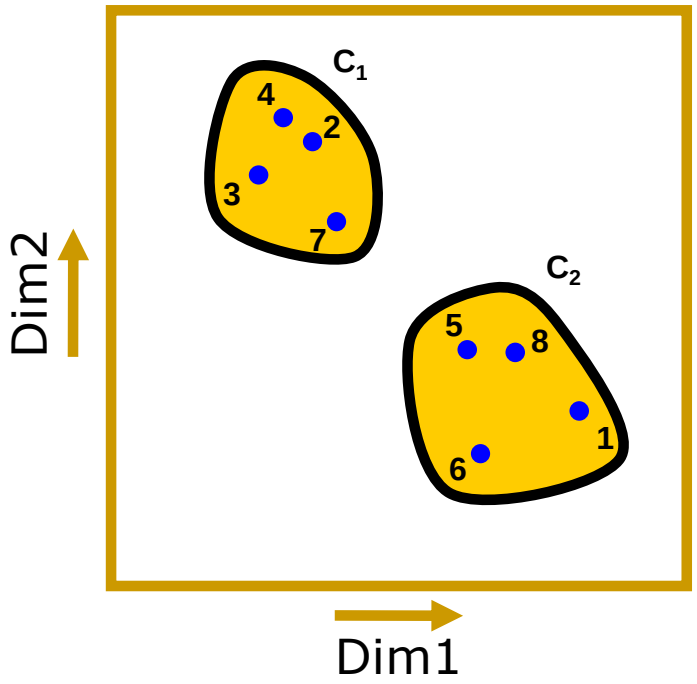


dendrogram



Join [object 1 and cluster 2] -> [cluster 2]
Repeat process

Hierarchical clustering



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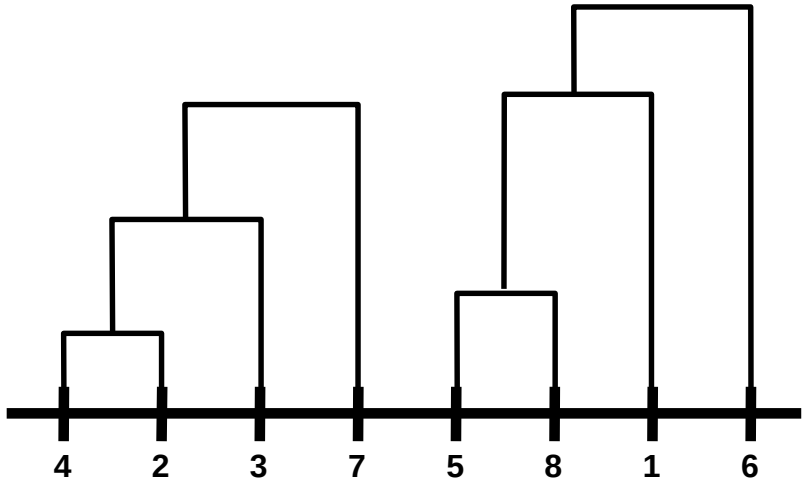
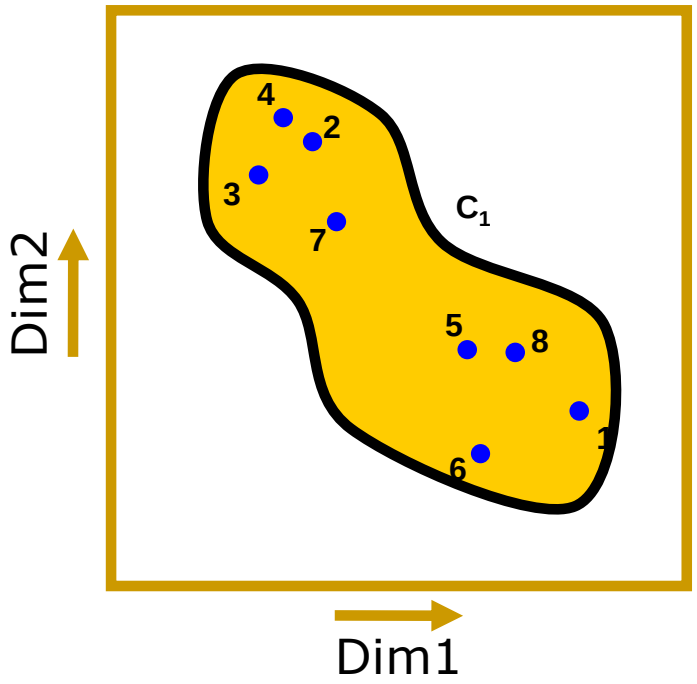
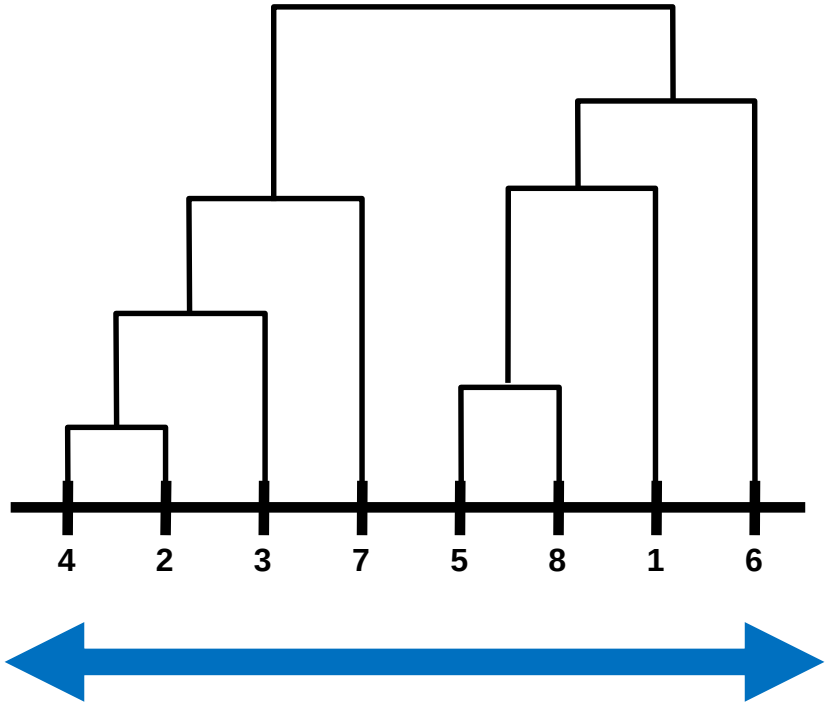


Join [object 6 and cluster 2] -> [cluster 2]
Repeat process

Hierarchical clustering

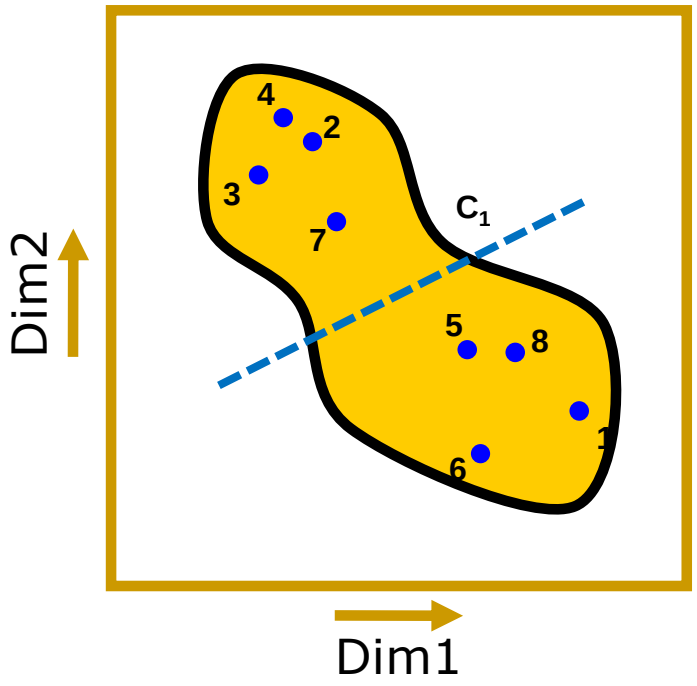


dendrogram

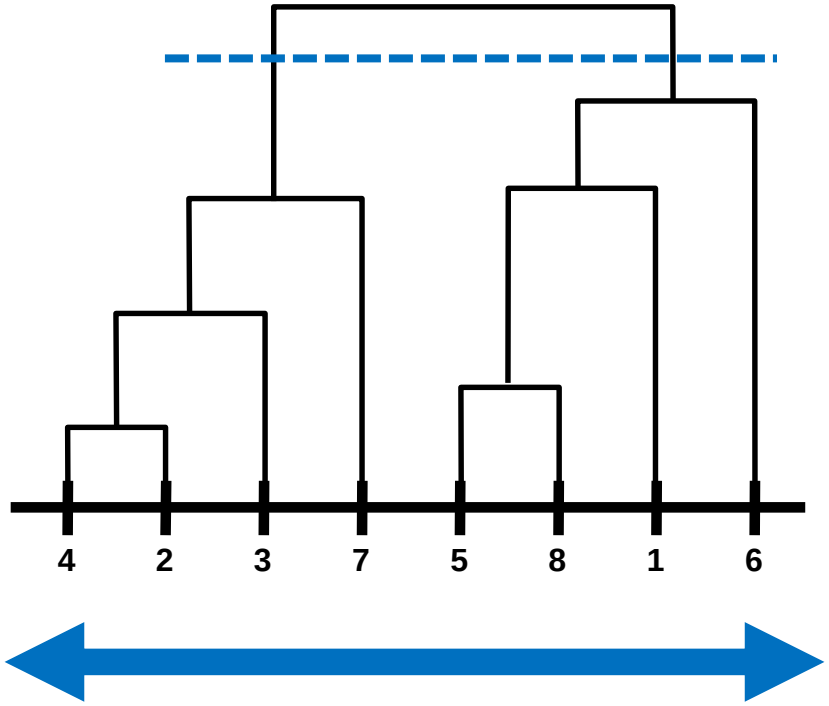


Join [cluster 1 and cluster 2] -> [cluster 1]
All in one cluster: FINISHED!

Hierarchical clustering



dendrogram

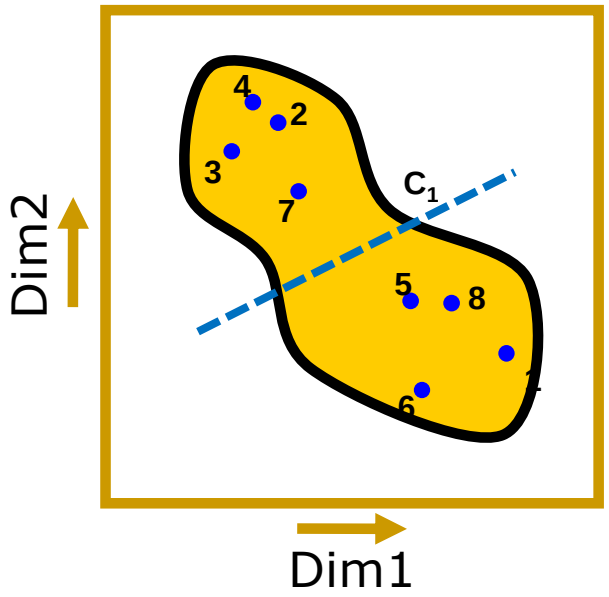


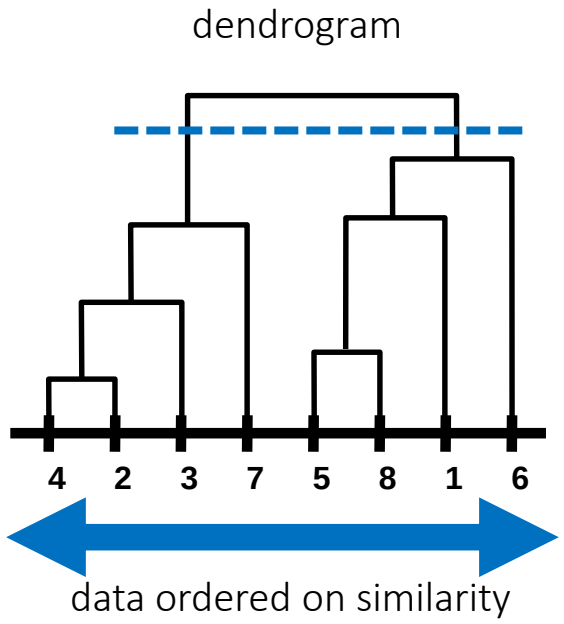
data ordered on similarity

Hierarchical clustering

Need to know:

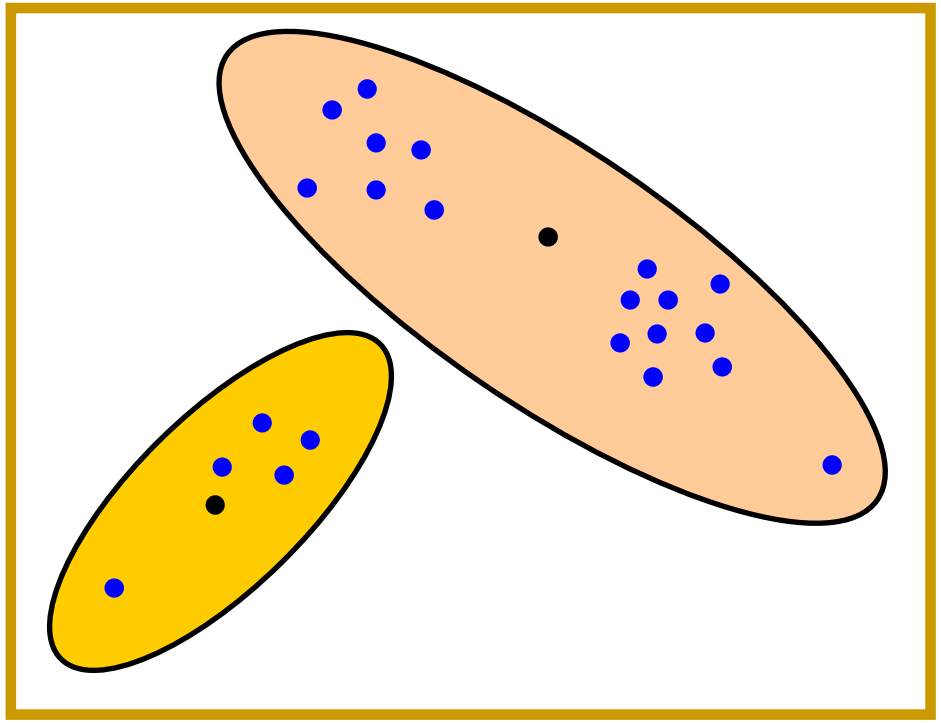
- Similarity between objects
- Similarity between clusters





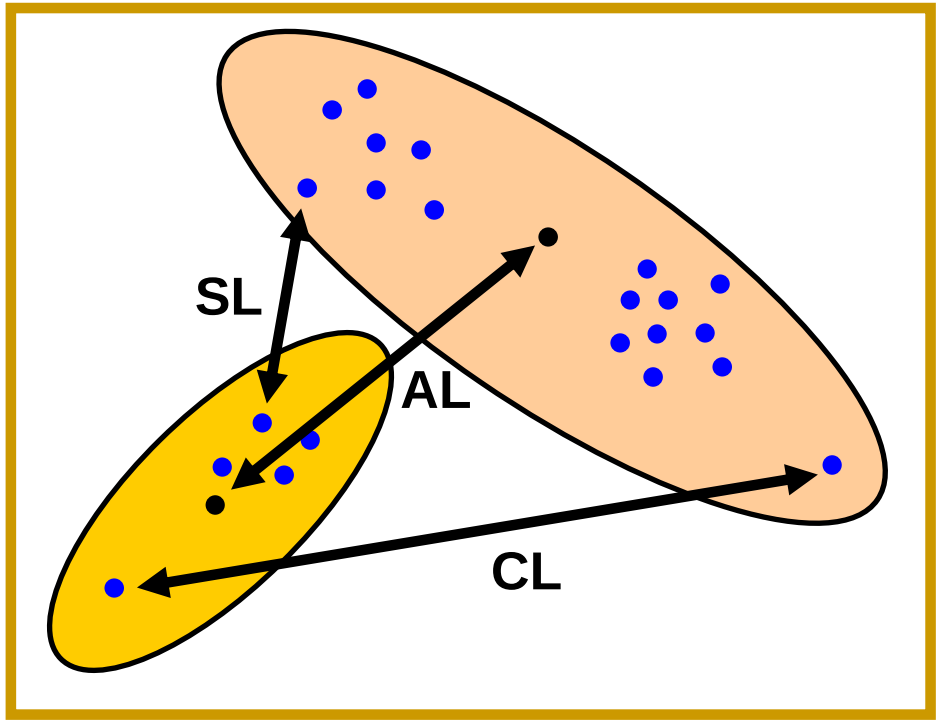
Hierarchical clustering

Similarity between clusters



Hierarchical clustering

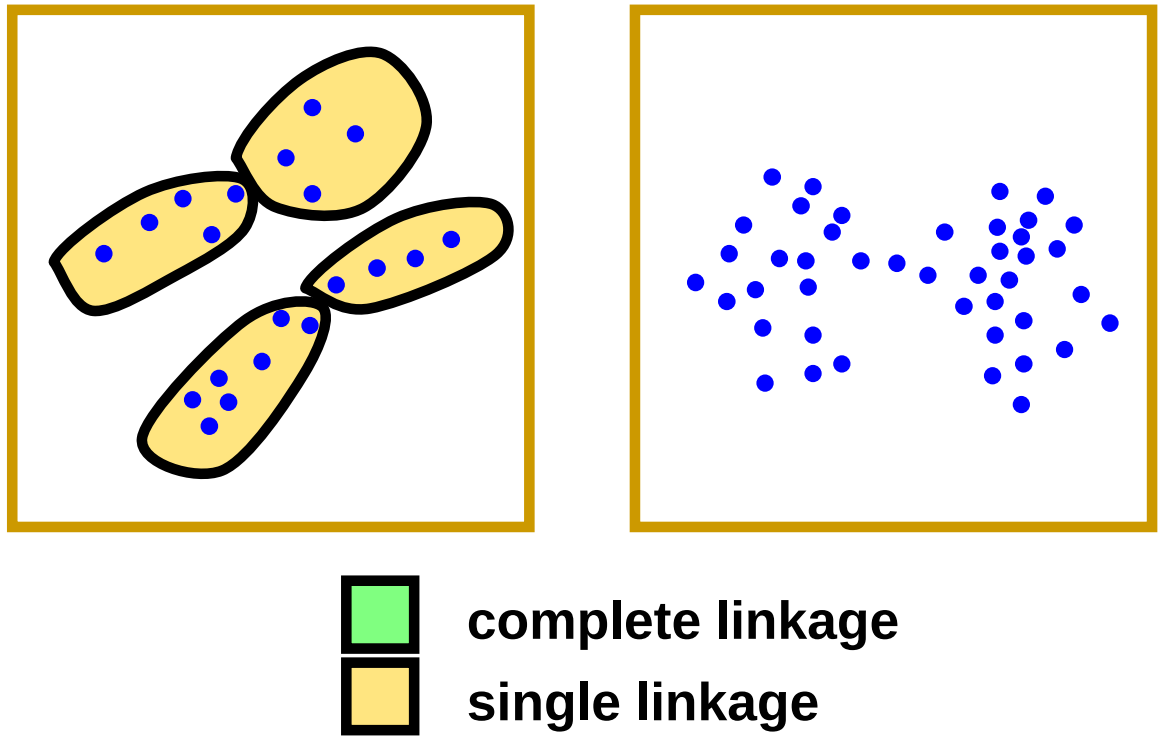
Similarity between clusters



- **Single linkage:** Closest objects
- **Complete linkage:** Furthest objects
- **Average linkage:** Average dissimilarity

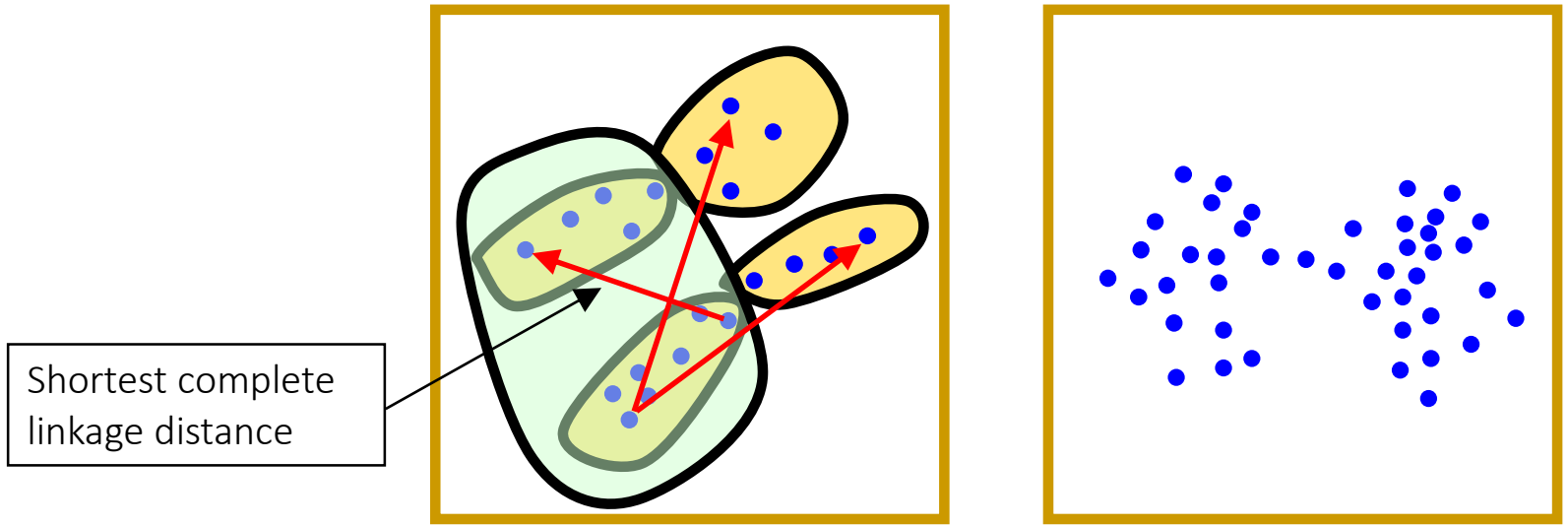
Hierarchical clustering

Similarity between clusters



Hierarchical clustering

Similarity between clusters

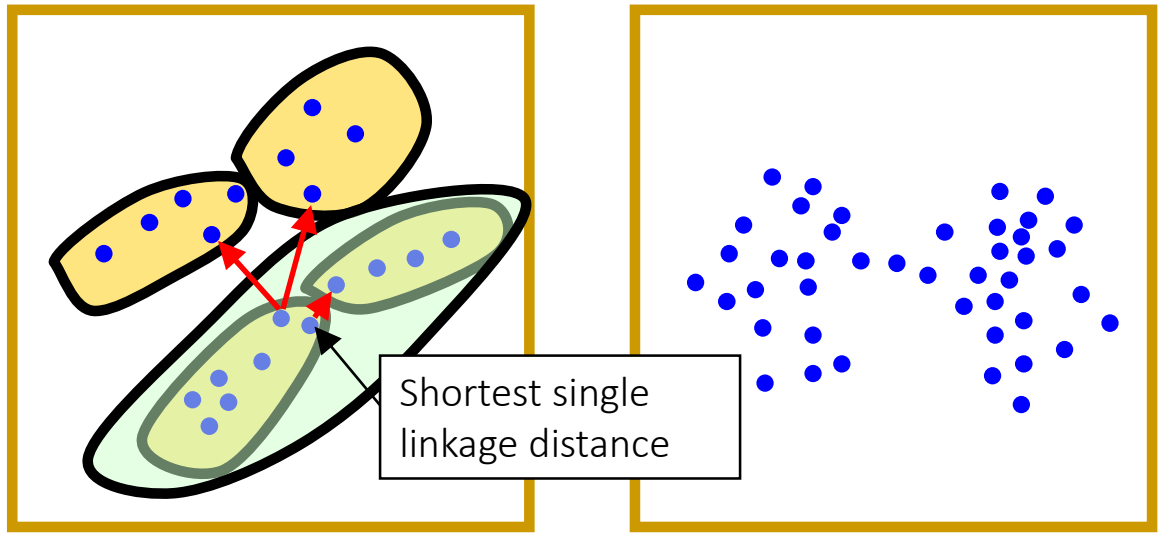


complete linkage

single linkage

Hierarchical clustering

Similarity between clusters



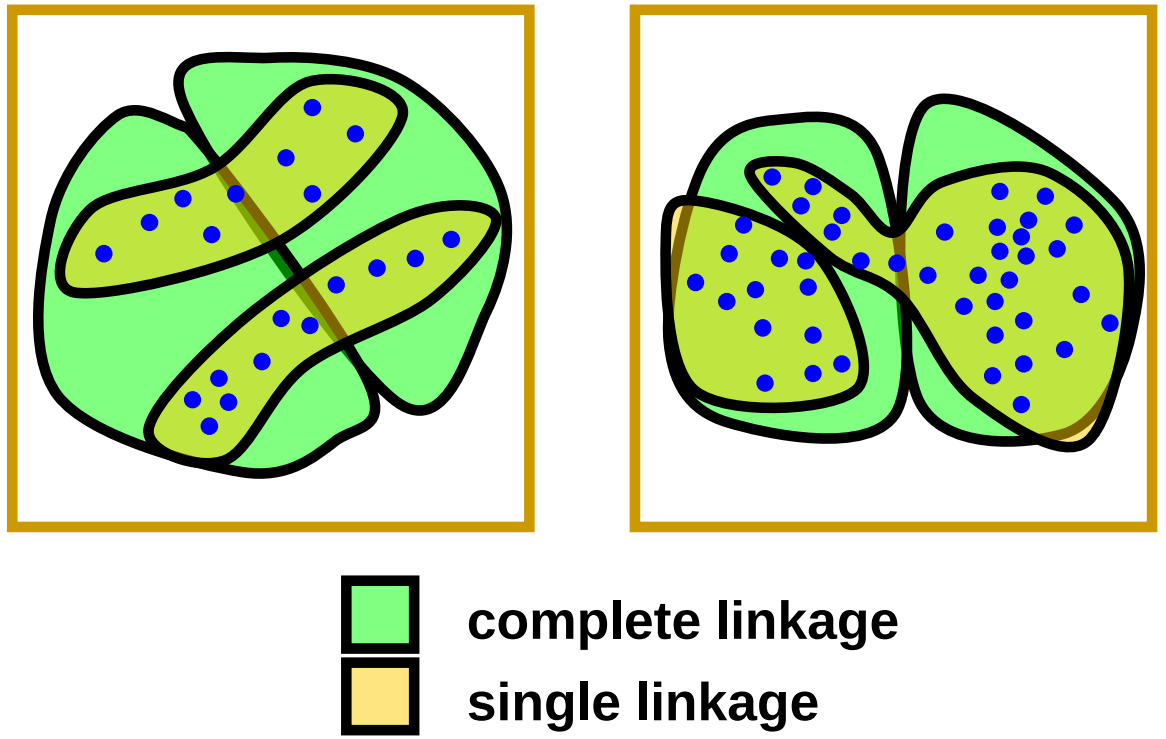
complete linkage

single linkage

Hierarchical clustering

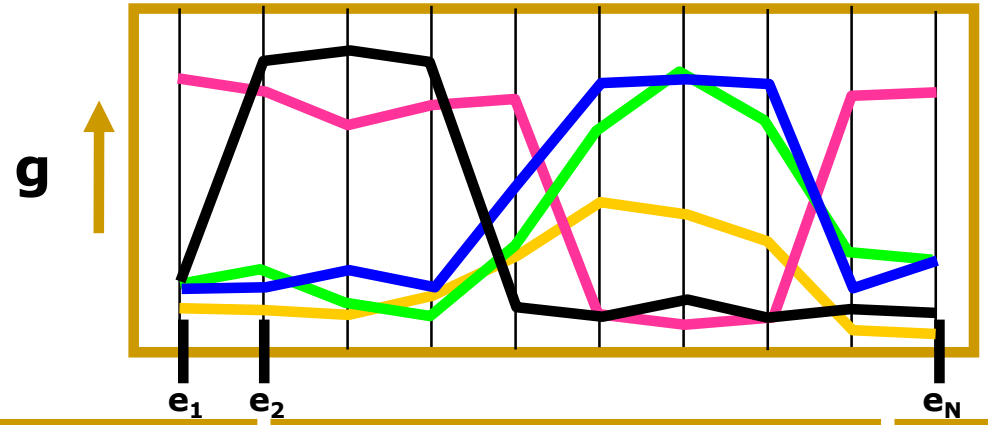
Similarity between clusters

- Single linkage -> long and “loose” clusters
- Complete linkage -> compact clusters



Hierarchical clustering

Similarity between objects



Euclidean distance

$$d(g_i, g_j) = \sqrt{(\sum((x_i - x_j)^2))}$$

d(●,●) < d(●,●)
d(●,●) << d(●,●)
d(●,●) << d(●,●)

Match exact shape

Pearson correlation

$$1 - \rho_{ij}$$

d(●,●) ≈ d(●,●)
d(●,●) << d(●,●)
d(●,●) << d(●,●)

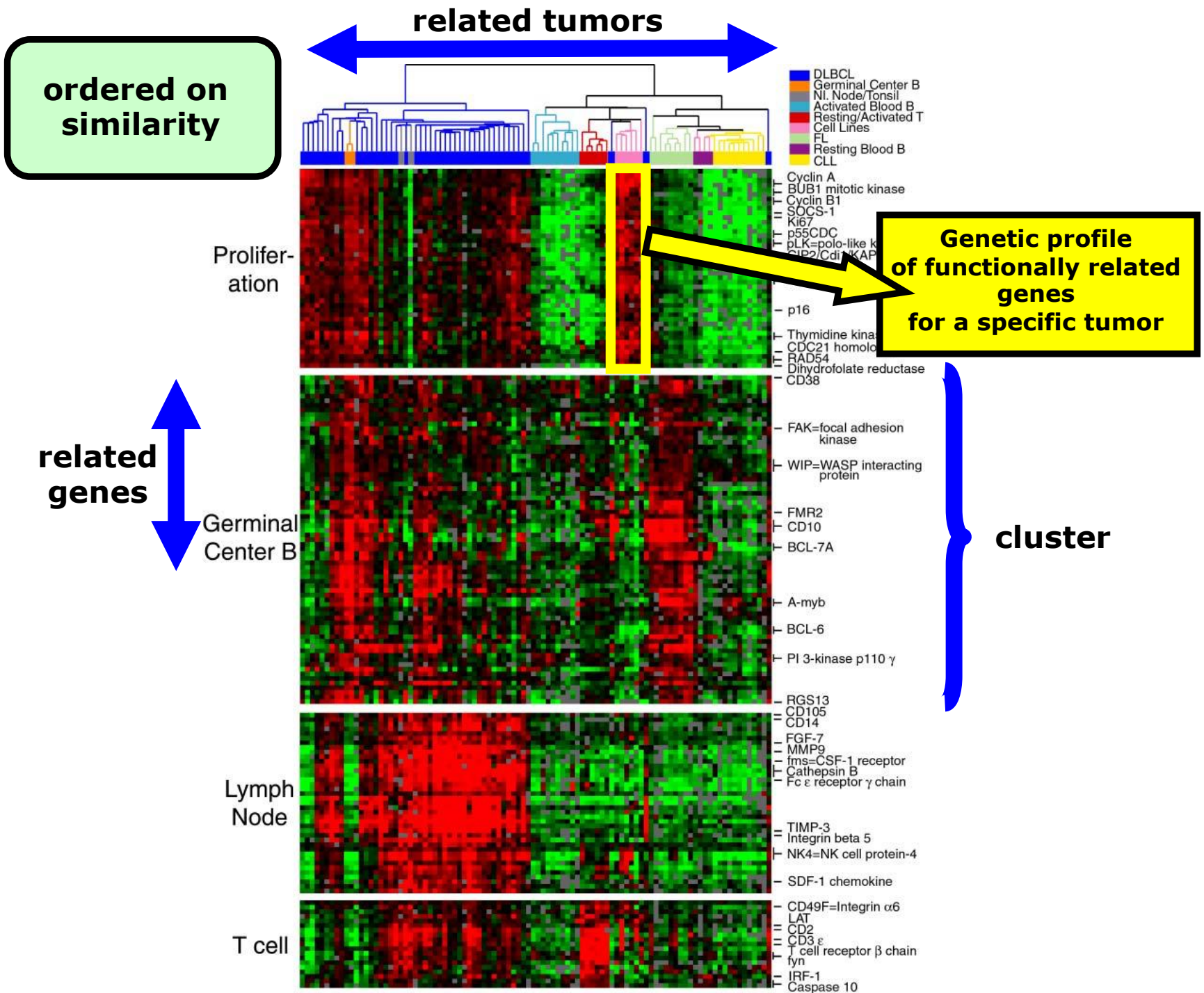
Ignore amplitude

Mixed Pearson correlation

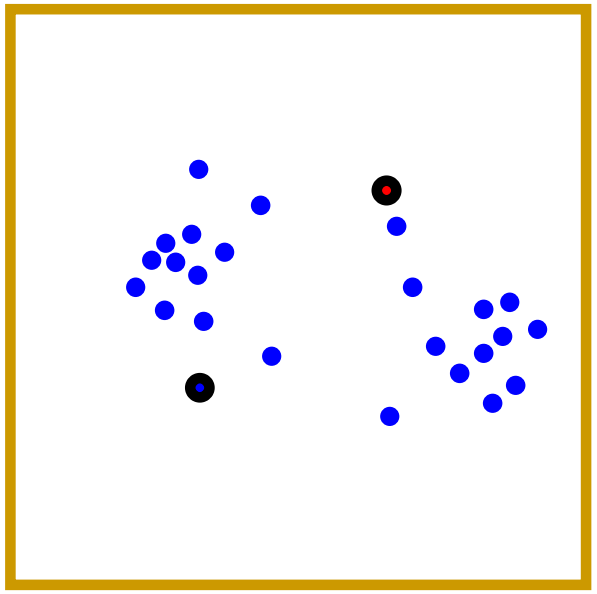
$$1 - |\rho_{ij}|$$

d(●,●) ≈ d(●,●)
d(●,●) ≈ d(●,●)
d(●,●) << d(●,●)

Ignore amplitude and sign

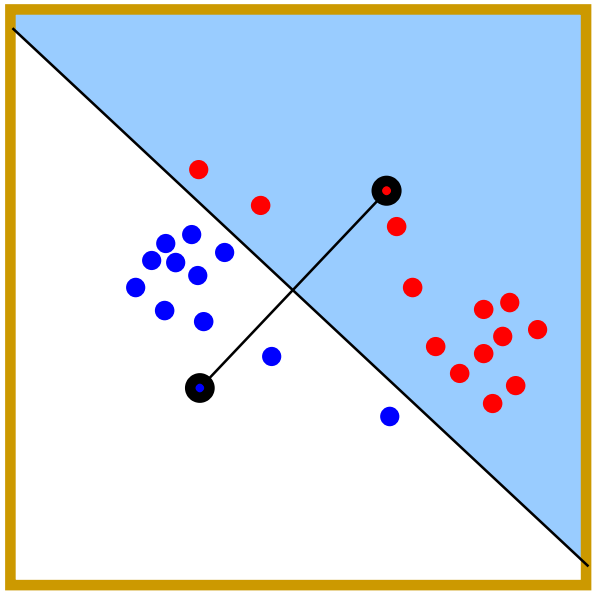

related tumors
ordered on similarity
DLBCL
Germinal Center B
NI. Node/Tonsil
Activated Blood B
Resting/Activated T
Cell Lines
FL
Resting Blood B
CLL
Cyclin A
BUB1 mitotic kinase
Cyclin B1
SOCS-1
Ki67
p55CDC
Prolifer-ation
p16
Thymidine kina
CDC21 homolo
RAD54
Dihydrofolate reductase
CD38
Genetic profile of functionally related genes for a specific tumor
related genes
FAK=focal adhesion kinase
WIP=WASP interacting protein
FMR2
CD10
Germinal Center B
BCL-7A
A-myb
BCL-6
PI 3-kinase p110 γ
cluster
RGS13
CD105
CD14
FGF-7
MMP9
fms=CSF-1 receptor
Cathepsin B
Fc ε receptor γ chain
Lymph Node
TIMP-3
Integrin beta 5
NK4=NK cell protein-4
SDF-1 chemokine
CD49F=Integrin α6
LAT
CD2
CD3 ε
T cell receptor β chain
T cell
fyn
IRF-1
Caspase 10

k-Means clustering



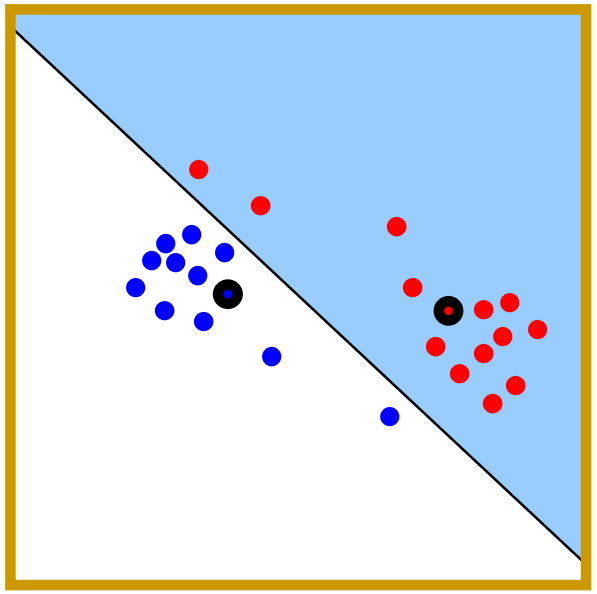
Choose randomly 2 prototypes

k-Means clustering



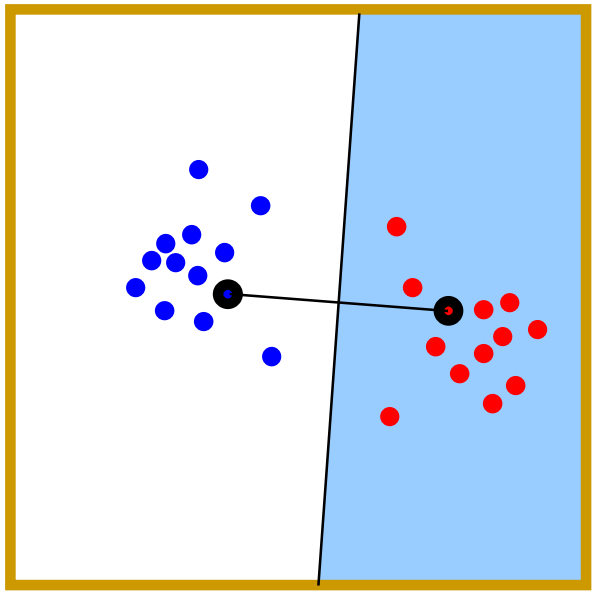
Assign objects to closest prototype
Blue area: cluster 1
White area: cluster 2

k-Means clustering



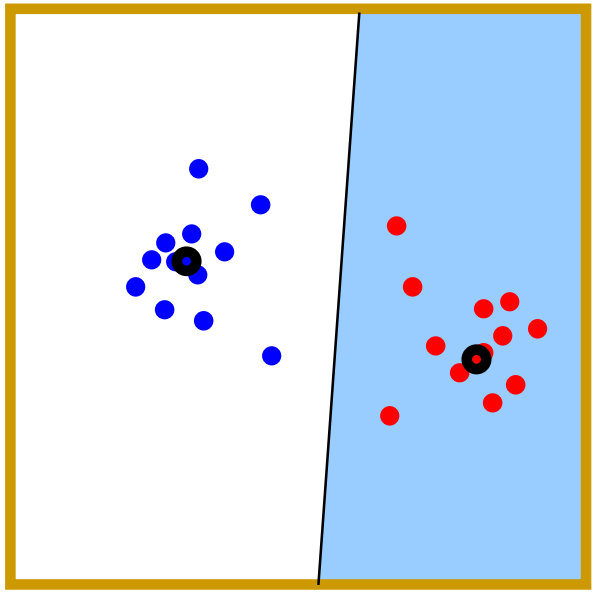
Calculate new cluster prototypes
By averaging objects

k-Means clustering



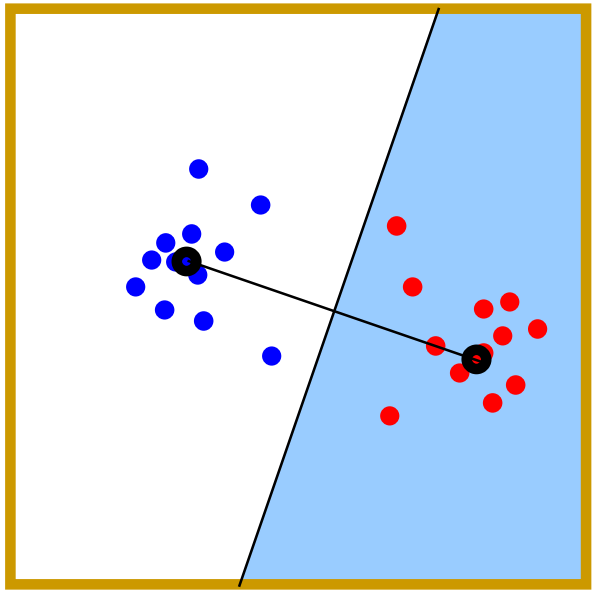
Re-assign objects to closest prototype
Blue area: cluster 1
White area: cluster 2

k-Means clustering



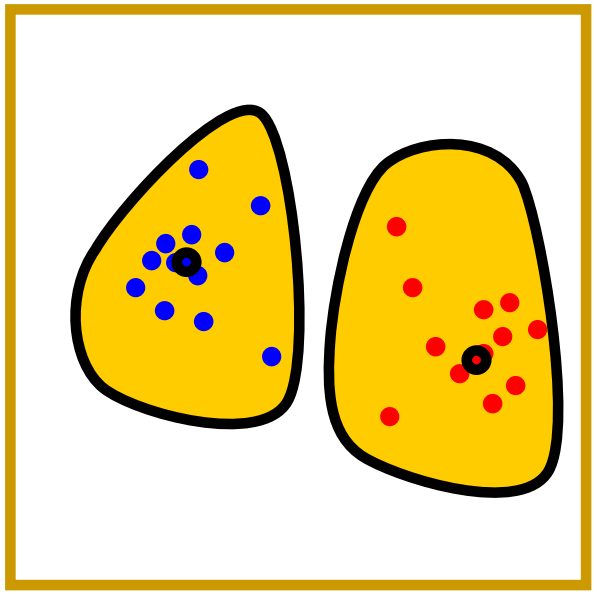
Re-calculate new cluster prototypes

k-Means clustering



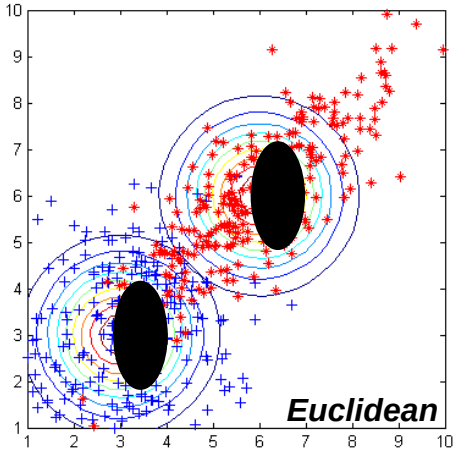
Re-assign objects to closest prototype
If no objects change cluster then finished

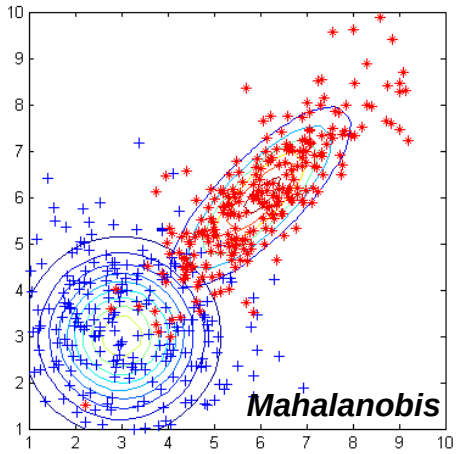
k-Means clustering



Establish clusters

K-means clustering: Parameters

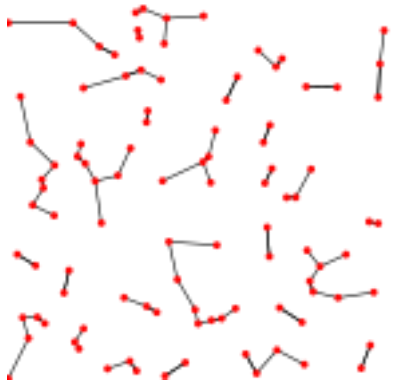




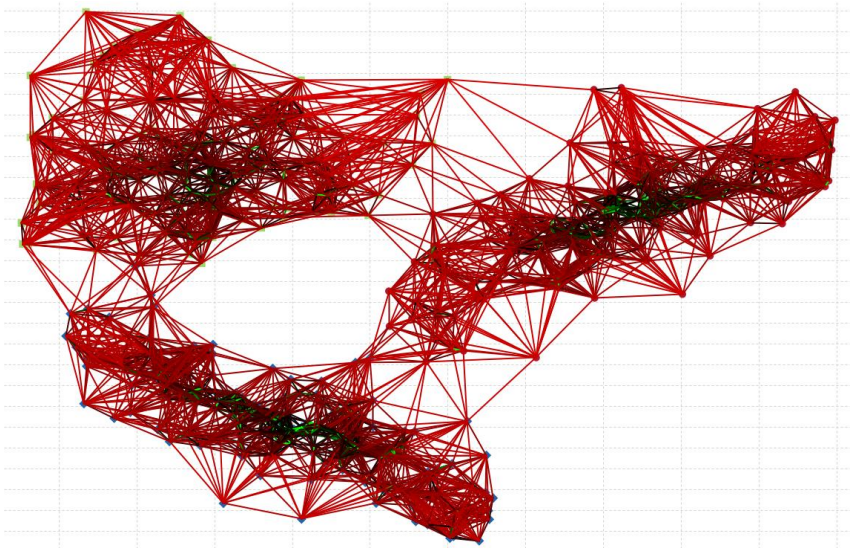
- K-means
  - Fixed number of clusters (need to know a priori)
  - Choice of distance measure
  - Prototype choice
- Distance measure
  - Euclidean: Round clusters
  - Mahalanobis: Elongated clusters
- Prototype choice
  - Point
  - Line etc.
- Number of clusters
  - Validate clustering!

Graph-based Clustering

- K-NN graph: Connect every node to its k-nearest nodes
- Find densely connected components



k=1



k=20

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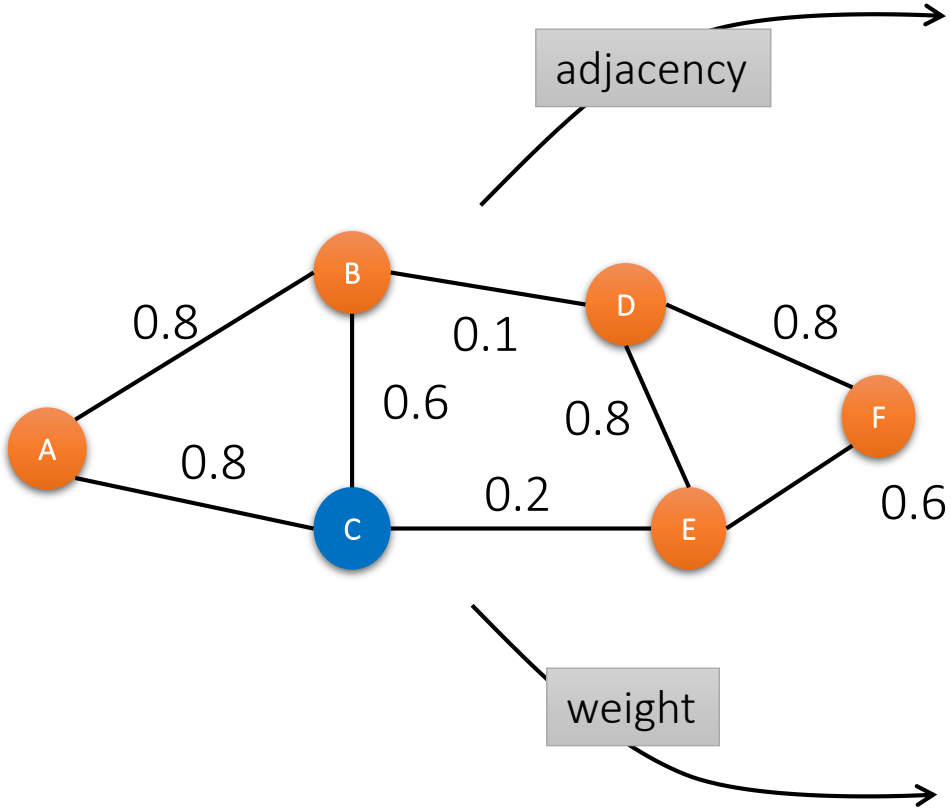
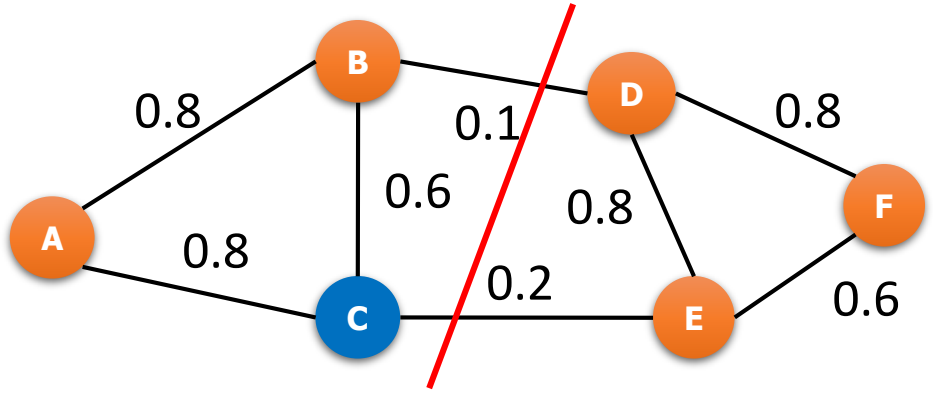
Graphs, adjacency and weight matrices



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

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

Spectral clustering (1)



- Minimise normalised cut
- Normalised cut between two clusters C_1 and C_2 :

$$NC(C_1,C_2) = \frac{\text{cut}(C_1,C_2)}{assoc(C_1,V)} + \frac{\text{cut}(C_2,C_1)}{assoc(C_2,V)} = 2 - \left(\frac{assoc(C_1,C_1)}{assoc(C_1,V)} + \frac{assoc(C_2,C_2)}{assoc(C_2,V)}\right)$$

- cut(C_1, C_2) = weight of links between C_1 and C_2
- cut(C_2, C_1) = same
- assoc(C_1,V) = total weight of links from nodes in C_1 to entire graph
- assoc(C_2,V) = total weight of links from nodes in C_2 to entire graph

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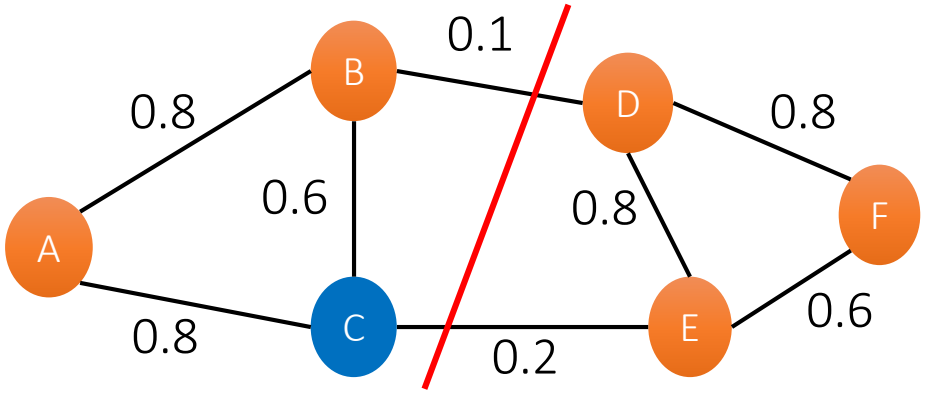
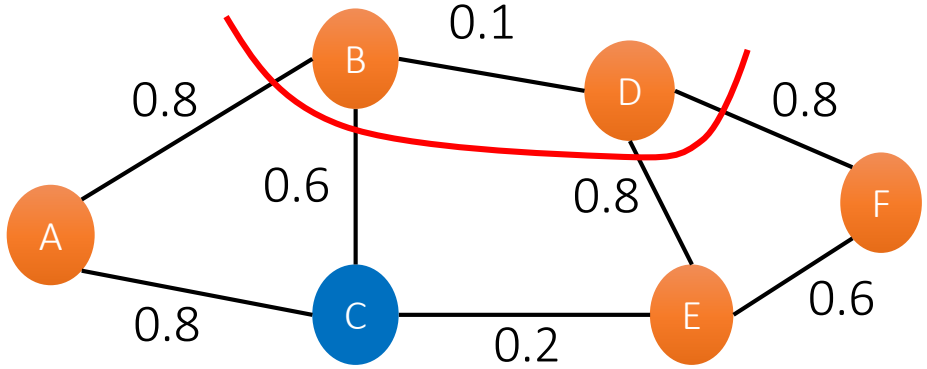
Normalized cut (example)

- 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



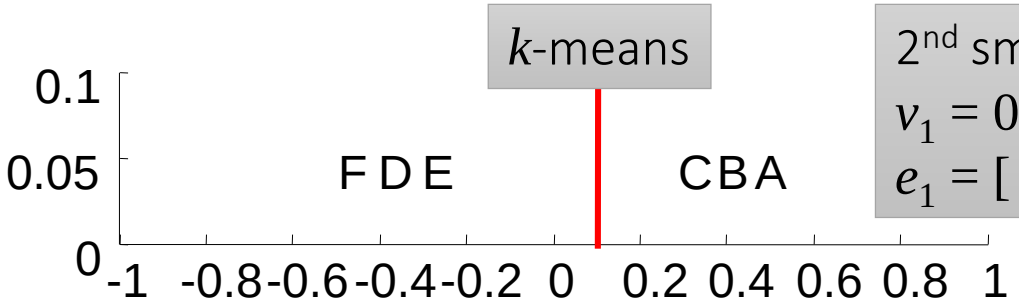
Spectral clustering (2)

(sum weights on diagonal – W)

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

$$\xrightarrow[\mathbf{L}=\mathrm{diag}(\mathbf{1}^T\mathbf{W})-\mathbf{W}]{\text{Laplacian}}$$

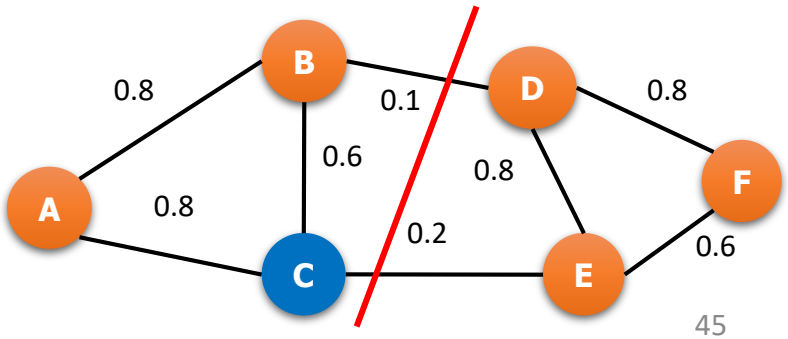
$$L = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 1.6 & -0.8 & -0.8 & 0 & 0 & 0 \\ -0.8 & 1.5 & -0.6 & -0.1 & 0 & 0 \\ -0.8 & -0.6 & 1.6 & 0 & -0.2 & 0 \\ 0 & -0.1 & 0 & 1.7 & -0.8 & -0.8 \\ 0 & 0 & -0.2 & -0.8 & 1.6 & -0.6 \\ 0 & 0 & 0 & -0.8 & -0.6 & 1.4 \end{pmatrix} \end{array}$$



2nd smallest eigenvalue:

$v_1 = 0.19$

$e_1 = [\ 0.44\ 0.41\ 0.37\ -0.40\ -0.37\ -0.45\ ]^T$



IEEE TRANSACTIONS ON PATTERN ANALYSIS AND MACHINE INTELLIGENCE, VOL. 22, NO. 8, AUGUST 2000

Normalized Cuts and Image Segmentation

Jianbo Shi and Jitendra Malik, *Member, IEEE*

Markov clustering (1)

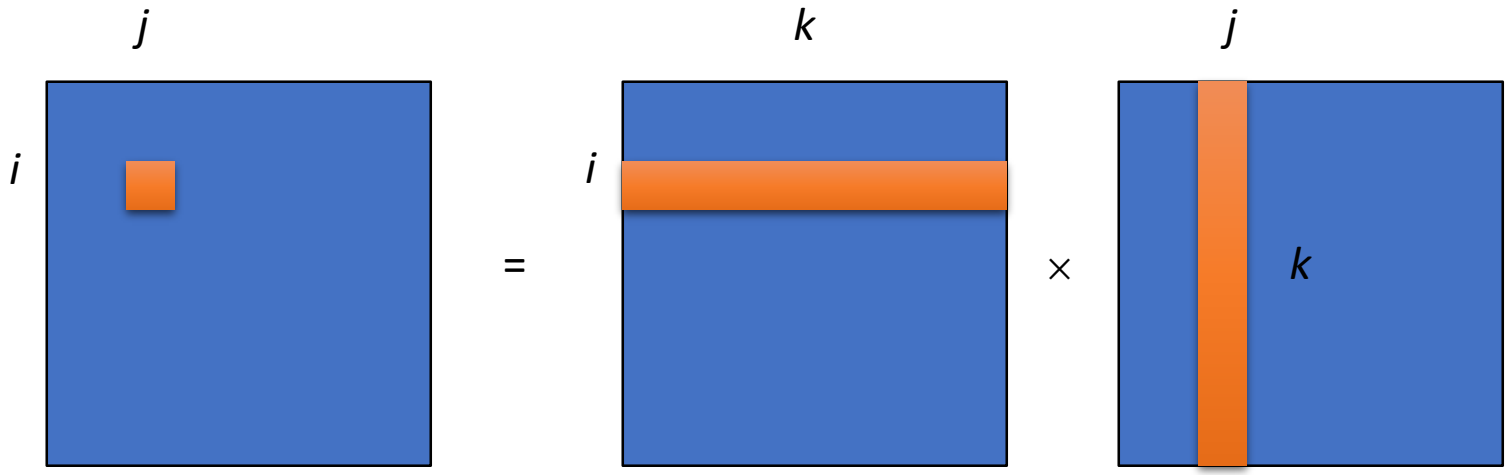
- Markov clustering
  - Random walks are Markov chains
  - Start with Markov matrix: $M_{ij} = p(j \rightarrow i)$
  - (optionally add loops (diagonal))

$$W = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 0.8 & 0.8 & 0 & 0 & 0 \\ 0.8 & 0 & 0.6 & 0.1 & 0 & 0 \\ 0.8 & 0.6 & 0 & 0 & 0.2 & 0 \\ 0 & 0.1 & 0 & 0 & 0.8 & 0.8 \\ 0 & 0 & 0.2 & 0.8 & 0 & 0.6 \\ 0 & 0 & 0 & 0.8 & 0.6 & 0 \end{pmatrix} \end{array} \xrightarrow[\mathbf{M} = \mathbf{W}\mathrm{diag}(\mathbf{1}^T \mathbf{W})^{-1}]{\text{Markov}} M = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0 & 0.53 & 0.5 & 0 & 0 & 0 \\ 0.5 & 0 & 0.38 & 0.06 & 0 & 0 \\ 0.5 & 0.4 & 0 & 0 & 0.12 & 0 \\ 0 & 0.07 & 0 & 0 & 0.5 & 0.57 \\ 0 & 0 & 0.12 & 0.47 & 0 & 0.43 \\ 0 & 0 & 0 & 0.47 & 0.38 & 0 \end{pmatrix} \end{array}$$

(sum columns = 1)

Markov clustering (2)

$M_{ij} = p(j \to i)$ probability of arriving in i from j in 1 step



$\left(M^2\right)_{ij} = \sum_k p(j \to k)p(k \to i)$ probability of arriving in i from j in 2 steps (etc.)

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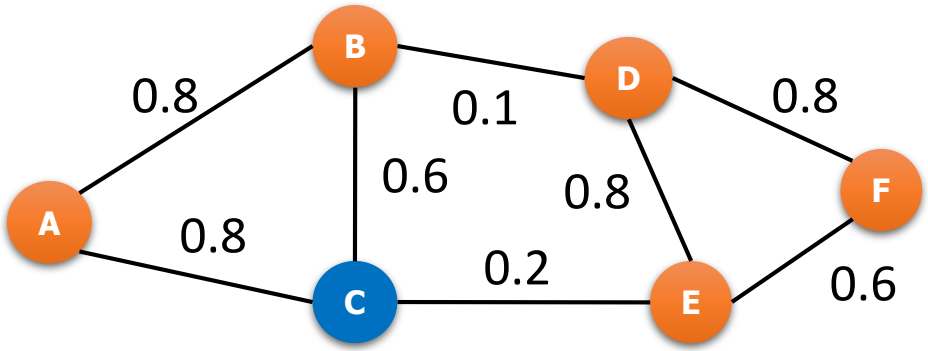
Markov clustering (3)



- Markov clustering:
  - Iterate
    1. $M = M^{\alpha}$
    2. $M_{ij} = M_{ij}^{\beta} \Big/ \sum_i M_{ij}^{\beta}$
- Step 1 : take walk of $\langle$ steps
- Step 2 : increase difference between small probabilities (<0.5) and large probabilities (>0.5) ; converges to maximum value in column
- E.g. for $\langle = \beta = 2$

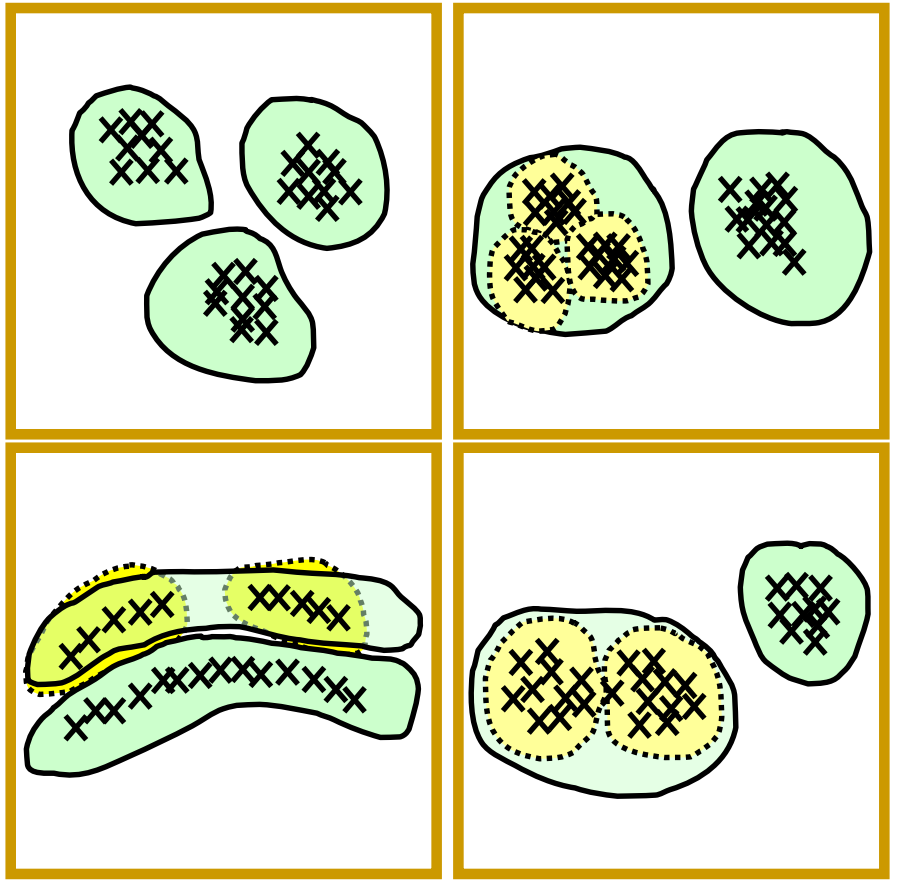
$$M = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} A & B & C & D & E & F \end{array} \\ \begin{pmatrix} 0.97 & 0.89 & 0.89 & 0.00 & 0.01 & 0.00 \\ 0.02 & 0.44 & 0.04 & 0.01 & 0.02 & 0.00 \\ 0.02 & 0.44 & 0.46 & 0.02 & 0.10 & 0.01 \\ 0.00 & 0.07 & 0.01 & 0.97 & 0.89 & 0.97 \\ 0.01 & 0.02 & 0.10 & 0.02 & 0.79 & 0.04 \\ 0.00 & 0.00 & 0.01 & 0.02 & 0.07 & 0.98 \end{pmatrix} \end{array}$$

Outline

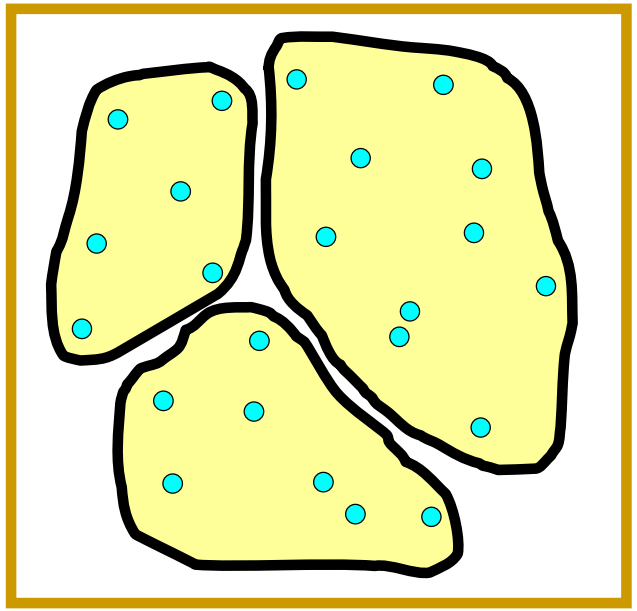
- Feature selection
- Introduction to clustering
  - Hierarchical clustering
  - *k*-Means clustering
  - Graph-based clustering
- **Validation**
- scRNA-seq clustering
  - Single Cell Consensus Clustering (SC3)
  - Seurat

Clustering is subjective!

- Principle choices
  - Similarity measure
  - Algorithm
- Different choice leads to different results
  - Subjectivity becomes reality
- Cluster process
  - Validate, interpret (generate hypothesis), repeat steps



Cluster Validation



- Cluster tendency

  Clustering IMPOSES structure even though data may not posses it

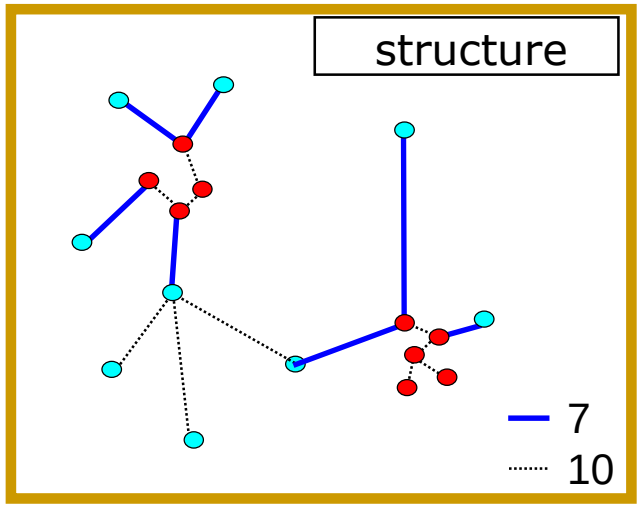
  Aim: Test whether data possesses structure

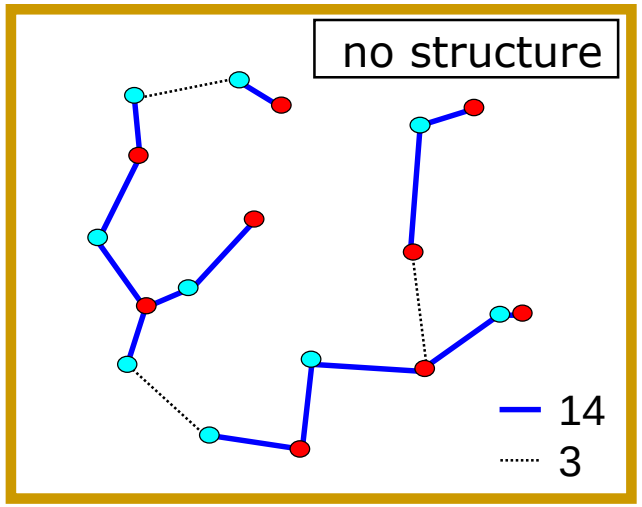
- Cluster validity

  Choices impose restrictions on for example shape

  Aim: Quantitative evaluation of the clustering results

Test for spatial randomness





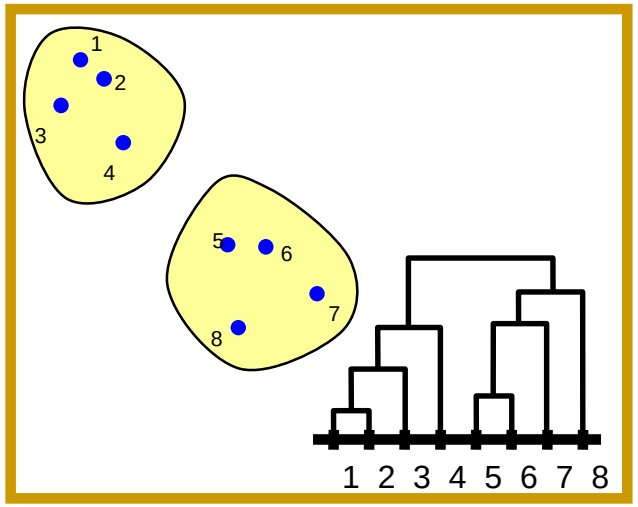
- Test

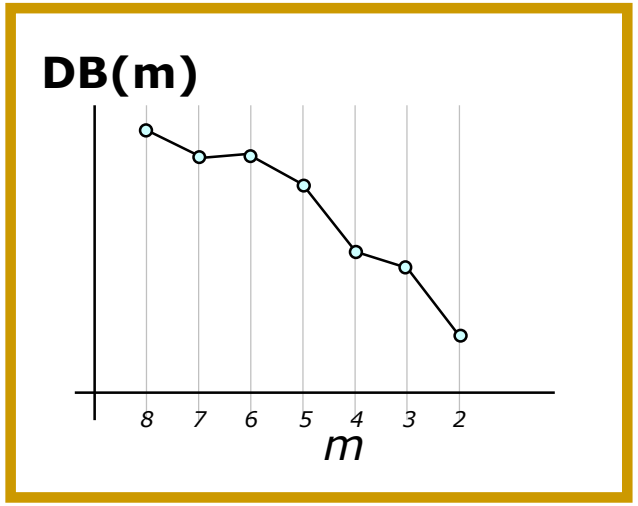
  If data (•) clusters frequently with random data (•) then data structureless

- Approach
  - Generate random vectors (**Y**) uniformly over observed region of data (**X**)
  - Find MST (single linkage HC) of **X** ∨ **Y**
  - Determine number of edges *q* that connect vectors of **X** with **Y**
  - If **X** contains clusters *q* should be small!

  (multiple random vs random measurements gives likelihood for *q*)

Davis-Bouldin index





- Test

  Select specific clustering according to a criteria

  For example: Davis-Bouldin index

- DB index

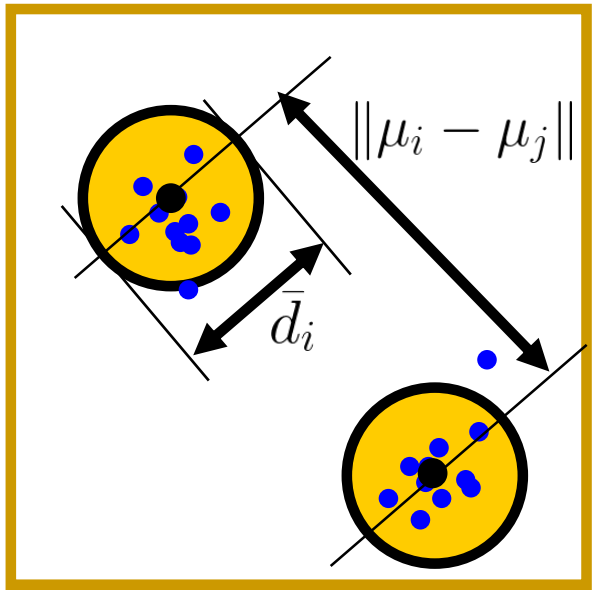
  For a specific clustering m, **DB(m)** : Average similarity of a cluster with its most similar cluster

- Approach

  Goal: Clusters to have minimal similarity

  Seek: Clustering that minimize *DB*(*m*) wrt *m*

Davis-Bouldin index



- Similarity cluster C_i and C_j

$$D_{i,j} = \frac{(\bar{d}_i + \bar{d}_j)}{\|\mu_i - \mu_j\|}$$

- $\bar{d}_i$:average distance within cluster *i*, μ_i :centroid of cluster *i*
- Most similar cluster to C_i

$$R_{i,j} = \max_{j \neq i} \{D_{i,j}\}$$

- DB index

$$DB = \frac{1}{k} \sum_{k=1}^{k} R_{i,j}$$

Silhouette score

- Measure similarity of object to its own cluster

$$s(i) = \frac{b(i) - a(i)}{max(a(i), b(i))}$$

with $a(i)$ being average distance to all objects in same cluster and $b(i)$ being closest object from all other clusters:

$$a(i) = \frac{1}{|C_i|} \sum_{\forall j} d(x_i, x_j) \qquad b(i) = min_{\forall j, j \notin C_i} d(x_i, x_j)$$

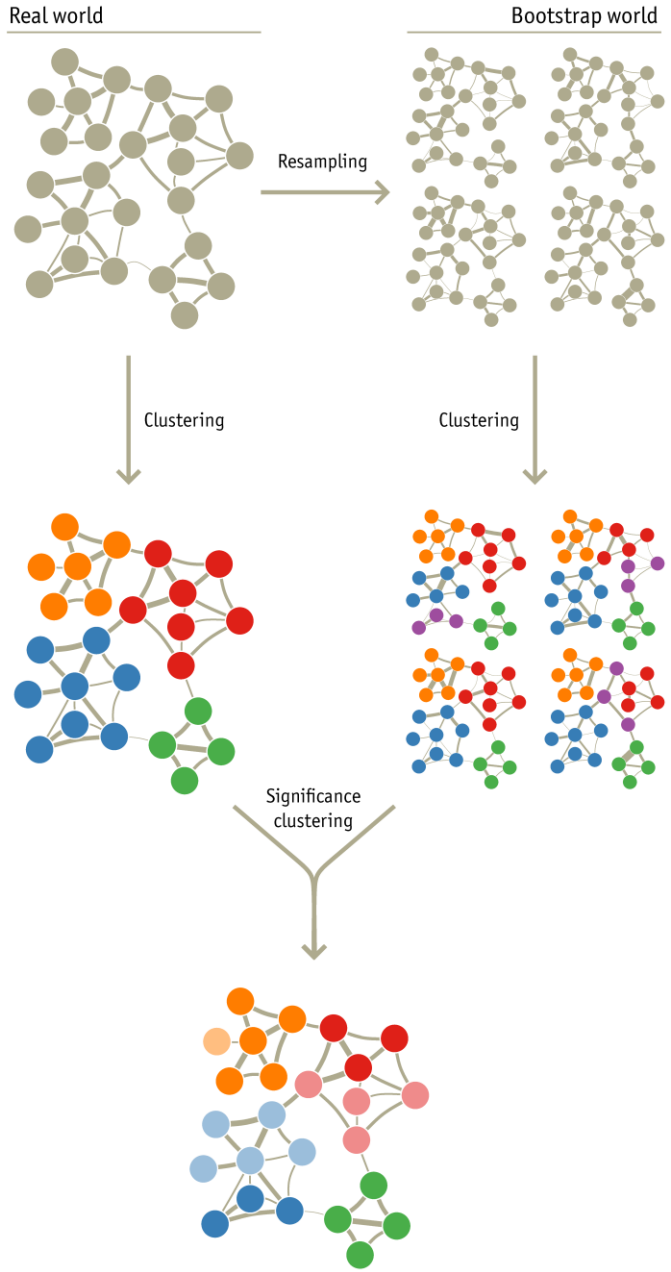
$-1 \leq s(i) \ll 1$; $s(i)$ is close to 1, if $a(i) \ll b(i)$; average distance within cluster much smaller than nearest objects

- Silhouette score is average of all these similarities

$$S = \frac{1}{N} \sum S(i)$$

Bootstrapping

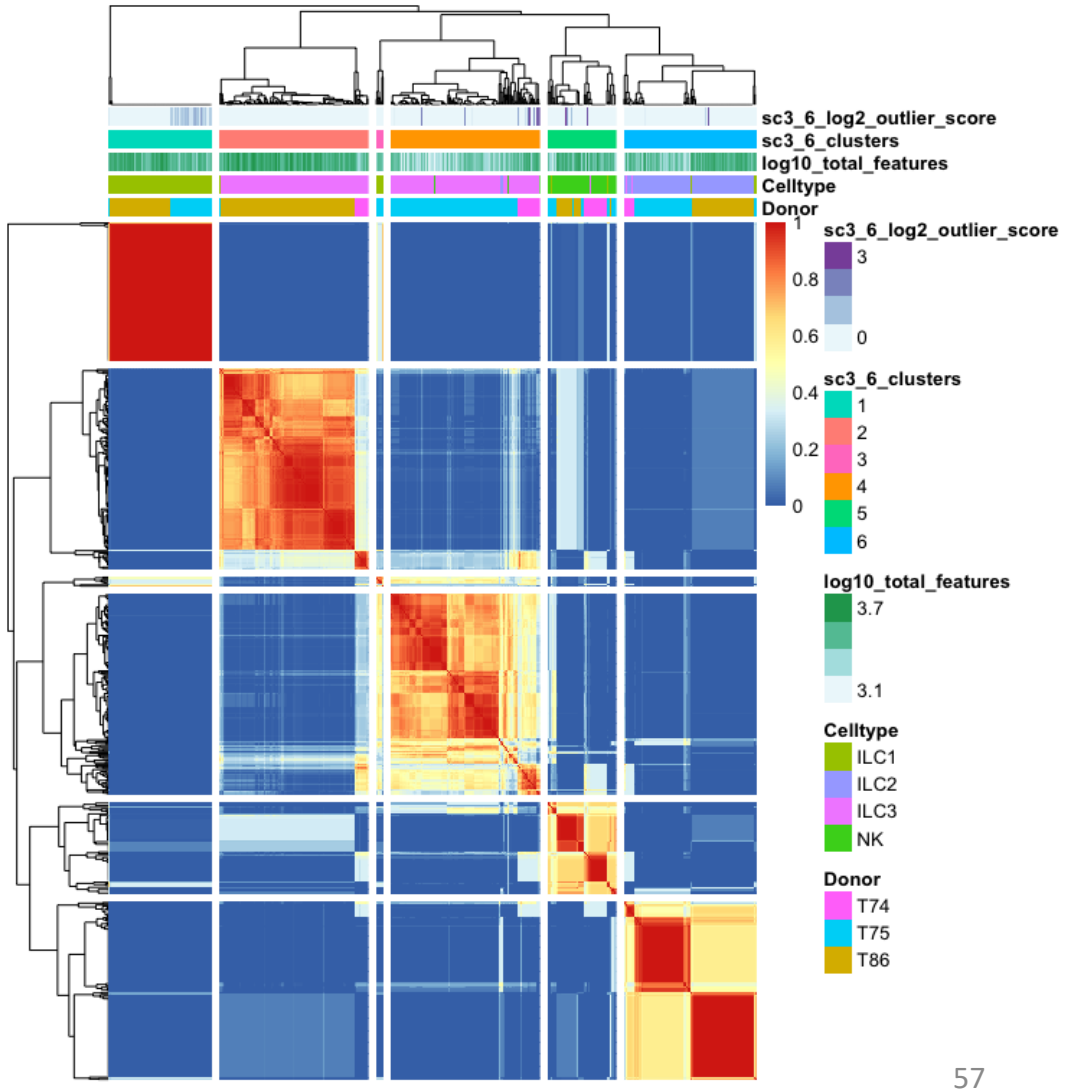
- How confident can you be that the clusters you see are real?
- You can always take a random set of cells from the same cell type and manage to split them into clusters.



Rosvall and Bergstrom (https://doi.org/10.1371/journal.pone.0008694)

Always check QC data

- Is what your splitting mainly related to batches, qc-measures (especially detected genes)?



From clusters to cell identities

- 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

Databases with celltype gene signatures

- PanglaoDB (https://panglaodb.se/)
  - Human: 295 samples, 72 tissues, 1.1 M cells
  - Mouse: 976 samples, 173 tissues, 4 M cells
  - Franzén et al (https://doi.org/10.1093/database/baz046)

- 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. (https://doi.org/10.1093/nar/gky900)

Outline

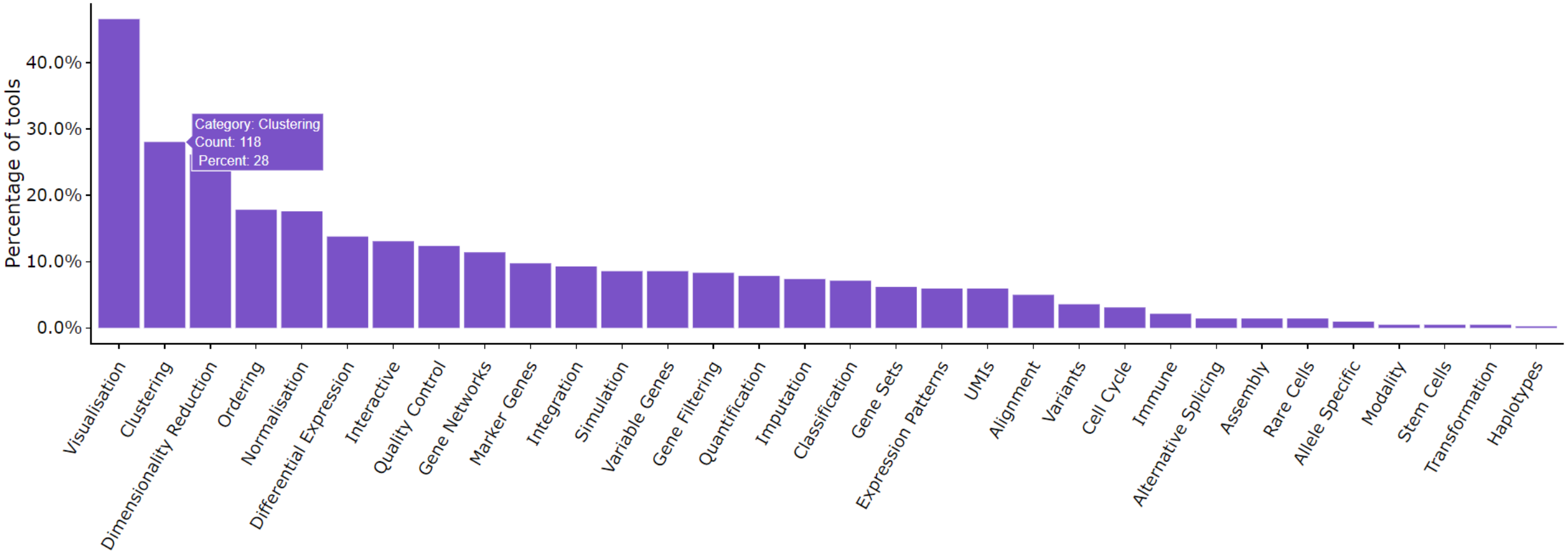
- Feature selection
- Introduction to clustering
  - Hierarchical clustering
  - *k*-Means clustering
  - Graph-based clustering
- Validation
- **scRNA-seq clustering**
  - Single Cell Consensus Clustering (SC3)
  - Seurat

scRNA-seq clustering methods

Name	Year	Method type	Strengths	Limitations
scanpy[4]	2018	PCA + graph-based	Very scalable	May not be accurate for small data sets
Seurat (latest)[3]	2016			
PhenoGraph[32]	2015			
SC3 (REF.[22])	2017	PCA + *k*-means	High accuracy through consensus, provides estimation of *k*	High complexity, not scalable
SIMLR[24]	2017	Data-driven dimensionality reduction + *k*-means	Concurrent training of the distance metric improves sensitivity in noisy data sets	Adjusting the distance metric to make cells fit the clusters may artificially inflate quality measures
CIDR[25]	2017	PCA + hierarchical	Implicitly imputes dropouts when calculating distances	
GiniClust[75]	2016	DBSCAN	Sensitive to rare cell types	Not effective for the detection of large clusters
pcaReduce[27]	2016	PCA + *k*-means + hierarchical	Provides hierarchy of solutions	Very stochastic, does not provide a stable result
Tasic et al.[28]	2016	PCA + hierarchical	Cross validation used to perform fuzzy clustering	High complexity, no software package available
TSCAN[41]	2016	PCA + Gaussian mixture model	Combines clustering and pseudotime analysis	Assumes clusters follow multivariate normal distribution
mpath[45]	2016	Hierarchical	Combines clustering and pseudotime analysis	Uses empirically defined thresholds and a priori knowledge
BackSPIN[26]	2015	Biclustering (hierarchical)	Multiple rounds of feature selection improve clustering resolution	Tends to over-partition the data
RaceID[23], RaceID2 (REF.[115]), RaceID3	2015	*k*-Means	Detects rare cell types, provides estimation of *k*	Performs poorly when there are no rare cell types
SINCERA[5]	2015	Hierarchical	Method is intuitively easy to understand	Simple hierarchical clustering is used, may not be appropriate for very noisy data
SNN-Cliq[80]	2015	Graph-based	Provides estimation of *k*	High complexity, not scalable

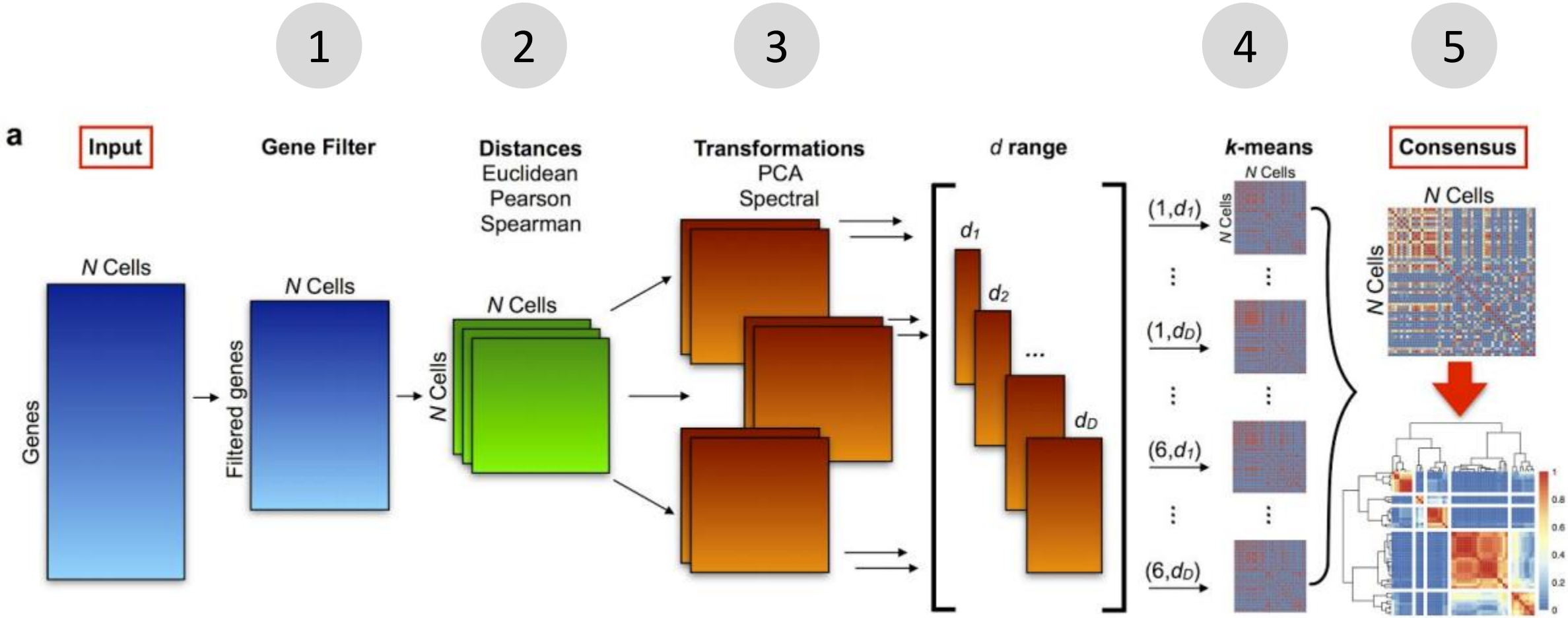
Kiselev et al. (https://doi.org/10.1038/s41576-018-0088-9)

scRNA-seq tools



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

Single Cell Consensus Clustering – SC3



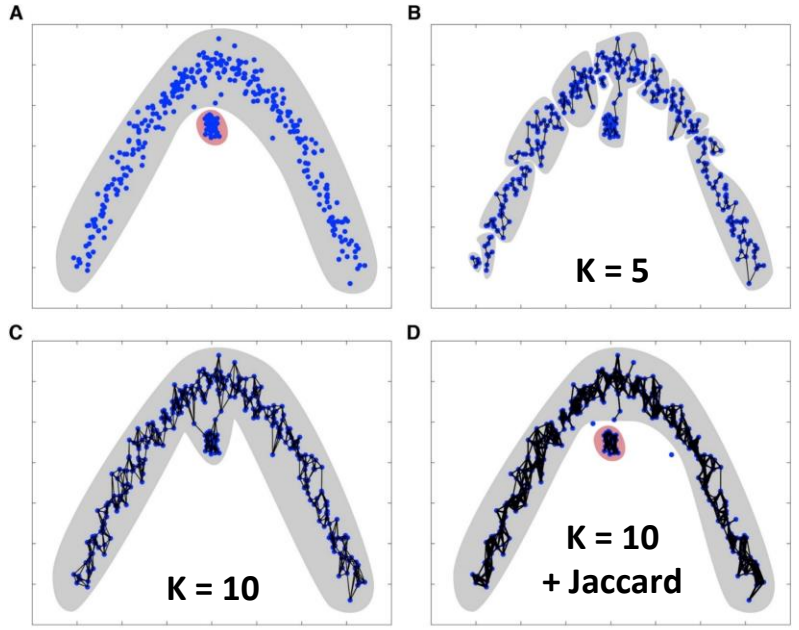
Kiselev et al. (https://doi.org/10.1038/nmeth.4236)

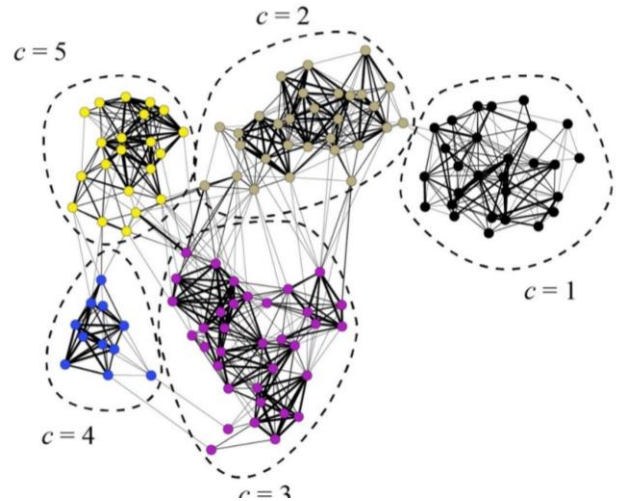
Seurat

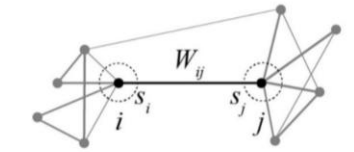
1) Construct KNN (k-nearest neighbor) graph based on the Euclidean distance in PCA space.

2) Refine the edge weights between any two cells based on the shared overlap in their local neighborhoods (Jaccard distance).

3) Cluster cells by optimizing for modularity (Louvain algorithm)







$$Q = \frac{1}{2m}\sum_{i,j}\left[W_{ij} - \frac{s_i s_j}{2m}\right]\delta(c_i, c_j)$$

Xu and Su (https://doi.org/10.1093/bioinformatics/btv088)
Levine et al. (https://doi.org/10.1016/j.cell.2015.05.047)

Comparing different clusterings

- Adjusted Rand Index (ARI)

Given a set S of n elements, and two groupings or partitions (e.g. clusterings) of these elements $X = \{X_1, X_2, \dots, X_r\}$ and $Y = \{Y_1, Y_2, \dots, Y_r\}$

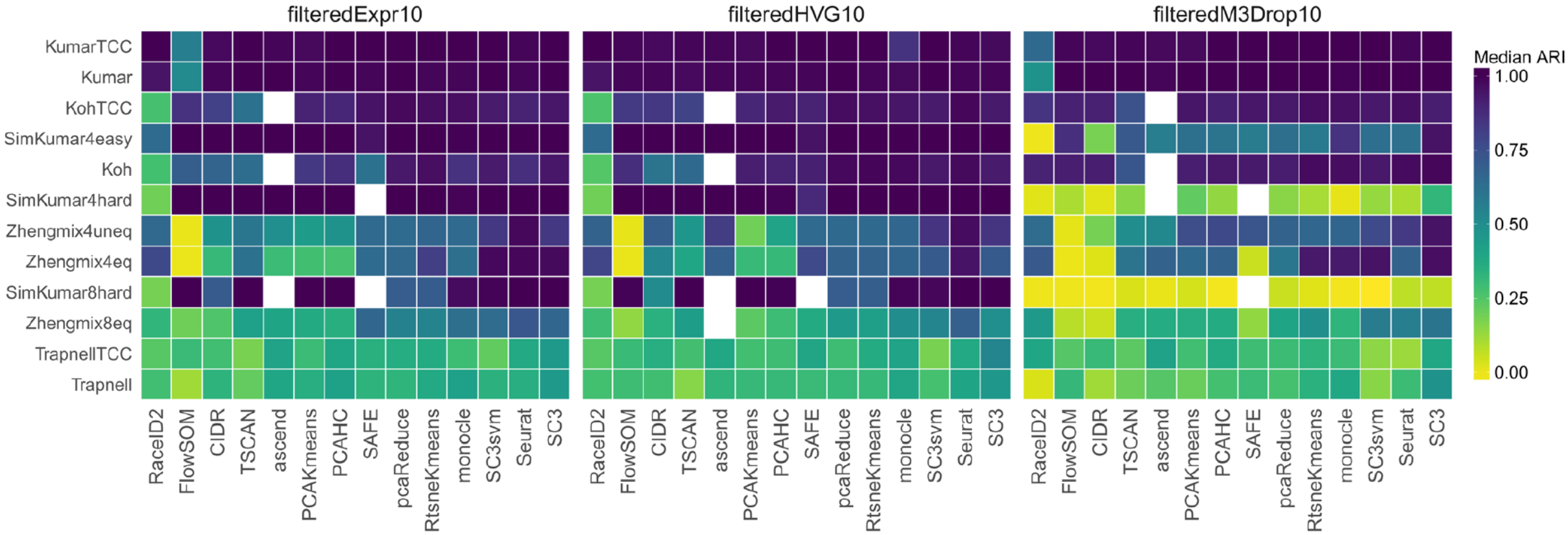
Confusion matrix/contingency table

$X \setminus Y$	Y_1	Y_2	$\dots$	Y_s	Sums
X_1	n_{11}	n_{12}	$\dots$	n_{1s}	a_1
X_2	n_{21}	n_{22}	$\dots$	n_{2s}	a_2
$\vdots$	$\vdots$	$\vdots$	$\ddots$	$\vdots$	$\vdots$
X_r	n_{r1}	n_{r2}	$\dots$	n_{rs}	a_r
Sums	b_1	b_2	$\dots$	b_s	

$$n_{ij} = |X_i \cap Y_j|$$

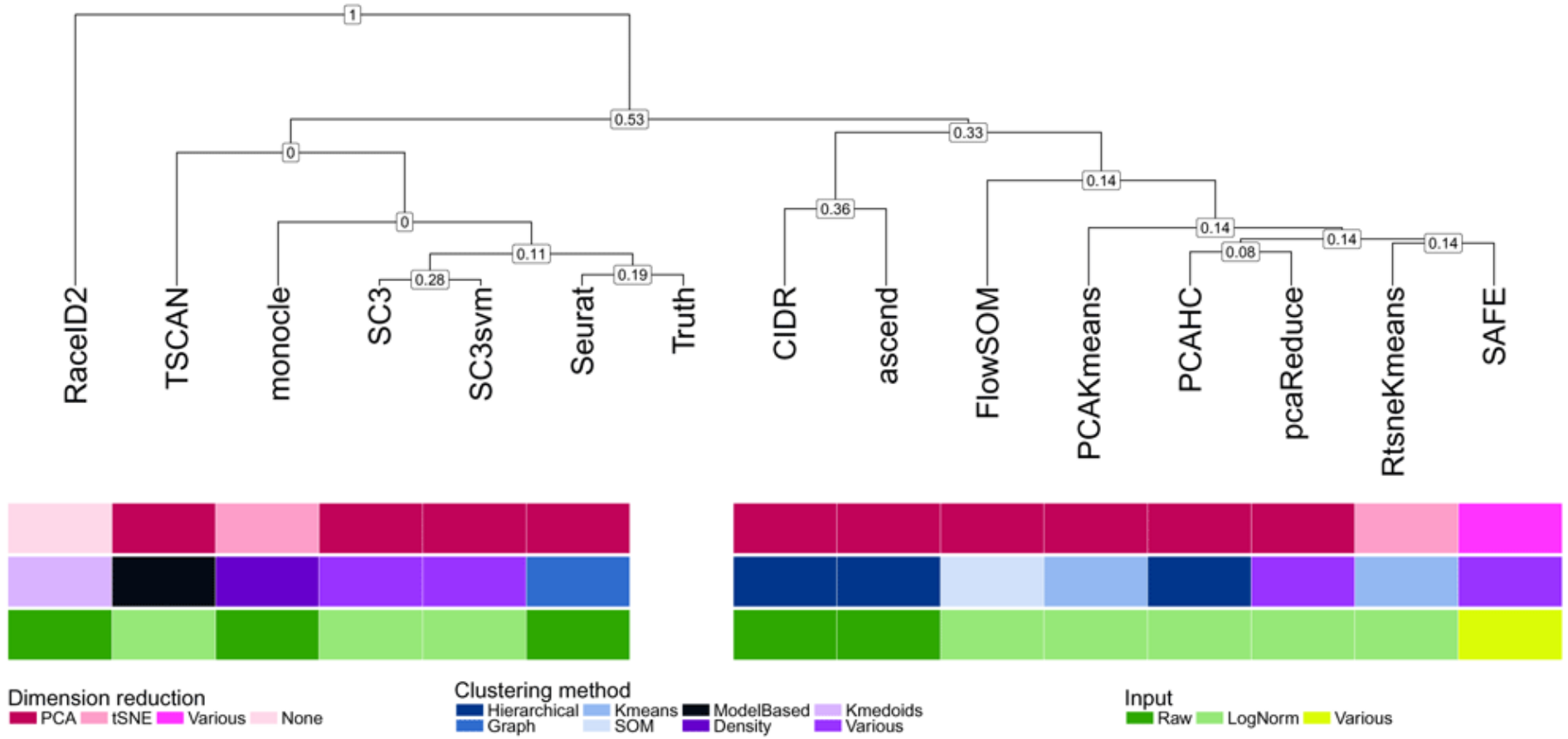
$$\overbrace{ARI}^{\text{Adjusted Index}} = \frac{\overbrace{\sum_{ij}\binom{n_{ij}}{2}}^{\text{Index}} - \overbrace{[\sum_i \binom{a_i}{2} \sum_j \binom{b_j}{2}]/\binom{n}{2}}^{\text{Expected Index}}}{\underbrace{\frac{1}{2}[\sum_i \binom{a_i}{2} + \sum_j \binom{b_j}{2}]}_{\text{Max Index}} - \underbrace{[\sum_i \binom{a_i}{2} \sum_j \binom{b_j}{2}]/\binom{n}{2}}_{\text{Expected Index}}}$$

Benchmarking scRNA-seq clustering methods



Duò et al. (https://doi.org/10.12688/f1000research.15666.2)

Benchmarking scRNA-seq clustering methods



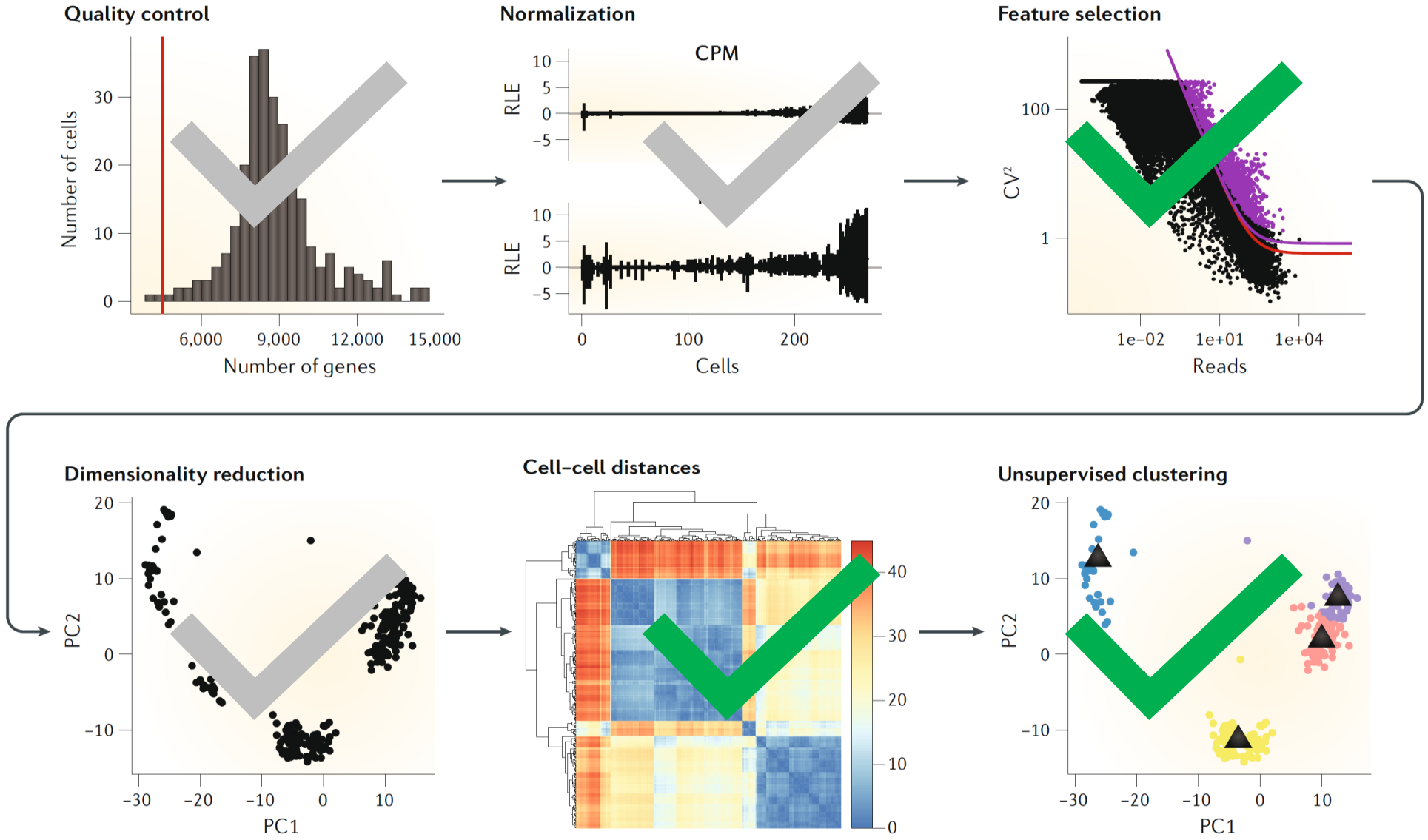
Duò et al. (https://doi.org/10.12688/f1000research.15666.2)

Challenges in clustering

- What is a cell type
  - *Validation using independent tech is important.*
  - *Commonly, spatial mapping (smFISH)*

- **Scalability**: in the last few years the number of cells in scRNA-seq experiments has grown by several orders of magnitude from ~10^2 to ~10^6
  - *Computational efficiency*
  - *Visual exploration, crowding problem*

Summary


Quality control
Number of cells
Number of genes
Normalization
CPM
RLE
Cells
Feature selection
CV²
Reads
Dimensionality reduction
PC2
PC1
Cell–cell distances
Unsupervised clustering
PC2
PC1

Clustering practical

- Feature selection (HVG)
- Dimensionality reduction: select principal components
- Hierarchical clustering: distances and linkage methods
- tSNE + *k*-Means
- Graph-based clustering

Resources

- Kiselev et al. ”Challenges in unsupervised clustering of single- cell RNA- seq data”

https://doi.org/10.1038/s41576-018-0088-9

- Duò et al. ” A systematic performance evaluation of clustering methods for single-cell RNA-seq data”

https://doi.org/10.12688/f1000research.15666.2

- Orchestrating Single-Cell Analysis with Bioconductor

https://osca.bioconductor.org/

- Hemberg single cell course: Analysis of single cell RNA-seq data

https://scrnaseq-course.cog.sanger.ac.uk/website/index.html

- Slides Åsa Björklund (NBIS, SciLifeLab)

https://github.com/NBISweden/workshop-scRNAseq/tree/master/slides2019