Bioinformatics Software Lab
Introduction to Single Cell Sequencing

Ivan Gesteira Costa, Mingbo Cheng, Zhijian Li, Martin Manolov
Institute for Computational Genomics

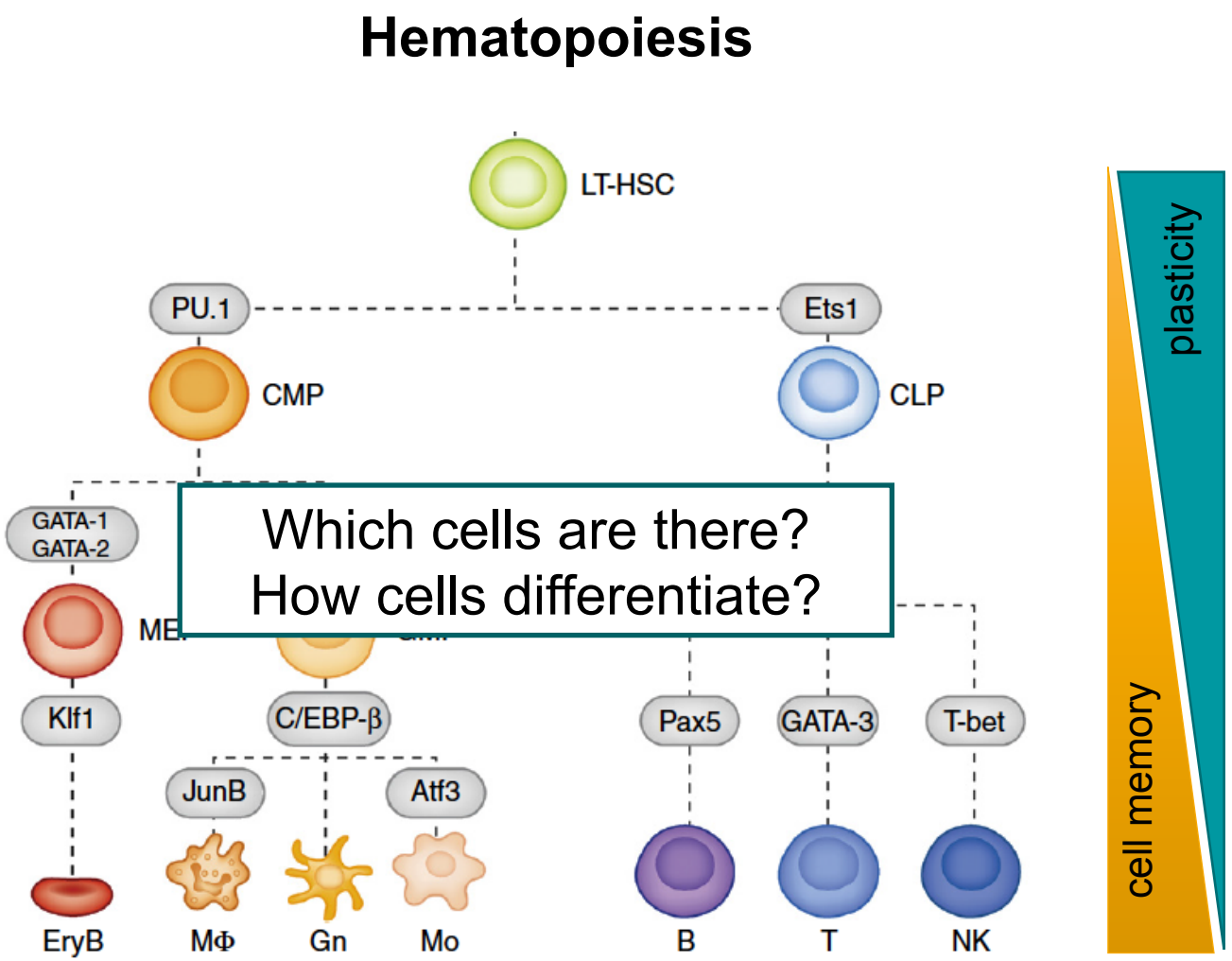
Institute for Computational Genomics
RWTH AACHEN UNIVERSITY

Objectives

1. basics of single cell sequencing
2. basic bioinformatics/computational problems
   - dimension reduction
   - clustering
   - data integration

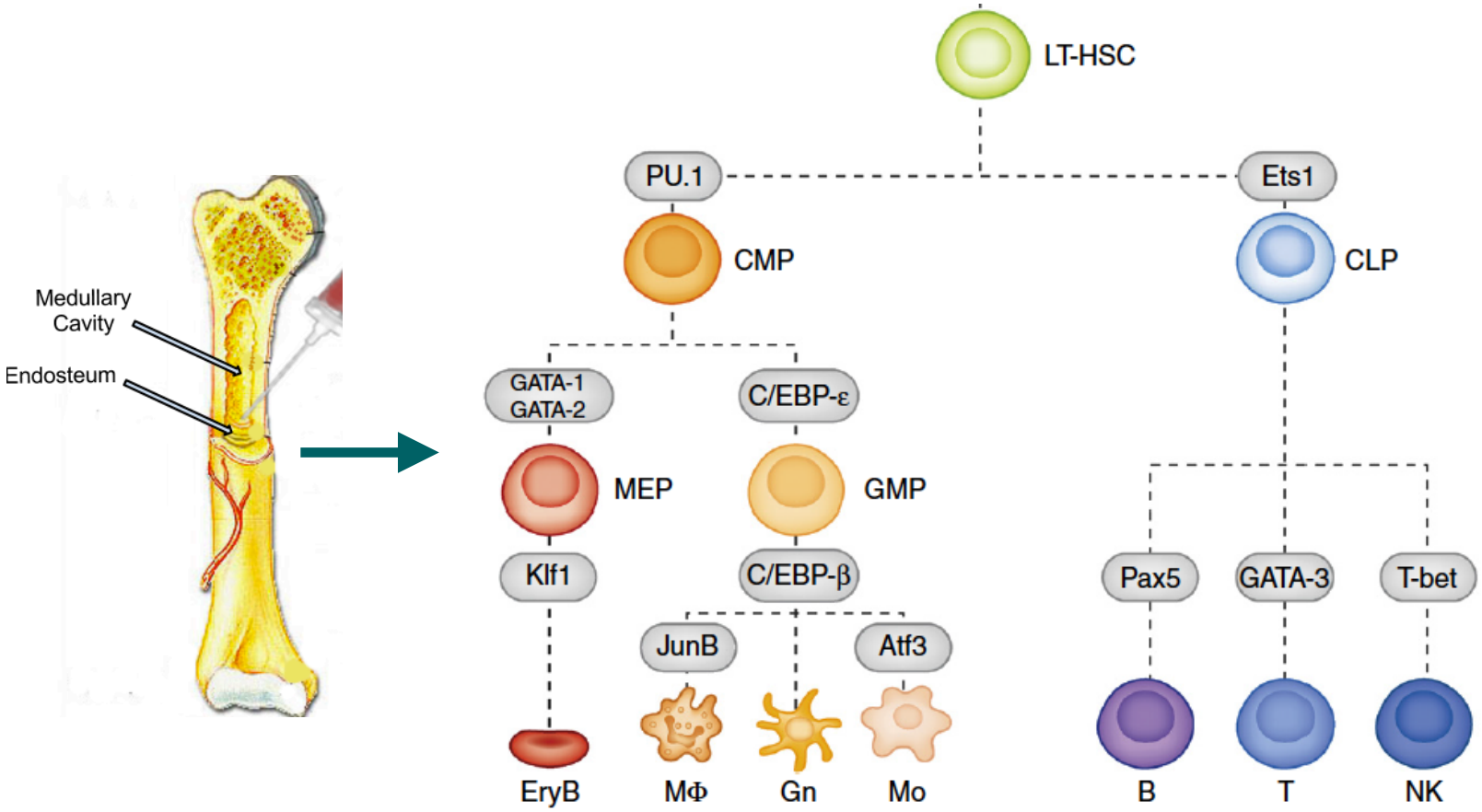
Expression at Single Cell Level

Cell Differentiation



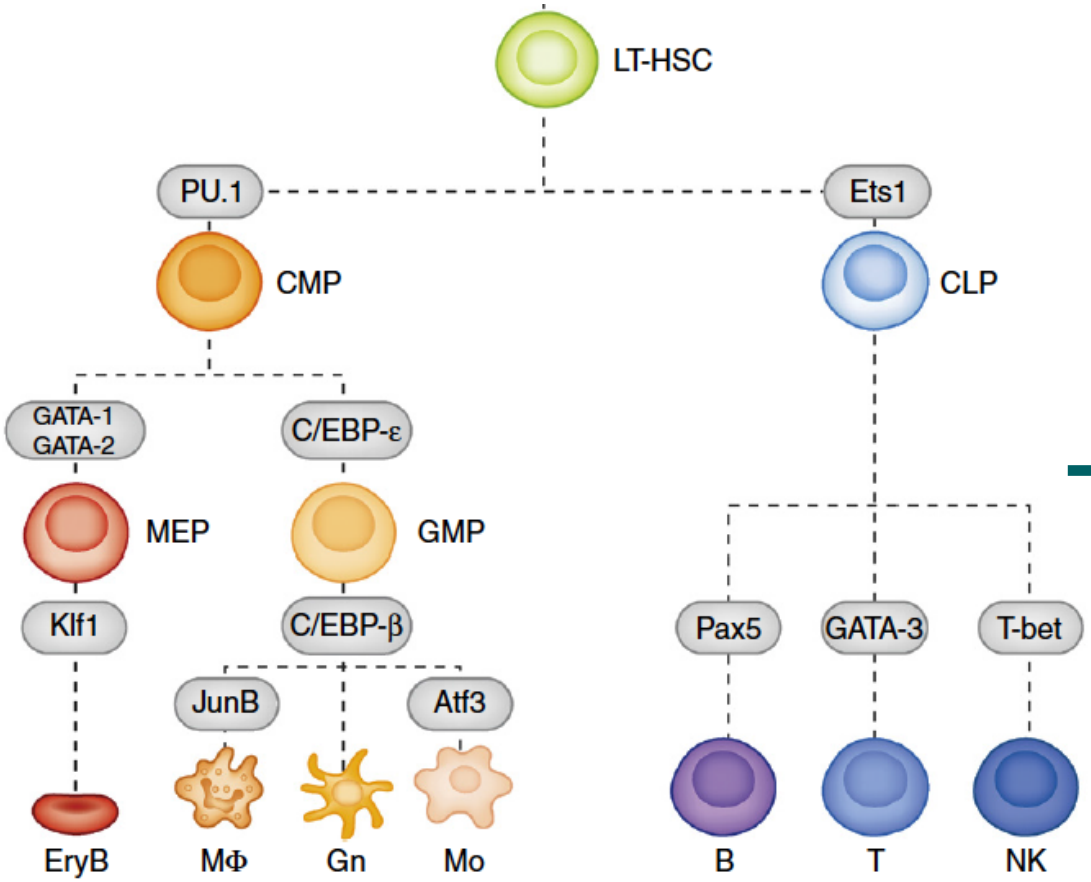
Source: Amit (2016), *Nature Immunoloy.*

Cell Differentiation



Source: Amit (2016), *Nature Immunoloy.*

Cell Differentiation & Gene Expression

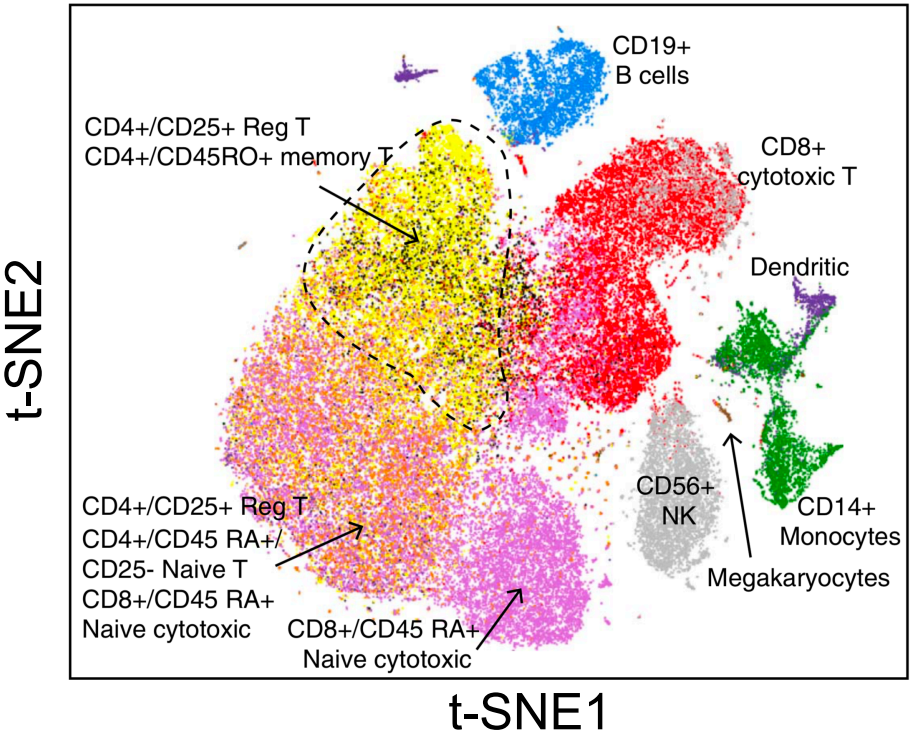


	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

Source: Amit (2016), *Nature Immunoloy.*

Gene Expression of Lymphoid Cells

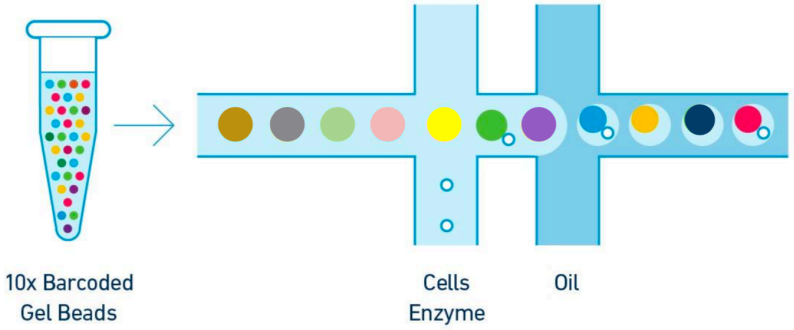
PBMCs from Humans

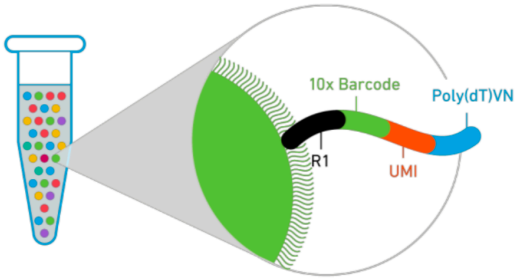


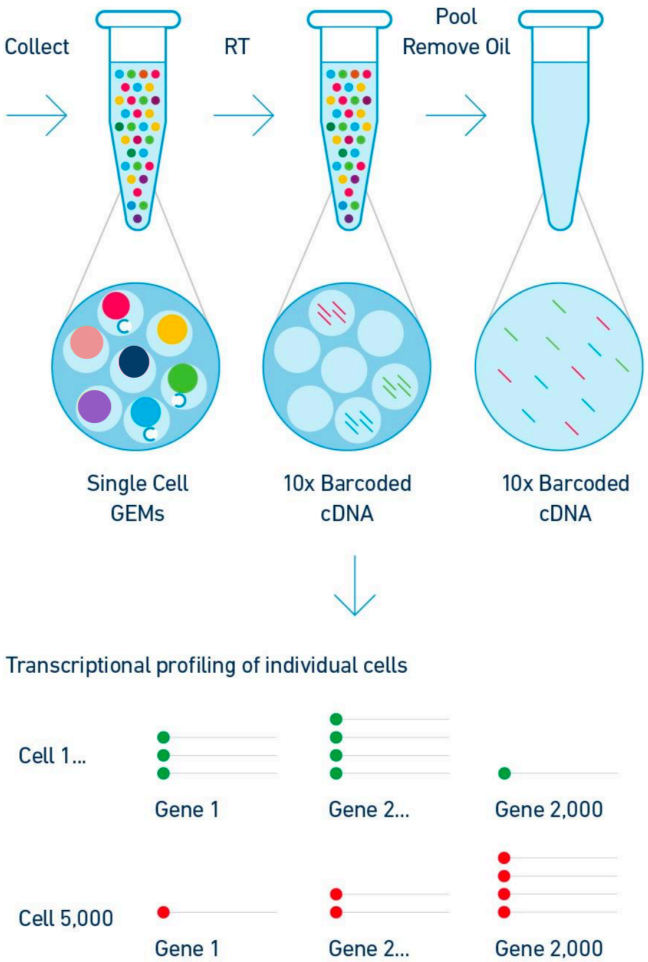
Single cell RNA-seq from 68k cells

Source: Zheng et al. 2017 & Buenrostro et al. 2018

Droplet based RNA single cell sequencing





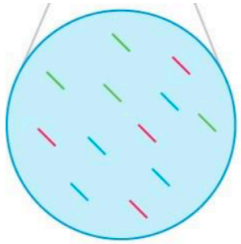


- Input: Single cells in suspension + 10x Gel Beads and Reagents
- Output: Digital gene expression profiles from every partitioned cell

Source: 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences



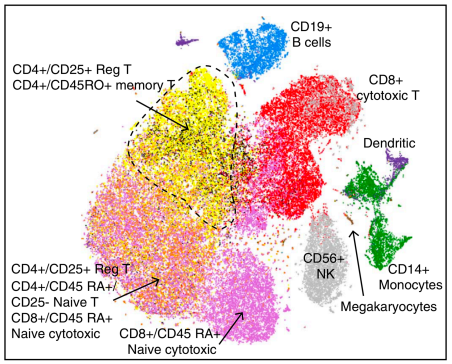
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering

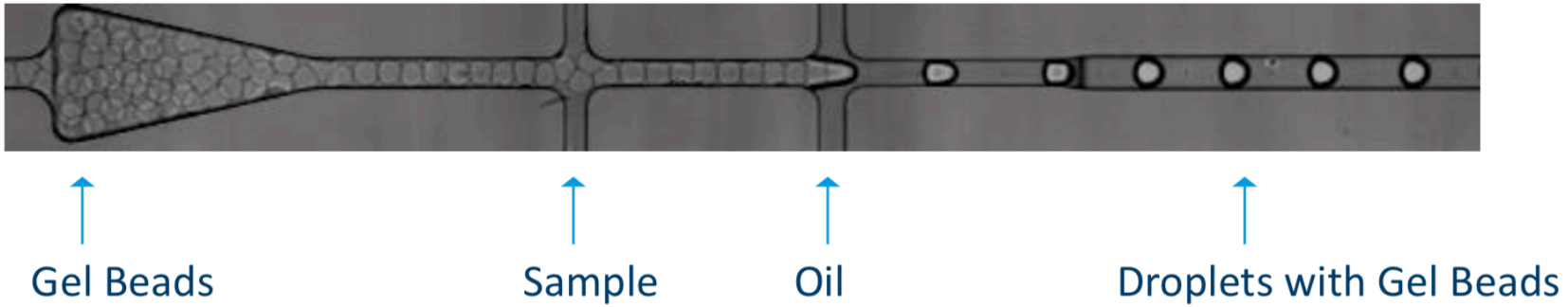


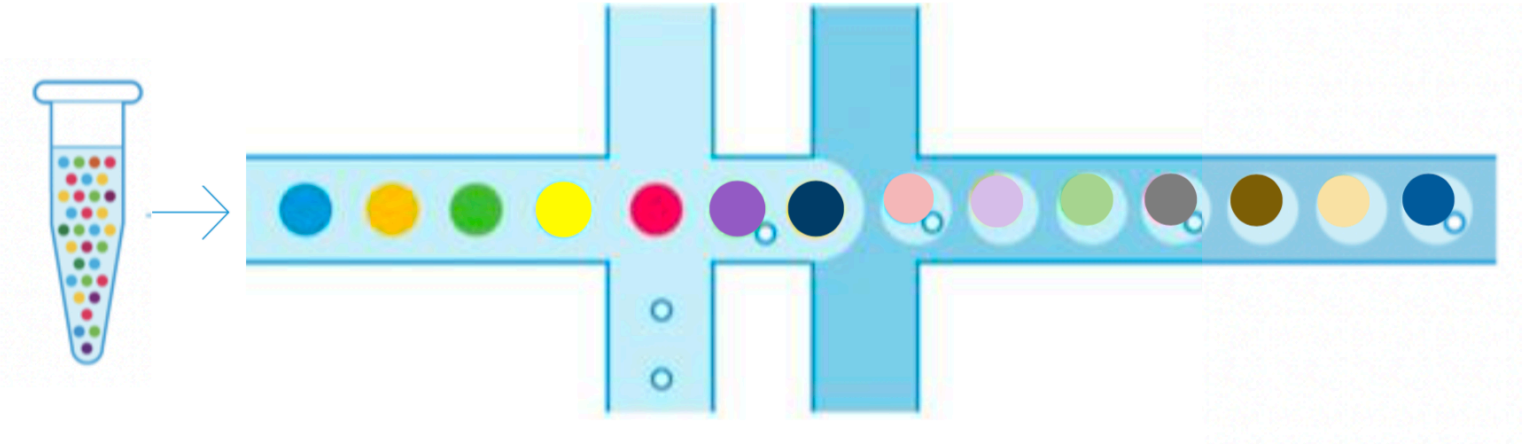
1. Alignment / Transcript count
2. Cell filtering
3. Removal of biological variation
4. Integration / Dimension reduction / cell clustering

cell ranger 10x genomics

Seurat -R

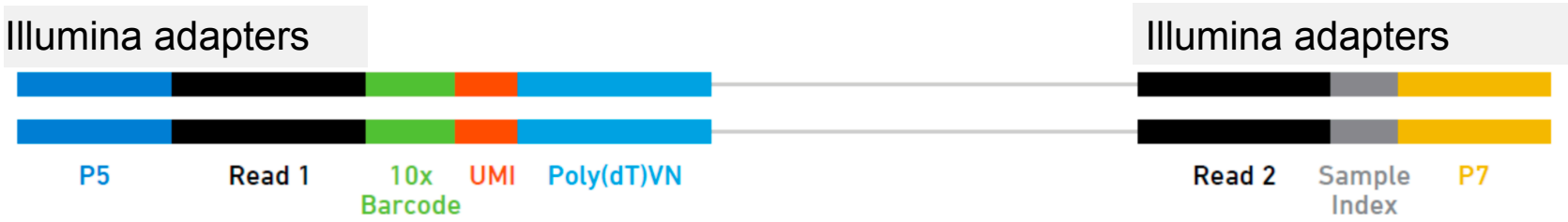
Droplet based RNA single cell sequencing





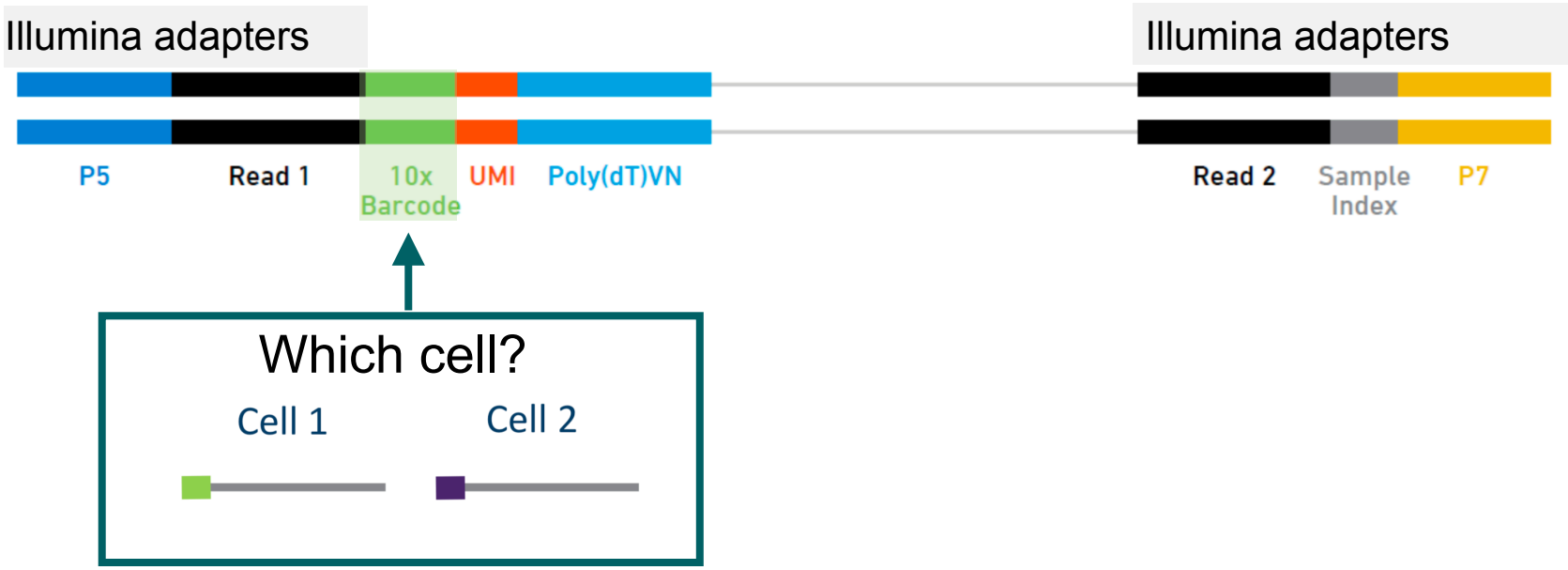
Source: 10x genomics

Basics Bioinformatics - Transcript Counts



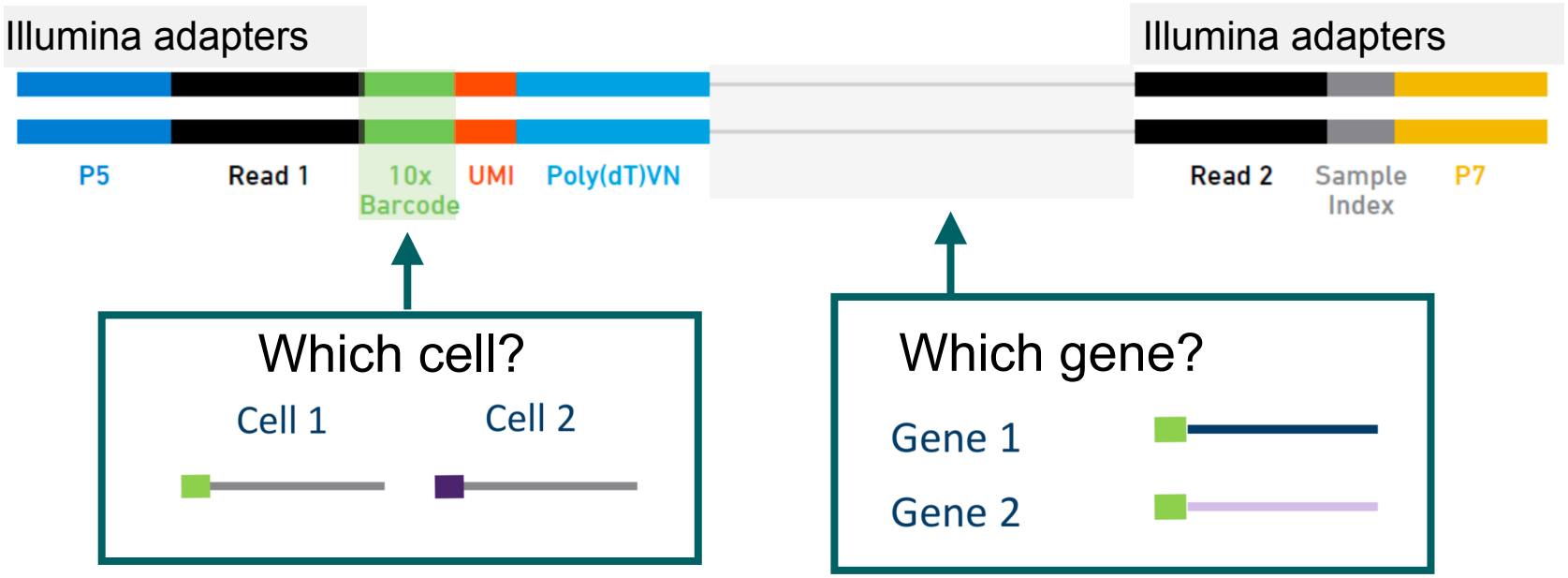
Source: 10x genomics

Basics Bioinformatics - Transcript Counts



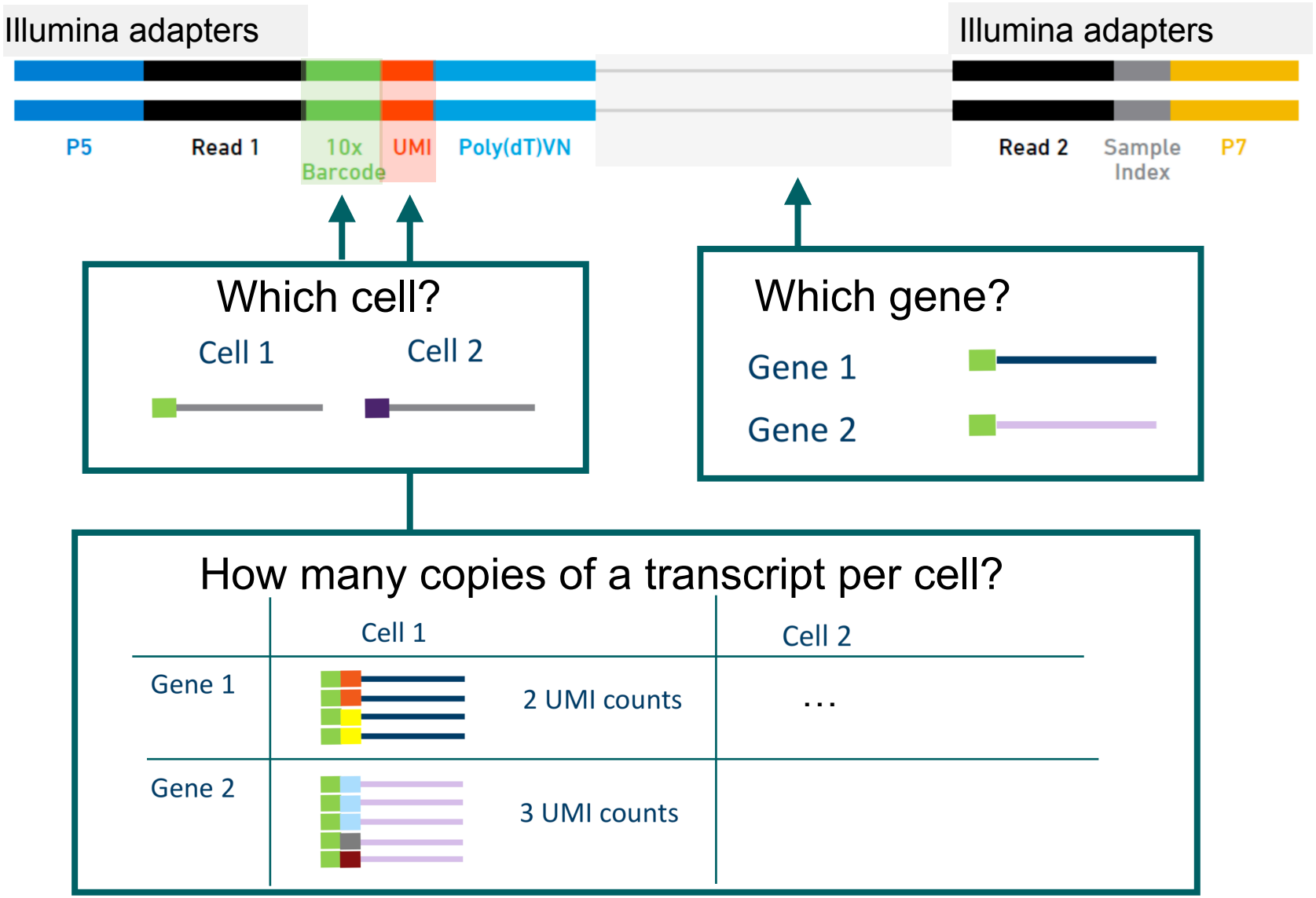
Source: 10x genomics

Basics Bioinformatics - Transcript Counts



Source: 10x genomics

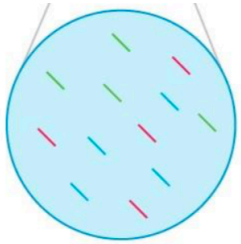
Basics Bioinformatics - Transcript Counts



Source: 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences



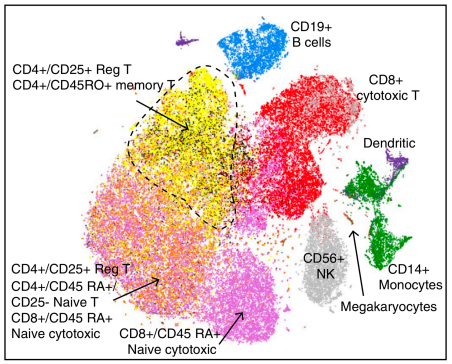
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering



1. Alignment / Transcript count

2. Cell filtering

3. Removal of biological variation

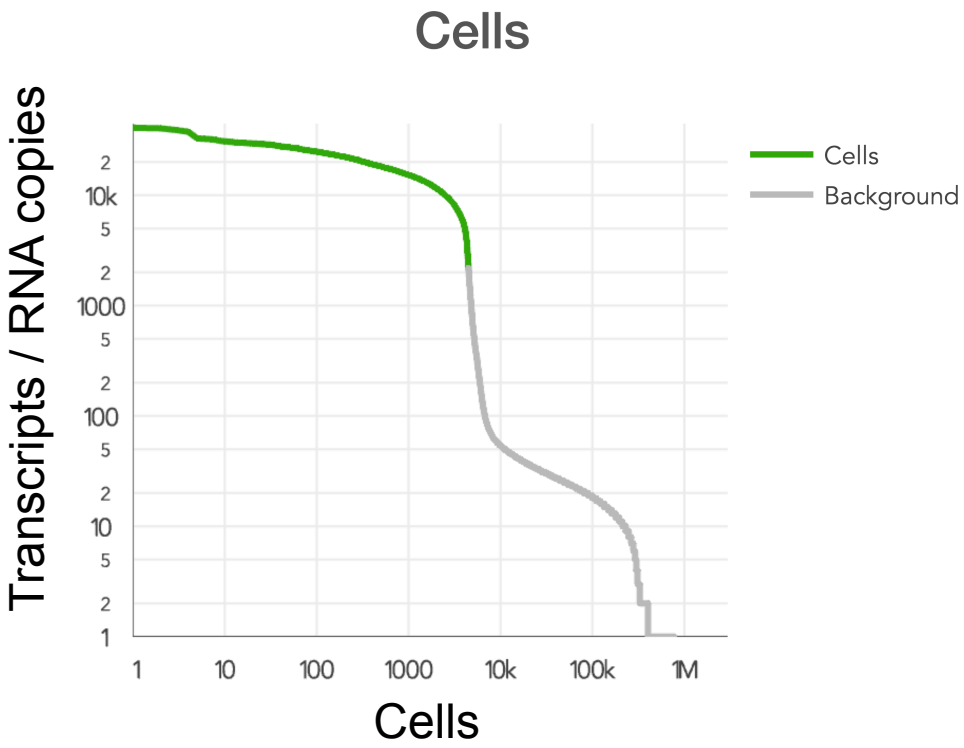
4. Integration / Dimension reduction / cell clustering

cell ranger 10x genomics

Seurat -R

Basics Bioinformatics - Cell Filtering

1. sum UMIs (copy of transcripts) per cell
2. consider cells with total UMI count > 99th of expected recovered cells



Estimated Number of Cells
4,495

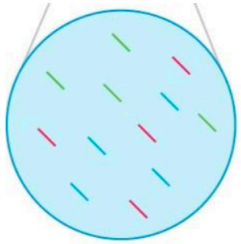
Post-Normalization Mean Reads per Cell
89,289

Median Genes per Cell
2,504

cell ranger - 10x genomics

Basics Bioinformatics - single cell RNA-seq

Sequences



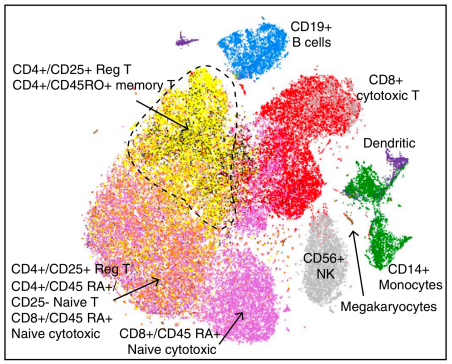
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering



1. Alignment / Transcript count

2. Cell filtering

3. Removal of biological variation

4. Integration / Dimension reduction / cell clustering

cell ranger 10x genomics

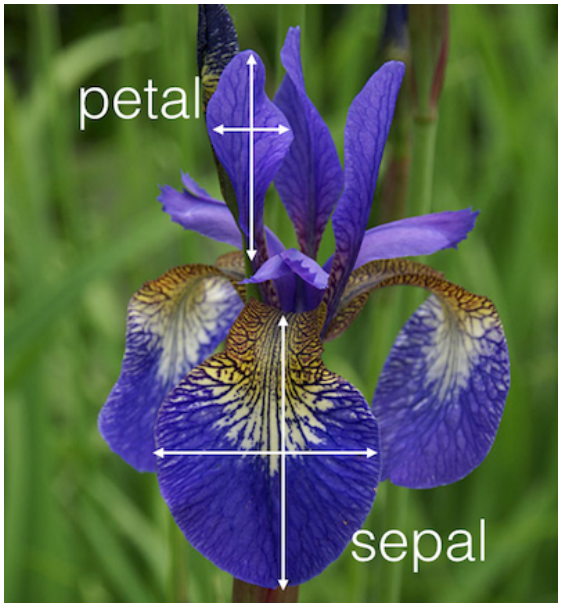
Seurat -R

High level analysis

Clustering & Dimension reduction

Clustering

- **Given a data description**
  - i.e. measurement of size of iris flowers
- **Find groups of similar observations**
  - i.e. iris flower sub-types

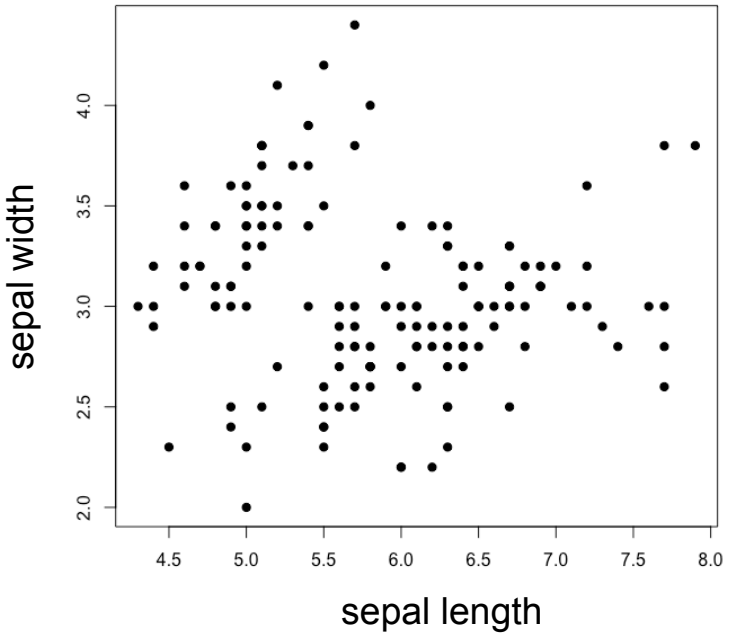


	Sepal Length	Sepal Width	Petal Length	Petal Width
Flower 1	5.1	3.5	1.4	0.2
Flower 2	4.9	3.0	1.4	0.2
Flower 3	4.7	3.2	1.3	0.2
Flower 4	4.6	3.1	1.5	0.2
…	…	…	…	…

Clustering

- **Given a data description**
  - i.e. measurement of size of iris flowers
- **Find groups of similar observations**
  - i.e. iris flower sub-types

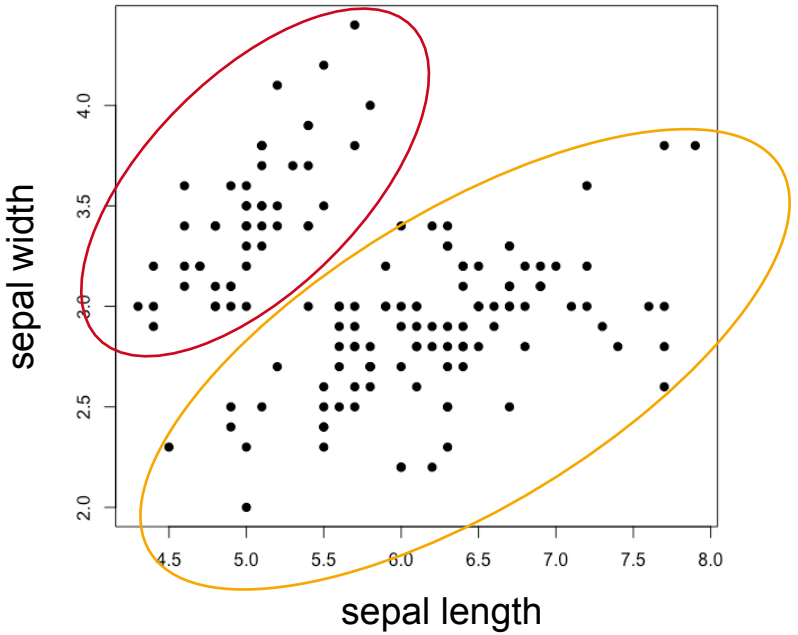
	Sepal Length	Sepal Width	Petal Length	Petal Width
Flower 1	5.1	3.5	1.4	0.2
Flower 2	4.9	3.0	1.4	0.2
Flower 3	4.7	3.2	1.3	0.2
Flower 4	4.6	3.1	1.5	0.2
…	…	…	…	…



Clustering

- **Given a data description**
  - i.e. measurement of size of iris flowers
- **Find groups of similar observations**
  - i.e. iris flower sub-types

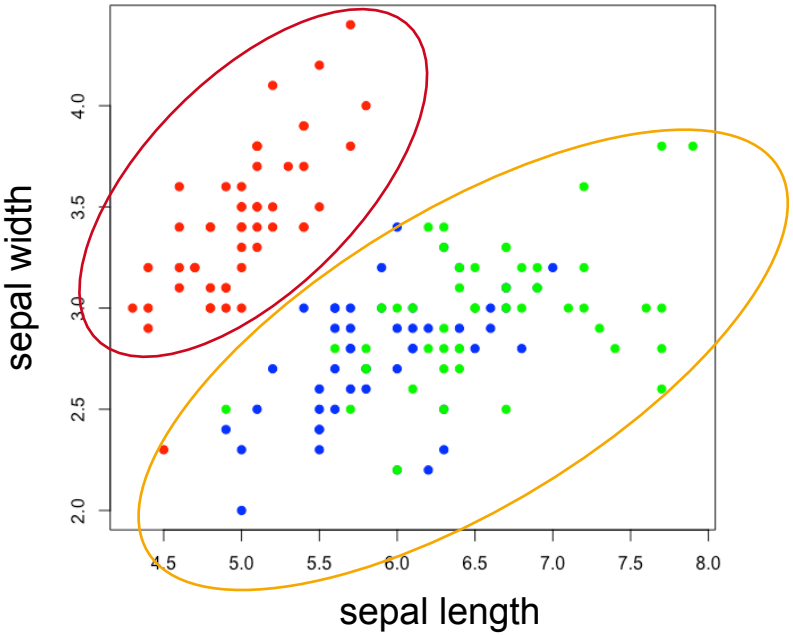
	Sepal Length	Sepal Width	Petal Length	Petal Width
Flower 1	5.1	3.5	1.4	0.2
Flower 2	4.9	3.0	1.4	0.2
Flower 3	4.7	3.2	1.3	0.2
Flower 4	4.6	3.1	1.5	0.2
…	…	…	…	…



Clustering

- **Given a data description**
  - i.e. measurement of size of iris flowers
- **Find groups of similar observations**
  - i.e. iris flower sub-types

Iris Setosa



Iris Virginia

Iris Versicolor

Clustering Formalism

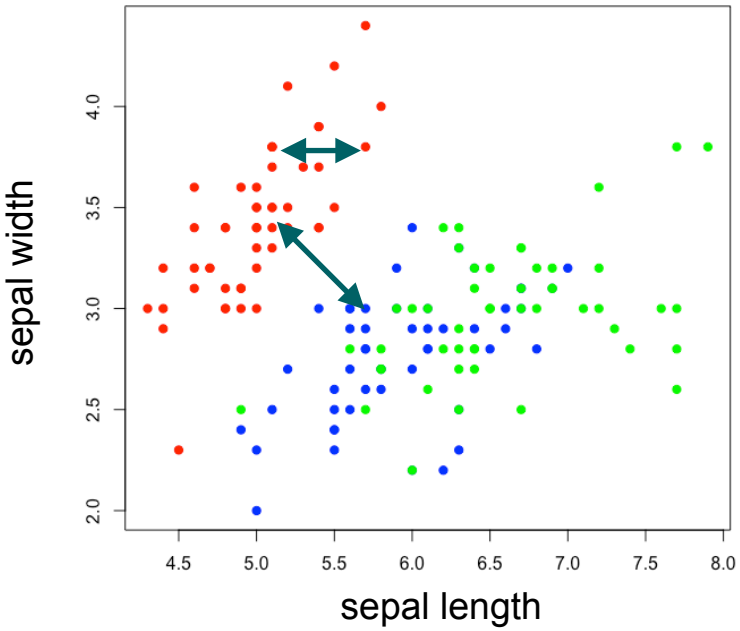
- **For a given data:**
  - Matrix X with N observations and L dimensions
    where x_i is a vector representing observation i

x_{11}	x_{12}	…	x_{1L}
x_{21}	x_{22}	…	x_{2L}
x_{31}	x_{32}	…	x_{3L}
…	…	…	…
x_{N1}	x_{N2}	…	x_{NL}

- **find groups of similar observations**
  - *vector* $Y = (y_1, \ldots, y_N)$
    where $y_i \in \{1, \ldots, K\}$ indicates the cluster of observation i

Distance

- **A important concept in clustering is a distance (similarity) between a pair of objects x_i and x_j**
  - Observations of a same group should be close in space

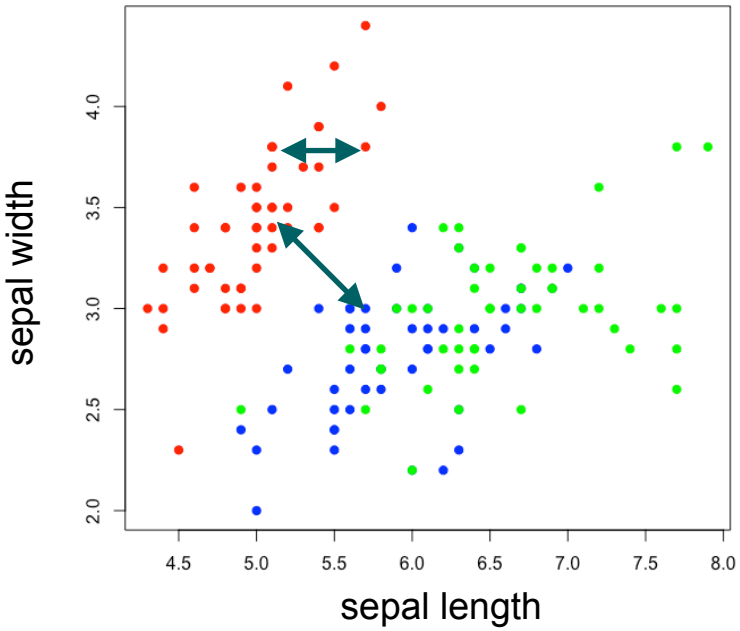


Euclidean distance
(sensitive to scale)

$$d(x_i, x_j) = \sqrt{\sum_{l=1}^{L} (x_{il} - x_{jl})^2}$$

Distance

- **A important concept in clustering is a distance (similarity) between a pair of objects x_i and x_j**
  - Observations of a same group should be close in space



Euclidean distance
(sensitive to scale)

$$d(x_i, x_j) = \sqrt{\sum_{l=1}^{L} (x_{il} - x_{jl})^2}$$

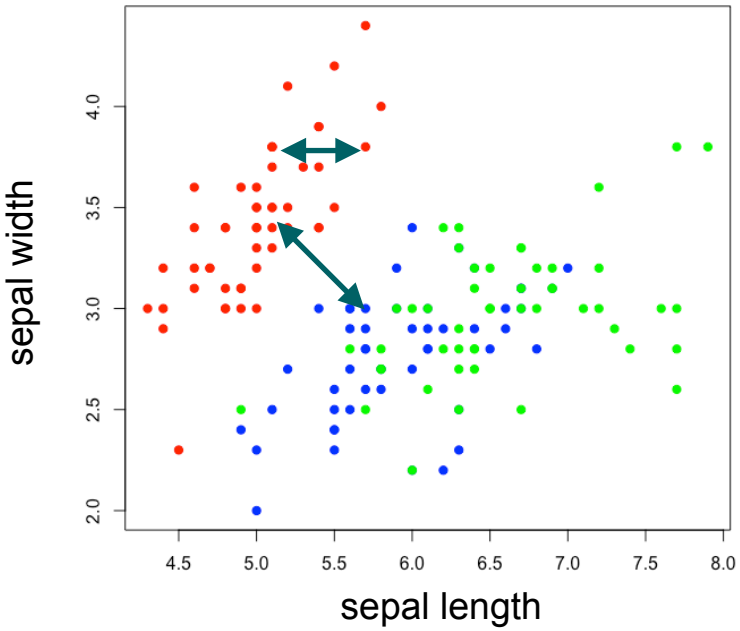
Pearson Correlation

(scale insensitive/ similarity)

$$d(x_i, x_j) = \frac{\sum_{l=1}^{L} (x_{il} - \bar{x}_i)(x_{jl} - \bar{x}_j)}{\sigma_i^2 \sigma_j^2}$$

Distance

- **A important concept in clustering is a distance (similarity) between a pair of objects x_i and x_j**
  - Observations of a same group should be close in space



Euclidean distance
(sensitive to scale)

$$d(x_i, x_j) = \sqrt{\sum_{l=1}^{L} (x_{il} - x_{jl})^2}$$

Pearson Correlation

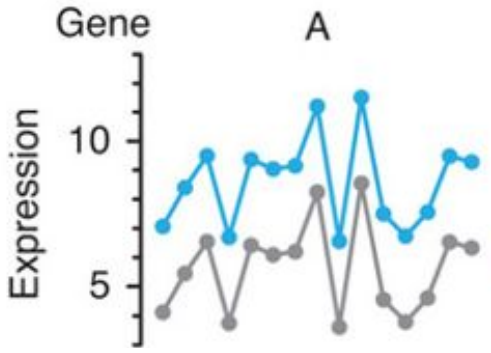
(scale insensitive/ similarity)

$$d(x_i, x_j) = \frac{\sum_{l=1}^{L} (x_{il} - \bar{x}_i)(x_{jl} - \bar{x}_j)}{\sigma_i^2 \sigma_j^2}$$

Distance and Scale

- **In some problems scale can be important!**
  - Similarly in changes are more important / not absolute values.

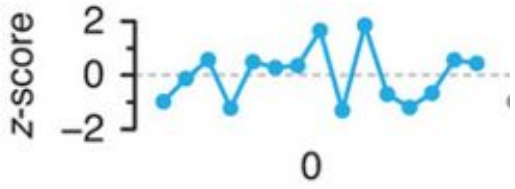
unscaled data



Euclidean - not similar

Correlation - similar

z-score normalised data



$$z = \frac{x_{ij} - \mu_i}{\sigma_i}$$

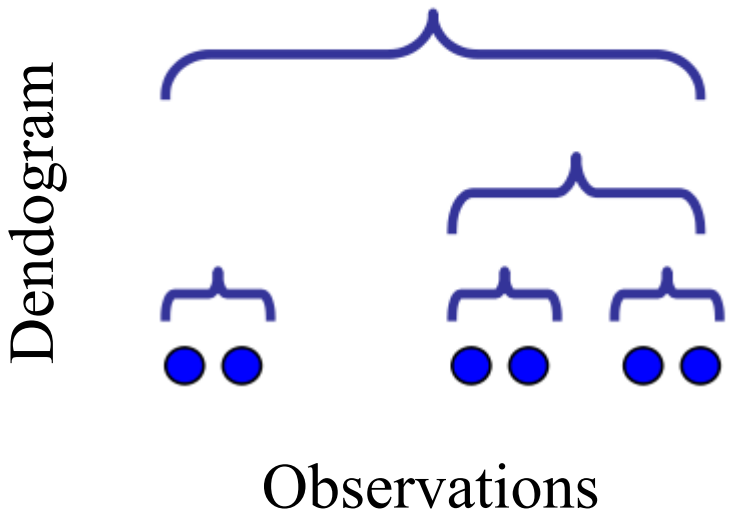
Euclidean - similar

Correlation - similar

Clustering Methods

- **Hierarchical methods**
  - Mostly bottom up
  - based on distance / simple to interpret
- **Partitional methods (k-means or mixture models)**
  - Mostly top down
  - Use models of groups, centroids
- **Graph based methods**
  - Use graph formalisms to represent data:
    - nodes are representations
    - edges weights represent distances
    - explore graph topologies

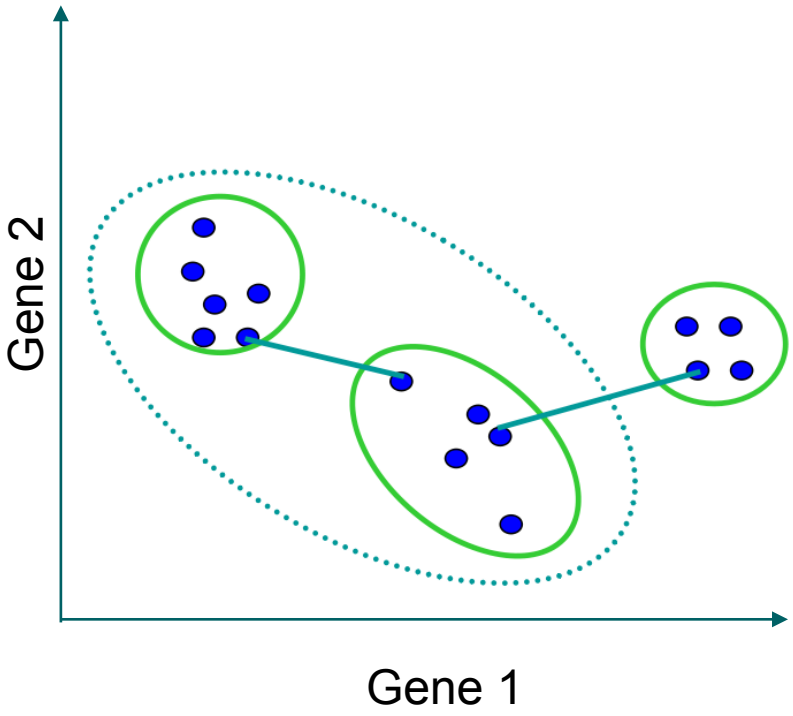
Hierarchical Clustering



- Botton up method
- Starting with a distance (similarity) matrix and each object as a group
- Repeat:
  - Joint two most similar groups
- Until the dendrogram has only one group

Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering



Single-Linkage

- Join two groups where two examples are close
- Find groups with linear shapes

Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

Distance Matrix

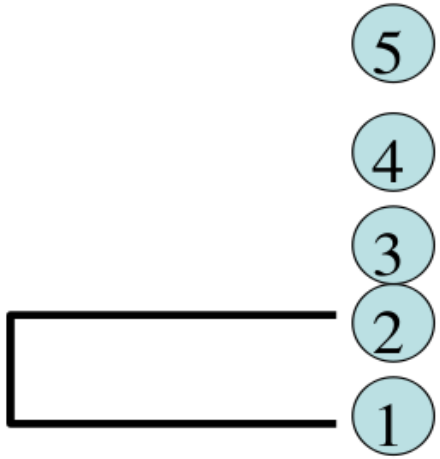
	1	2	3	4	5
1	0				
2	2	0			
3	6	3	0		
4	10	9	7	0	
5	9	8	5	4	0

Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

Distance Matrix

	1	2	3	4	5
1	0				
2	2	0			
3	6	3	0		
4	10	9	7	0	
5	9	8	5	4	0



Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

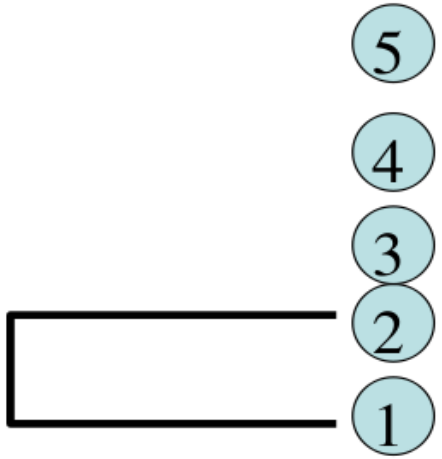
Distance Matrix

$$\begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array} \begin{array}{c} \begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & & \\ 2 & 0 & & & \\ 6 & 3 & 0 & & \\ 10 & 9 & 7 & 0 & \\ 9 & 8 & 5 & 4 & 0 \end{bmatrix} \end{array} \Rightarrow \begin{array}{c} \\ (1,2) \\ 3 \\ 4 \\ 5 \end{array} \begin{array}{c} \begin{array}{cccc} (1,2) & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & \\ 3 & 0 & & \\ 9 & 7 & 0 & \\ 8 & 5 & 4 & 0 \end{bmatrix} \end{array}$$

$$d_{(1,2),3} = \min\{d_{1,3}, d_{2,3}\} = \min\{6,3\} = 3$$

$$d_{(1,2),4} = \min\{d_{1,4}, d_{2,4}\} = \min\{10,9\} = 9$$

$$d_{(1,2),5} = \min\{d_{1,5}, d_{2,5}\} = \min\{9,8\} = 8$$



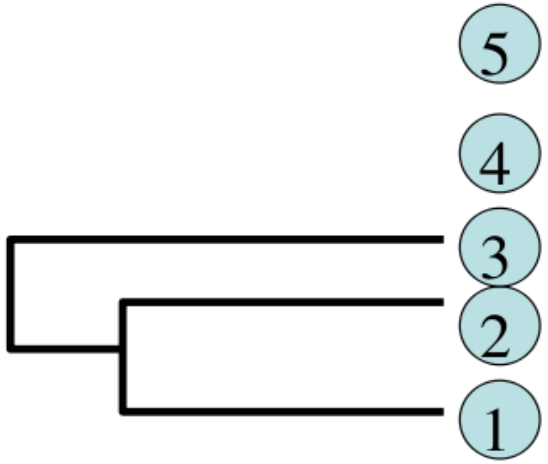
Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

$$\begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array}\begin{array}{c} \begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & & \\ 2 & 0 & & & \\ 6 & 3 & 0 & & \\ 10 & 9 & 7 & 0 & \\ 9 & 8 & 5 & 4 & 0 \end{bmatrix} \end{array} \Rightarrow \begin{array}{c} \\ (1,2) \\ 3 \\ 4 \\ 5 \end{array}\begin{array}{c} \begin{array}{ccccc} (1,2) & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & & \\ 3 & 0 & & \\ 9 & 7 & 0 & \\ 8 & 5 & 4 & 0 \end{bmatrix} \end{array} \Rightarrow \begin{array}{c} \\ (1,2,3) \\ 4 \\ 5 \end{array}\begin{array}{c} \begin{array}{ccc} (1,2,3) & 4 & 5 \end{array} \\ \begin{bmatrix} 0 & & \\ 7 & 0 & \\ 5 & 4 & 0 \end{bmatrix} \end{array}$$

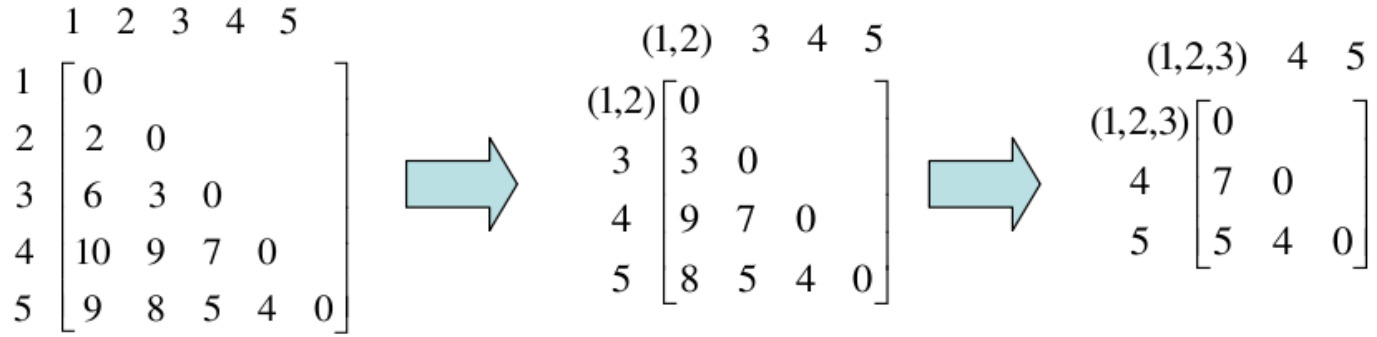
$$d_{(1,2,3),4} = \min\{d_{(1,2),4}, d_{3,4}\} = \min\{9,7\} = 7$$

$$d_{(1,2,3),5} = \min\{d_{(1,2),5}, d_{3,5}\} = \min\{8,5\} = 5$$

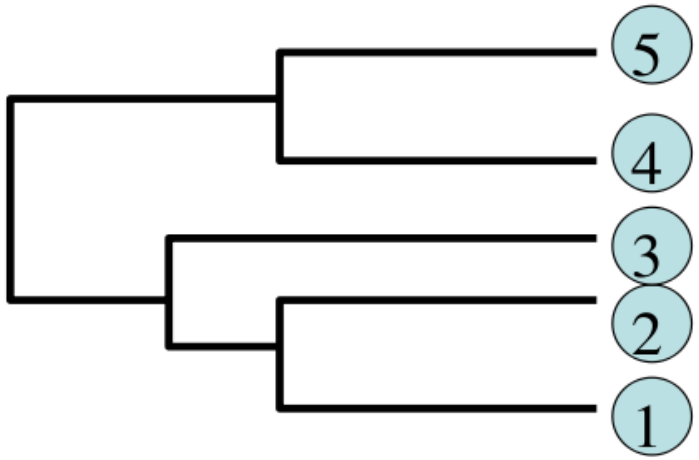


Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering

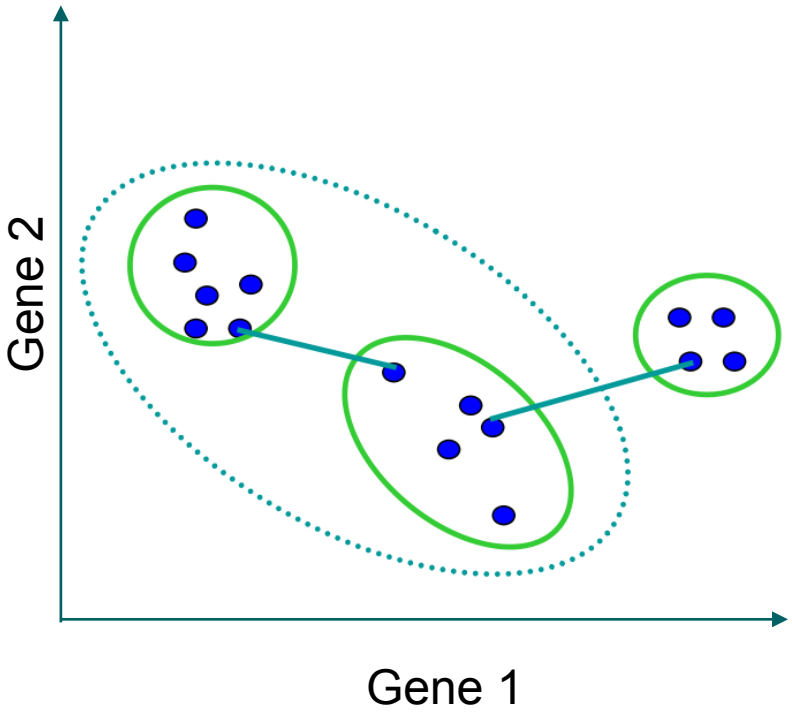


$$d_{(1,2,3),(4,5)} = \min\{d_{(1,2,3),4}, d_{(1,2,3),5}\} = 5$$



Source: Slides adapted from Ziv-Bar Joseph

Hierarchical Clustering



Single-Linkage

- Groups with closest genes
- linear shapes

Complete-Linkage

- Closest groups with more far genes
- Compact clusters

Average Linkage

- Groups with closest centroids (middle)
- Outlier robust

Source: Slides adapted from Ziv-Bar Joseph

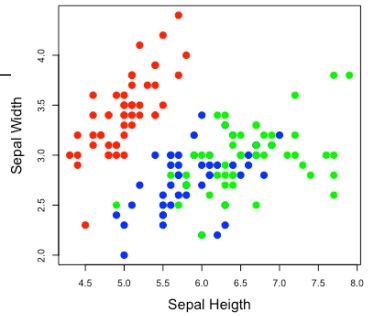
Hierarchical Clustering

Which linkage?

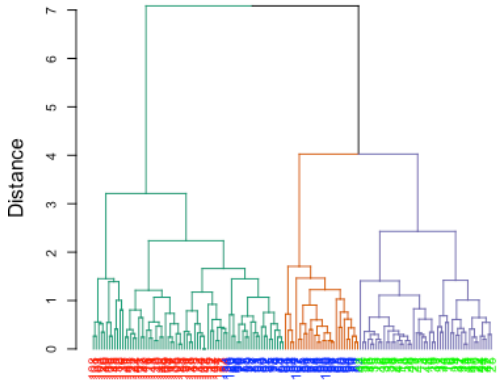
Which distance?

Hierarchical Clustering of Iris

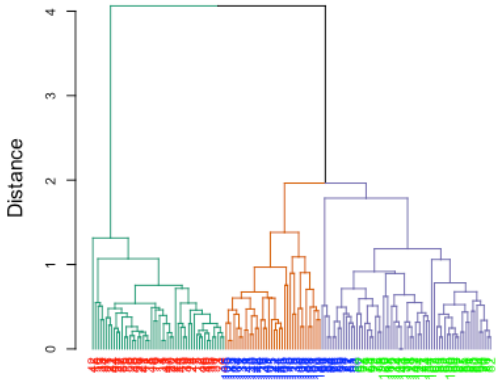
True labels



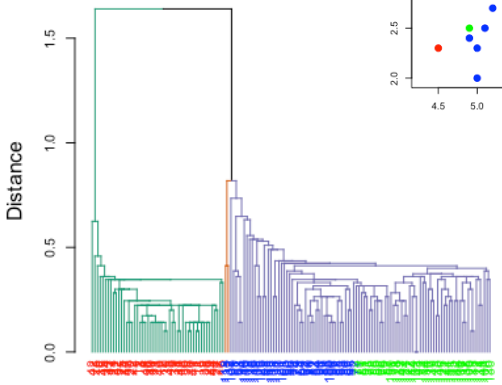
Euclidean distance



Complete



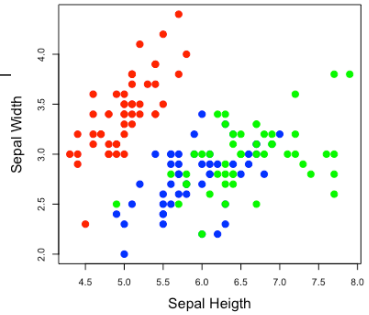
Average



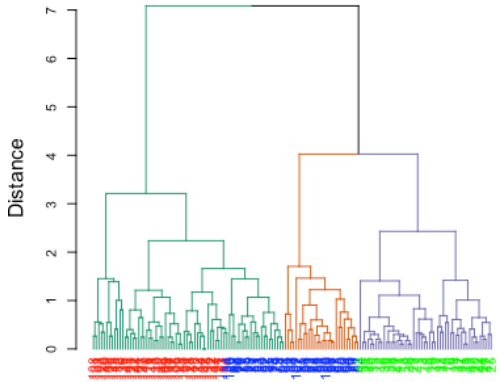
Single

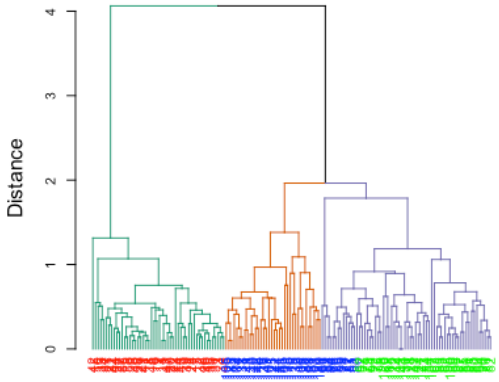
Hierarchical Clustering of Iris

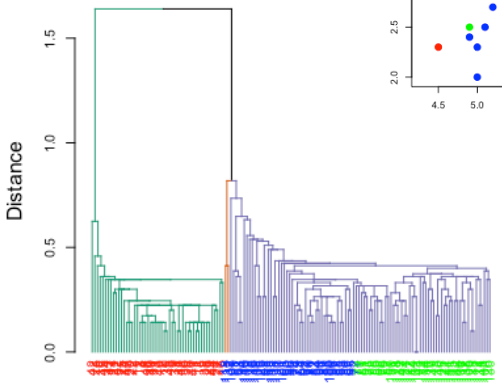
True labels



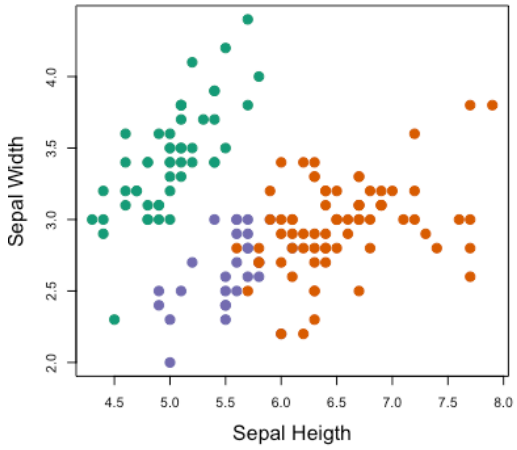
Euclidean distance



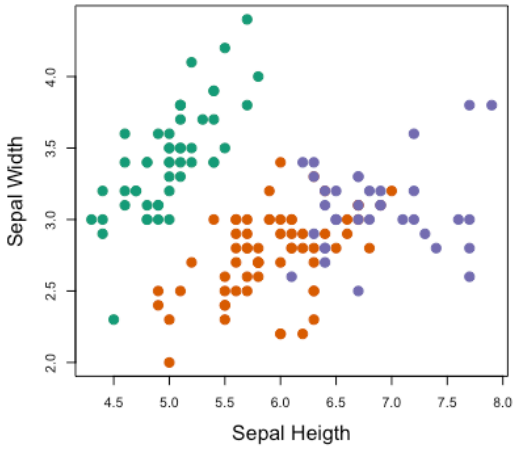




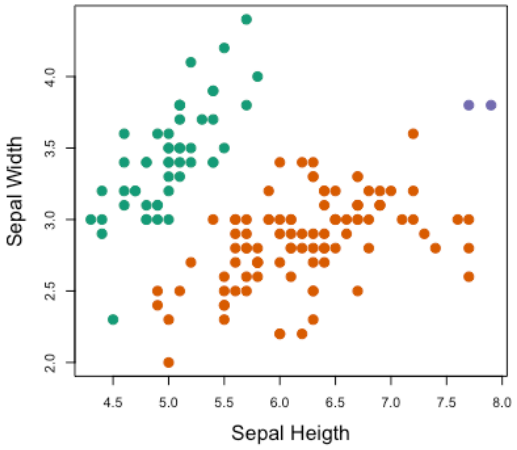
Complete



Average



Single

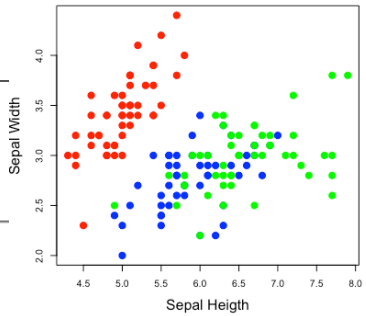


Institute for Computational Genomics

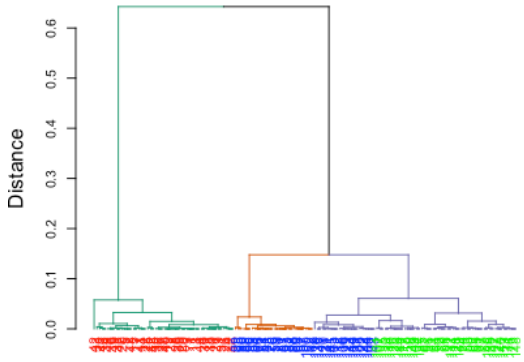
RWTH AACHEN UNIVERSITY

Hierarchical Clustering of Iris

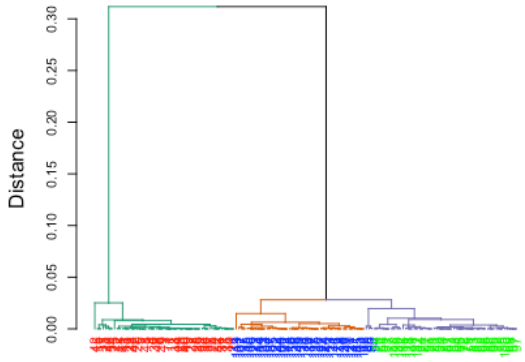
True labels



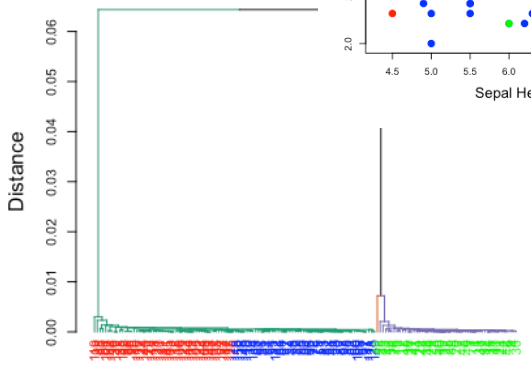
Pearson distance



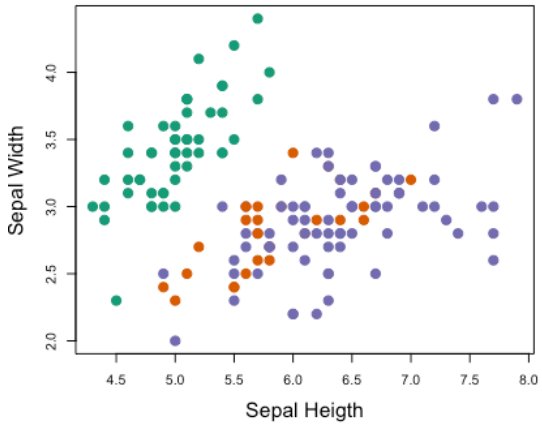
Complete

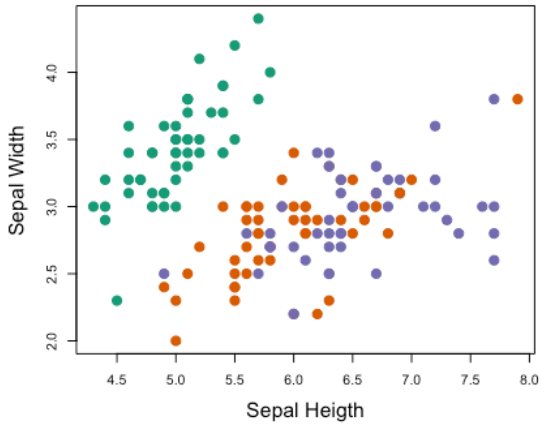


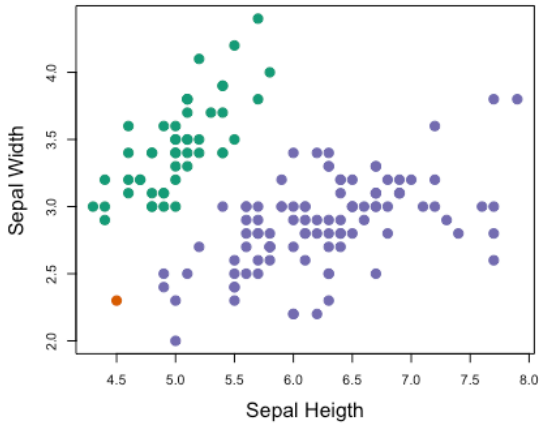
Average



Single







- Hierarchical cluster is sensitive to noise/outliers
- High computational cost O(n^2)

Institute for Computational Genomics

RWTH AACHEN UNIVERSITY

K-means

Iterative algorithm using **centroids** as cluster representations

Requires specification of number of clusters (***K***)

Algorithm:

Start cluster (Y) randomly

Repeat for a number of iterations

- estimate centroid (m_k) for each cluster

$$m_k = \frac{\sum_{i=1}^{N} 1(y_i = k) x_i}{\sum_{i=1}^{N} 1(y_i = k)}$$

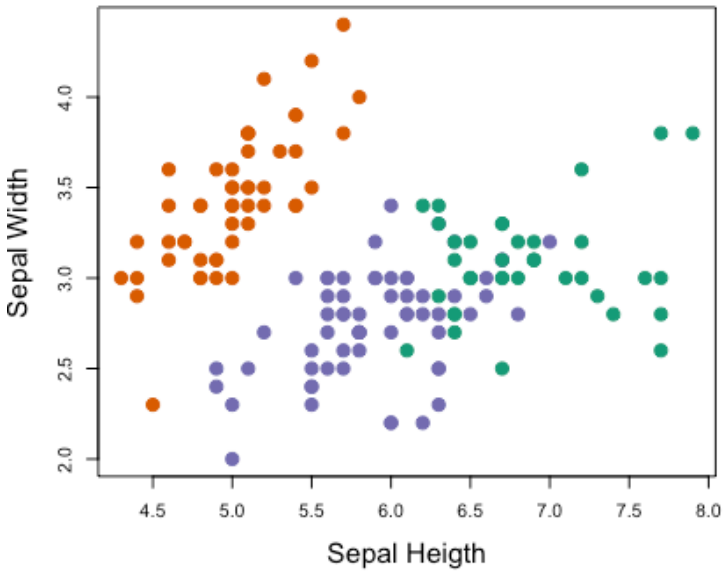
- Assign objects to closest centroid:

$$y_i = \text{argmin}_k \, d(x_i, m_k)$$

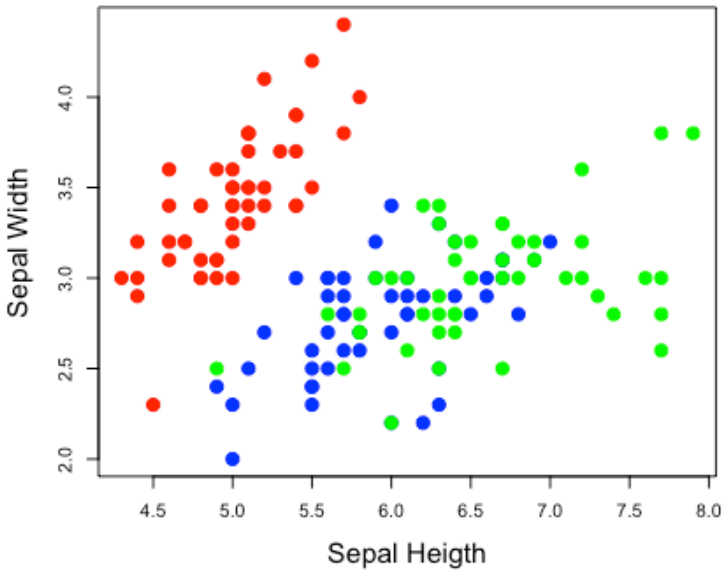
* convergence is only guaranteed for Euclidean distance

K-means on Iris

K-means solutions



True labels



- K-means tends to find spherical clusters
- Sensitive to initialisation

Resume / Clustering Methods

- K-means and hierarchical clustering

  - standard algorithms with standard performance on simple clustering problems

- State-of-art methods explore characteristics of the data (images, genomic data, text) at hand as type of features, dimensionality)

- Further issues:
  - Validation:
    - How many clusters is present in the data?
    - Which is the best method?
  - Data dimensionality:
    - distances do not work well on high dimension
    - visualisation is easier in low level space

More details on clustering

- Hastie, Tibshirani and Friedman, The Elements of Statistical Learning, Chapter 14
- Bishop, Pattern Recognition and Machine Learning, Chapter 9

Cluster Validation

- How to evaluate clustering results? Which is the best method? How many clusters?
- Internal/relative validation:
  - Measure of cluster coherence:
    - Distance within a cluster -> small (compactness)
    - Distance between clusters -> high (separation)
  - Stability measures:
    - Cluster data in part of the data and compare results
- External validation:
  - Compare clusters with class labels (iris data)
    - Not possible in real word problems!

Silhouette - Internal Index

The silhouette for a given object *i* is defined as:

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

where

a(i) –mean distance of *i* to objects on same cluster **(compactness)**

d(i,k) – mean distance of *i* to objects of cluster *k* (not own)

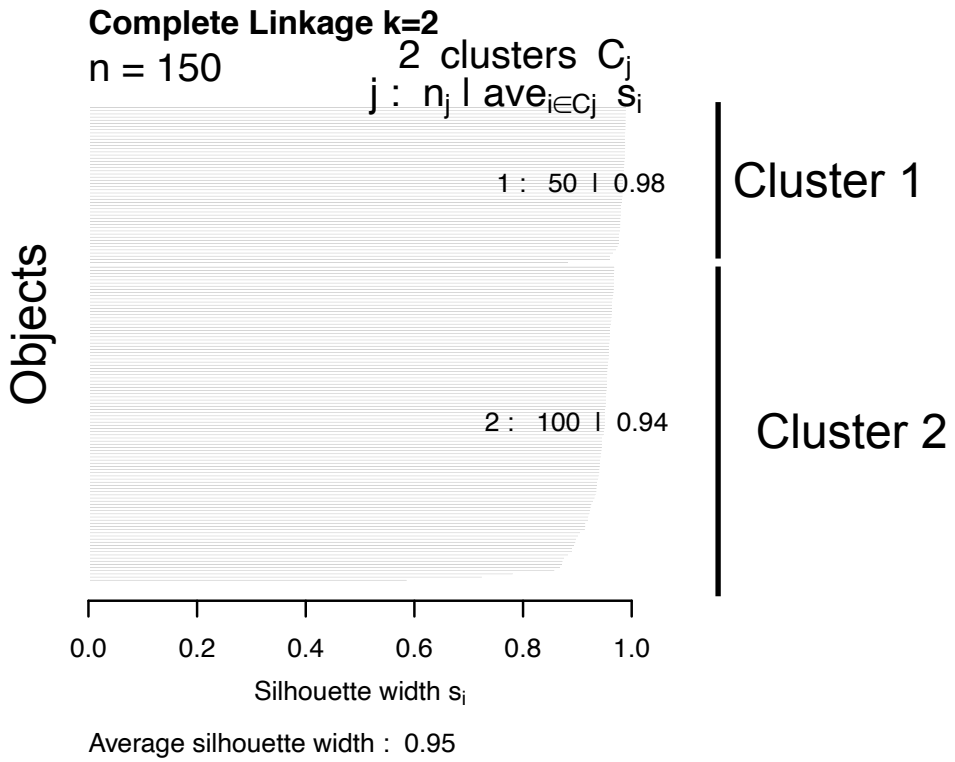
b(i) = min_k *(d(i,k))* **(separation)**

Average of *s(i)* -> quality of all results or clusters

Value of 1 indicate perfect solutions!

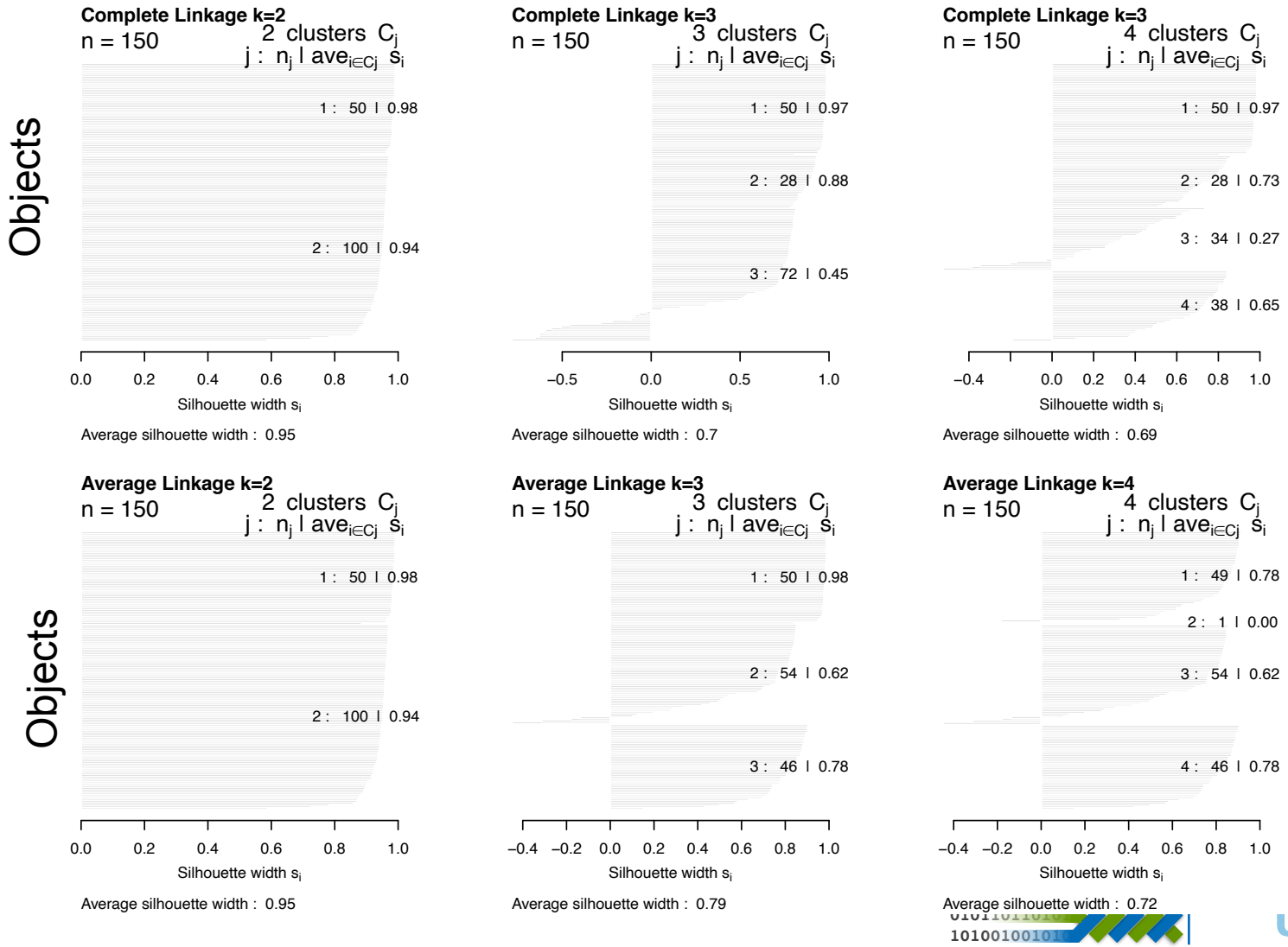
Silhouette - Internal Index / Iris

- silhouette values for hierarchical clustering with Pearson



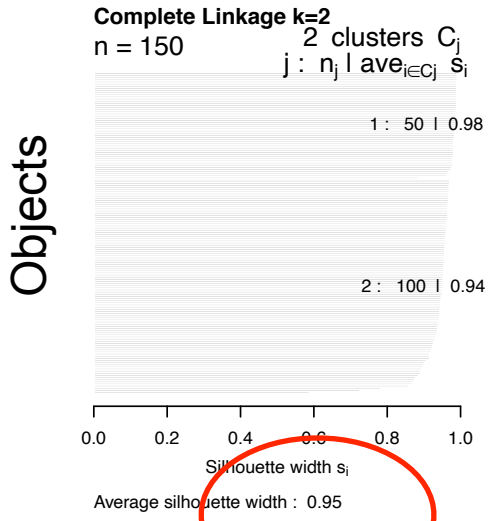
Silhouette - Internal Index / Iris

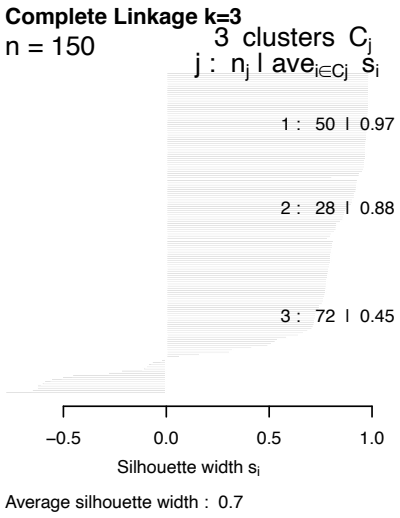
- silhouette values for hierarchical clustering with Pearson

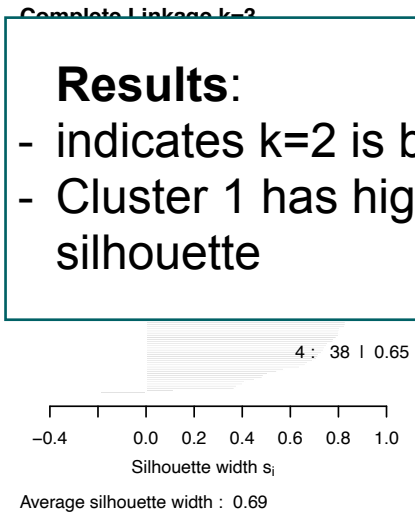


Silhouette - Internal Index / Iris

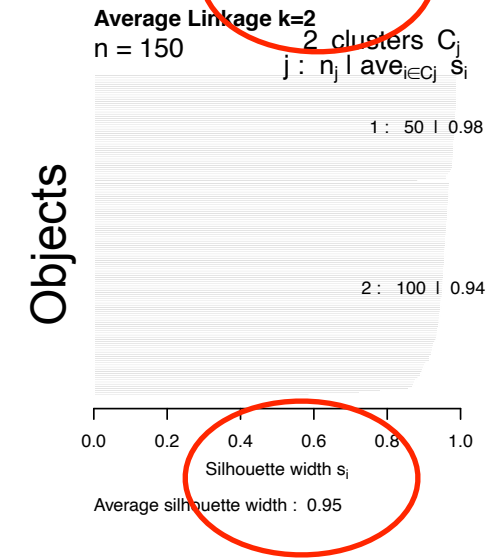
- silhouette values for hierarchical clustering with Pearson

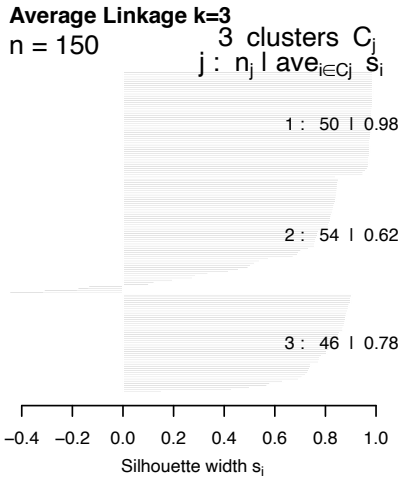


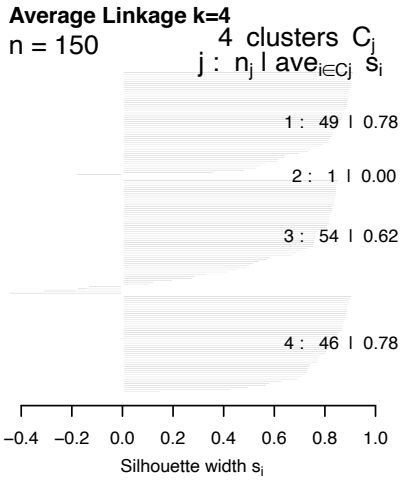




Results:
- indicates k=2 is best
- Cluster 1 has high silhouette



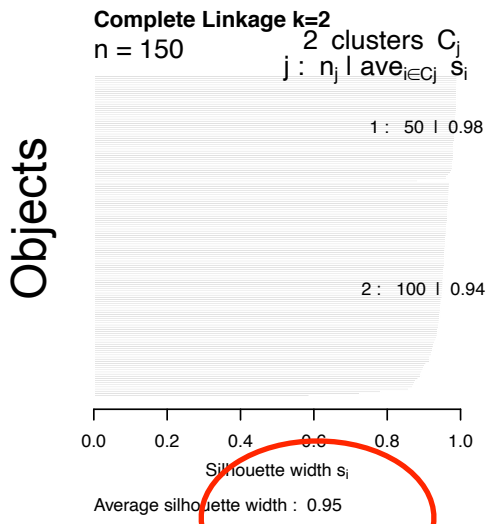


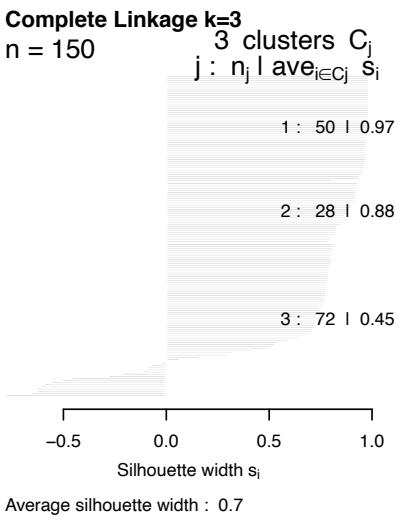


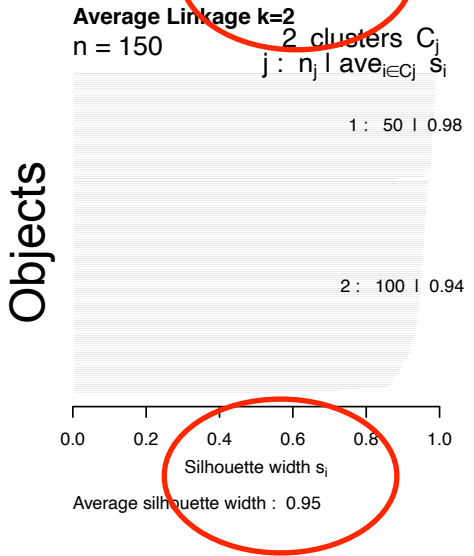
RWTH AACHEN UNIVERSITY

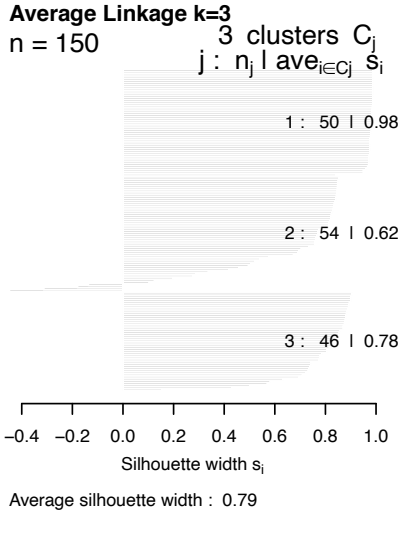
Silhouette - Internal Index / Iris

- silhouette values for hierarchical clustering with Pearson

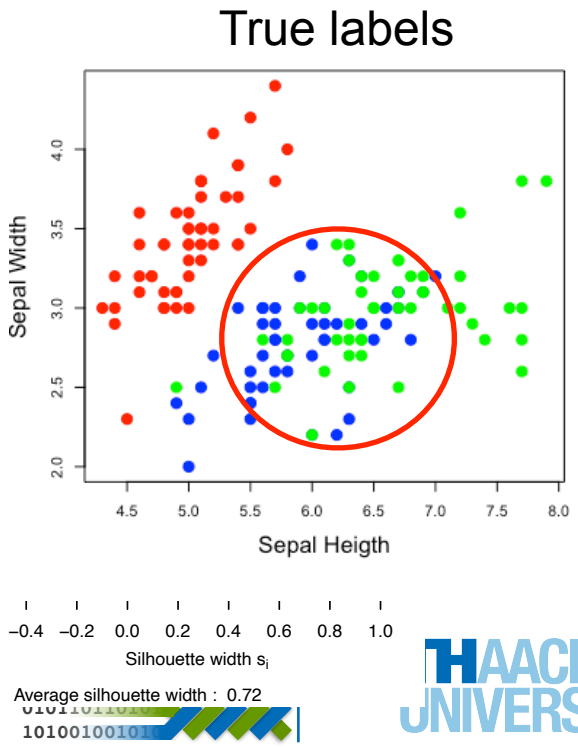








Results:
- indicates k=2 is best
- Cluster 1 has high silhouette



Gap statistic - Internal Index

For a given solution with *K clusters*

$$W_K = \sum_{k=1}^{K} \sum_{y_i=k} \sum_{y_j=k} ||x_i - x_j||^2$$

W_K - measures cluster compactness
W_K - tends to 0 for increasing K

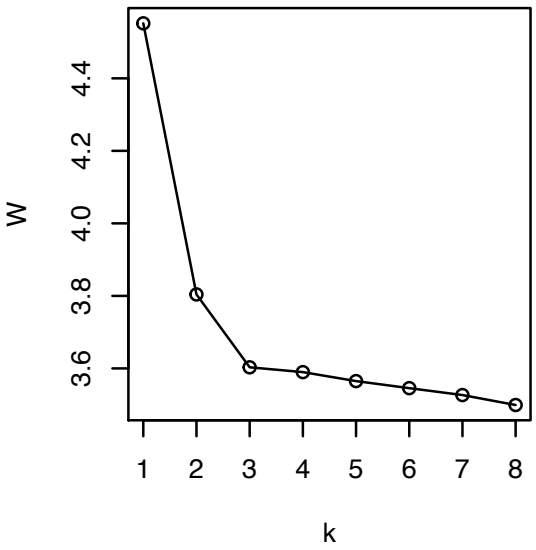
The Gap Statistic consider clustering of random data *W**

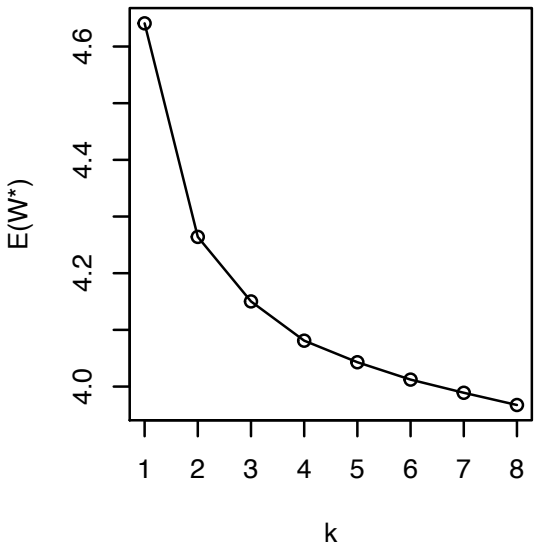
$$GAP(k) = E_r[logW_K^*] - logW_K$$

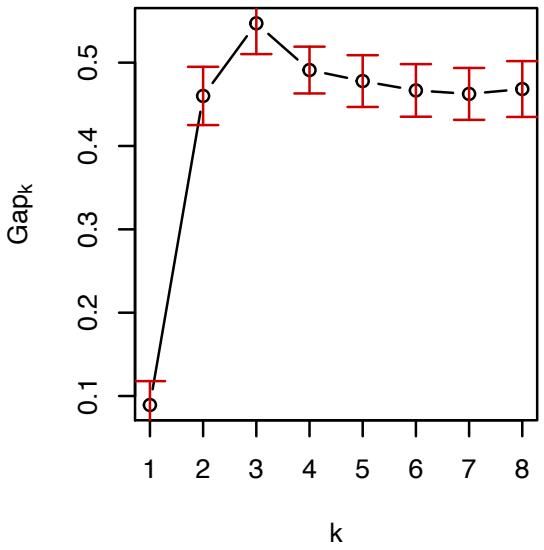
where *W** estimated from clustering random points at the same data space of *X*

Gap statistic - Iris

- GAP statistics for Iris / Average Linkage with Pearson



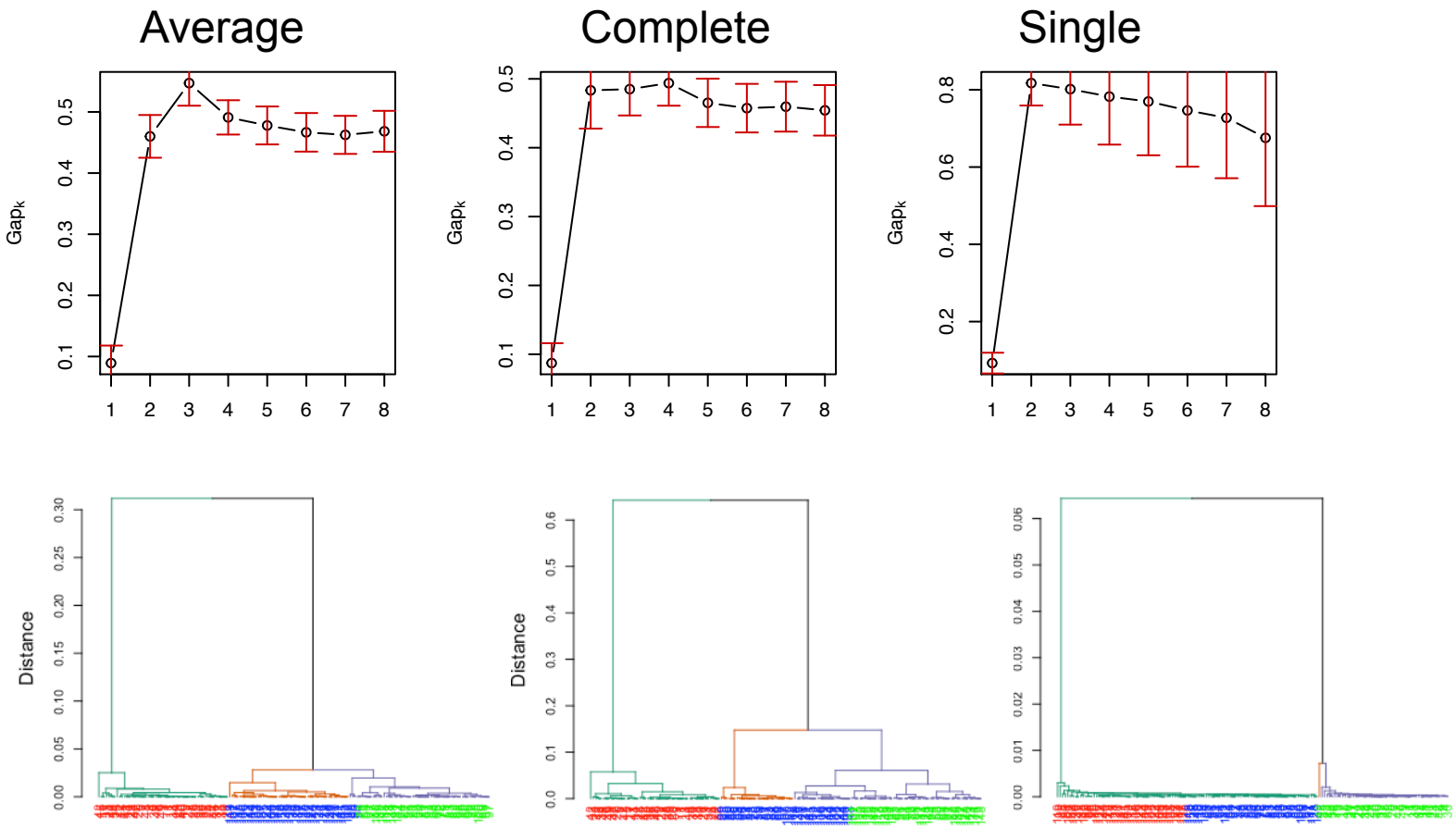




3 clusters has highest Gap !!!

Gap statistic - Iris

- GAP statistics for distinct linkage methods



Resume / Validation

- Help detection of number of clusters / real clusters
  - Do not work perfectly!
- GAP statistics is widely used
  - Requires *r* data randomisations
    - high computational costs
    - random datasets uniformly distributed (unreal assumption)
- Expert interpretation is important!

Dimension Reduction

- Distances lose meaning at high dimensional space (curse of dimensionality)

- Unspecific Filtering (without class labels):
  - Keep variables with highest variance
    - ***rational****: im*portant features change values across groups

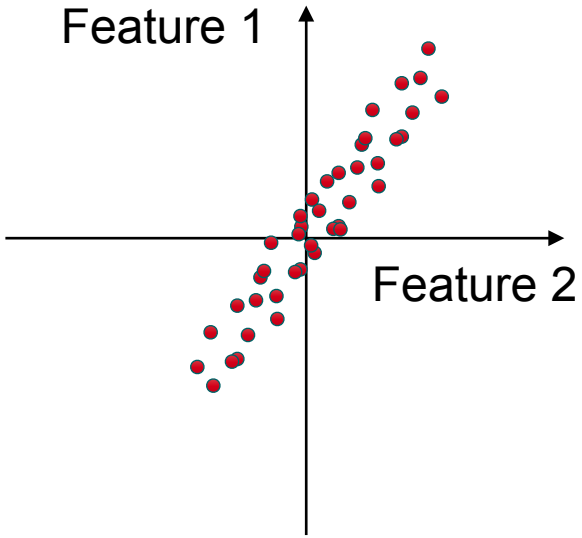
- Dimensionality Reduction by Transformation:
  - linear: principal component analysis (PCA)
  - Non-linear / manifold learning: t-SNE & UMAP

Principal Component Analysis

- For a data *X*, find linear combination of features (*w)* capturing most of data variance

$$\mathbf{w}_{(1)} = \arg\max_{\|\mathbf{w}\|=1} \{\|\mathbf{X}\mathbf{w}\|^2\}$$

- Can be solved by linear algebra / eigen vector decomposition



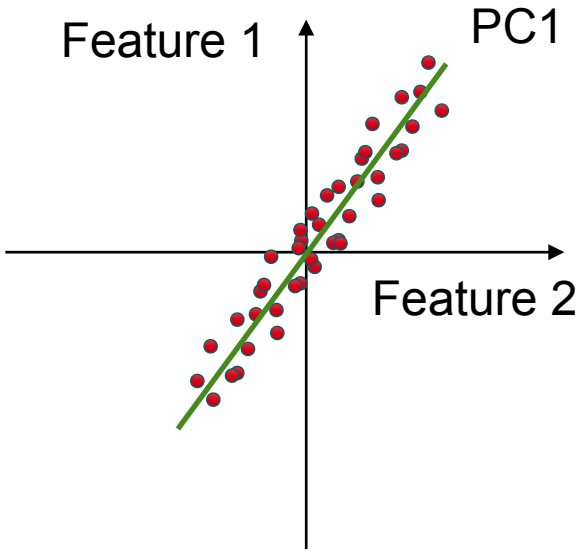
Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

Principal Component Analysis

- For a data *X*, find linear combination of features (*w)* capturing most of data variance

$$\mathbf{w}_{(1)} = \arg\max_{\|\mathbf{w}\|=1} \{\|\mathbf{X}\mathbf{w}\|^2\}$$

- Can be solved by linear algebra / eigen vector decomposition



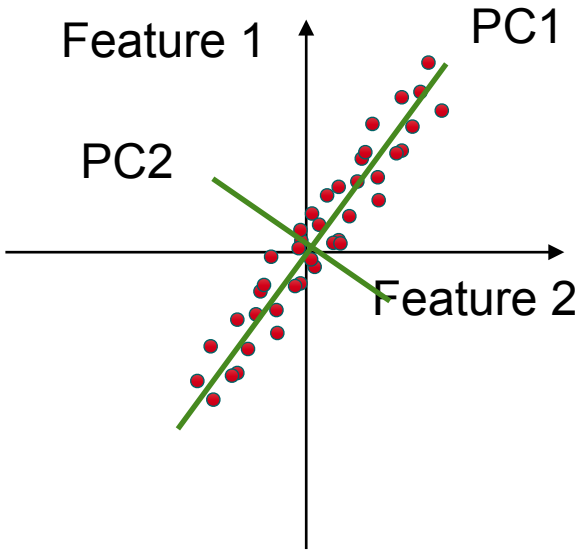
Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

Principal Component Analysis

- For a data *X*, find linear combination of features (*w)* capturing most of data variance

$$\mathbf{w}_{(1)} = \arg\max_{\|\mathbf{w}\|=1} \{\|\mathbf{X}\mathbf{w}\|^2\}$$

- Can be solved by linear algebra / eigen vector decomposition



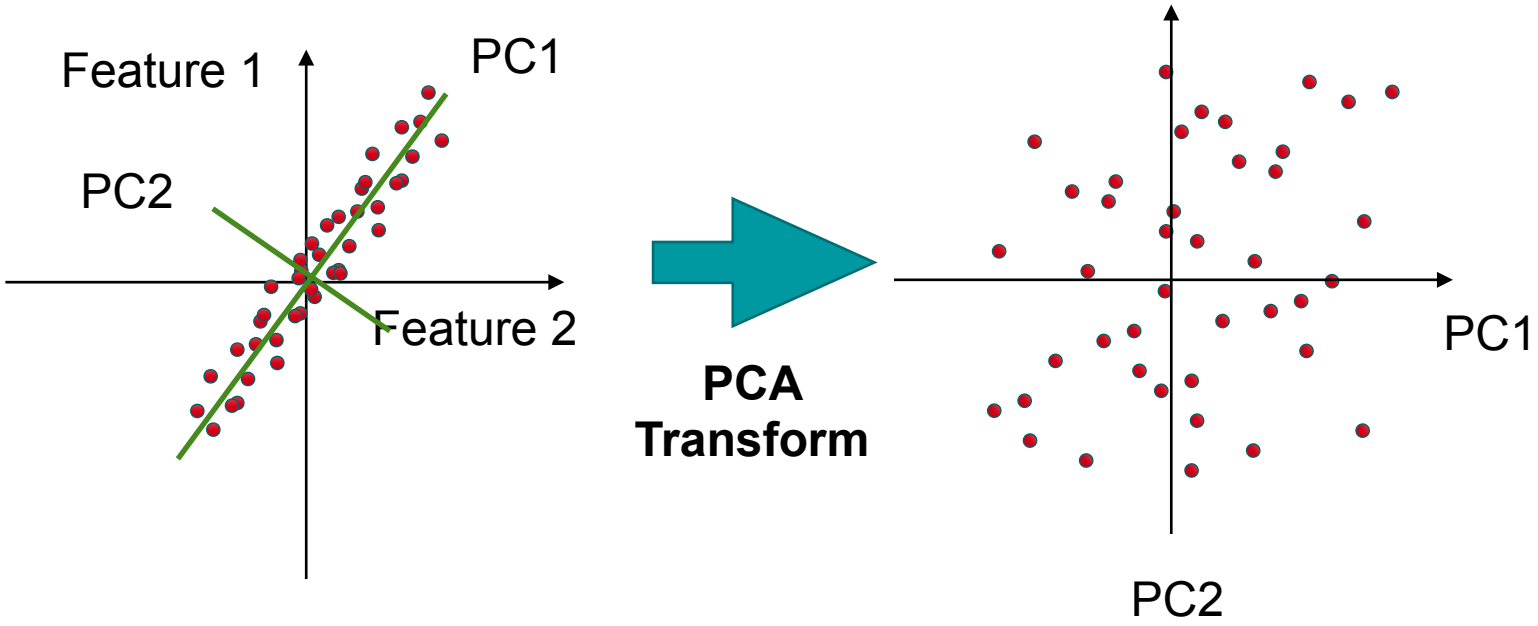
Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

Principal Component Analysis

- For a data *X*, find linear combination of features (*w)* capturing most of data variance

$$\mathbf{w}_{(1)} = \arg\max_{\|\mathbf{w}\|=1} \{\|\mathbf{X}\mathbf{w}\|^2\}$$

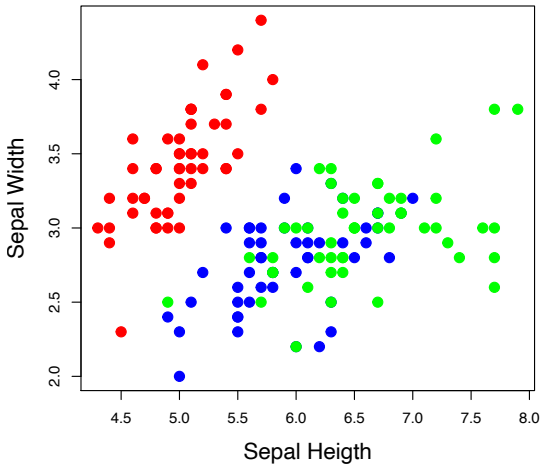
- Can be solved by linear algebra / eigen vector decomposition

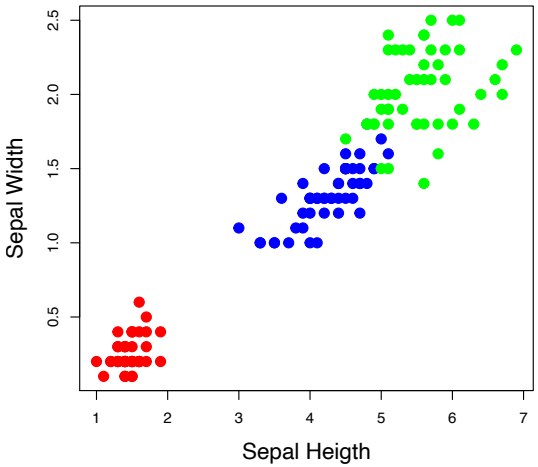


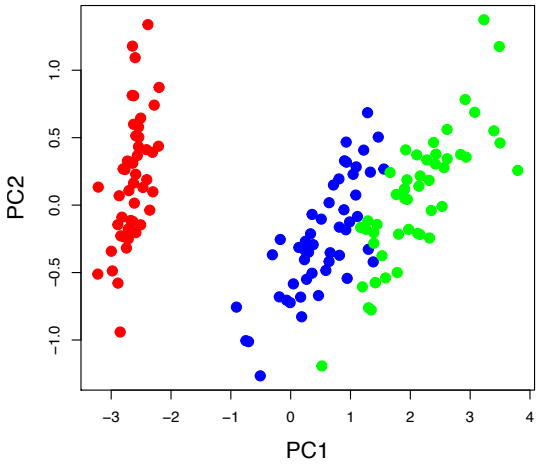
Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

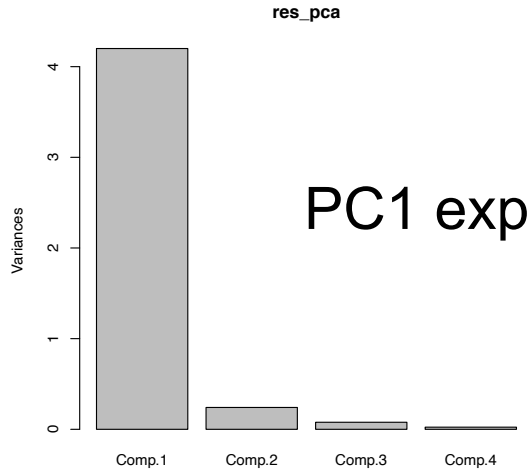
PCA - Iris

- Original iris data had 4 variables





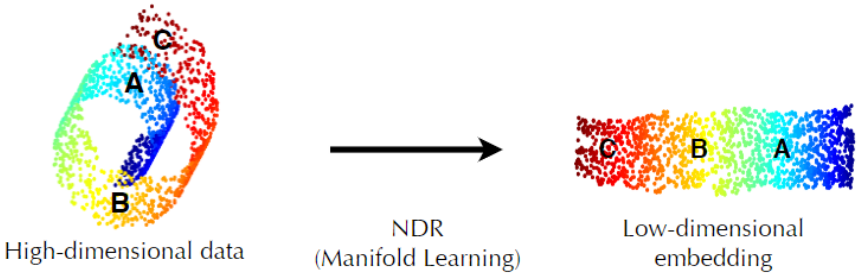




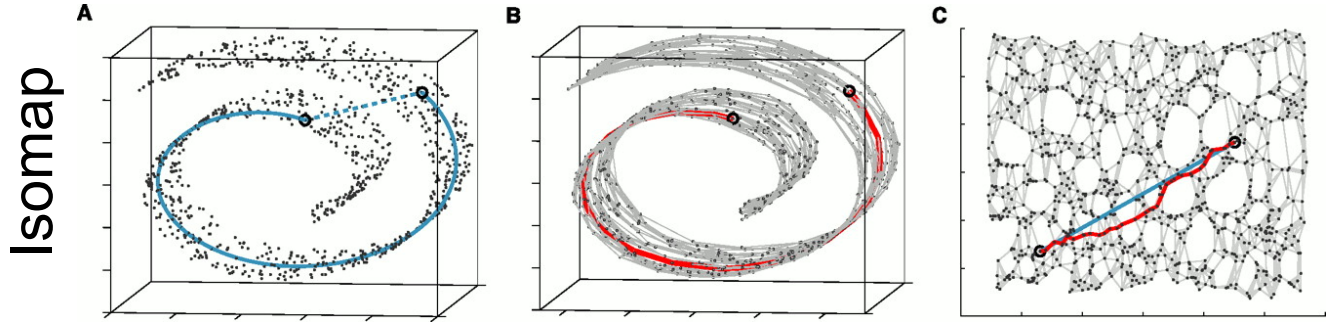
PC1 explains most of variance

Non-linear / Manifold methods

- Data might be distributed at particular regions of a high dimensional space



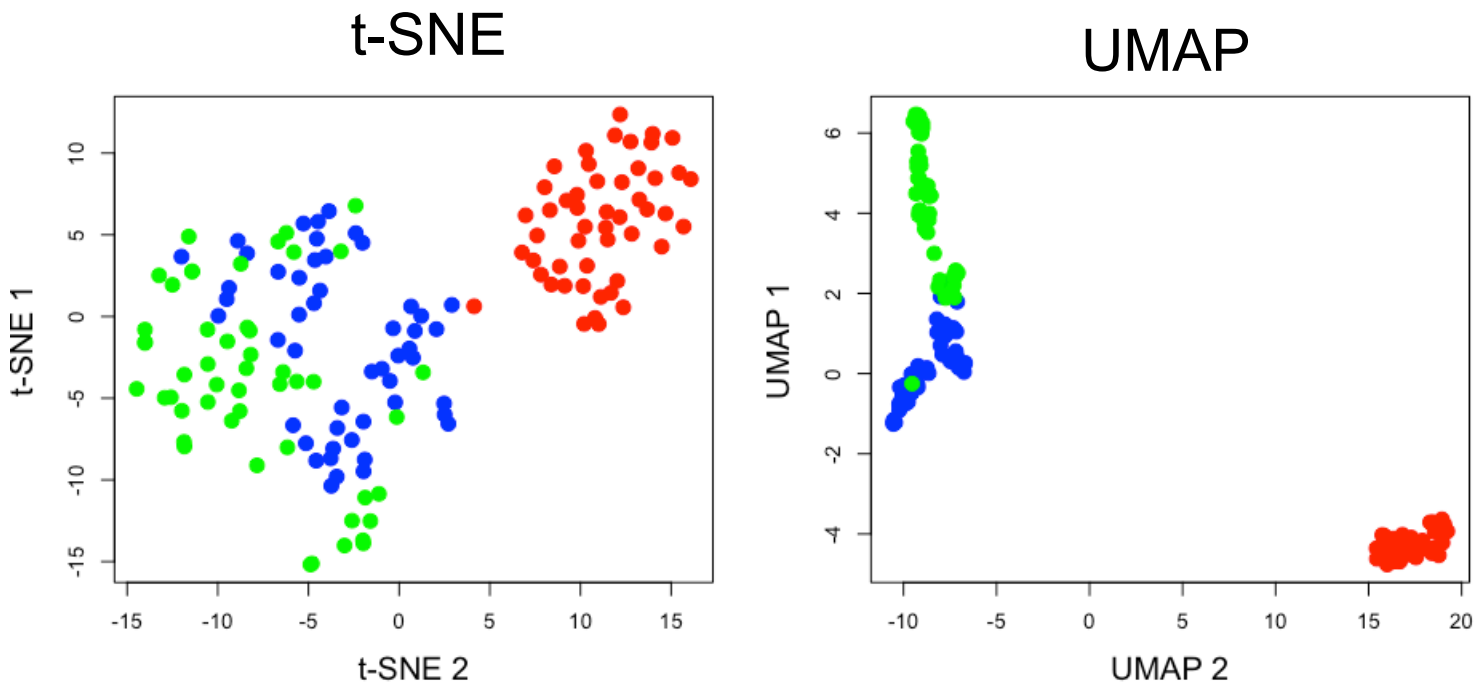
- Manifold methods use topological distance (nearest neighbour graphs)



- t-SNE and UMAP are newer/widely used methods

Adapted from Tenembaum, et al. 2000

Manifold learning and IRIS



- Nice low dimensional visualisation of the data
- Caution: These methods fail capturing global structures (distance between clusters!)

See for more details: https://www.youtube.com/watch?v=9iol3Lk6kyU&t=350s

Resume / Dimension Reduction

- PCA analysis is a wide spread technique to reduce dimension!
  - Loses importance of individual variables
- Manifold methods
  - Nice low dimensional representation of data
  - Require parametrisation and loose global distance information

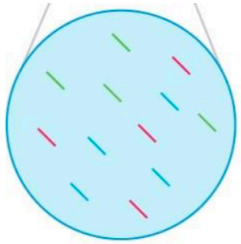
Complete course on manifolds/dimension reduction:

https://www.youtube.com/watch?v=evGm6IJKrDI&t=4421s

Expression at Single Cell Level

Basics Bioinformatics - single cell RNA-seq

Sequences



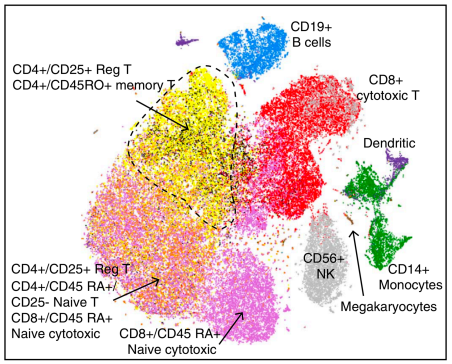
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering



1. Alignment / Transcript count

2. Cell filtering

3. Removal of biological variation

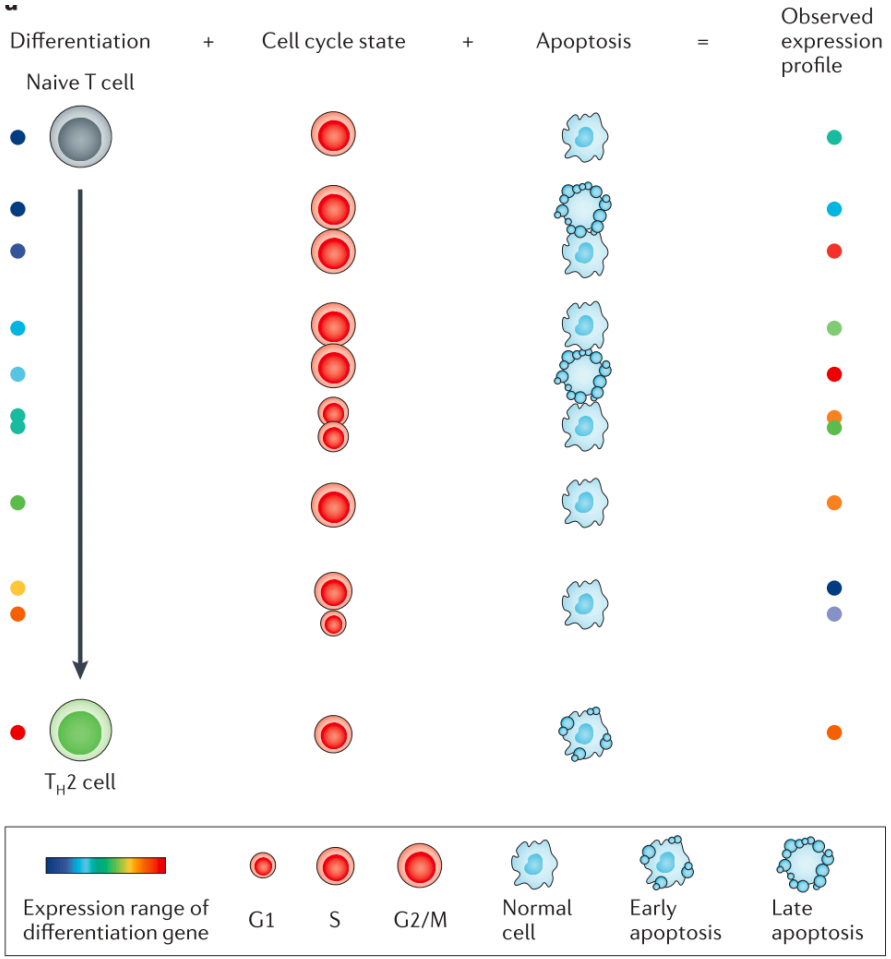
4. Integration / Dimension reduction / cell clustering

cell ranger 10x genomics

Seurat -R

Removal of Unwanted Biological Variation

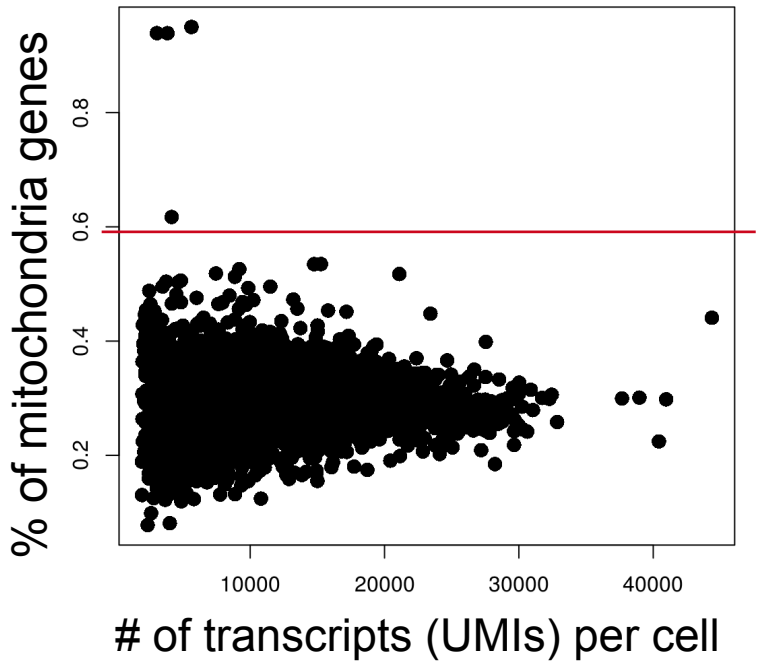
some biological changes might not be of interest for your study



Source: Stegle et al. 2015

Removal of Unwanted Biological Variation

Cells with high % of mitochondria genes

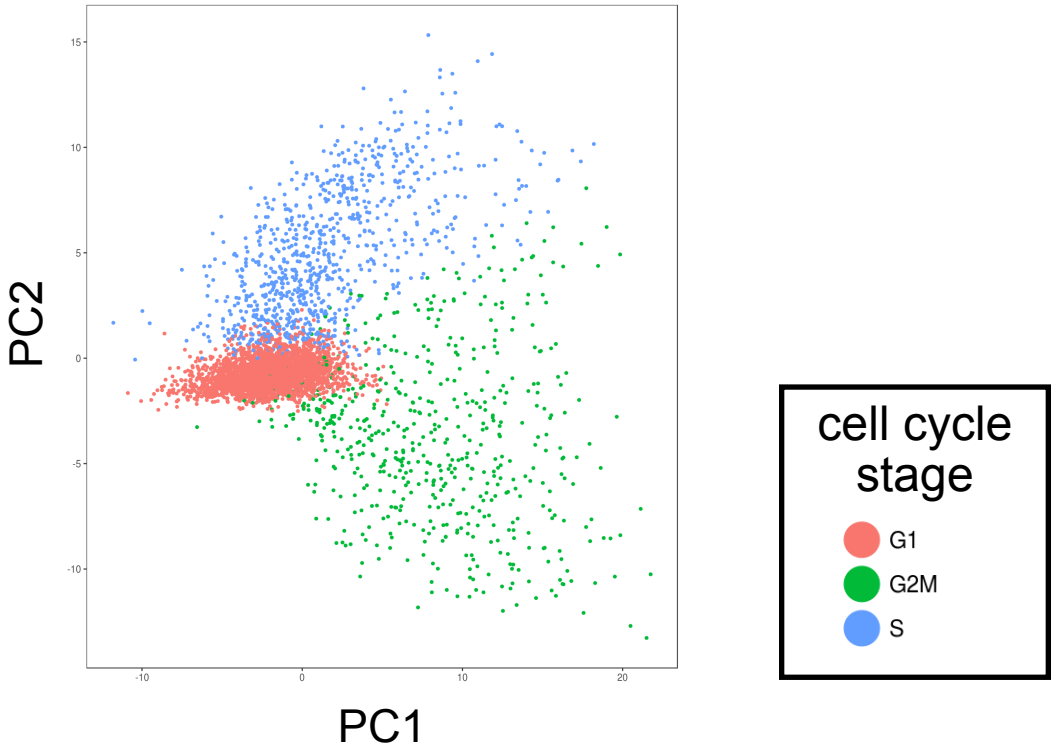


Data: dendritic cells of Peyer patches
with Torrow & Hornef UK Aachen

Removal of Unwanted Biological Variation

Remove genes associated to cell cycle

PCA based on cell cycle genes

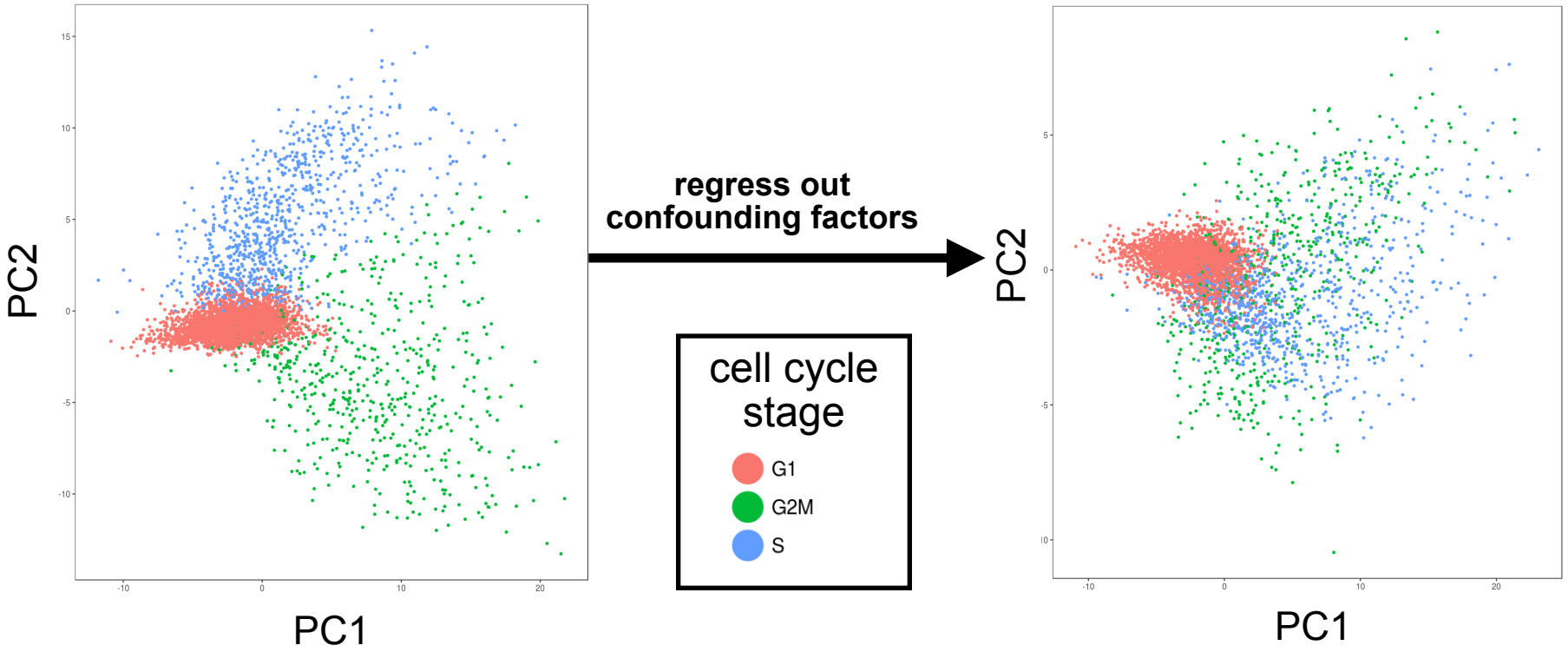


Data: dendritic cells of Peyer patches with Torrow & Hornef UK Aachen

Removal of Unwanted Biological Variation

Remove genes associated to cell cycle

PCA based on cell cycle genes



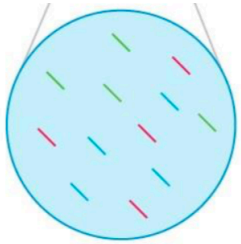
Other confounding factors: experimental replicates, individual variation

Data: dendritic cells of Peyer patches
with Torrow & Hornef UK Aachen

Institute for
Computational Genomics
RWTH AACHEN
UNIVERSITY

Basic Bioinformatics - single cell RNA-seq

Sequences



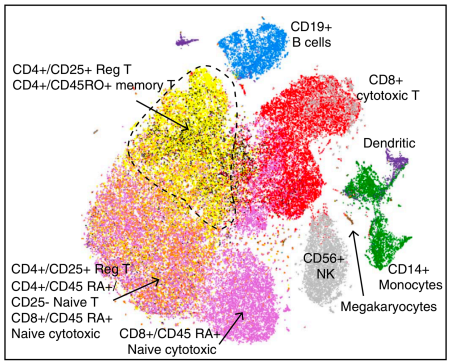
10x Barcoded cDNA

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
...			

Clustering



1. Alignment / Transcript count

2. Cell filtering

3. Removal of biological variation

4. Integration / Dimension reduction / cell clustering

cell ranger 10x genomics

Seurat -R

Basic Bioinformatics - Dimension Reduction

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

- High dimension matrix:
  - 4945 cells vs. 17328 genes
- Sparse matrix:
  - 50% zeros (90k reads per cell)

Data: dendritic cells of Peyer patches with Torrow & Hornef UK Aachen

Basic Bioinformatics - Dimension Reduction

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

Reduction with t-SNE or PCA

t-SNE matrix

t-SNE Scores

	Cell 1	Cell 2	...
t-SNE1	3.1	0.3	
t-SNE2	-2.1	2.1	

- High dimension matrix:
  - 4945 cells vs. 17328 genes
- Sparse matrix:
  - 50% zeros (90k reads per cell)

PCA - distance preserving
- used for clustering

t-SNE - local neighbourhood preserving
- used for visualisation

Basic Bioinformatics - Dimension Reduction

Expression matrix

Read counts

	Cell 1	Cell 2	...
Gene 1	25	918	
Gene 2	0	456	
Gene 3	20	342	
Gene 4	0	214	
...			

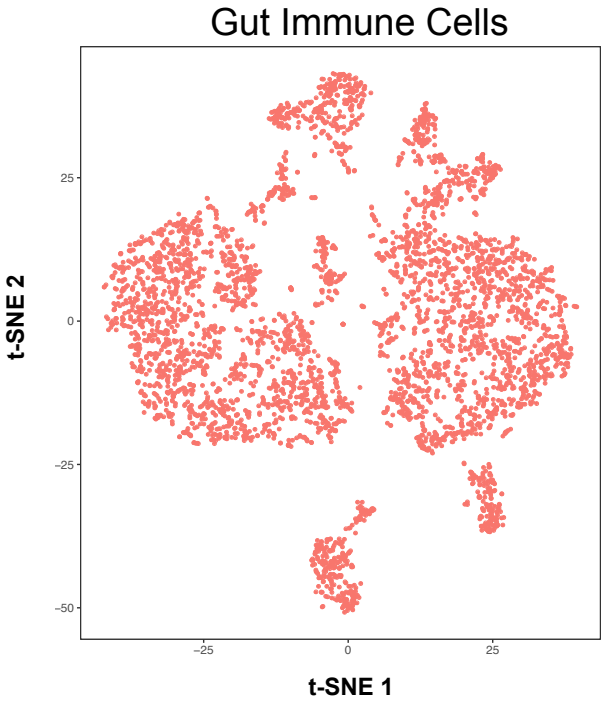
Reduction with t-SNE or PCA

t-SNE matrix

t-SNE Scores

	Cell 1	Cell 2	...
t-SNE1	3.1	0.3	
t-SNE2	-2.1	2.1	

- High dimension matrix:
  - 4945 cells vs. 17328 genes
- Sparse matrix:
  - 50% zeros (90k reads per cell)



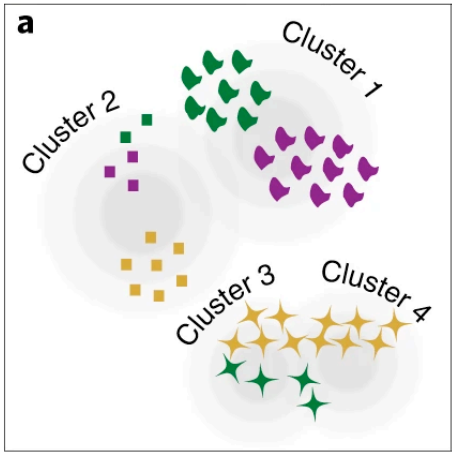
PCA - distance preserving
- used for clustering

t-SNE - local neighbourhood preserving
- used for visualisation

Basic Bioinformatics - Integration

- Usually single cell experiments are performed over distinct conditions
  - comparing disease vs. normal / treated vs. Untreated
- distinct experiments have batch effects, i.e. sequencing depth, cell capture

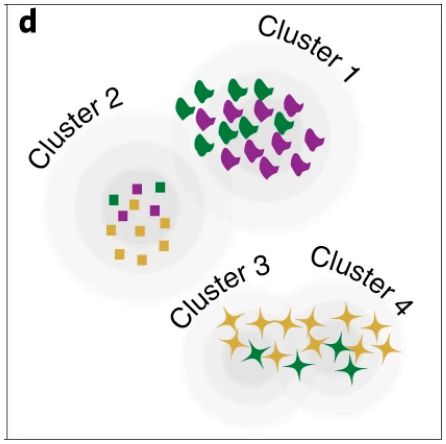
naive integration



- condition specific clusters

- Canonical correlation analysis
- Centroid based correction
- Mutual Information

integrated data

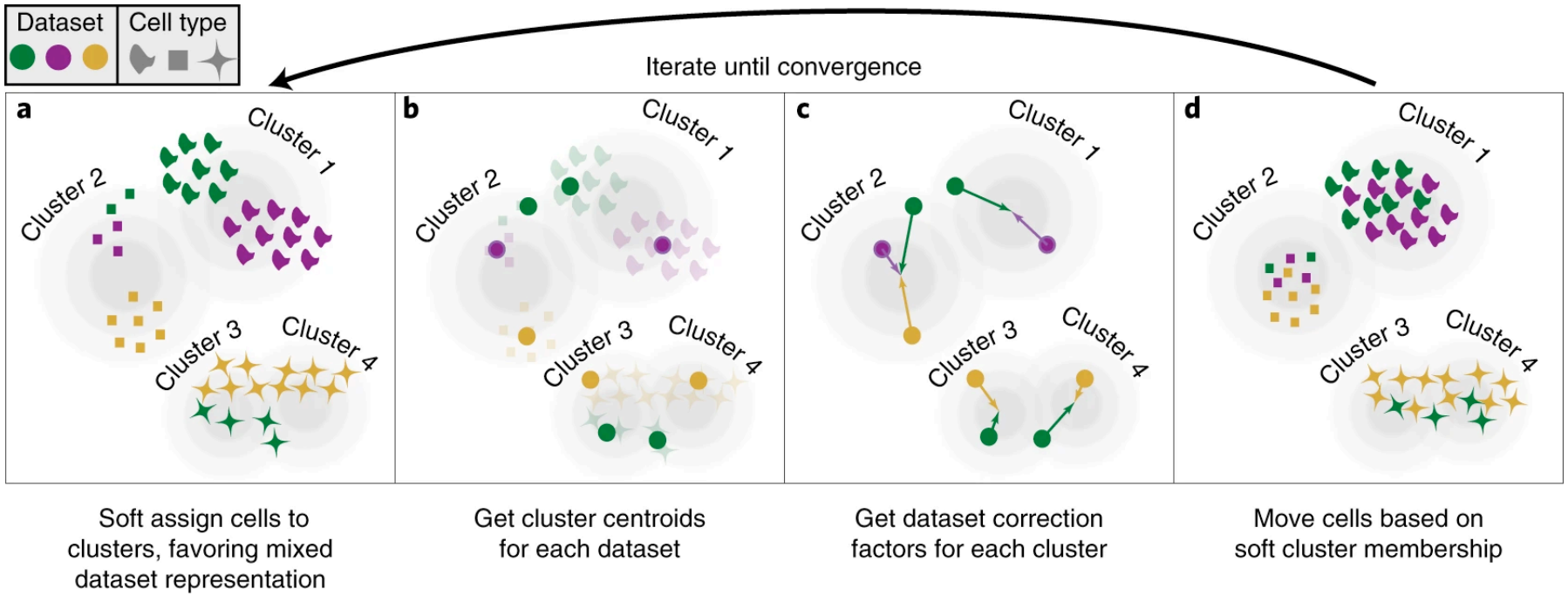


- same/similar cells in same cluster

Adapted from: Korsunsky et al. 2019

Basic Bioinformatics - Integration

- Harmony explores centroids from fuzzy clustering and linear correction models for data integration

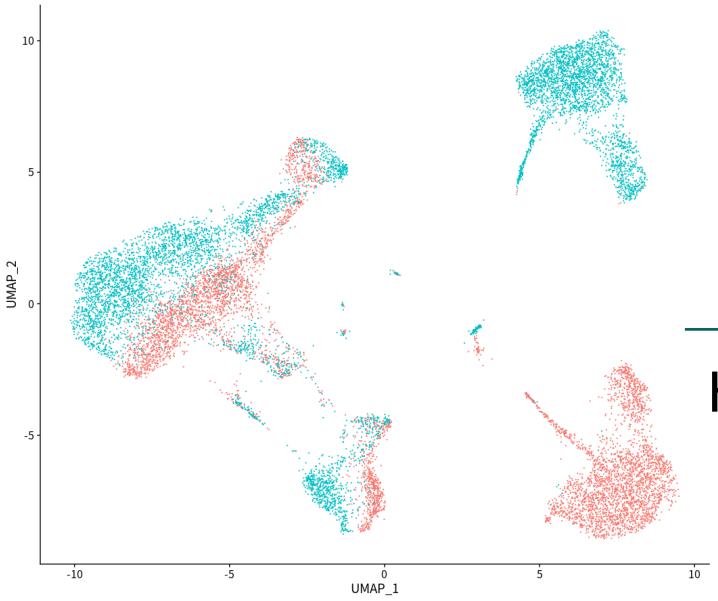


Adapted from: Korsunsky et al. 2019

Basic Bioinformatics - Integration

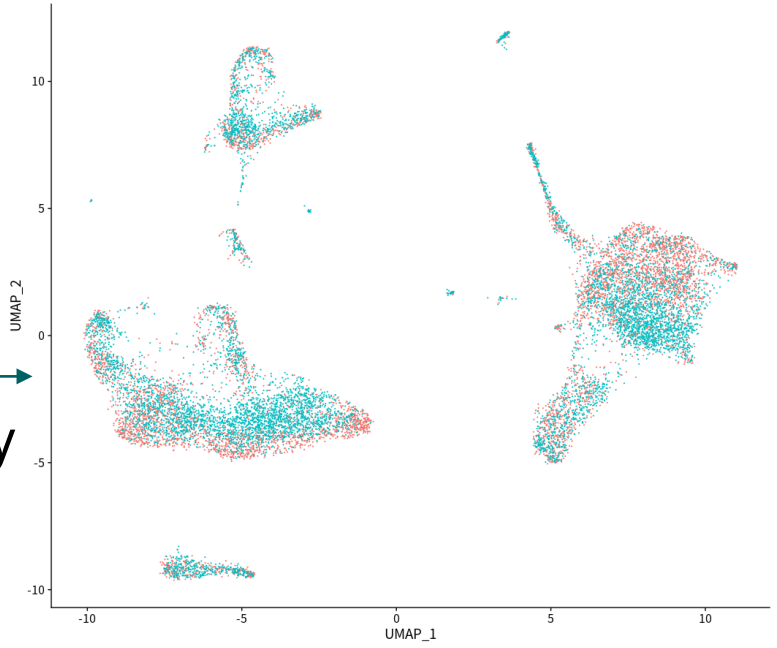
- Real world example: blood cells after stimulation

Raw data



Harmony

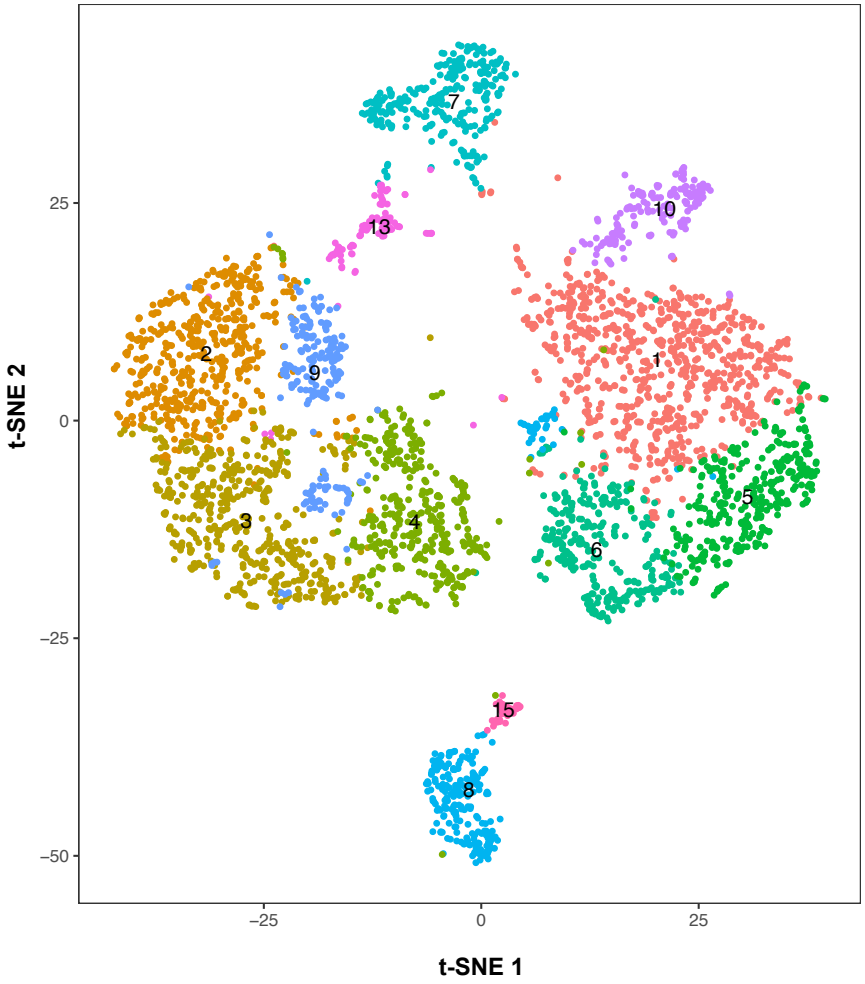
Integrated data



Adapted from: Korsunsky et al. 2019

Basics Bioinformatics - Clustering

Gut Immune Cells - 12 groups

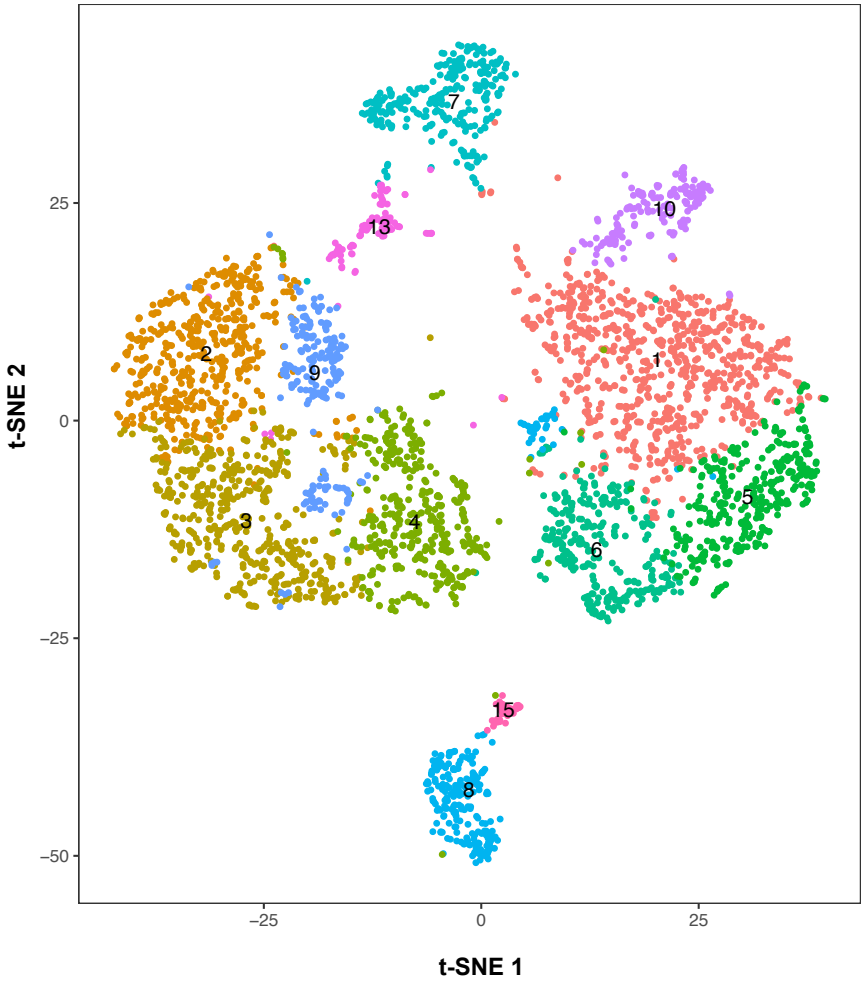


Clustering - identify cells with similar expression patterns
- based on PCA (20 dimension)

How to identify cell types?

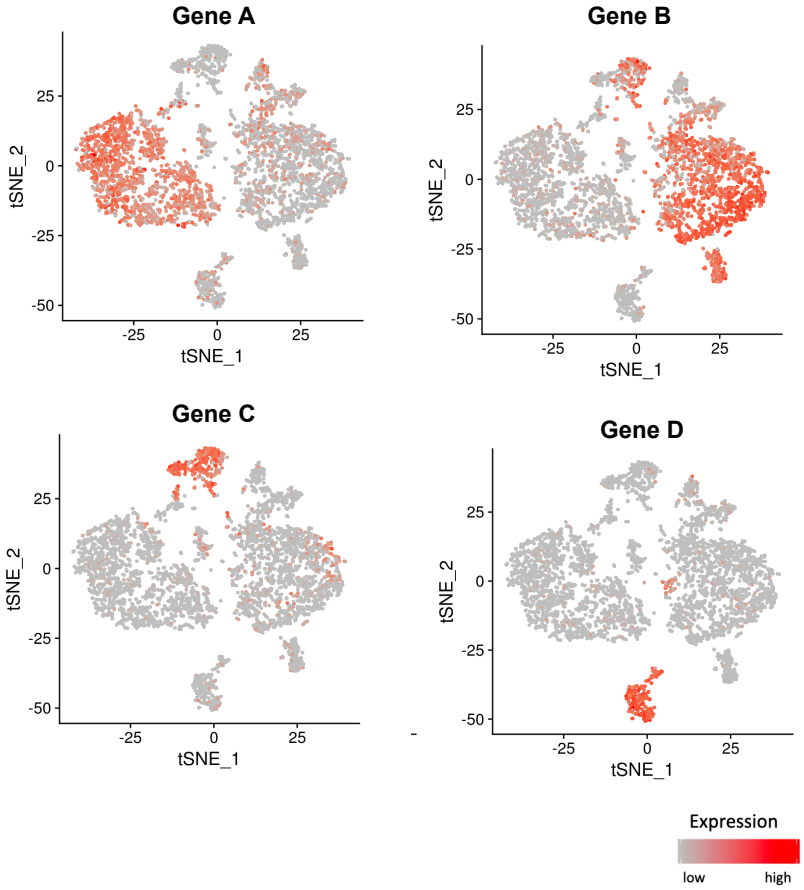
Cell Identity with an Expert

Gut Immune Cells - 12 groups



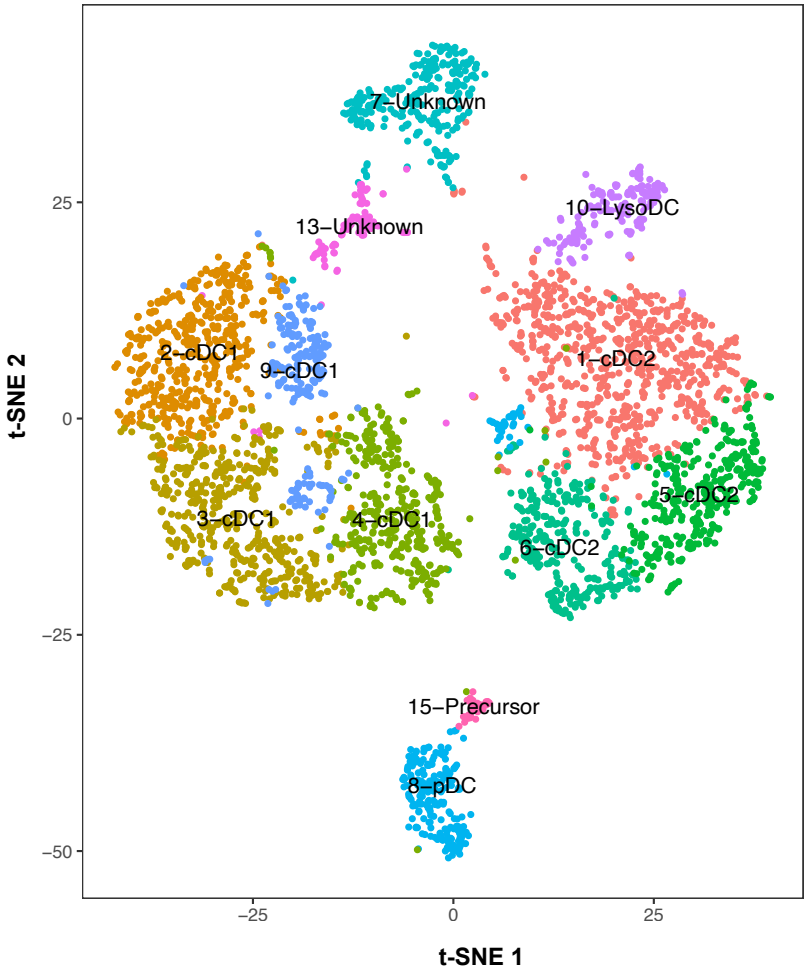
Check expression of:

1. known genes



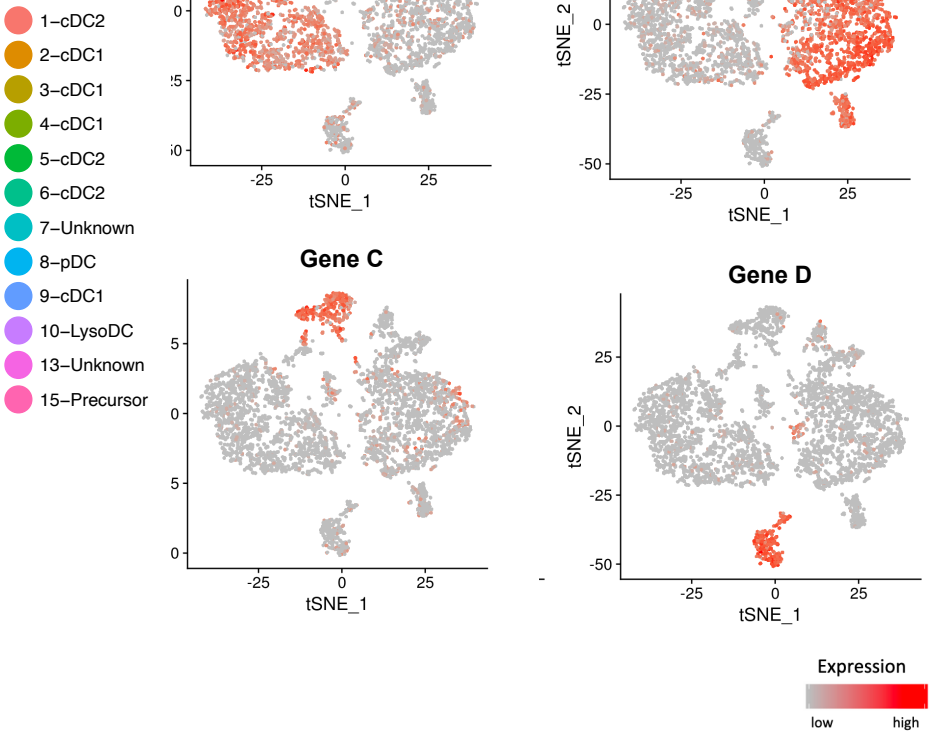
Cell Identity with an Expert

Gut Immune Cells - 12 groups



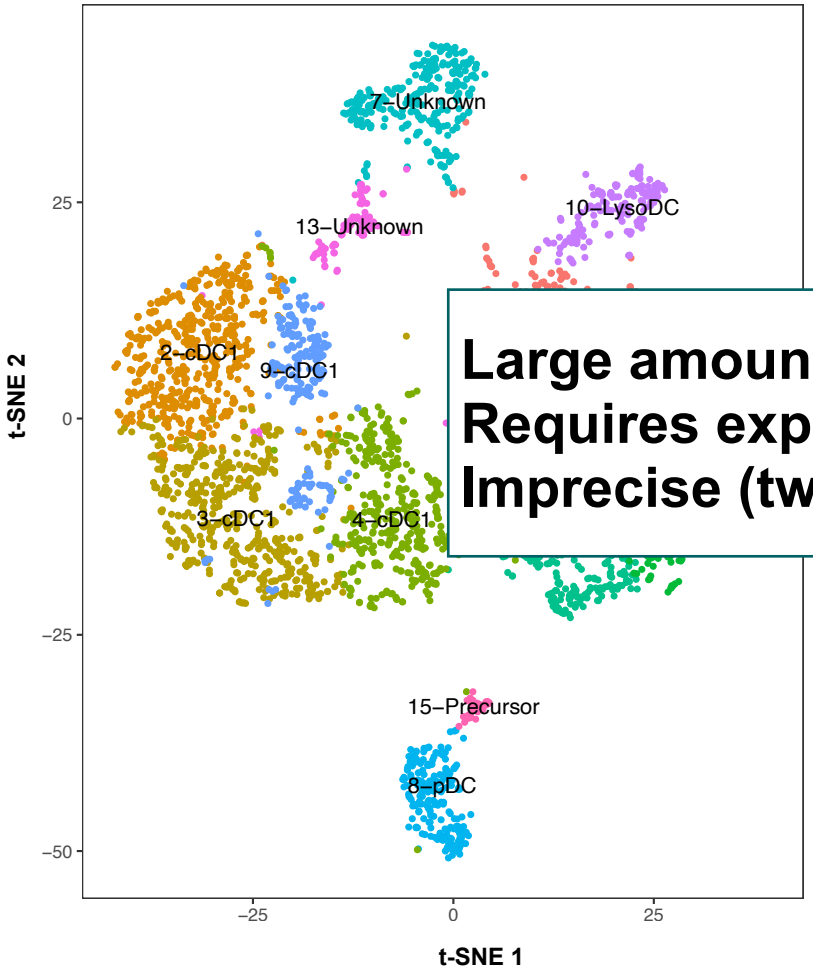
Check expression of:

1. known genes



Cell Identity with an Expert

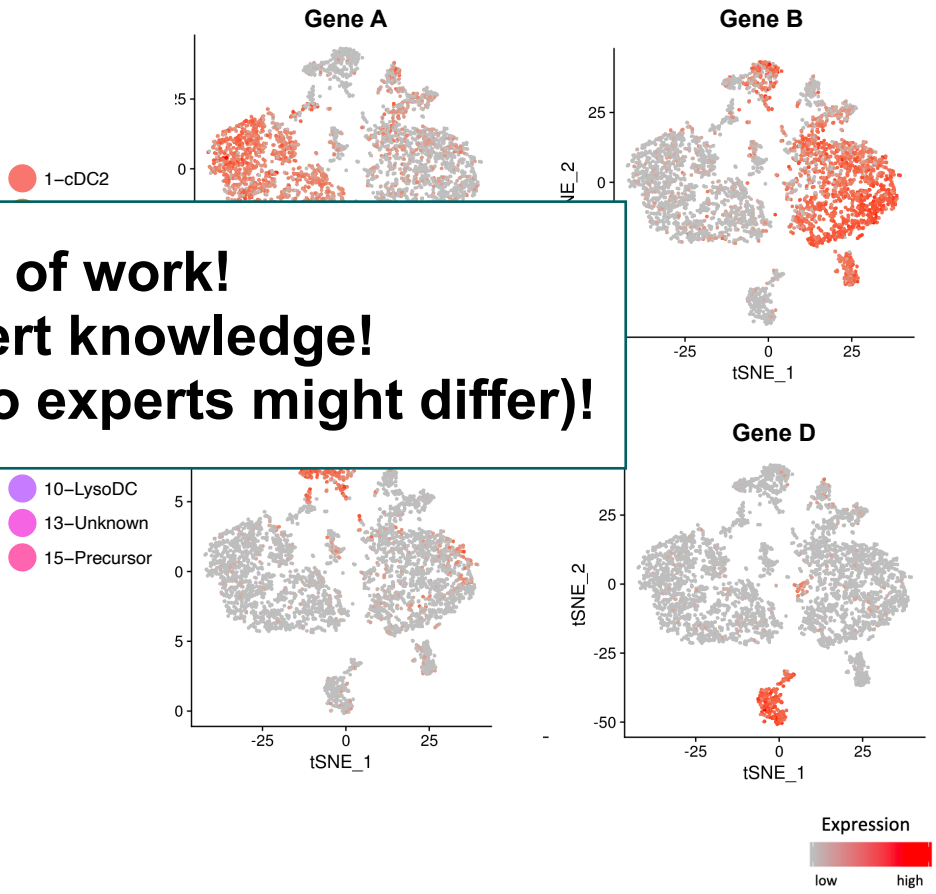
Gut Immune Cells - 12 groups



Check expression of:

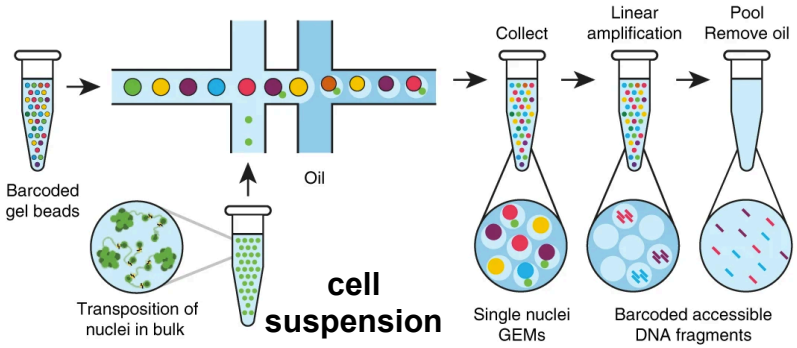
1. known genes

Large amount of work!
Requires expert knowledge!
Imprecise (two experts might differ)!



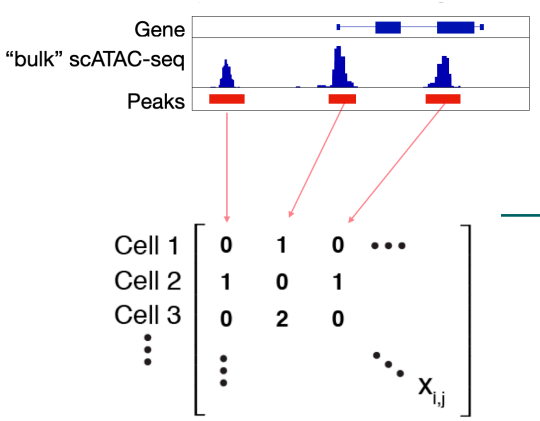
Open chromatin with scATAC-seq

Droplet based scATAC-seq

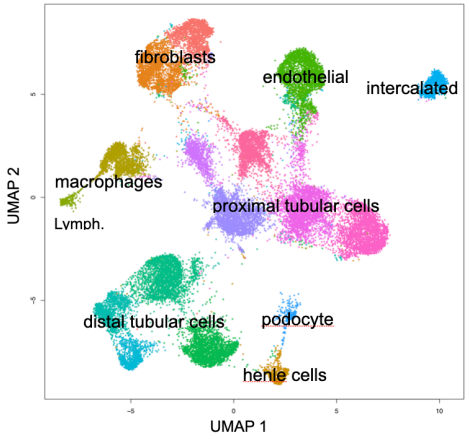


Adapted from Satpathy,
Nature biotechnology, 2019

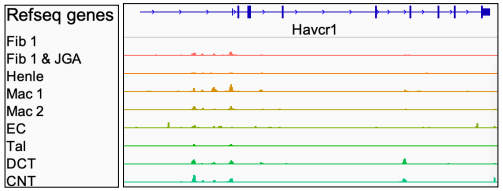
Open Chromatin Matrix

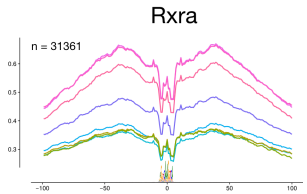


UMAP / Clustering



cluster pseudo bulk ATAC-seq





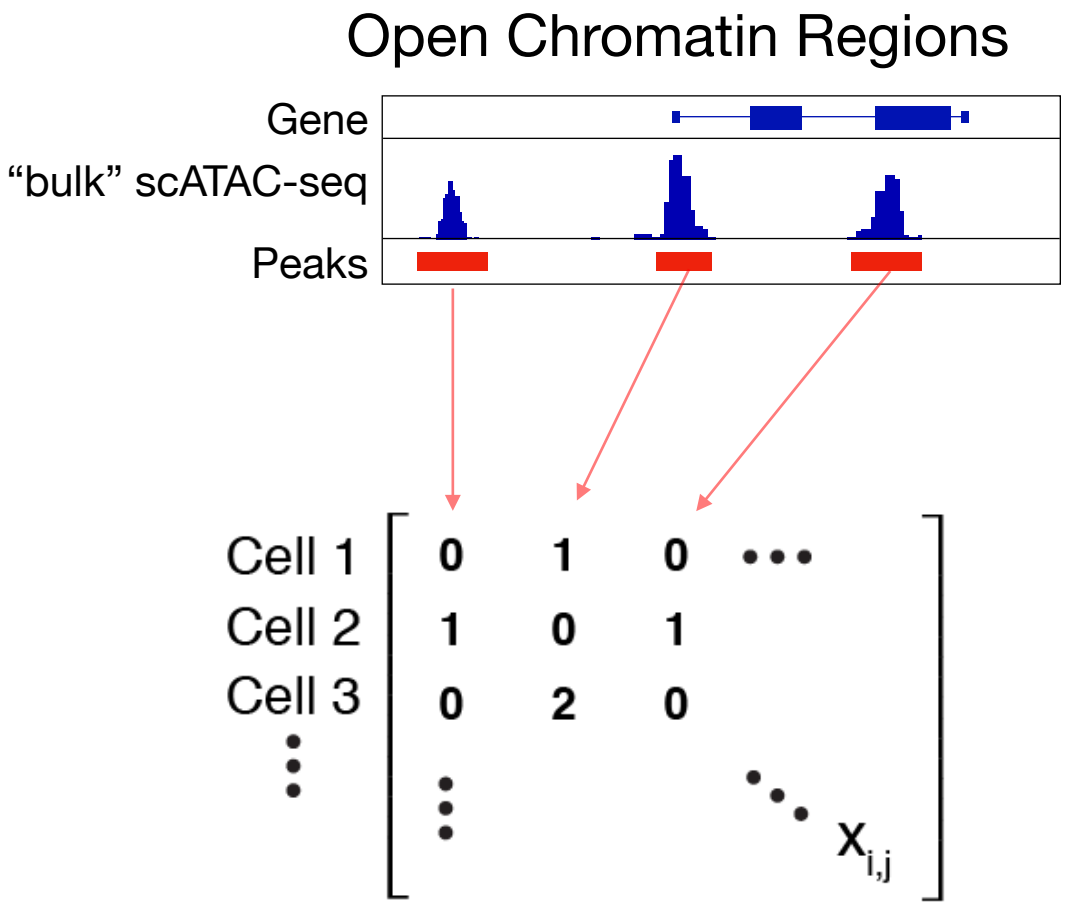
Footprinting & TF Activity

Li, …, **Kramann, Costa**, Biorvx, https://doi.org/10.1101/865931.

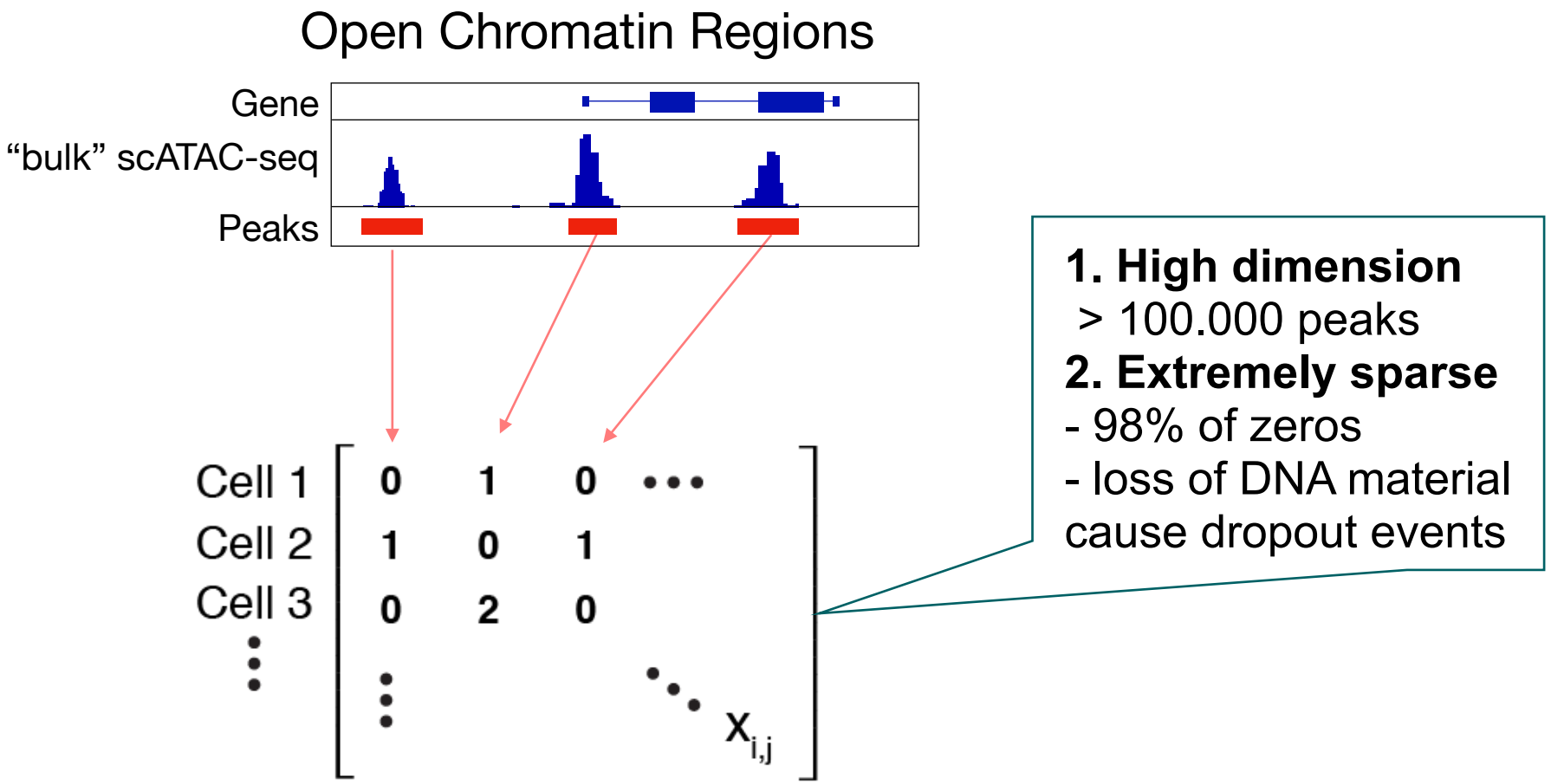
Institute for Computational Genomics
01011011010
10100100101

RWTH AACHEN UNIVERSITY

Computational Challenges - Single Cell ATAC



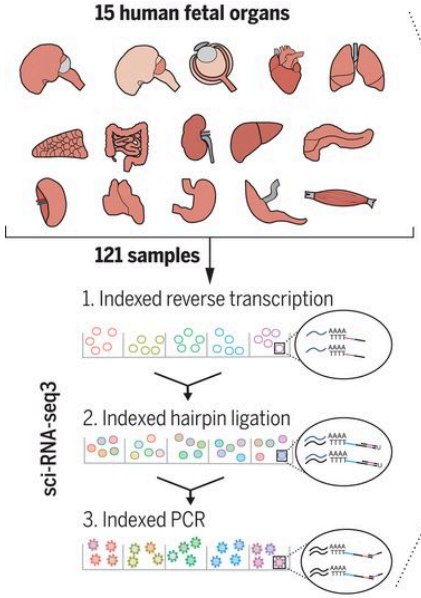
Computational Challenges - Single Cell ATAC



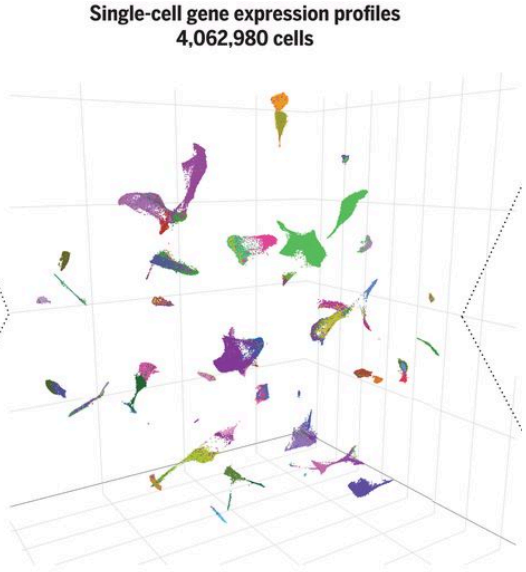
Resume / Single cell clustering

- Finding groups of single cells require complex pipeline:
  - Cell filtering
  - Normalisation
  - Artefact removal
  - Dimension reduction
  - Integration
  - Clustering
  - Cell annotation / visualisation
- Open points:
  - How to deal with large data sets (millions of cells)?
  - How to detect cells of rare populations?
  - How to deal with sparsity of scATAC seq data?

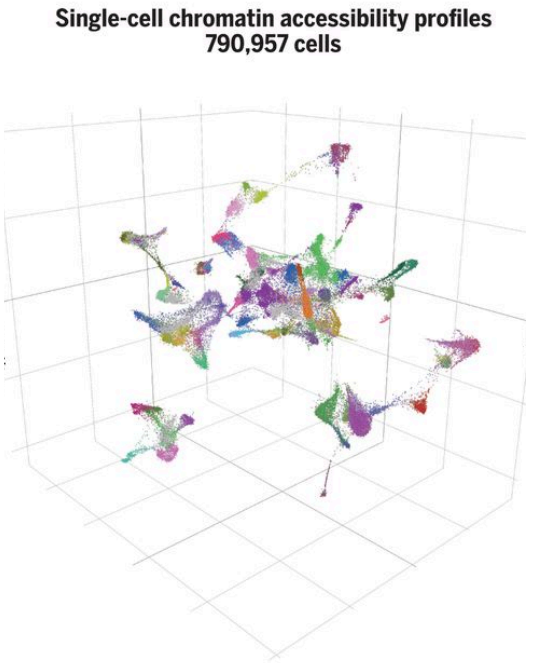
Clustering of cells / Human Fetal Cell Atlas



scRNA-seq



scATAC-seq



https://descartes.brotmanbaty.org/

- Open points:
  - How to deal with large data sets (millions of cells)?
  - How to detect cells of rare populations?
  - How to deal with sparsity of scATAC seq data?

Adapted from Donke et al. 2020.

Calendar

Today – Single cell sequencing / Practical Course

3.05.2021 – Project Description / Introduction to HPC clusters and GPUs

10.05.2021 – 5.7.2021 – Project development

12.07.2021 – Project Presentation

Thank you!