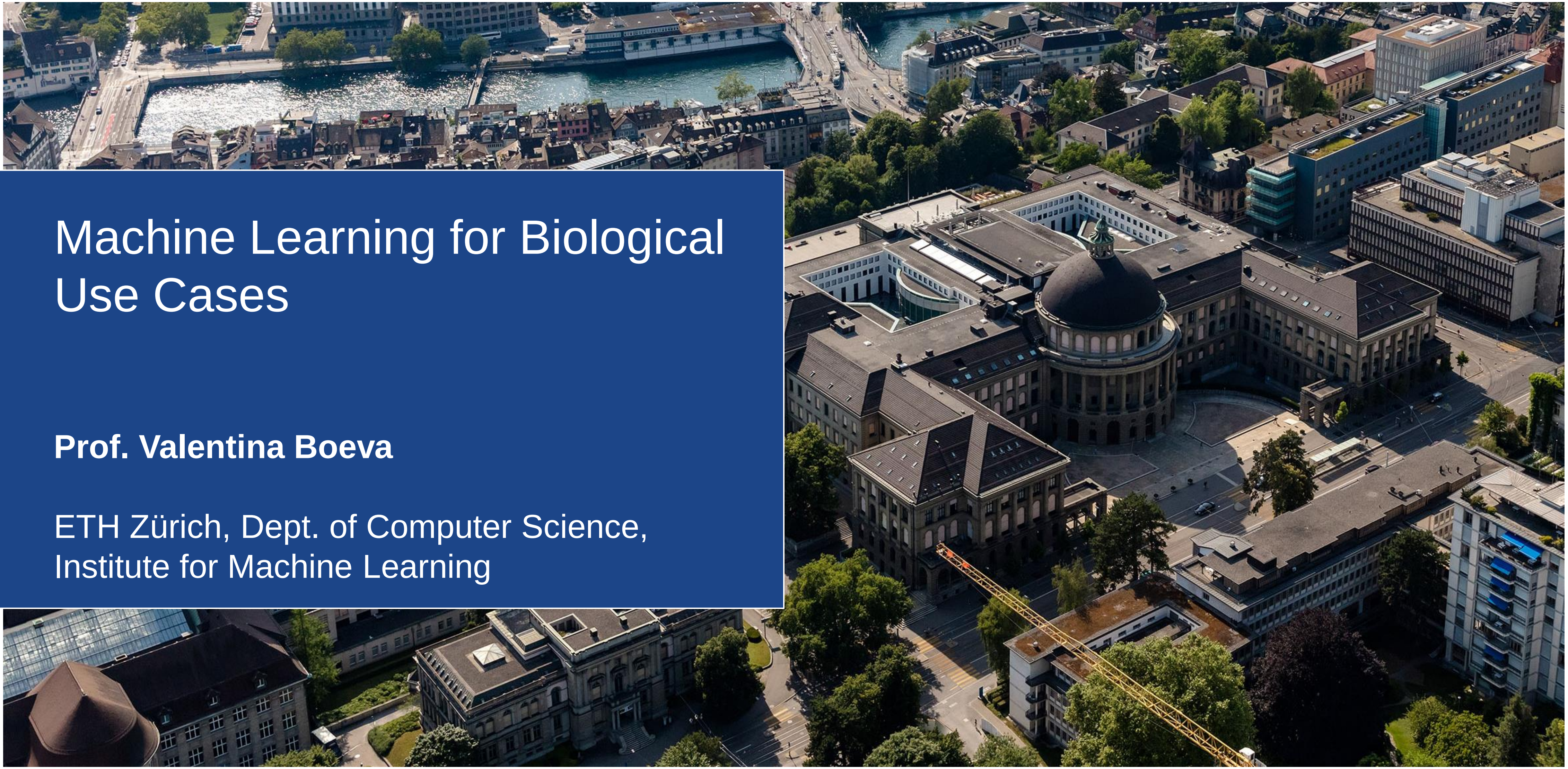
Dept. Computer Science,
Inst. for Machine Learning

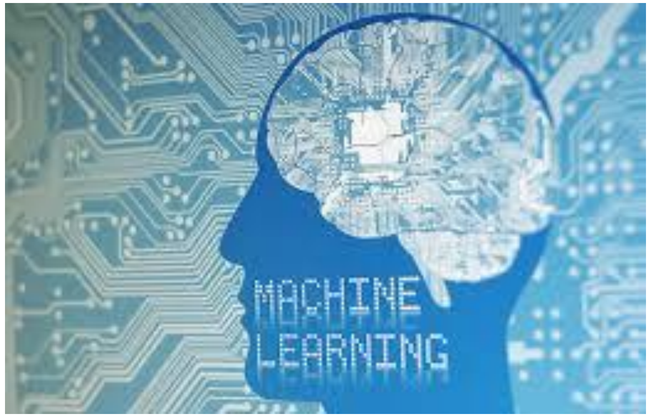


Machine Learning for Biological Use Cases

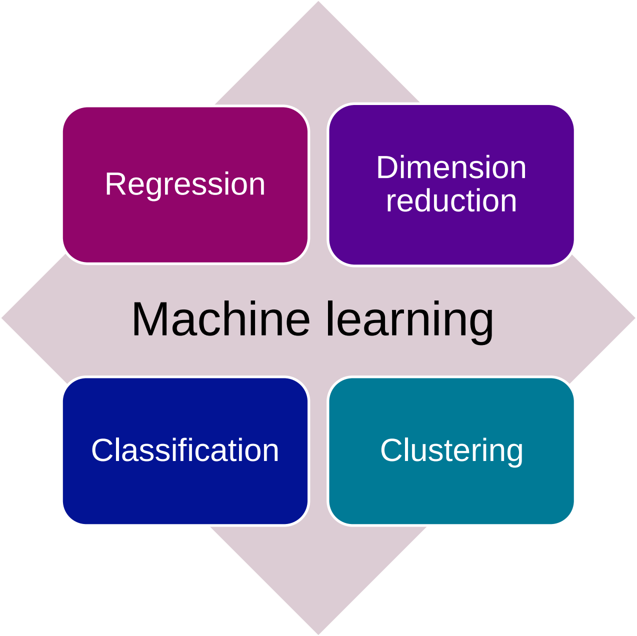
Prof. Valentina Boeva

ETH Zürich, Dept. of Computer Science,
Institute for Machine Learning

Machine learning



Map of classical machine learning methods



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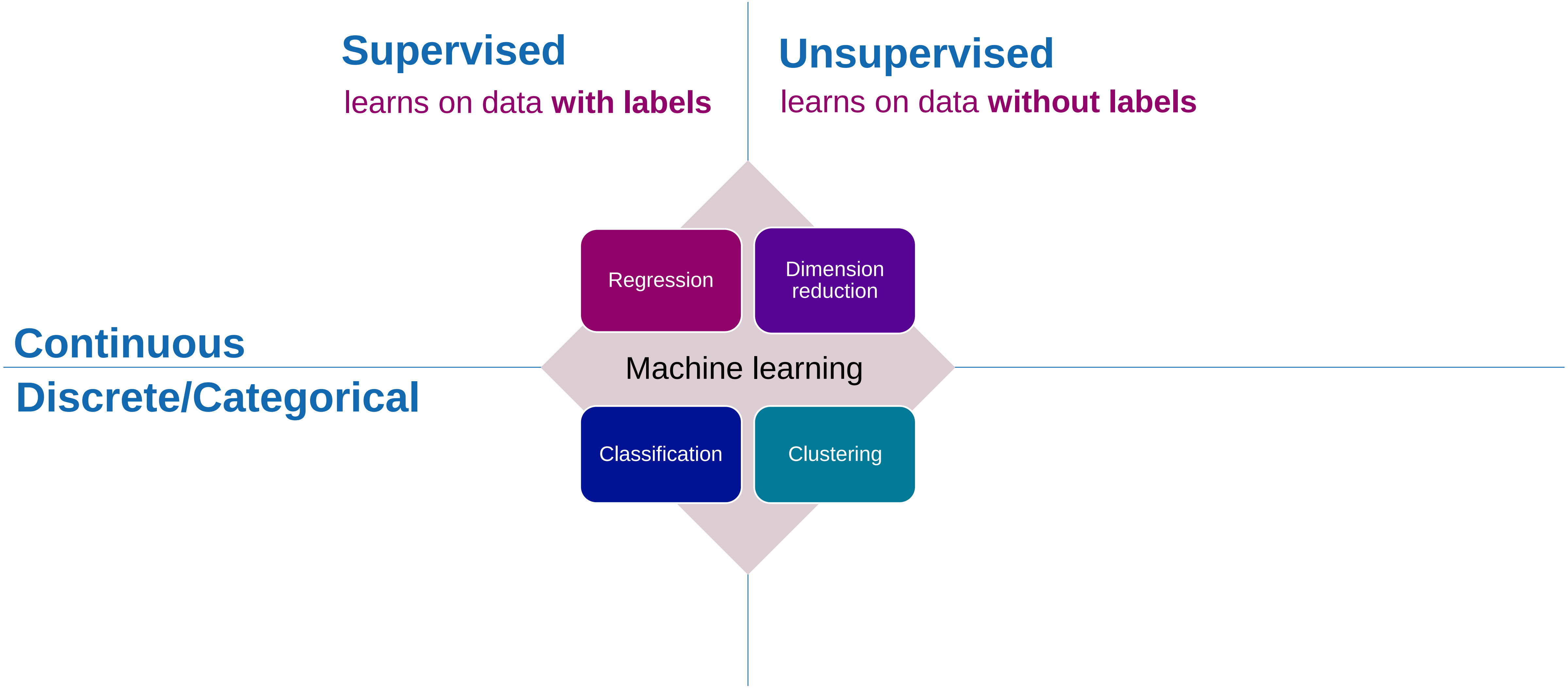

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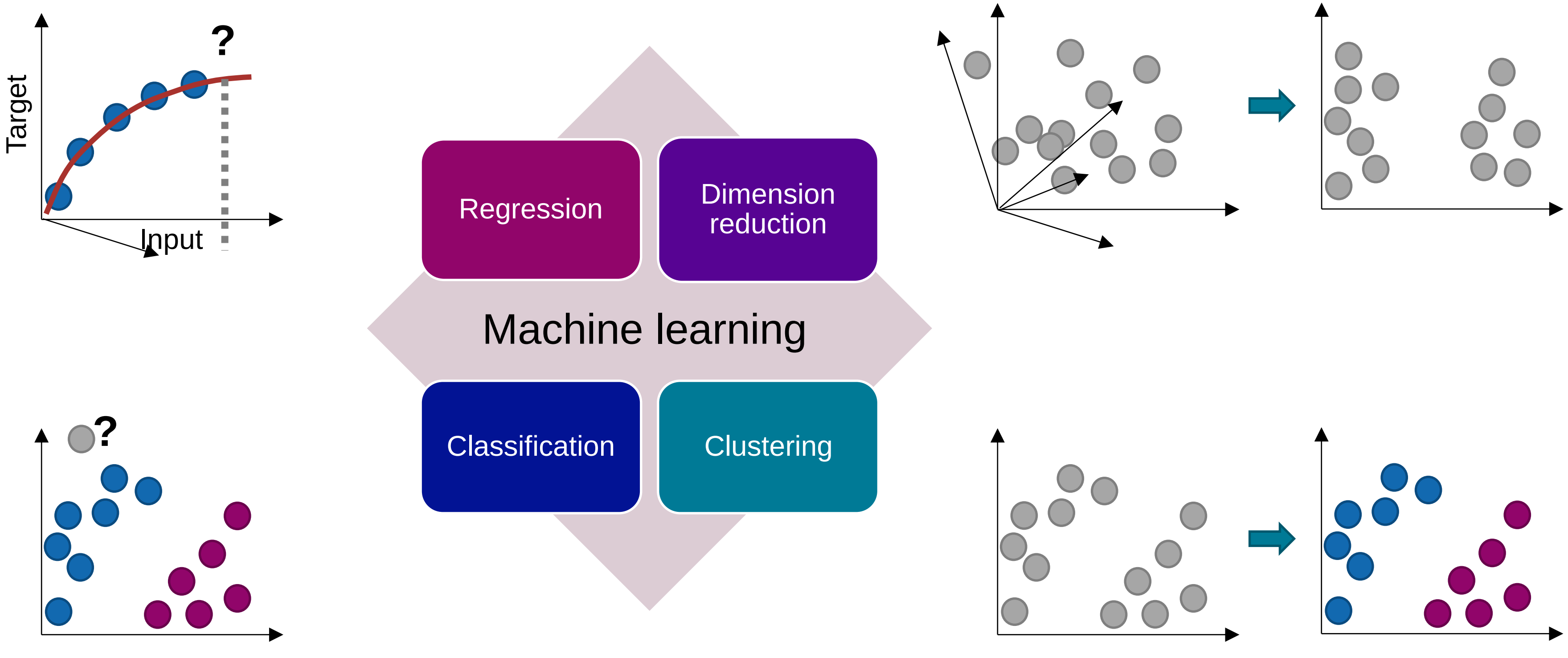


Regression

Dimension reduction

Machine learning

Classification

Clustering

Map of classical machine learning methods

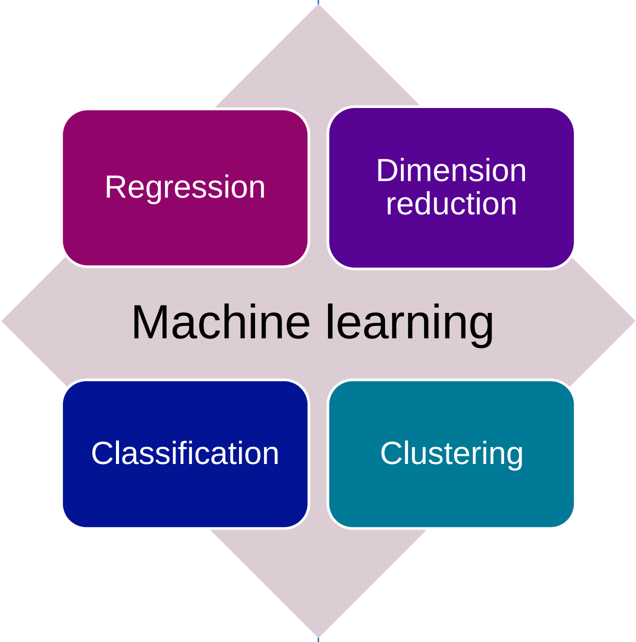
Supervised

learns on data **with labels**

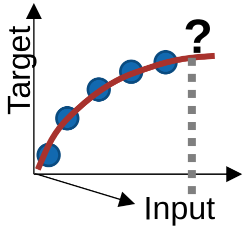
Unsupervised

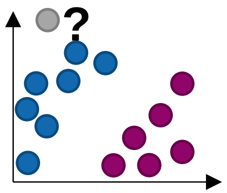
learns on data **without labels**

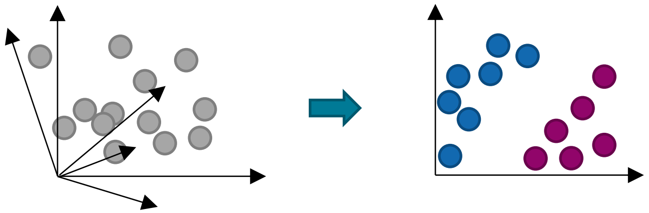
- Automatize decisions
  - Primary diagnosis
  - Choice of treatment
- Predict future
  - Treatment response
  - Side effects
- Detect the most “important” features



- Understand data structure
- Visualize data in 2D/3D
- Detect hidden features







Types of input data in molecular biology and genetics

Type of data:

- **Genomic**:
  - Variants common in general population (SNPs)
  - Rare variants
    - Single nucleotide variants, SNV (a.k.a. mutations)
    - Structural aberrations (e.g., translocations, amplifications)
    - Copy number profiles
- **Transcriptomic**:
  - RNA-seq (or expression microarrays) – bulk and single cell
  - mi-RNA
- **Epigenetic**:
  - DNA methylation (sequencing or methylation arrays)
  - Histone modifications (ChIP-seq) and open chromatin (ATAC-seq, DHS-seq)
- **Proteomic:**
  - RPPA (bulk), CyTOF (single cell)

Context:

- Common diseases (Alzheimer, asthma, hypertension,…)
- Genetic syndromes (Down syndrome, CHARGE syndrome, …)
- Cancer

Type of samples:

- Blood samples
- Saliva samples
- Tissue samples
- Maternal blood samples

Omics data are high dimensional

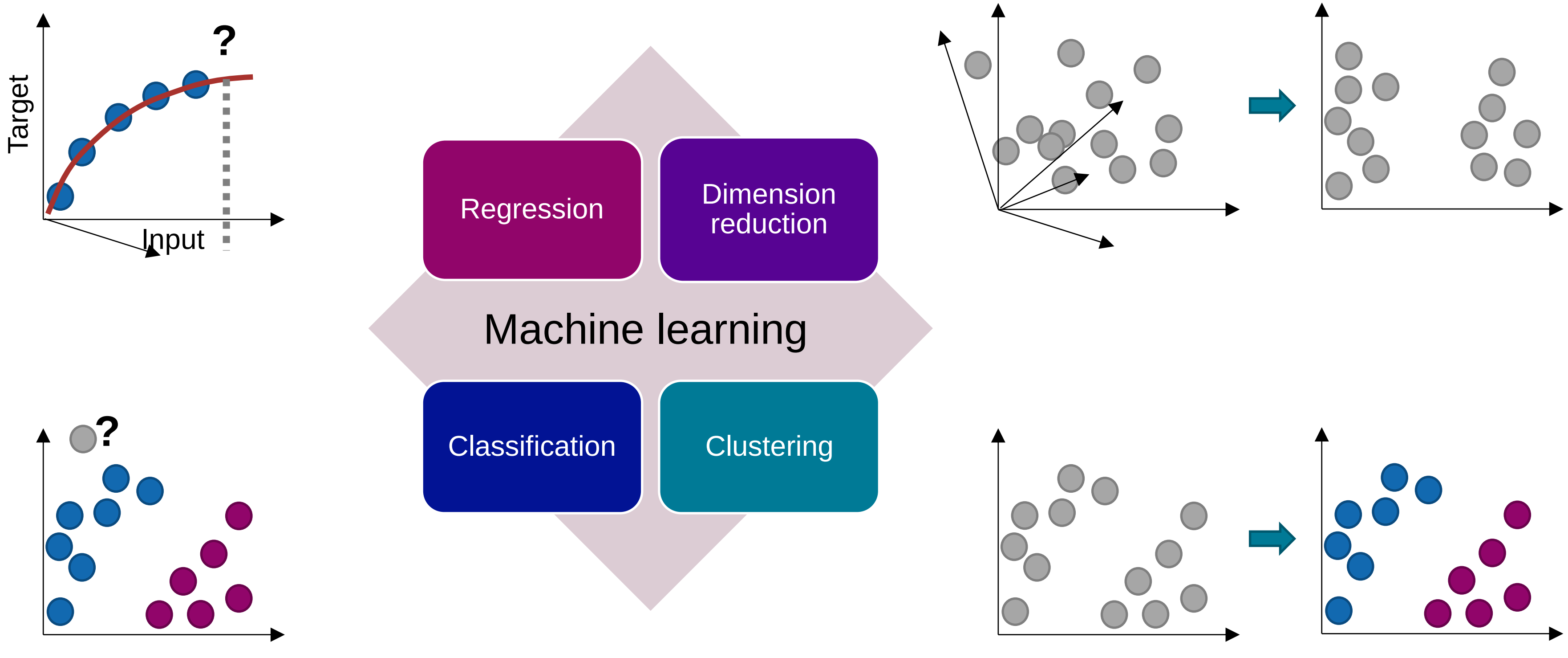
SNPs	Mutations (SNVs)	Structural variants	Copy number alterations	mRNA expression	miRNA expression	DNA methylation
3M-100M	~10-10K	~1-1000	~10-25K	~25K	~1000	27K-28M

+ sometimes these data is complemented with proteomics data (expression of hundreds of proteins)

Full –omics dataset millions of observations per patient:

Great challenge to avoid over-fitting and perform feature selection!

Map of classical machine learning methods



Regression

Dimension reduction

Machine learning

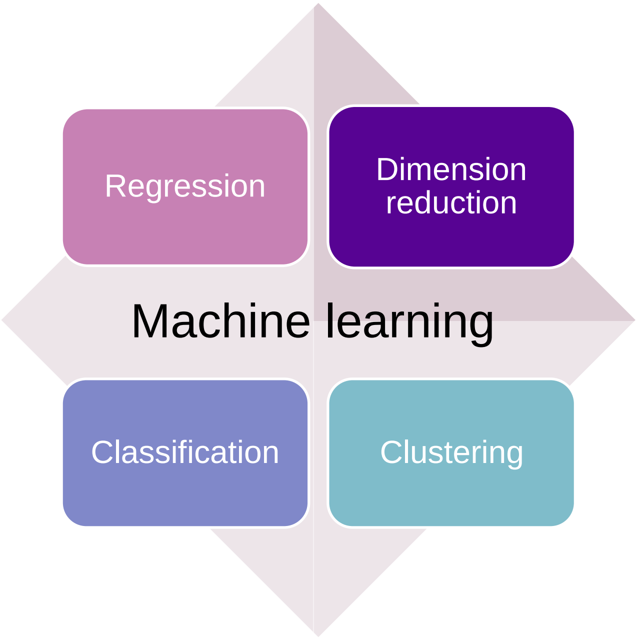
Classification

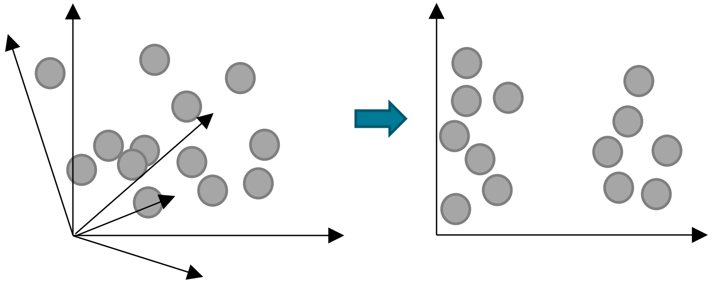
Clustering

Map of machine learning methods

Unsupervised

learns on data **without labels**





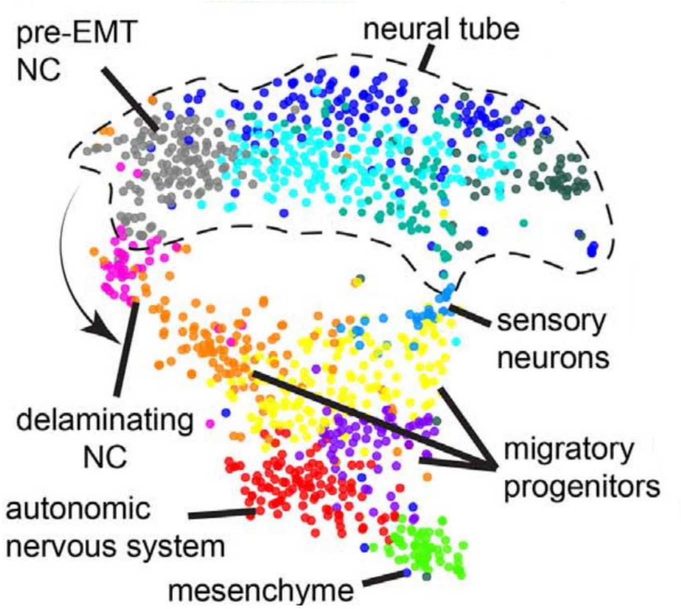
Examples: Dimension reduction and clustering

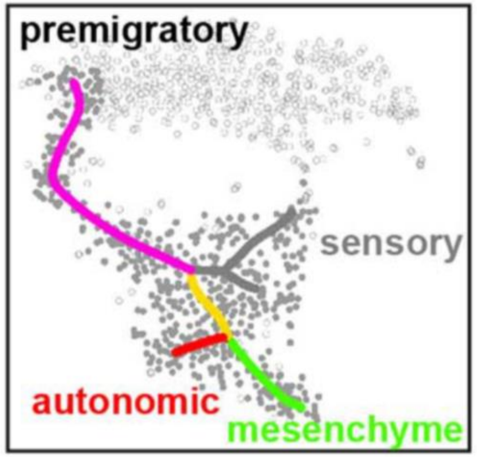
- Single cell transcriptomics: development, cell type heterogeneity and cancer

- Bulk transcriptomics and epigenetics (cancer)

- Bulk transcriptomics: Effect of mutations

tSNE





Spatiotemporal structure of cell fate decisions in murine neural crest. Soldatov et al., *Science* 364, 971 (2019)

Examples: Dimension reduction and clustering

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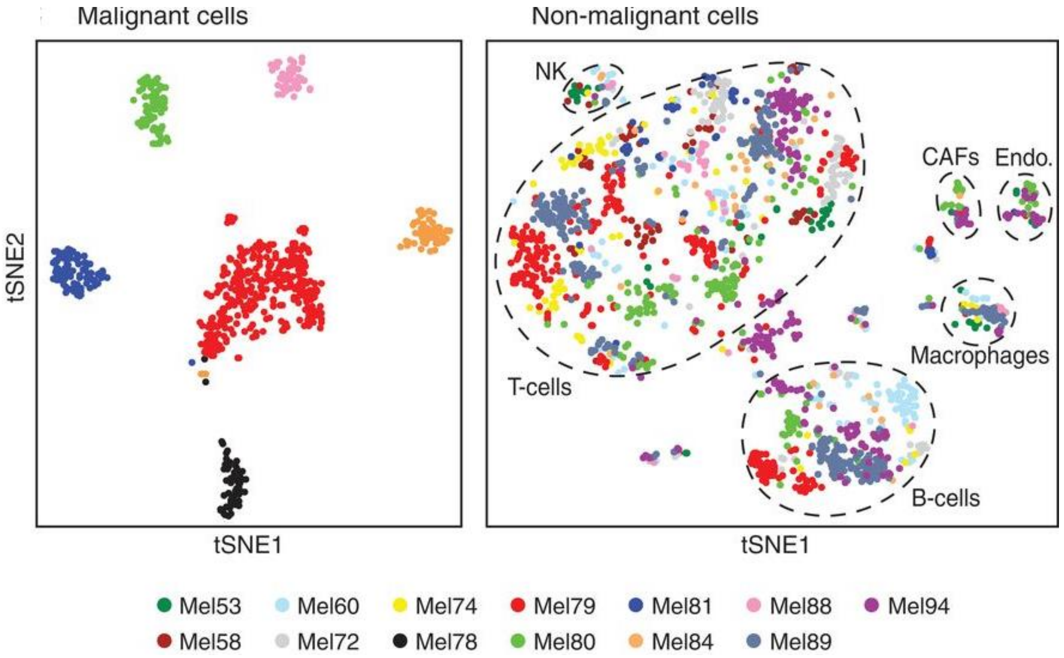


Clusters called by DBScan

Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Tirosh et al. *Science*. 2016 Apr 8;352(6282):189-96.

Examples: Dimension reduction and clustering

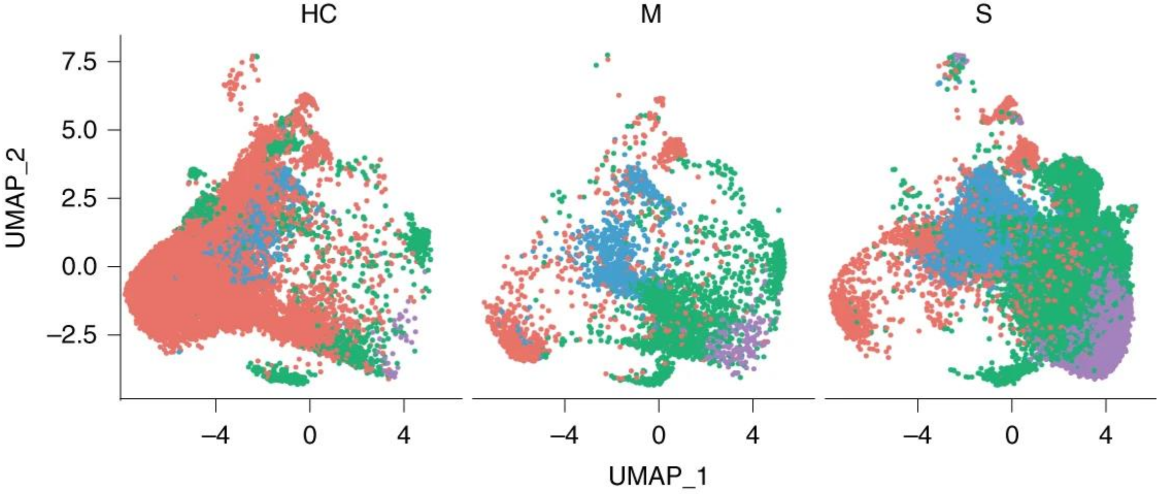
- Single cell transcriptomics: development, cell type heterogeneity and cancer

- Bulk transcriptomics and epigenetics (cancer)

- Bulk transcriptomics: Effect of mutations

Bronchoalveolar lavage fluid macrophages from patients with varying severity of COVID-19 and from healthy people:

● Group 1	*FCN1*^{hi}	classic M1-like macrophages
● Group 2	*FCN1*^{lo}*SPP1*$^{+}$	classic M1-like macrophages
● Group 3	*SPP1*$^{+}$	M2-like macrophages
● Group 4	*FABP4*$^{+}$	alveolar macrophages



Severe cases: Presence of proinflammatory monocyte-derived macrophages

Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Liao et al., *Nature Medicine*. 2020

Examples: Dimension reduction and clustering

- Single cell transcriptomics: development, cell type heterogeneity and cancer
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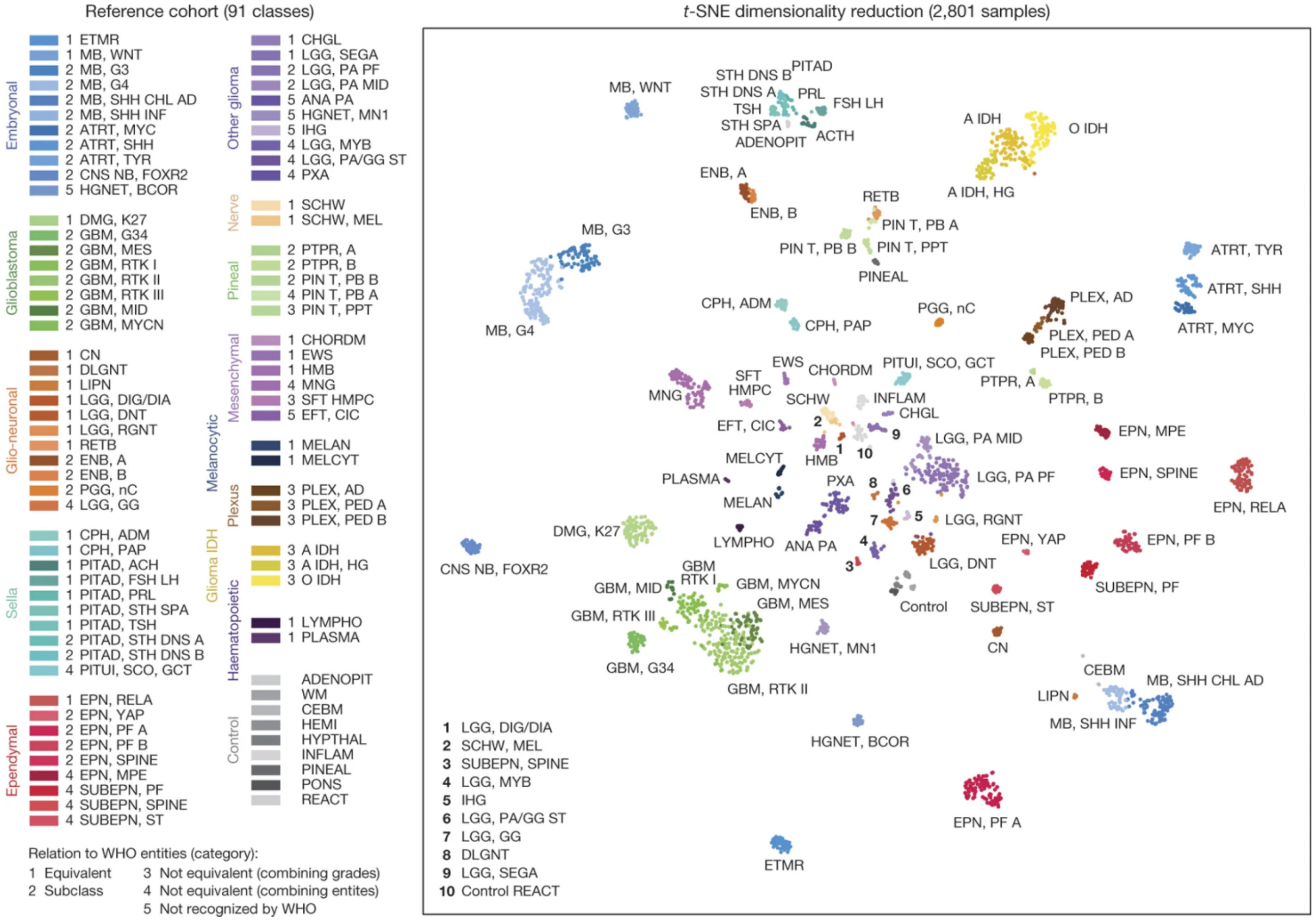
- Bulk transcriptomics: Effect of mutations

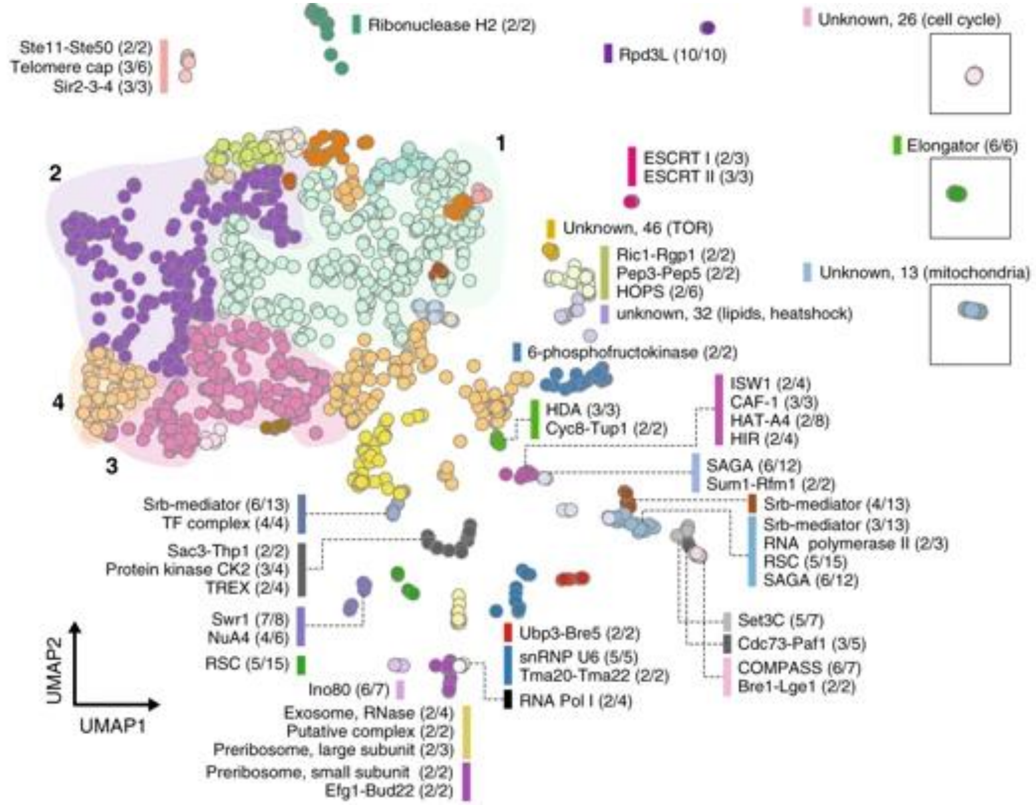


DNA methylation-based classification of central nervous system tumours. Capper et al. *Nature* 2018

Examples: Dimension reduction and clustering

- Single cell transcriptomics: development, cell type heterogeneity and cancer
- Bulk transcriptomics and epigenetics (cancer)
- Bulk transcriptomics: Effect of mutations

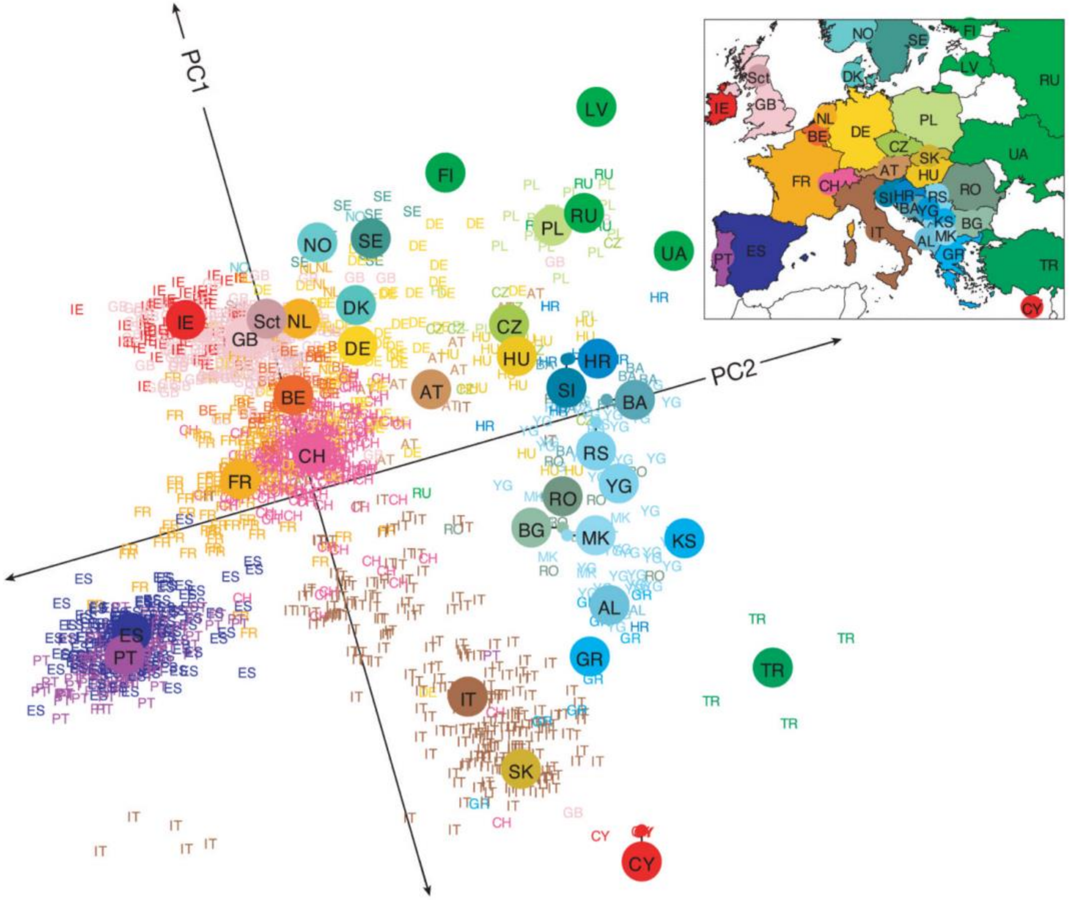
Transcript profiles of 1484 single gene deletions of *Saccharomyces cerevisiae* (baker's yeast)



Dimensionality reduction by UMAP to visualize physical and genetic interactions. Michael W. Dorrity et al., *Nature Comm.* 2020

Examples: Dimension reduction and clustering

- Population genetics



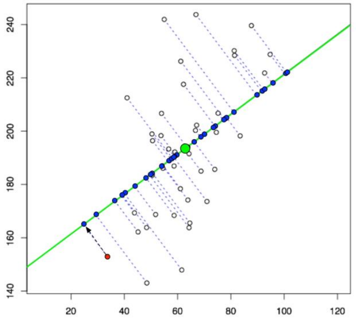
PCA

«Genes mirror geography within Europe» Novembre et al, *Nature*, 2008

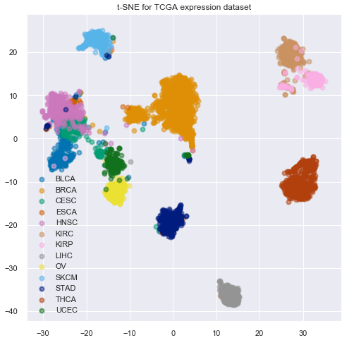
Methods for dimension reduction

Most widely used methods:

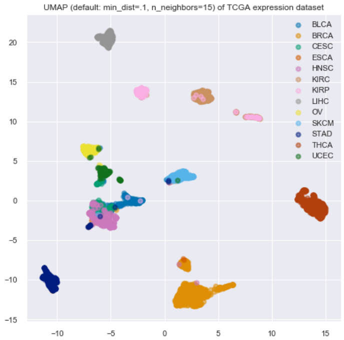
- Principal component analysis (PCA)
- t-distributed Stochastic Neighbor Embedding (tSNE) – check!
- Uniform Manifold Approximation and Projection (UMAP) – check!



PCA



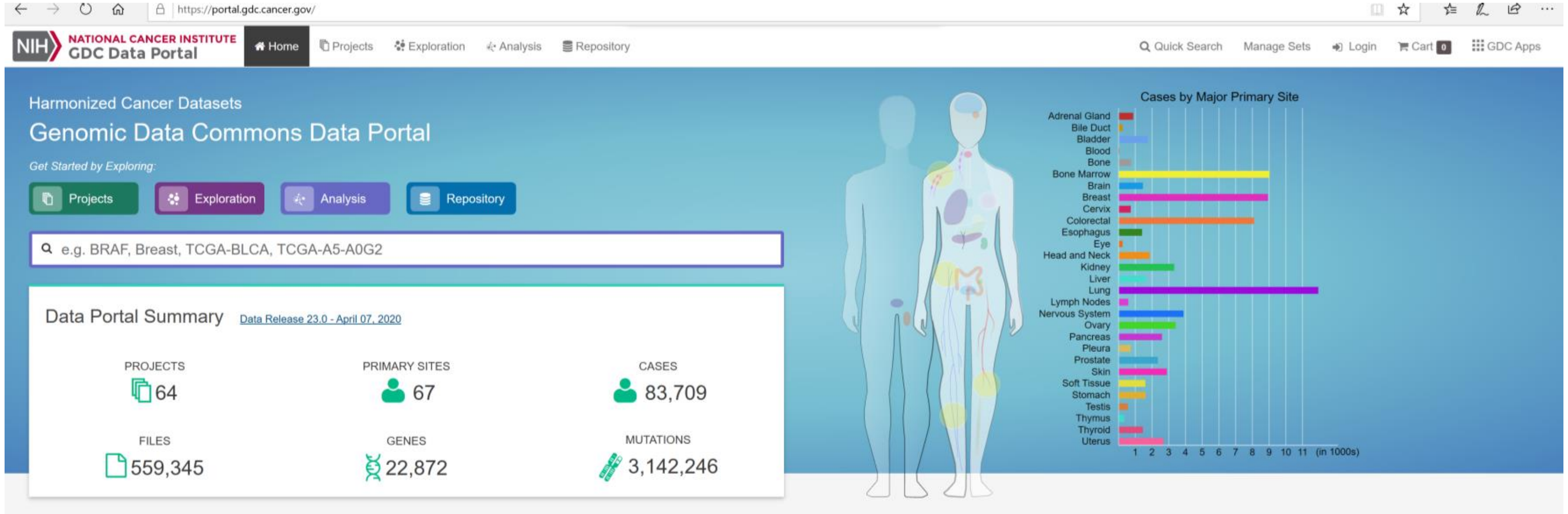
t-SNE



UMAP

Hands-on: gene expression data from several cancer types

- https://github.com/BoevaLab/Teaching
- Input: The Cancer Genome Atlas (TCGA) mRNA expression data



https://portal.gdc.cancer.gov/

Harmonized Cancer Datasets

Genomic Data Commons Data Portal

Get Started by Exploring:

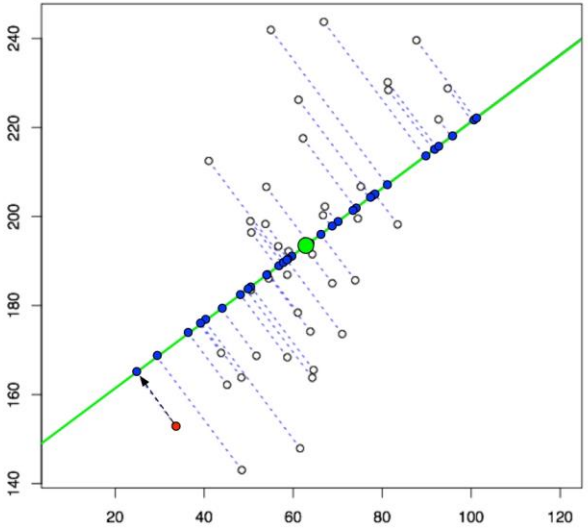
e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2

Data Portal Summary Data Release 23.0 - April 07, 2020

PROJECTS	PRIMARY SITES	CASES
64	67	83,709
FILES	GENES	MUTATIONS
559,345	22,872	3,142,246

Principal component analysis (PCA)

- **PCA**: an **orthogonal linear transformation** that transforms the data to a new coordinate system such that the greatest variance by some scalar projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, and so on.



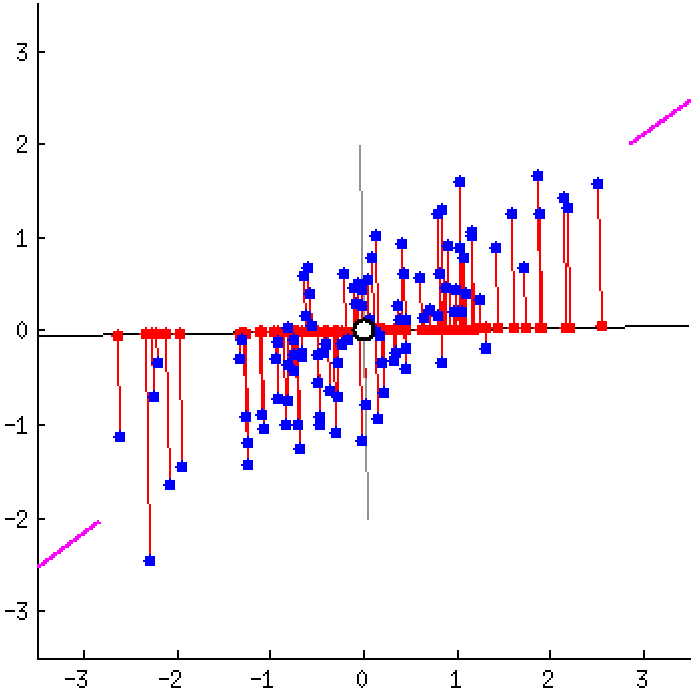
From https://liorpachter.wordpress.com/2014/05/26/what-is-principal-component-analysis/

Principal component analysis (PCA)

The **first** principal component:
the line that maximizes the variance (the average of the squared distances from the projected points (red dots) to the origin “**o**”).

The **second** principal component is calculated in the same way, with the condition that it is perpendicular to the first principal component and that it accounts for the next highest variance.

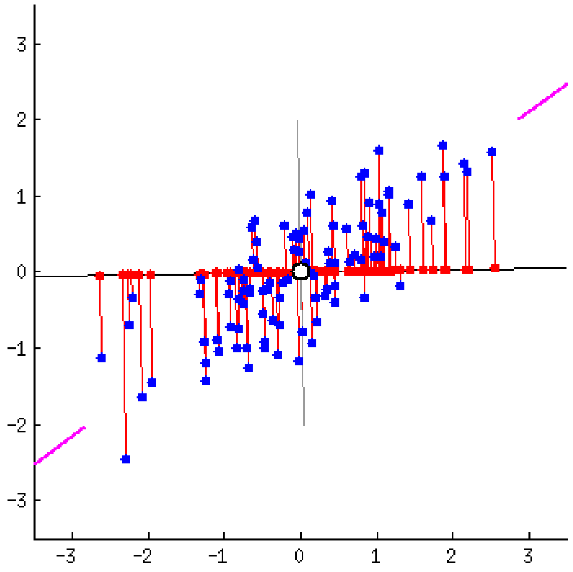
Etc.



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

Principal component analysis (PCA)

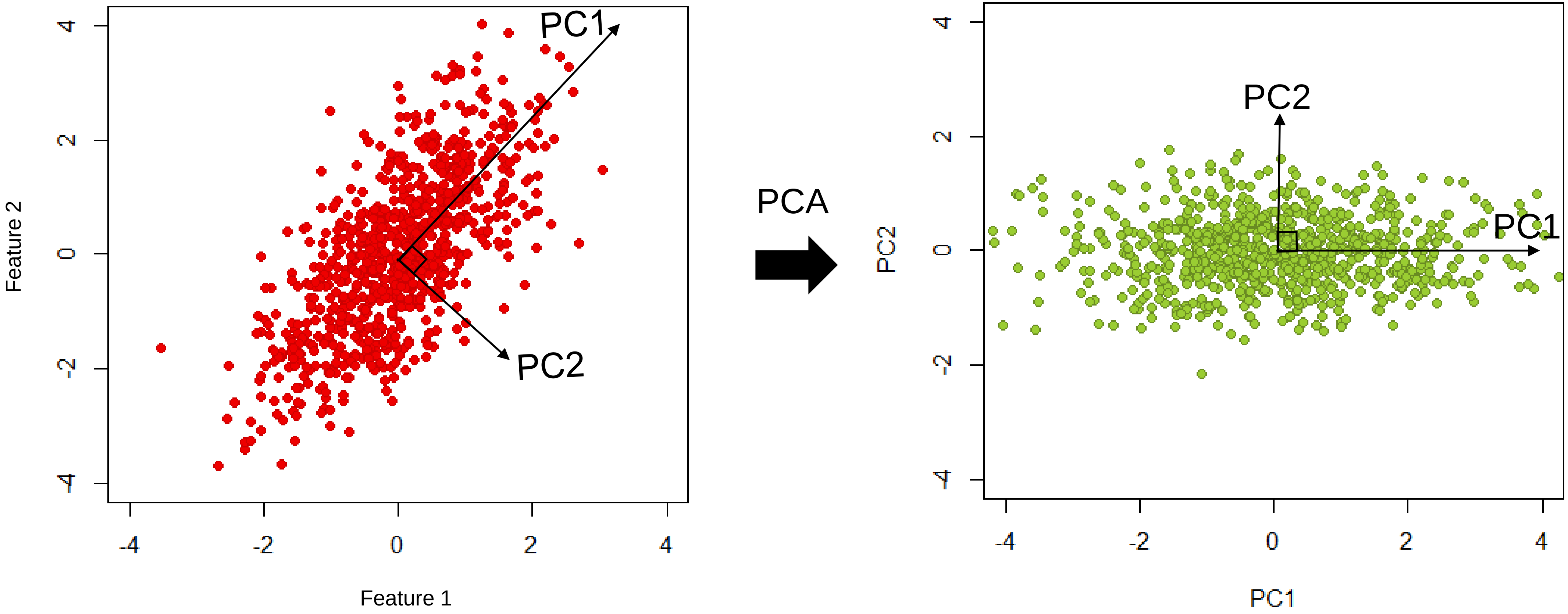
- Given *n* points in $\mathbb{R}^p$, principal components analysis consists of choosing a dimension $k < p$ and then finding the affine space of dimension *k* with the property that the squared distance of the points to their orthogonal projection onto the space is minimized.



Principal component analysis (PCA)

Generally, one standardizes the data along each dimension before applying PCA.

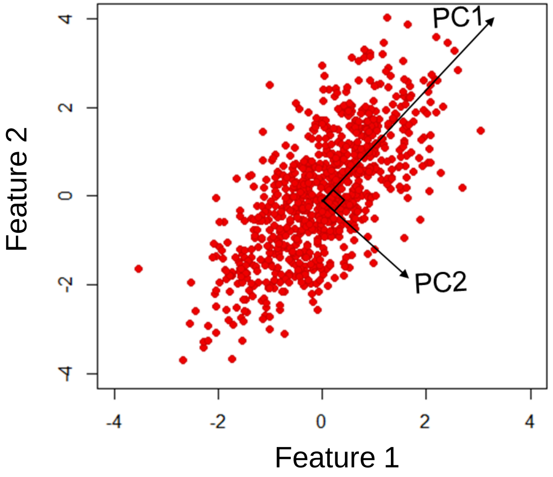
PCA: an orthogonal linear transformation



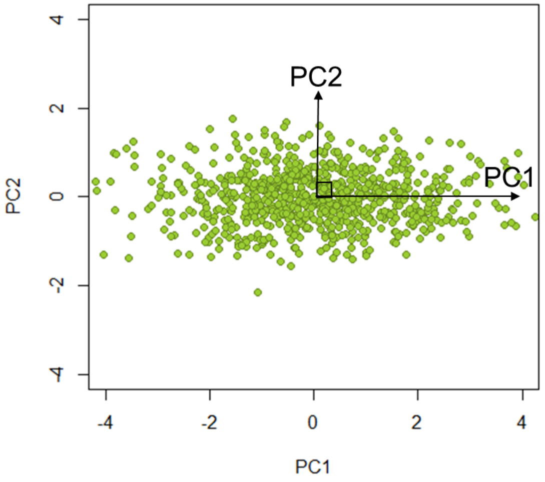
Principal component analysis (PCA)

Generally, one standardizes the data along each dimension before applying PCA.

Q: How will look the PCA transformation of a PCA?



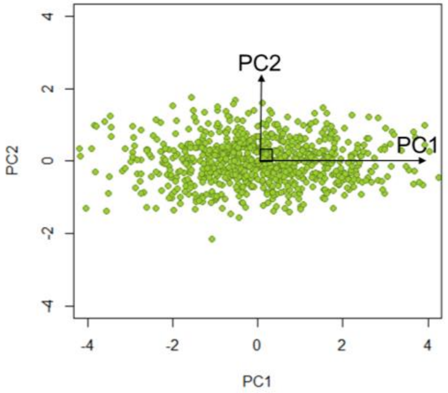
PCA



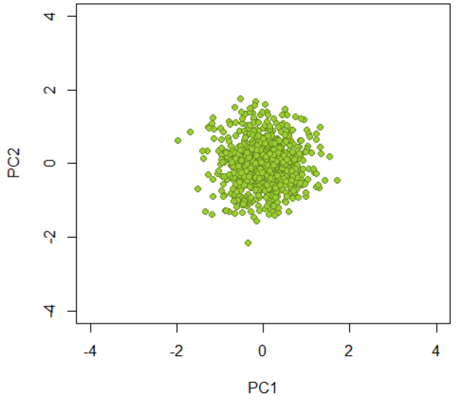
?

PCA

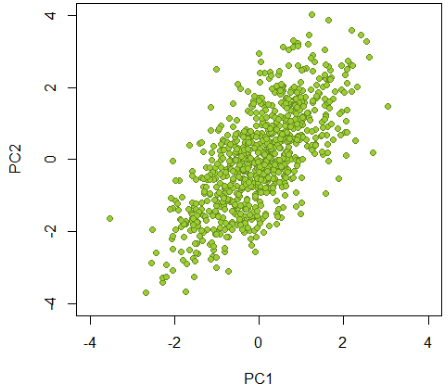
(1) the same



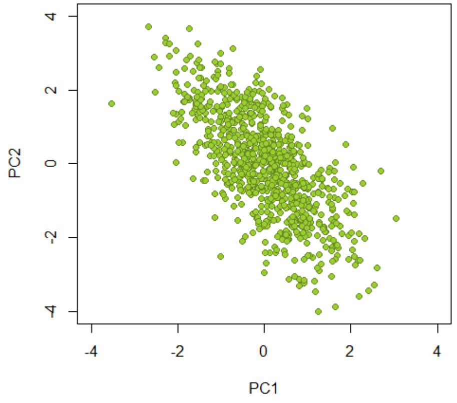
(2) the “ball”



(3) as original set



(4) Inverted original



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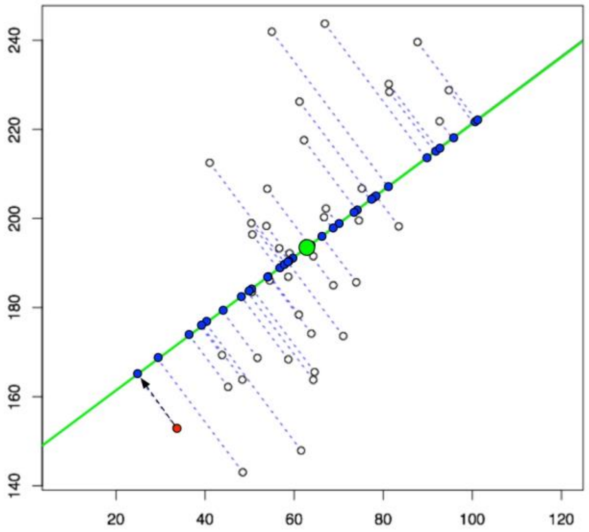
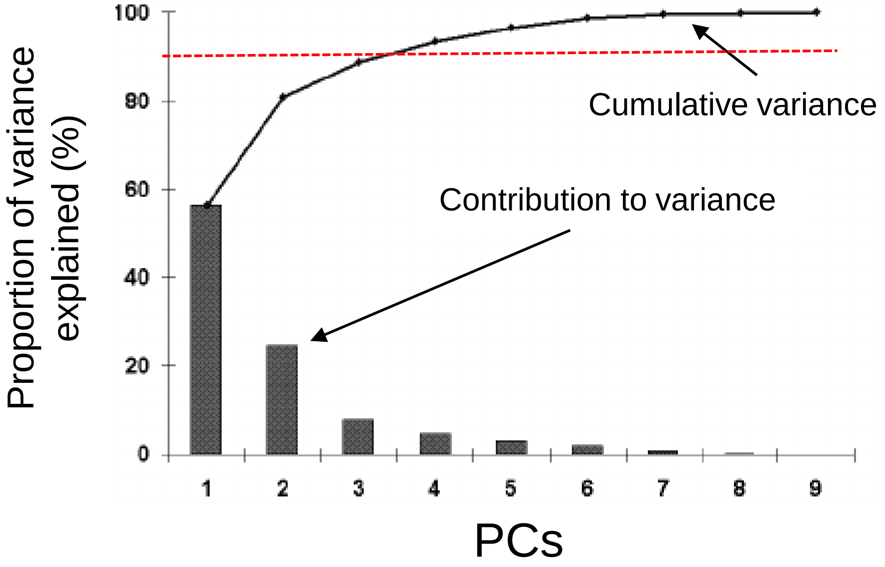


Given n points in R^p, principal components analysis consists of choosing a dimension $k < p$ and then finding the affine space of dimension k with the property that the squared distance of the points to their orthogonal projection onto the space is minimized.

From https://liorpachter.wordpress.com/2014/05/26/what-is-principal-component-analysis/

Principal component analysis (PCA)

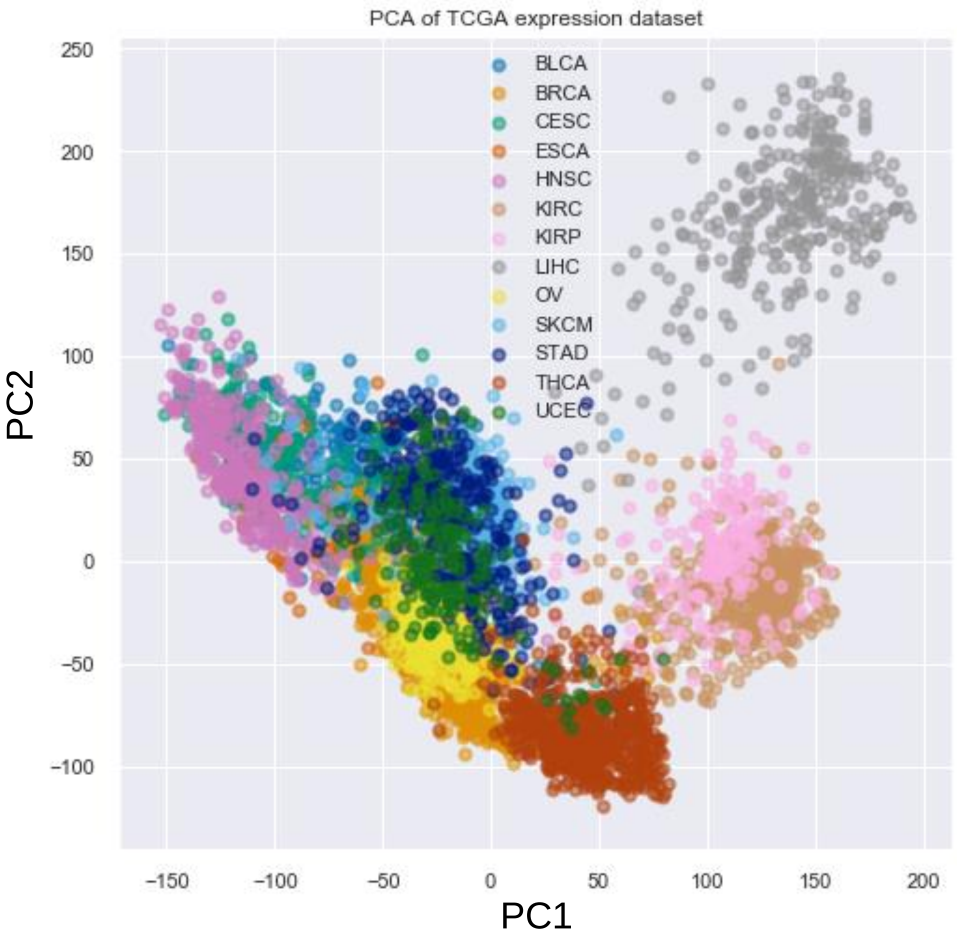
- PCA is a deterministic method, the only parameter one can choose is *k* for how many principal components to keep.
- Choosing *k* based on the proportion of the variance explained.



$Var\_explained_k = \frac{\lambda_k}{\sum_i \lambda_i}$, where λ_k is k^{th} eigenvalue.

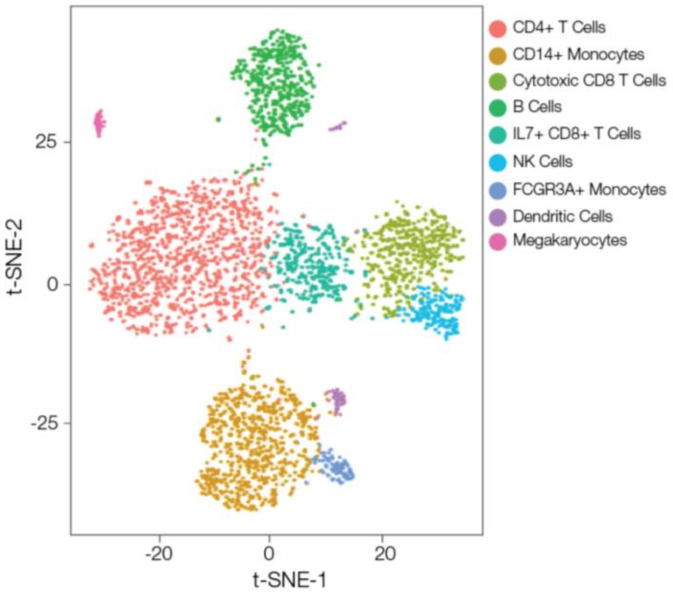
Let’s go to the Jupiter Notebook to see the result of PCA on out toy data set

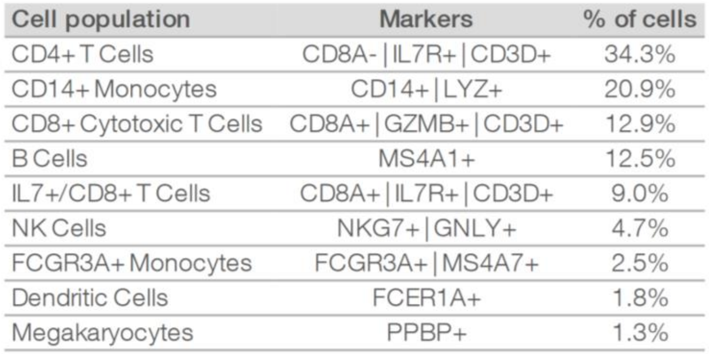
- https://github.com/BoevaLab/Teaching
- Input: The Cancer Genome Atlas (TCGA) mRNA expression data



t-distributed Stochastic Neighbor Embedding (tSNE)

- tSNE: nonlinear dimensionality reduction technique, converts similarities between data points to joint probabilities and tries to minimize the Kullback-Leibler divergence between the joint probabilities of the low-dimensional embedding and the high-dimensional data.





Cell population	Markers	% of cells
CD4+ T Cells	CD8A- | IL7R+ | CD3D+	34.3%
CD14+ Monocytes	CD14+ | LYZ+	20.9%
CD8+ Cytotoxic T Cells	CD8A+ | GZMB+ | CD3D+	12.9%
B Cells	MS4A1+	12.5%
IL7+/CD8+ T Cells	CD8A+ | IL7R+ | CD3D+	9.0%
NK Cells	NKG7+ | GNLY+	4.7%
FCGR3A+ Monocytes	FCGR3A+ | MS4A7+	2.5%
Dendritic Cells	FCER1A+	1.8%
Megakaryocytes	PPBP+	1.3%

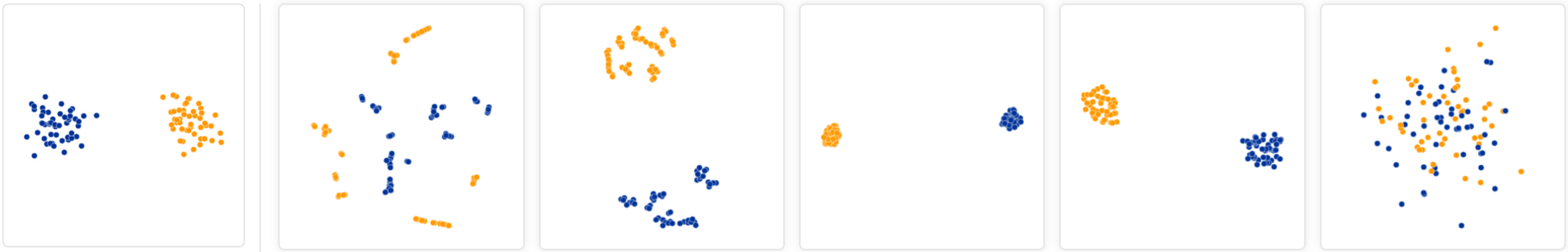
- t-SNE has a cost function that is not convex, i.e., with different initializations we can get different results.

First introduced by van der Maaten & Hinton paper from 2008.

t-distributed Stochastic Neighbor Embedding (tSNE)

tSNE method’s the most important parameter:

- **Perplexity**, scikit-learn recommended range: [5, 50], default: 30



Original

Perplexity: 2
Step: 5,000

Perplexity: 5
Step: 5,000

Perplexity: 30
Step: 5,000

Perplexity: 50
Step: 5,000

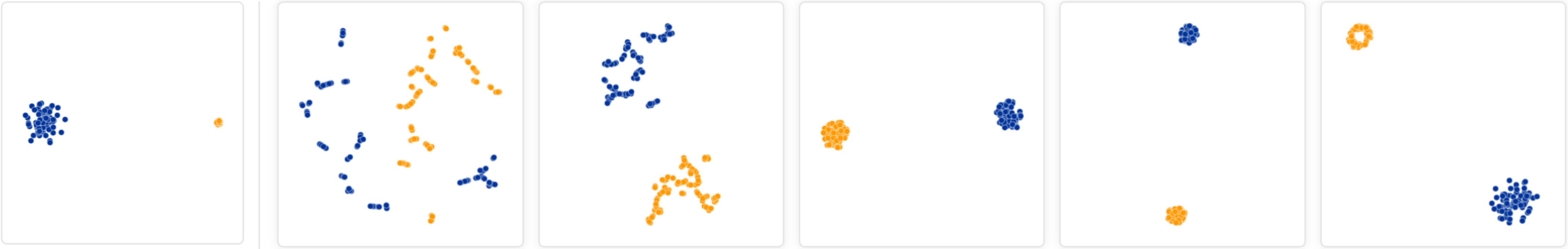
Perplexity: 100
Step: 5,000

From https://distill.pub/2016/misread-tsne/

t-distributed Stochastic Neighbor Embedding (tSNE)

tSNE method’s particularities:

- Cluster sizes in a t-SNE plot mean nothing



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Perplexity: 2
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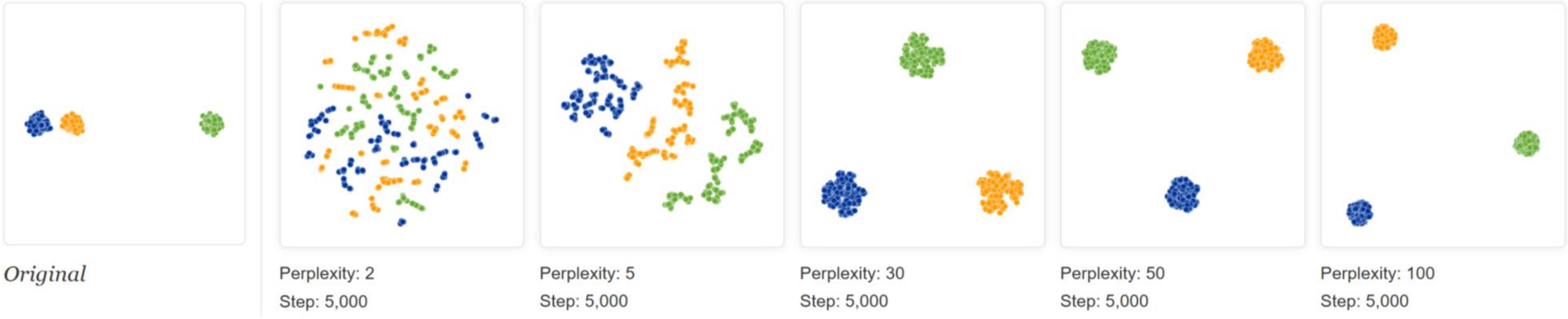


Original

Perplexity: 2
Step: 5,000

Perplexity: 5
Step: 5,000

Perplexity: 30
Step: 5,000

Perplexity: 50
Step: 5,000

Perplexity: 100
Step: 5,000

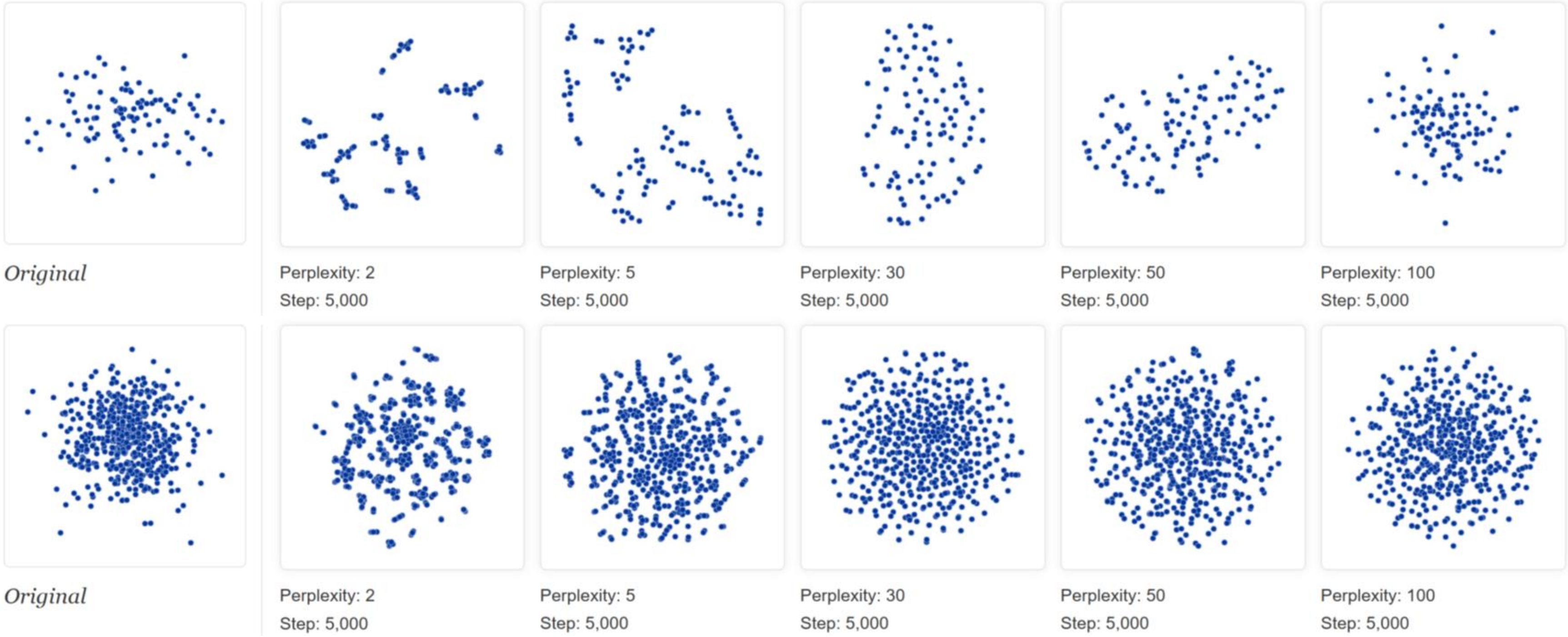
200 points/cluster

From https://distill.pub/2016/misread-tsne/

t-distributed Stochastic Neighbor Embedding (tSNE)

tSNE method's particularities:

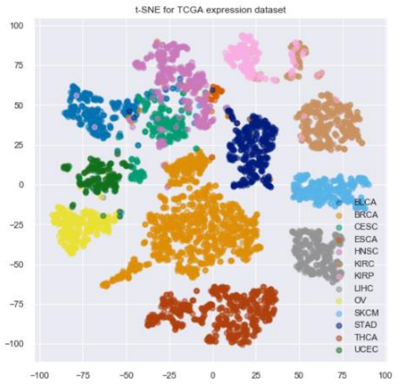
- Cluster sizes in a t-SNE plot mean nothing
- Distances between clusters might not mean anything
- Random noise does not always look random, and sometimes one can see some shapes



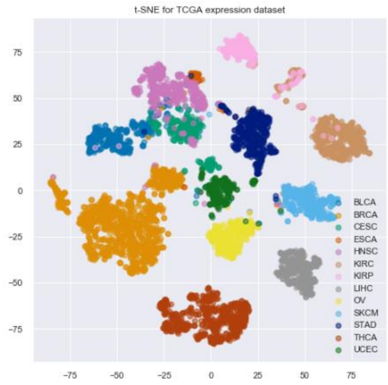
From https://distill.pub/2016/misread-tsne/

What would you choose as the best value of perplexity in this example?

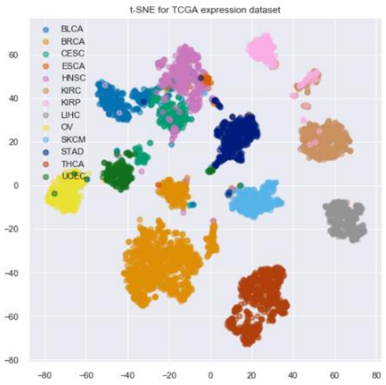
●	BLCA	Bladder
●	BRCA	Breast
●	CESC	Cervical squamous cell
●	ESCA	Esophageal
●	HNSC	Head and Neck squamous cell
●	KIRC	Kidney clear cell
●	KIRP	Kidney papillary
●	LIHC	Liver
●	OV	Ovarian
●	SKCM	Skin Cutaneous Melanoma
●	STAD	Stomach
●	THCA	Thyroid
●	UCEC	Uterine Corpus Endometrial



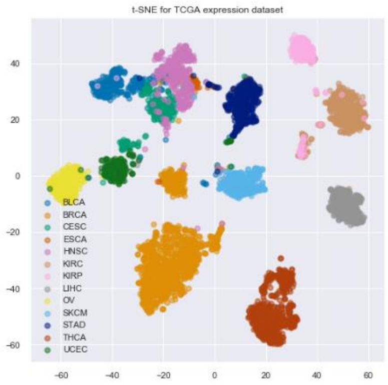
Perplexity: 10



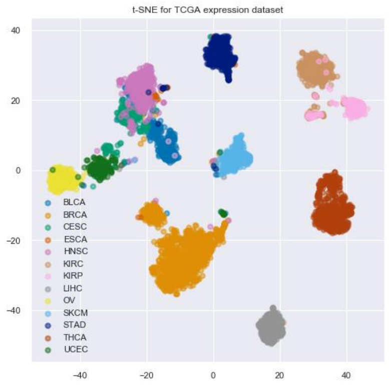
20



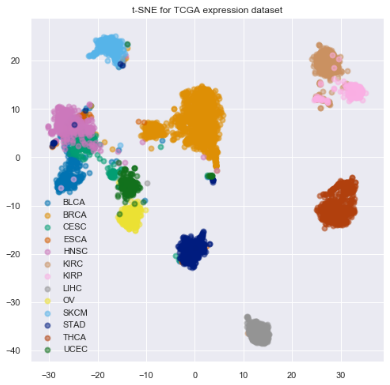
30



50



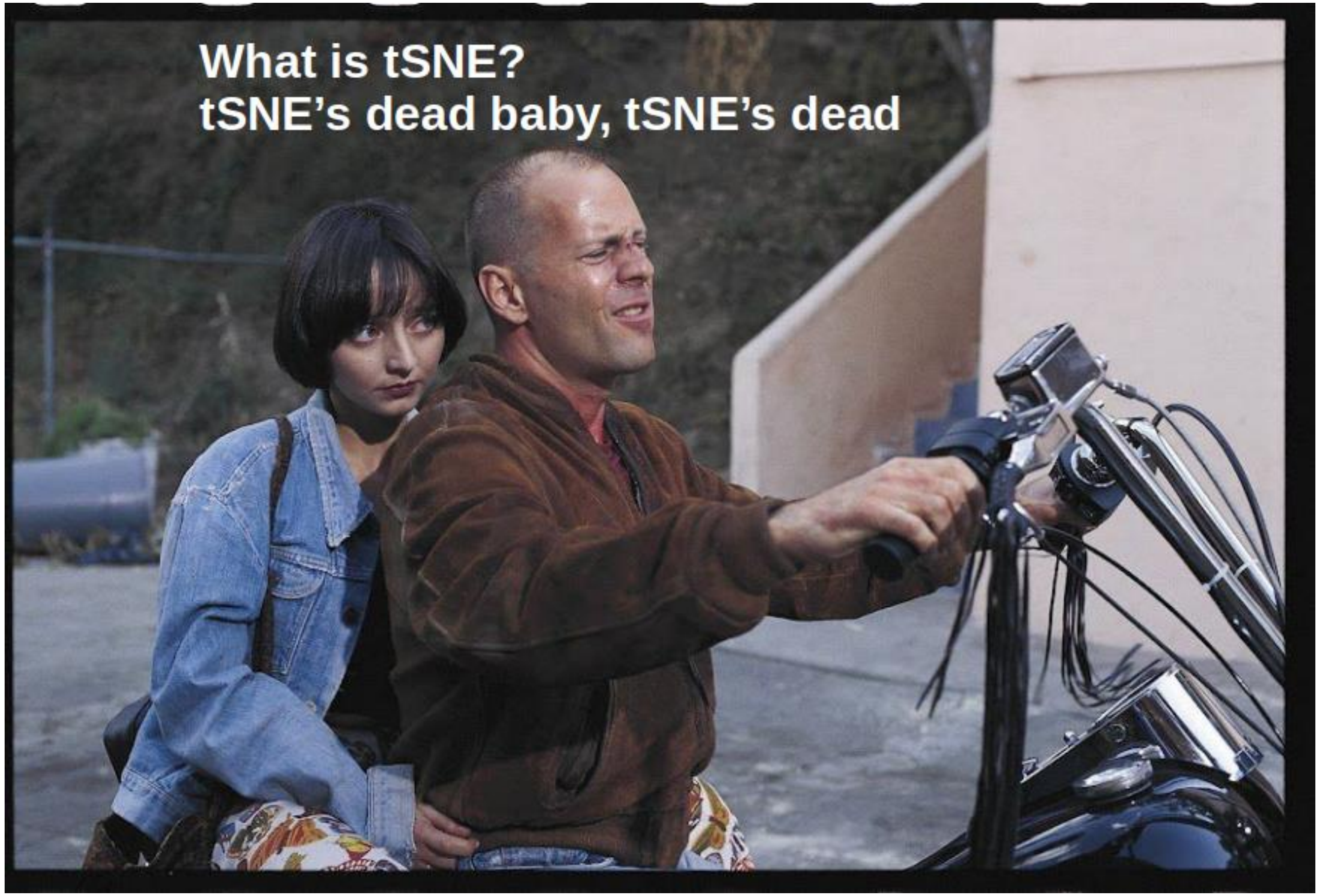
100



200

Cancer type abbreviations: https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations

Uniform Manifold Approximation and Projection (UMAP)

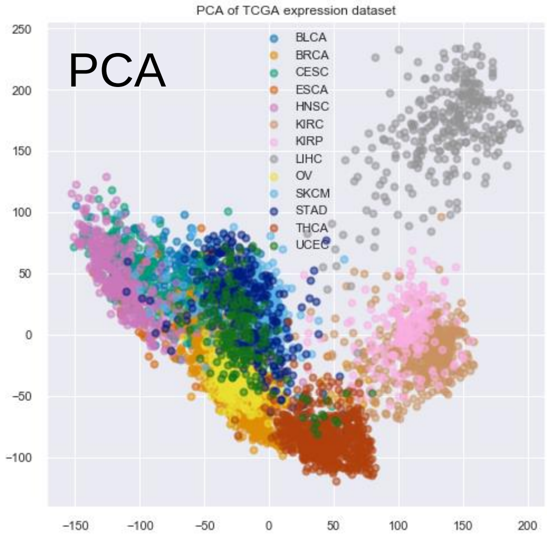


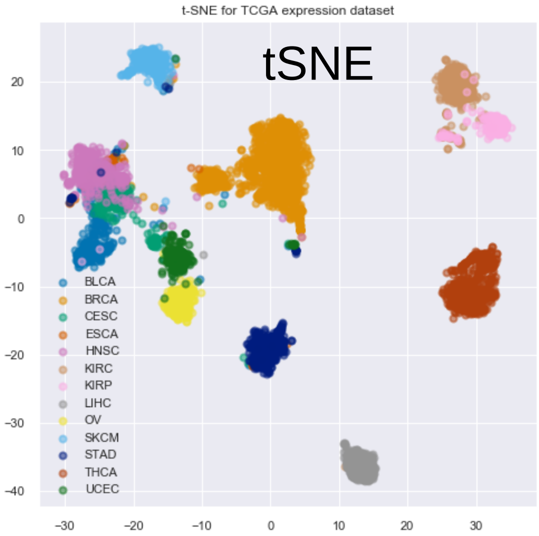
From https://towardsdatascience.com/how-exactly-umap-works-13e3040e1668

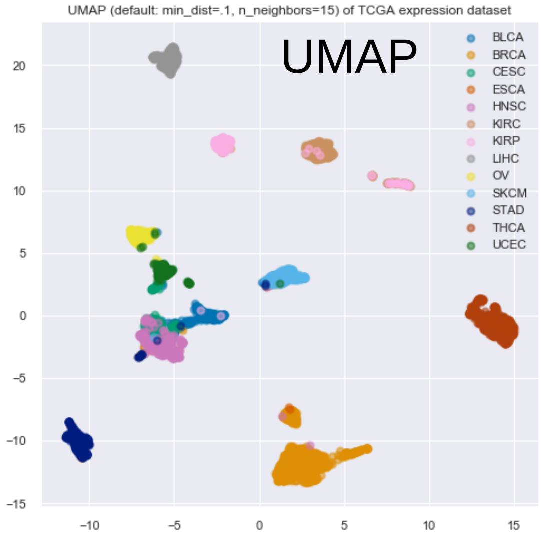
Uniform Manifold Approximation and Projection (UMAP)

UMAP: nonlinear dimensionality reduction technique. Idea is similar to tSNE, but

- Much faster
- Not limited to the first 2-3 dimensions
- Uses binary cross-entropy as a cost function instead of the KL-divergence
- Better preserves global structure
- Uses the number of nearest neighbors and min-dist correction instead of perplexity







First introduced by McInnes, L, Healy, J, ArXiv e-prints 1802.03426, 2018

Uniform Manifold Approximation and Projection (UMAP)

UMAP’s the most important parameters:

min_dist, recommended range: [0.1, 1], default: 0.1

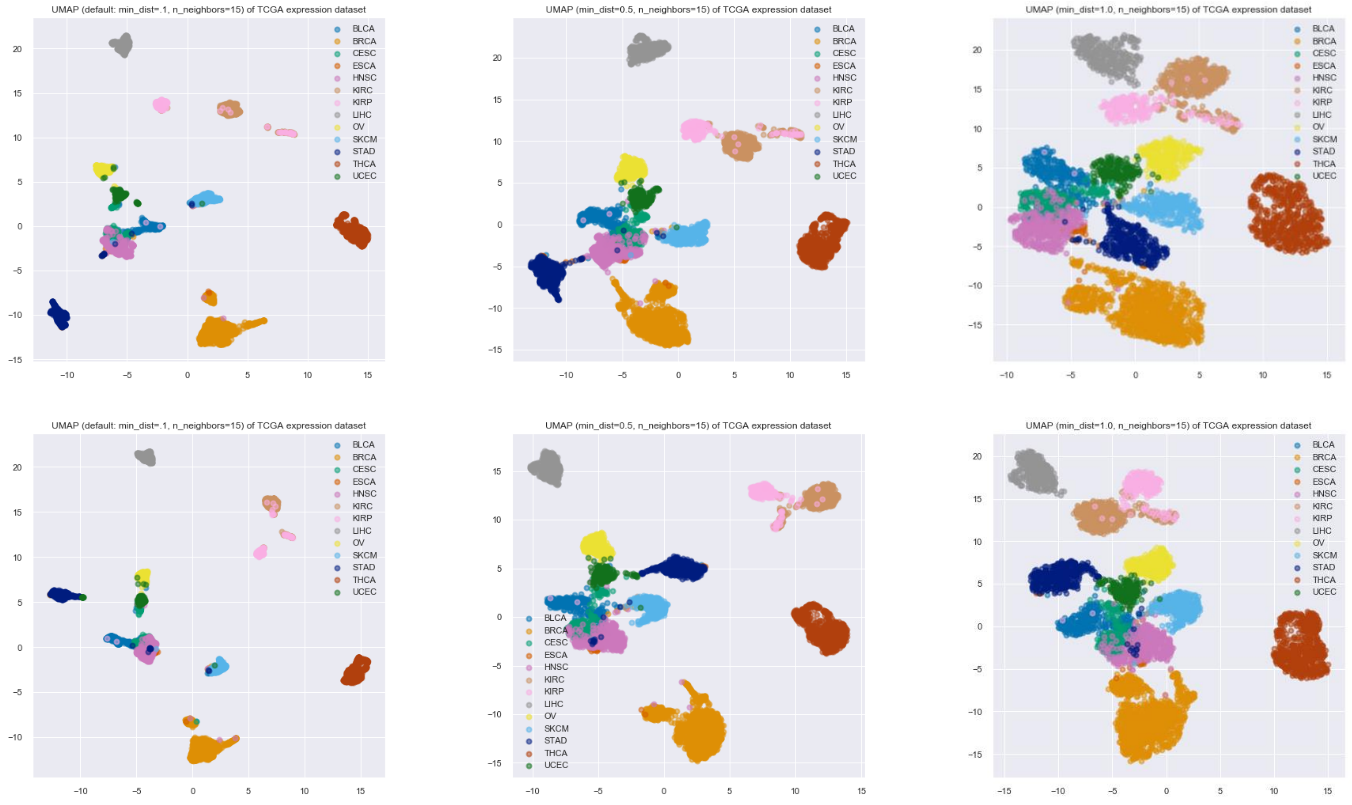
- Controls how tightly the embedding is allowed compress points together. Larger values ensure embedded points are more evenly distributed, while smaller values allow the algorithm to optimise more accurately with regard to local structure. Sensible values are in the range 0.001 to 0.5, with 0.1 being a reasonable default.

n_neighbors, recommended range: [2, 100], default: 15

- Determines the number of neighboring points used in local approximations of manifold structure. Larger values will result in more global structure being preserved at the loss of detailed local structure. In general, this parameter should often be in the range 5 to 50, with a choice of 10 to 15 being a sensible default.

From https://distill.pub/2016/misread-tsne/

What would you choose as the best value of **min_dist** and **n_neighbors** for this example?



n_neighbors: 15

n_neighbors: 50

Min_dist: 0.1

0.5

1

● BLCA	Bladder
● BRCA	Breast
● CESC	Cervical squamous cell
● ESCA	Esophageal
● HNSC	Head and Neck squamous cell
● KIRC	Kidney clear cell
● KIRP	Kidney papillary
● LIHC	Liver
● OV	Ovarian
● SKCM	Skin Cutaneous Melanoma
● STAD	Stomach
● THCA	Thyroid
● UCEC	Uterine Corpus Endometrial

Cancer type abbreviations: https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations

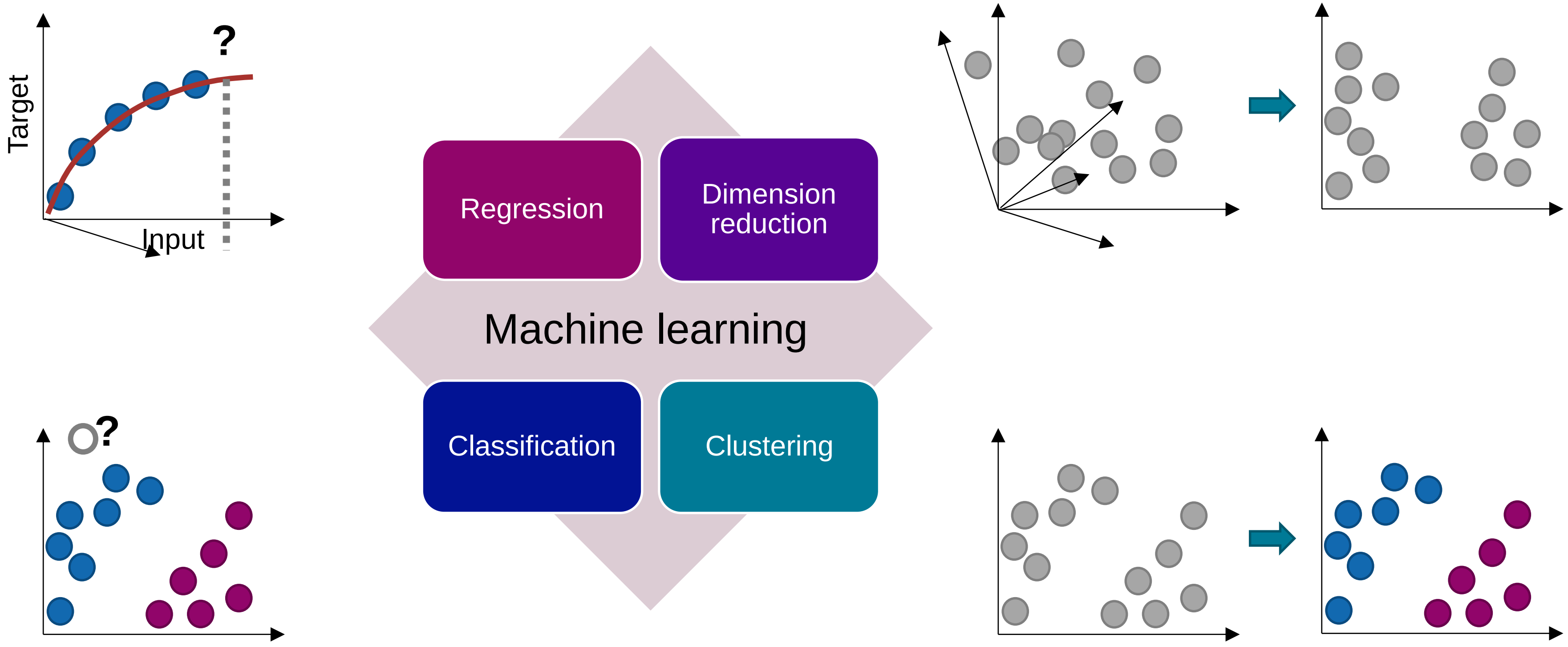
Take home message: dimensionality reduction

- One should often try different methods with different parameters to choose the one that fits the best to our expectations from the data

- Random noise does not always look random, and sometimes one can see shapes

- Projections on the first *n* principal components can be used as input (instead of the original *X*) to other dimension reduction methods such as tSNE to reduce execution time.

Map of classical machine learning methods



Regression

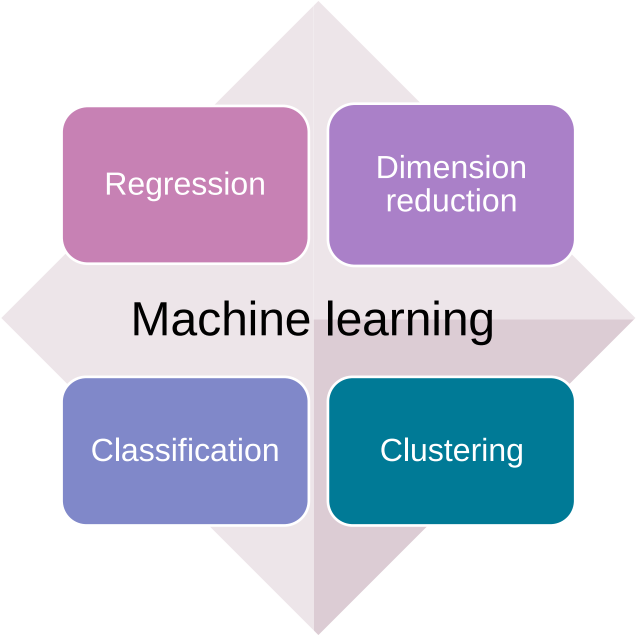
Dimension reduction

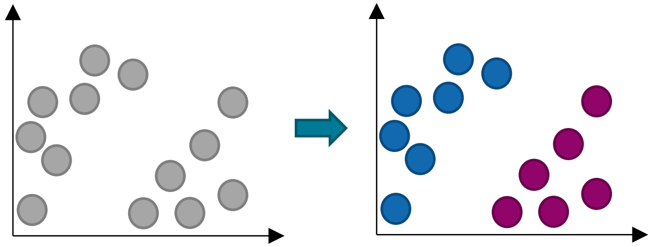
Machine learning

Classification

Clustering

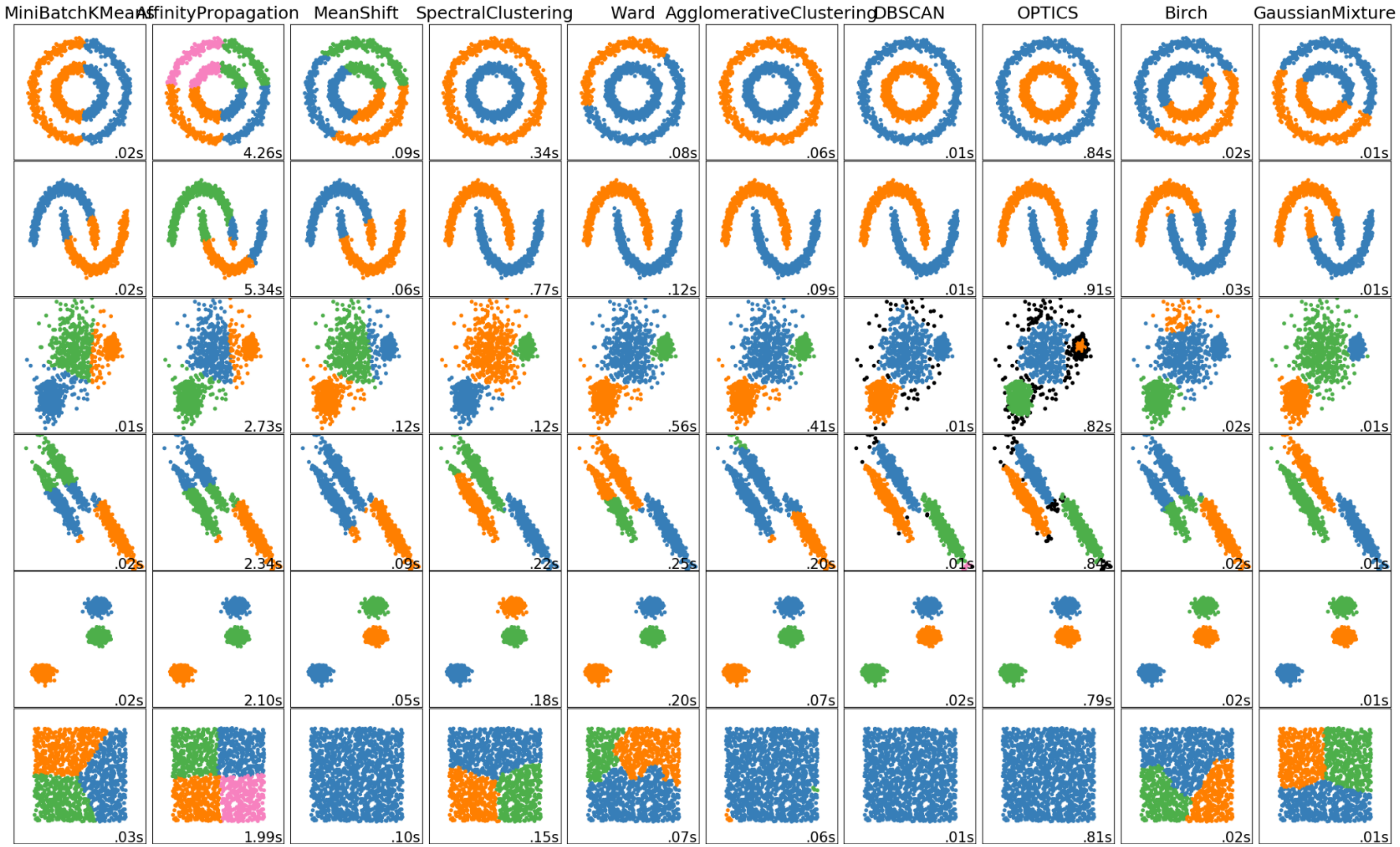
Map of classical machine learning methods





Clustering methods

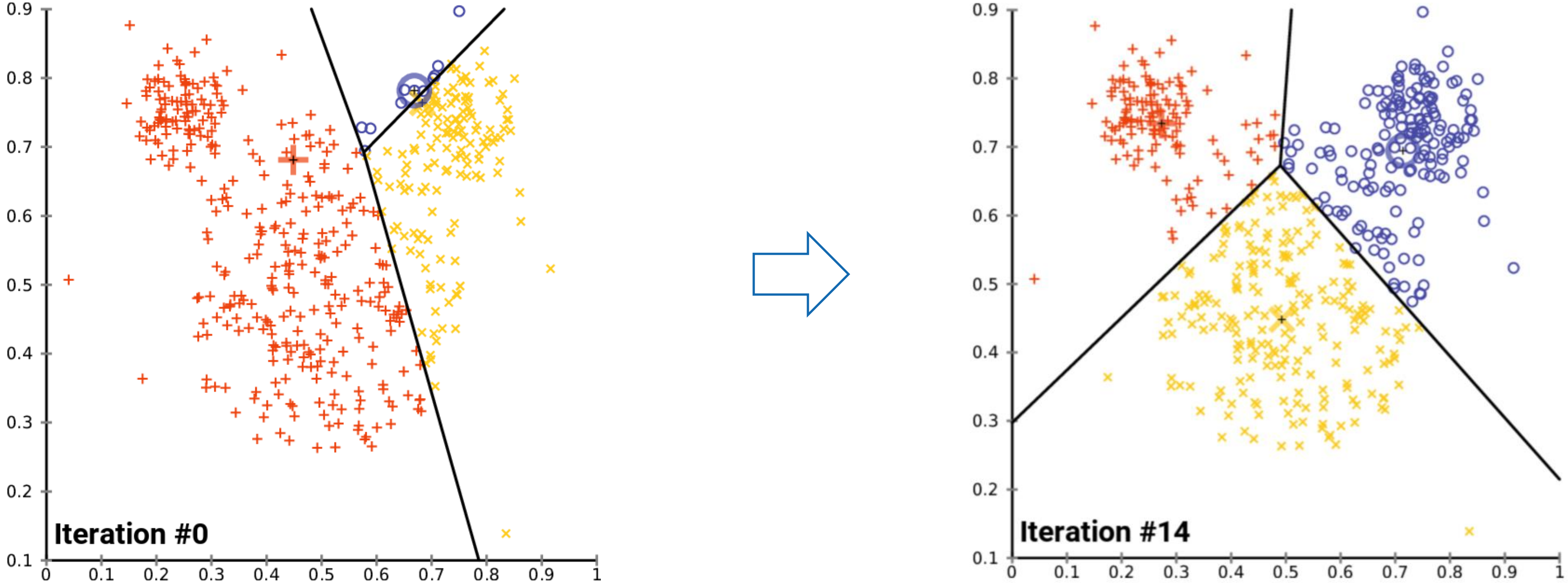
- K-means
- Gaussian mixture models
- Spectral clustering
- Hierarchical clustering



From https://scikit-learn.org/stable/modules/clustering.html

K-means

- k-means: aims to partition n observations into k clusters in which each observation belongs to the cluster with the nearest mean (cluster centers)

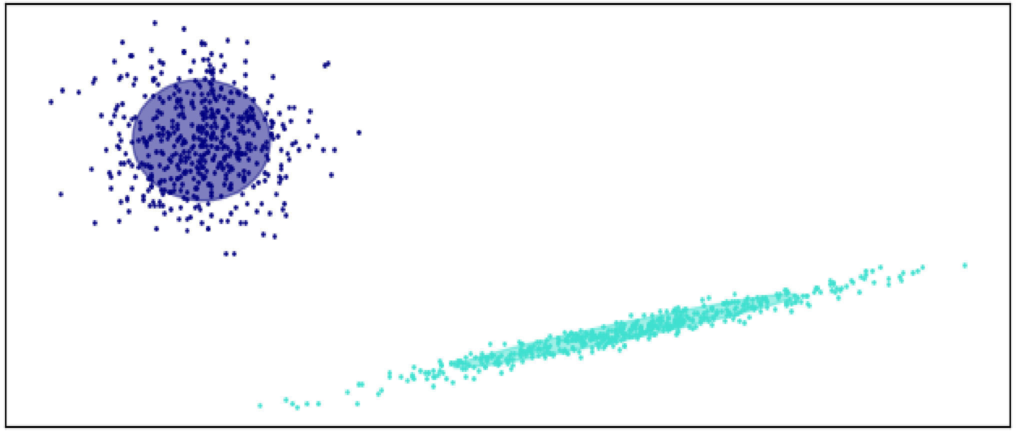


- k-means clustering tends to find clusters of comparable spatial extent

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

Gaussian mixture models (GMM)

GMM: probabilistic model that assumes all the data points are generated from a mixture of a finite number of Gaussian distributions with unknown parameters



From https://scikit-learn.org/stable/modules/mixture.html

Gaussian mixture models (GMM)

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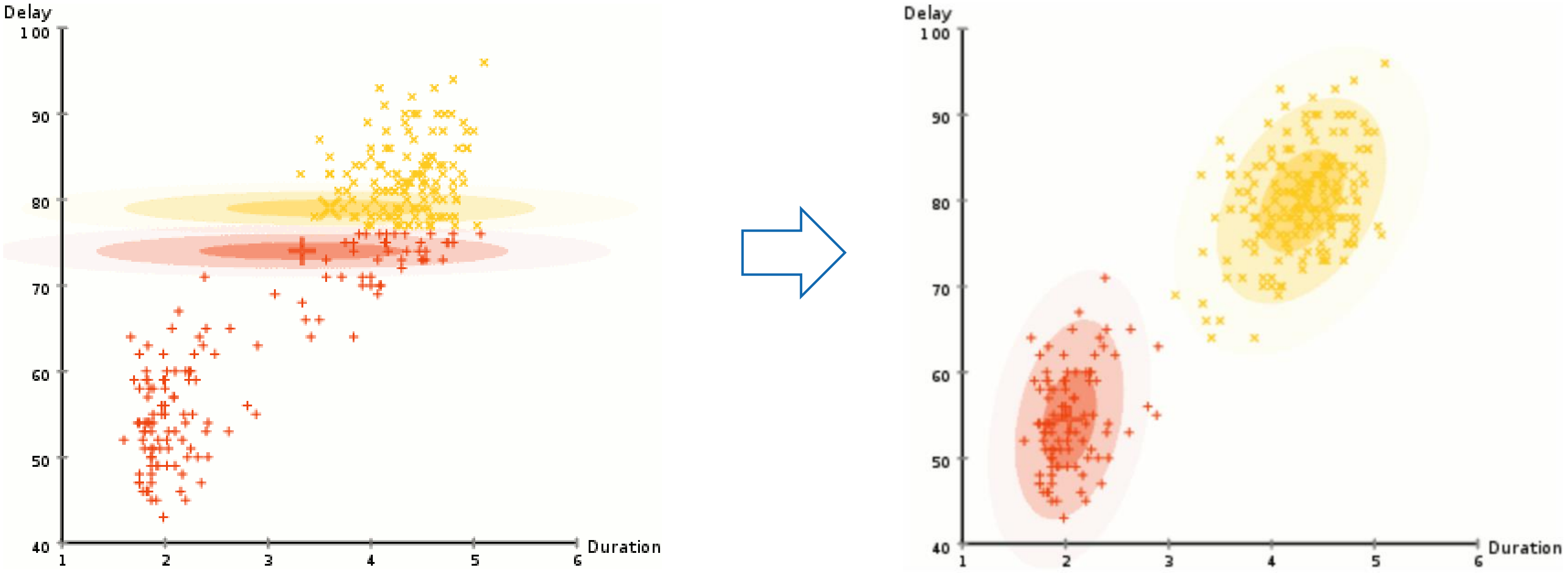
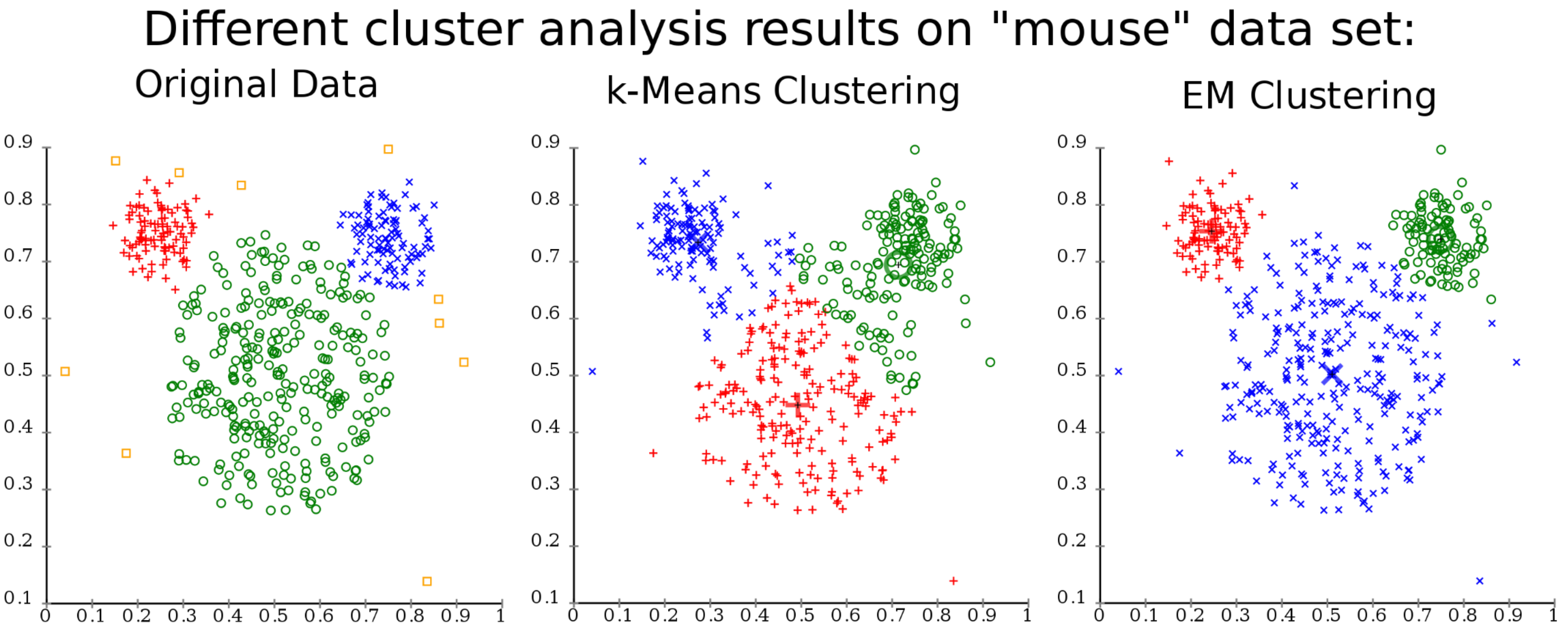


From http://primo.ai/index.php?title=Expectation%E2%80%93Maximization_(EM)_Clustering_using_Gaussian_Mixture_Models_(GMM) k-means clustering tends to find clusters of comparable spatial extent, while the GMM expectation-maximization mechanism allows clusters to have different shapes.



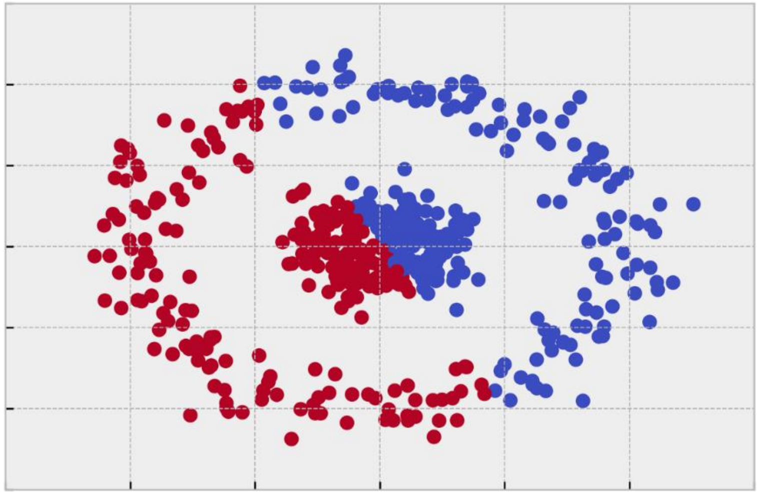
Also, GMMs support mixed membership

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

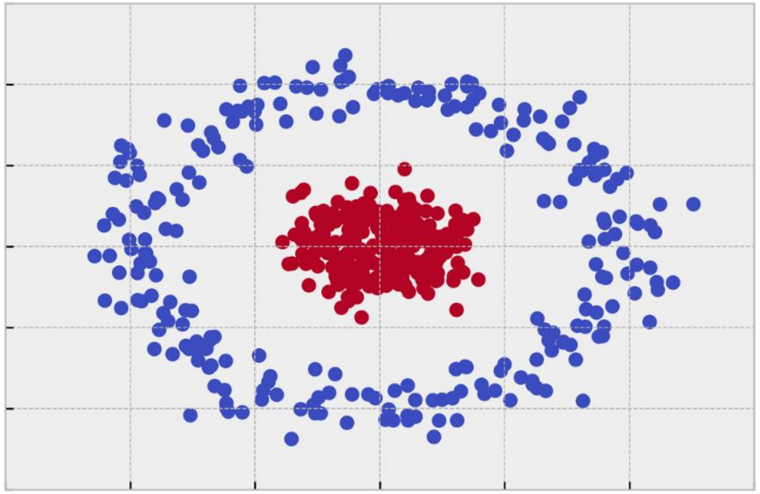
Spectral clustering: relies on the assumption that “close” points should belong in the same cluster

- Spectral clustering: uses a standard clustering method (e.g., k-means) on relevant eigenvectors of a Laplacian matrix L of symmetric data similarity matrix A. – can also use k-nearest neighbors graphs for construction of A.
- $L := D - A$, where D is the diagonal matrix, such as $D_{ii} = \sum_j A_{ij}$.

K-Means Circles



Spectral Circles

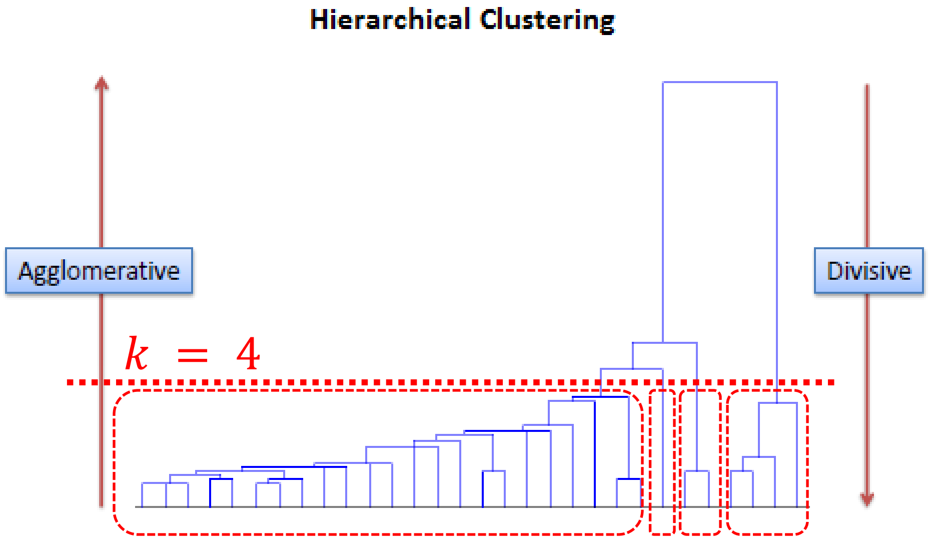


From https://towardsdatascience.com/spectral-clustering-aba2640c0d5b

Hierarchical clustering

- Hierarchical clustering: family of clustering algorithms that build nested clusters by merging or splitting them successively. This hierarchy of clusters is represented as a tree (or dendrogram).

- **Agglomerative approach**: This is a "bottom-up" approach: each observation starts in its own cluster, and pairs of clusters are merged as one moves up the hierarchy.
- **Divisive approach**: This is a "top-down" approach: all observations start in one cluster, and splits are performed recursively as one moves down the hierarchy.

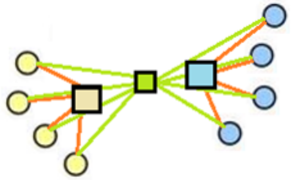


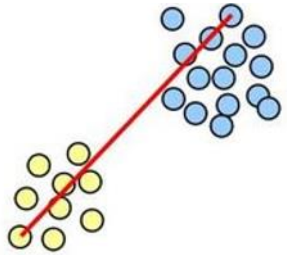
From https://www.saedsayad.com/clustering_hierarchical.htm

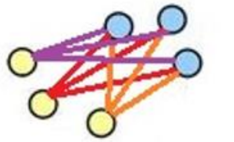
Hierarchical clustering: important parameters

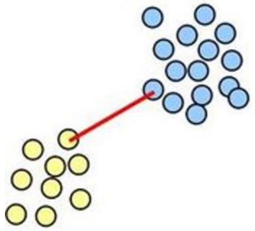
You choose:

- **Distance metric** (between observations): Euclidean, Squared Euclidean, Manhattan, Maximum
- **Linkage criterium** (distance between sets of observations):
  - “**Ward**” minimizes the sum of squared differences within all clusters (within-cluster variance). It is a variance-minimizing approach and in this sense is similar to the k-means objective function but tackled with an agglomerative hierarchical approach.
  - “**Maximum**” or “complete linkage” minimizes the maximum distance between observations of pairs of clusters.
  - “**Average linkage**” minimizes the average of the distances between all observations of pairs of clusters.
  - “**Single linkage**” minimizes the distance between the closest observations of pairs of clusters.





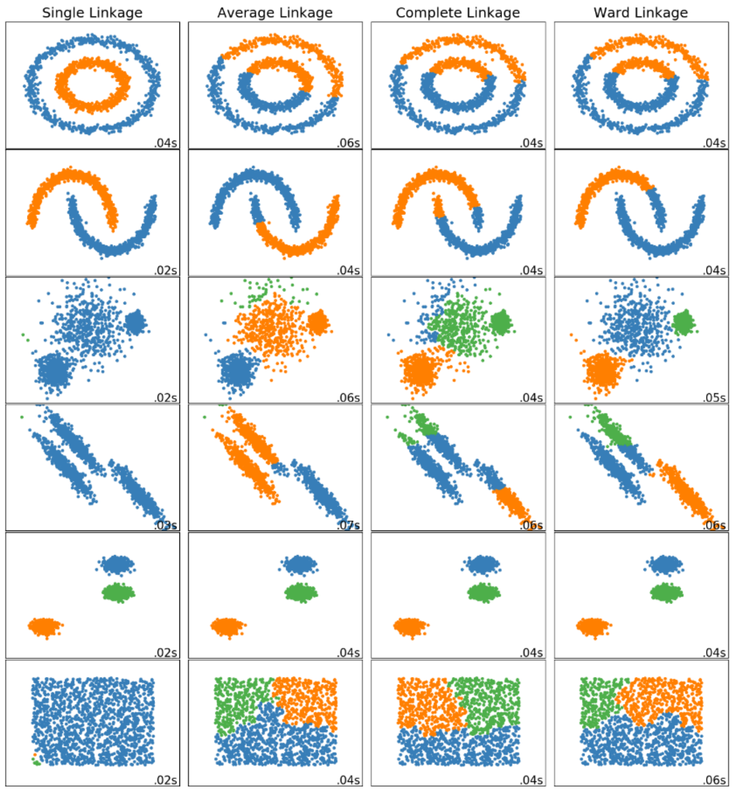


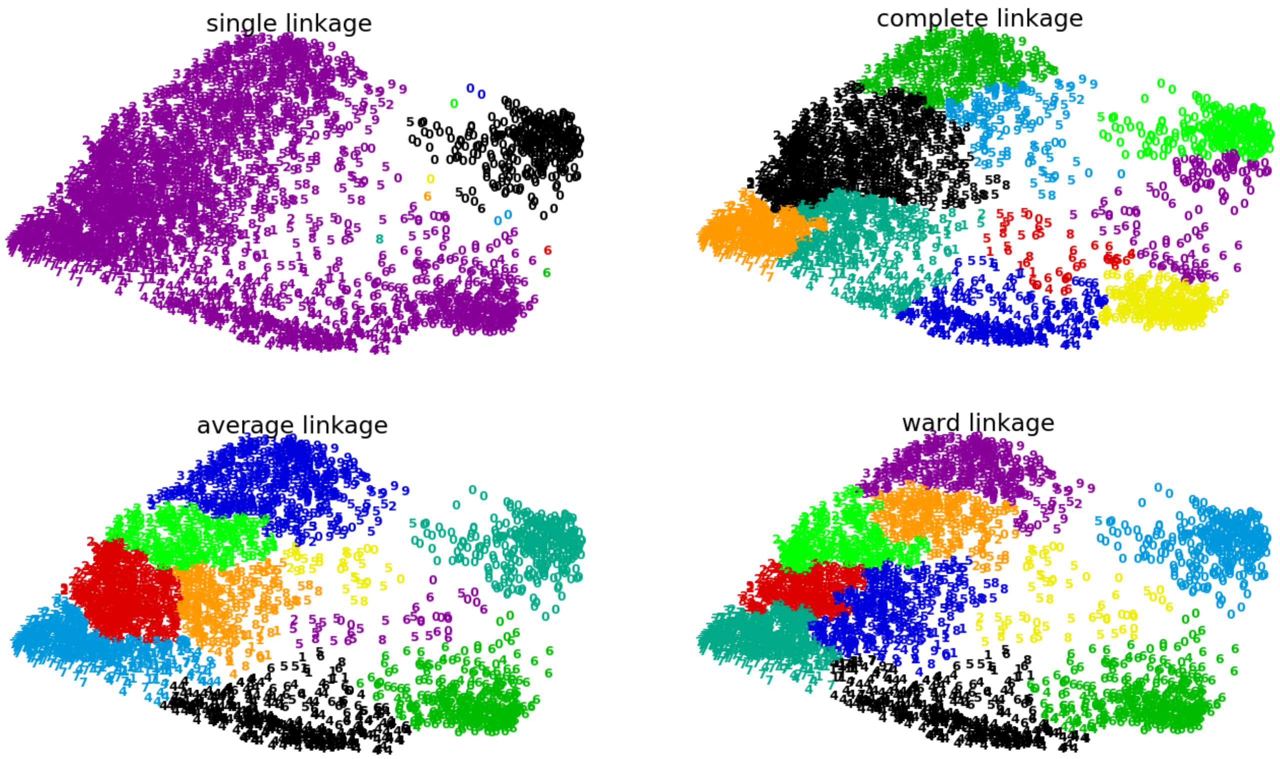


https://blog.tdwi.eu/hierarchical-clustering-in-python/

Hierarchical clustering: important parameters

- **Linkage criterium** (distance between sets of observations):



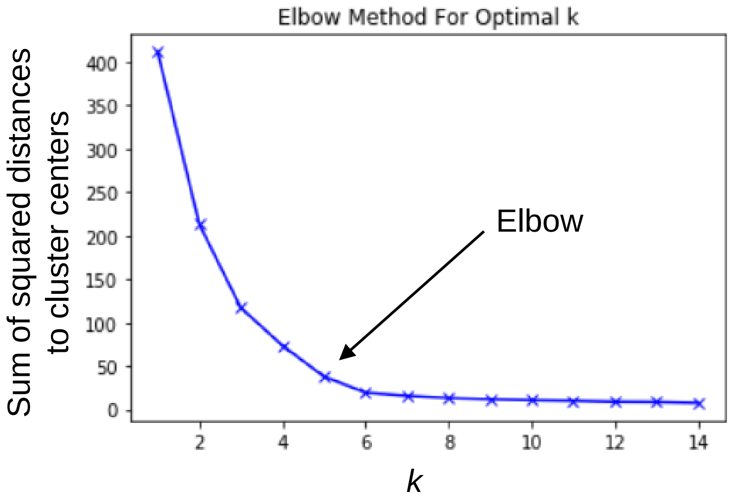


“Ward” gives the most regular cluster sizes

From https://scikit-learn.org/stable/modules/clustering.html#hierarchical-clustering
https://scikit-learn.org/stable/auto_examples/cluster/plot_digits_linkage.html

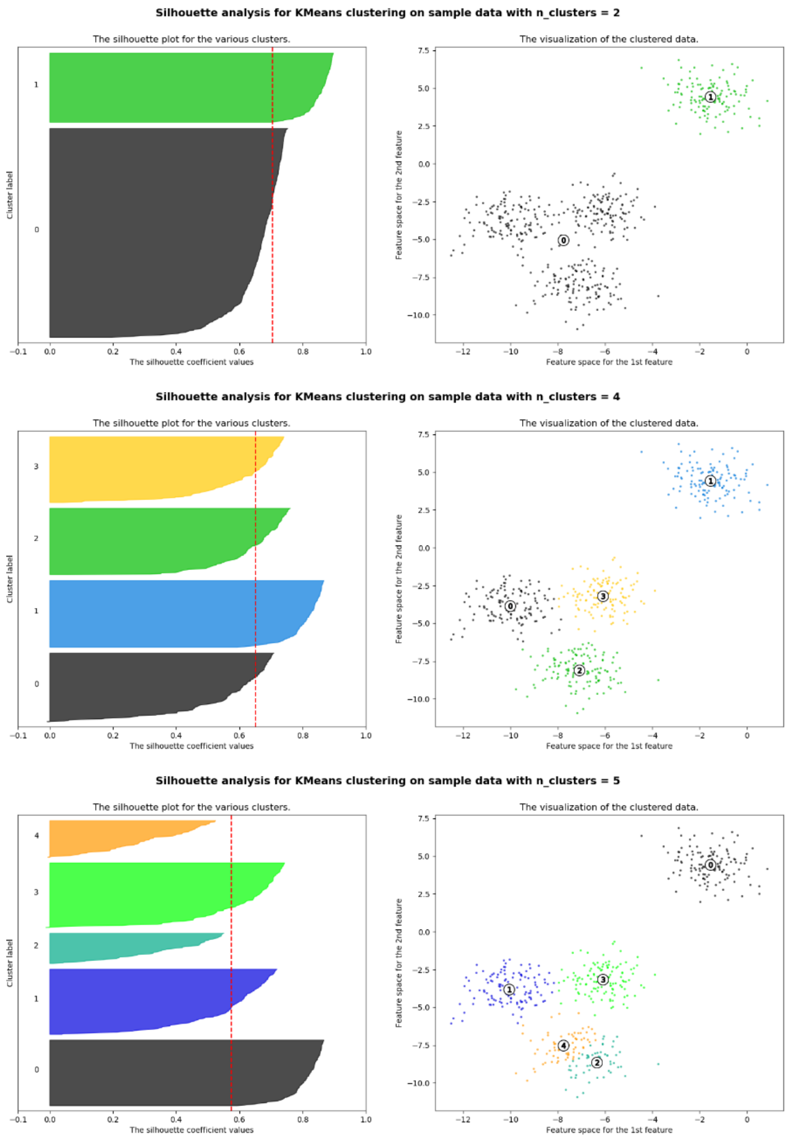
How to choose the best number of clusters k?

- Elbow method (for sum of squared distances to cluster centers)
  - How find the “elbow”? By eye or https://github.com/arvkevi/kneed
- Silhouette analysis
- BIC and AIC



How to choose the best number of clusters k?

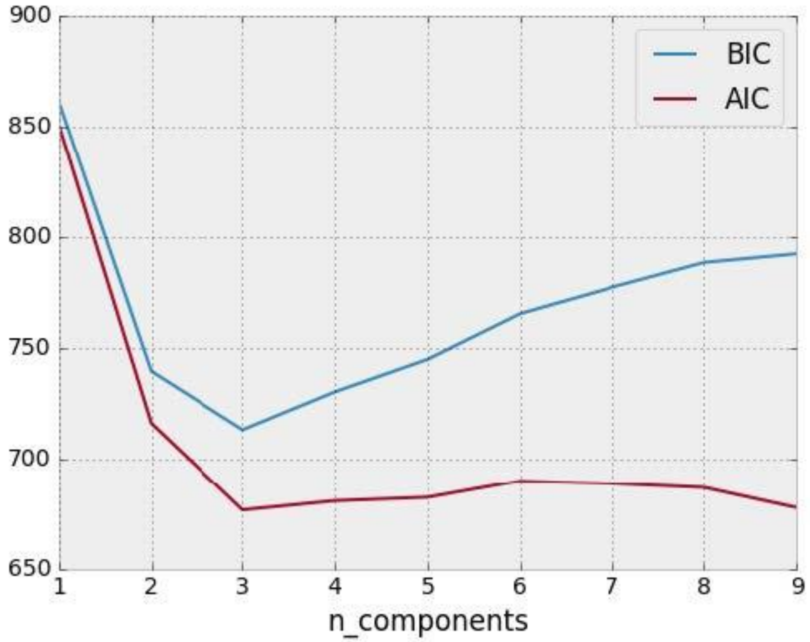
- Elbow method
- Silhouette analysis
  - Plots silhouette score: a measure of how close each point in one cluster is to points in the neighboring clusters, in [-1, 1].
  - Values near +1 indicate that the sample is far away from the neighboring clusters. A value of 0 indicates that the sample is on very close to the decision boundary between two neighboring clusters and negative values indicate that those samples might have been assigned to the wrong cluster.



From https://scikit-learn.org/stable/auto_examples/cluster/plot_kmeans_silhouette_analysis.html

How to choose the best number of clusters k?

- Elbow method
- Silhouette analysis
- Akaike information criterion (AIC) or Bayesian information criterion (BIC)

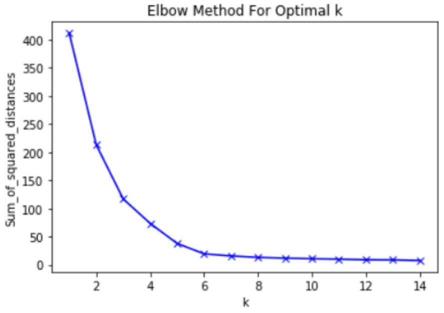


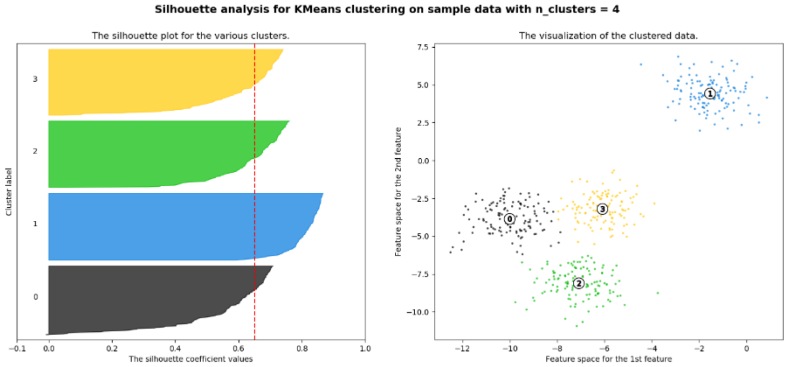
  - The BIC generally penalizes free parameters more strongly than the AIC

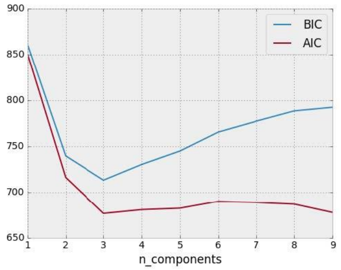
From https://sites.northwestern.edu/msia/2016/12/08/k-means-shouldnt-be-our-only-choice/

How to choose the best number of clusters k?

- Elbow method
- Silhouette analysis
- BIC and AIC

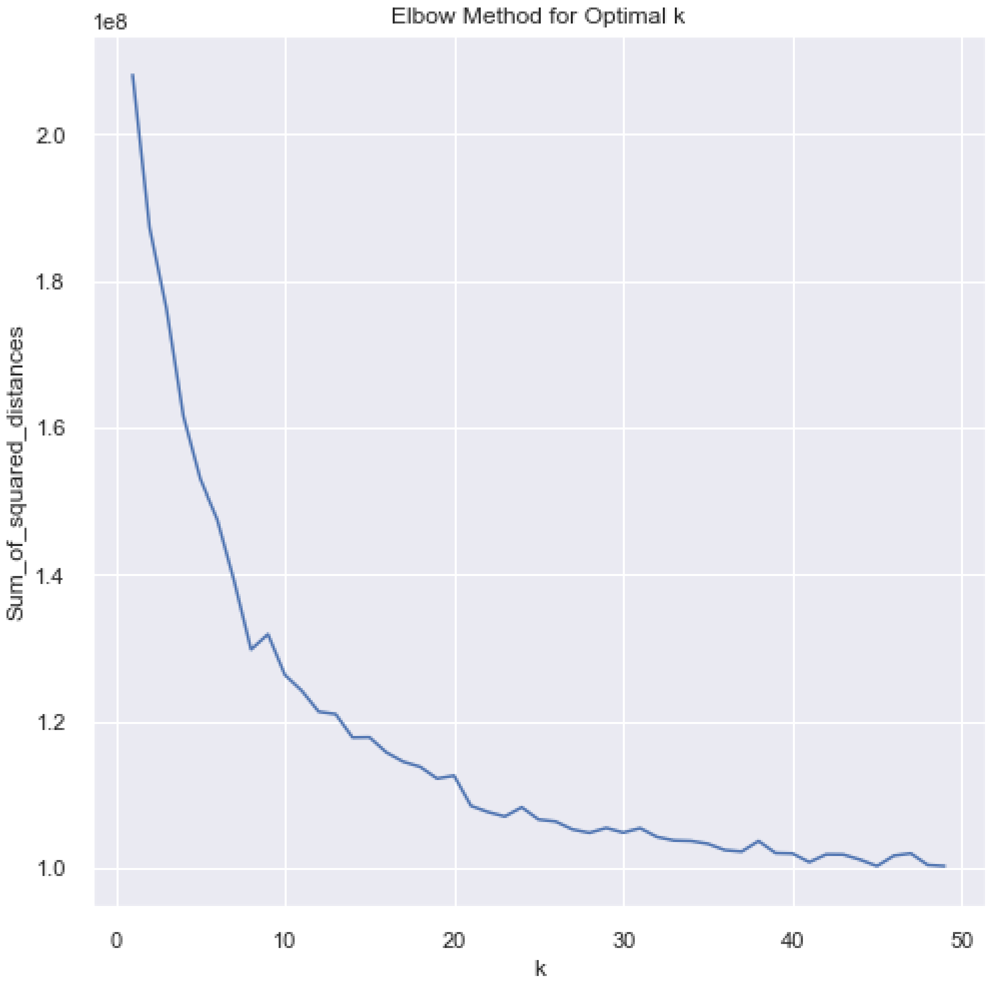




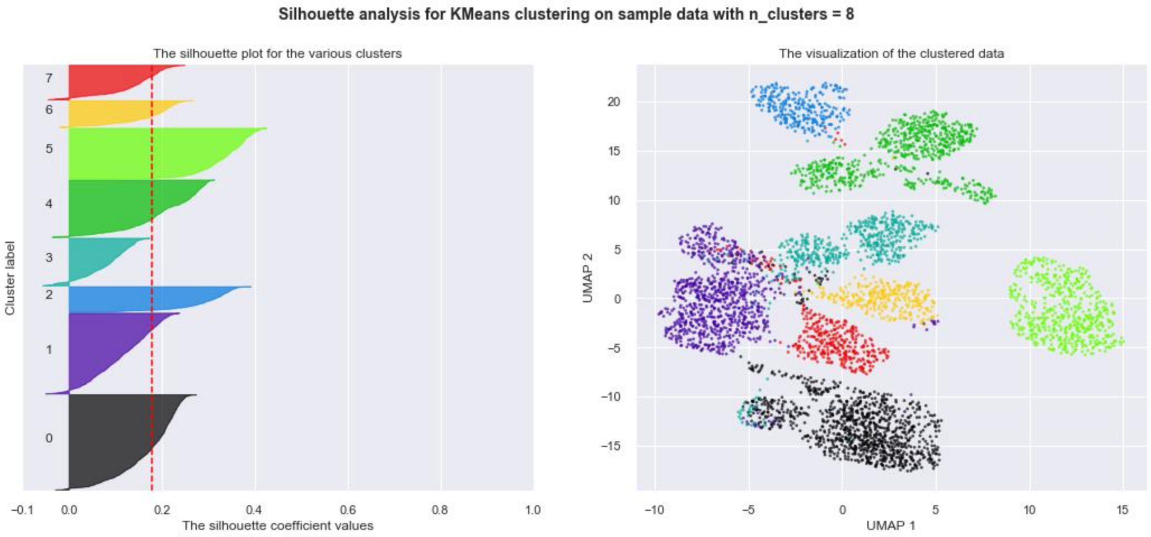


Let's go to our hands on exercise to see how it works!

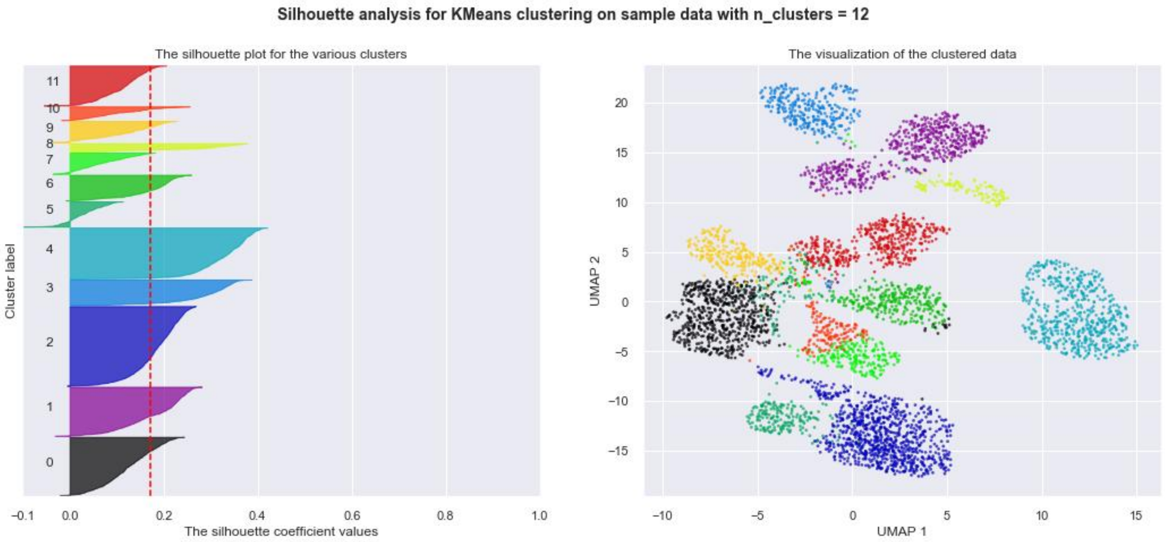
Which value of k would you choose?
(Mini-batch k-means clustering method)



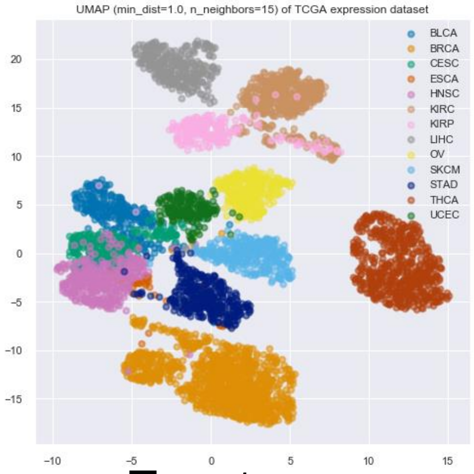
Which value of k would you choose?



$k = 8$



$k = 12$

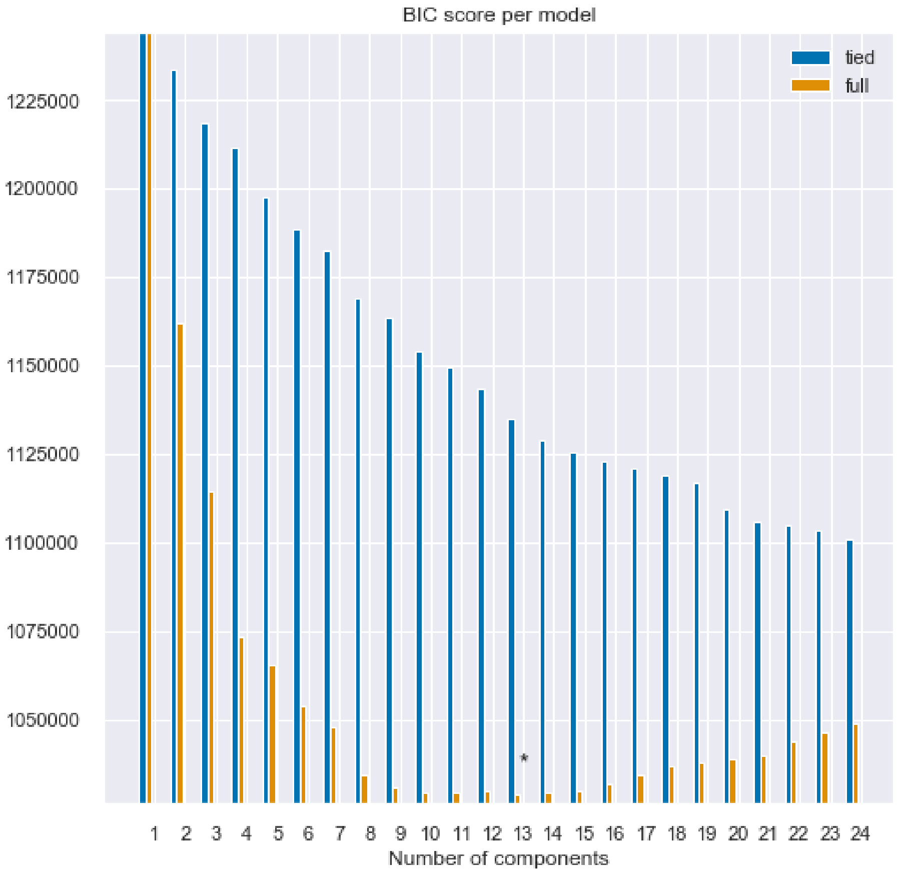


True types

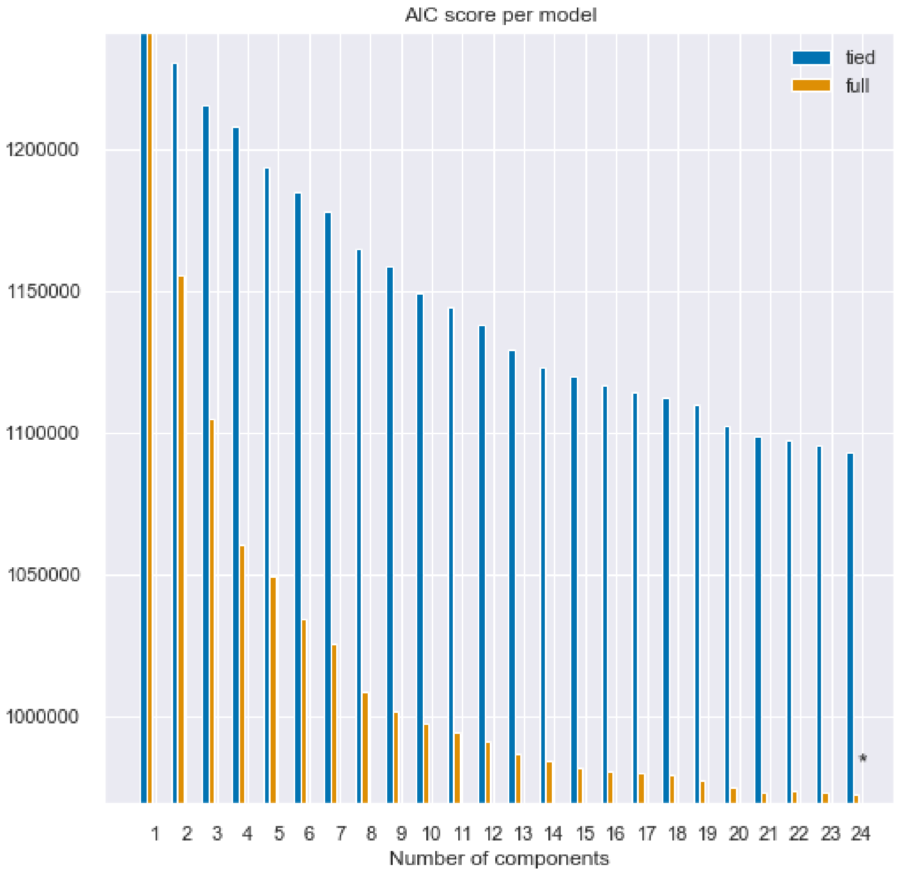
●	BLCA	Bladder
●	BRCA	Breast
●	CESC	Cervical squamous cell
●	ESCA	Esophageal
●	HNSC	Head and Neck squamous cell
●	KIRC	Kidney clear cell
●	KIRP	Kidney papillary
●	LIHC	Liver
●	OV	Ovarian
●	SKCM	Skin Cutaneous Melanoma
●	STAD	Stomach
●	THCA	Thyroid
●	UCEC	Uterine Corpus Endometrial

Which value of k would you choose? (Gaussian mixture model)

BIC

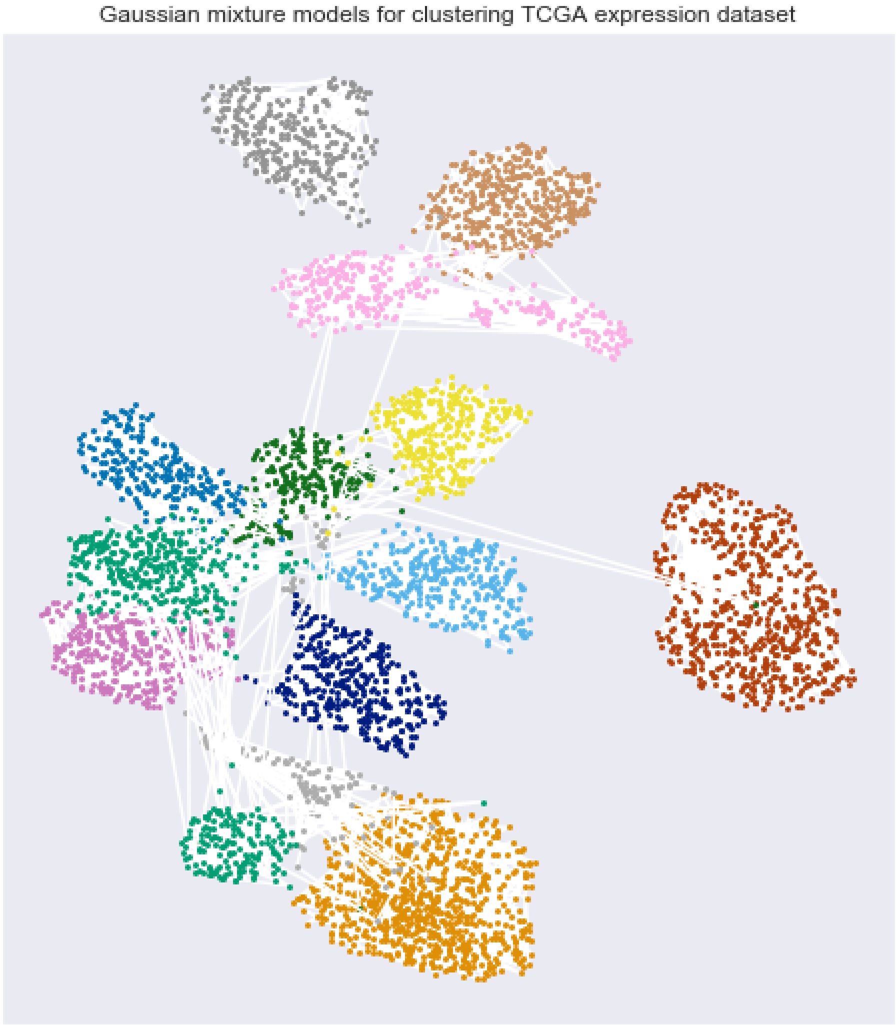


AIC

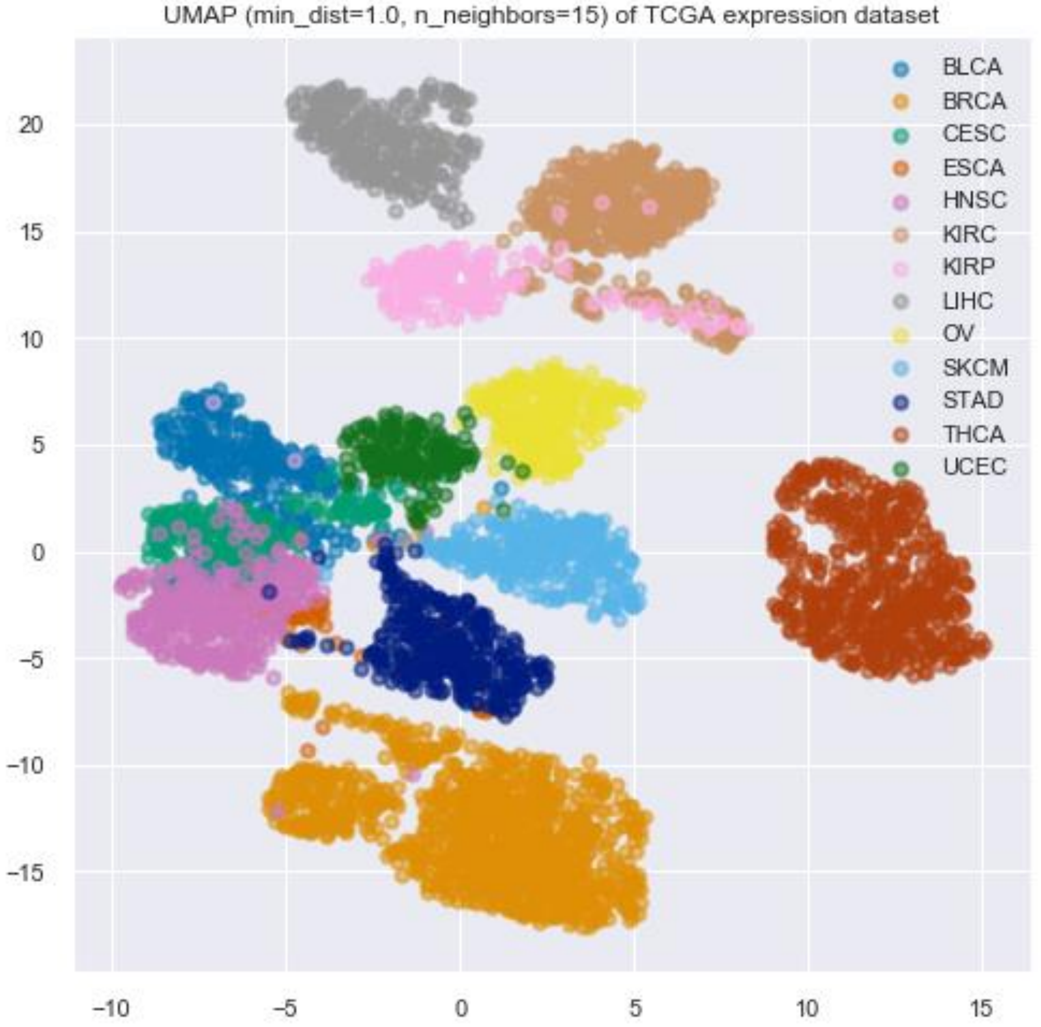


Which value of k would you choose? (Gaussian mixture model)

Best solution according to BIC: *k*=13



True labels



NMI and ARI allows to see which clustering model is the best on our data set

When true labels are available (rarely the case though):

NMI = Normalized Mutual Information

ARI = Adjusted rand index

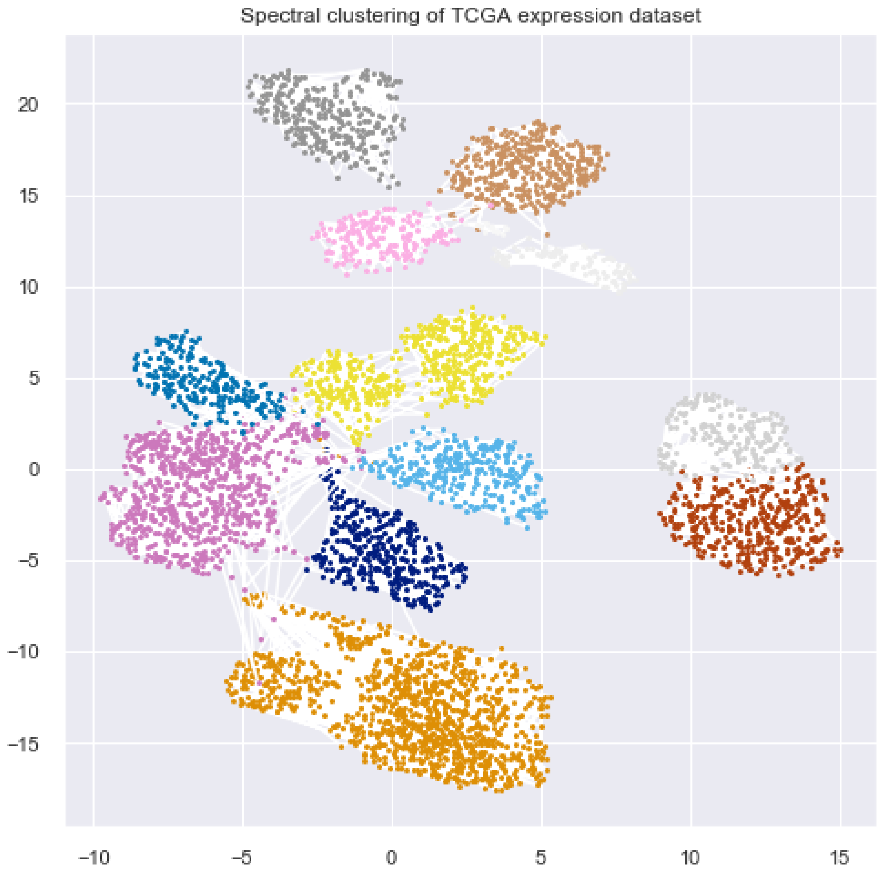
measure mutual dependence between the true and predicted labels

(can be also used as a measure of similarity between two clusterings)

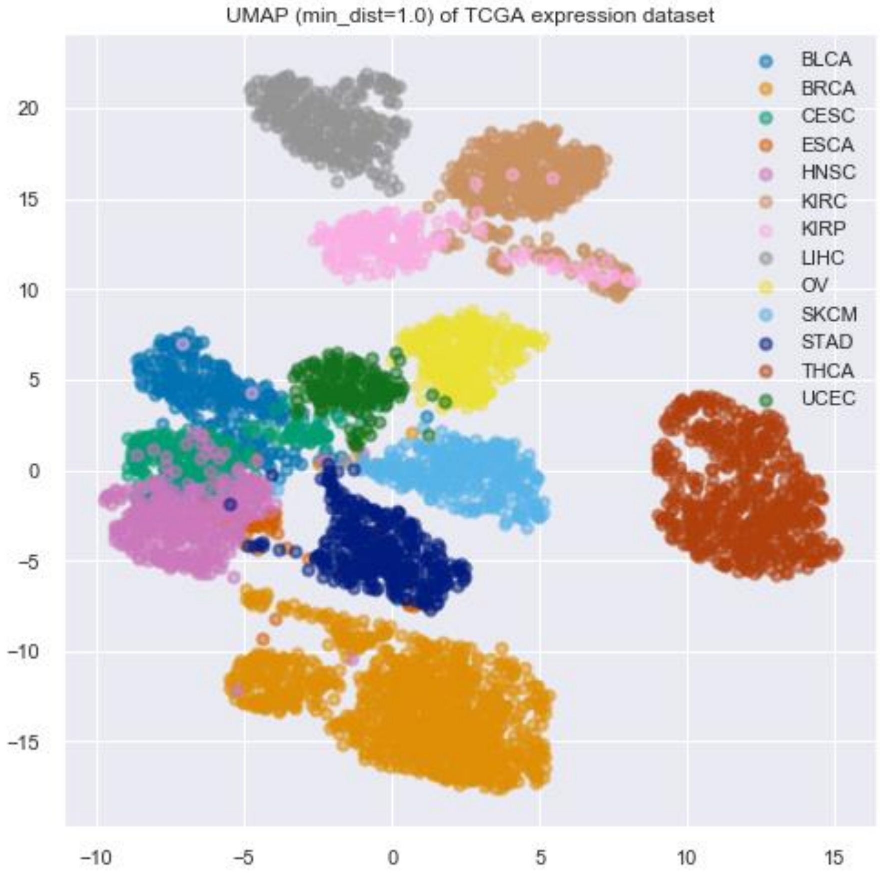
Method	***k***	**ARI**	**NMI**
Mini-batch k-means	8	0.7479	0.8102
Mini-batch k-means	19	0.7426	0.8179
Gaussian mixture model	13	0.7600	0.8479
Spectral clustering	**13**	**0.8148**	**0.8671**
Hierarchical clustering (linkage='ward’)	13	0.7394	0.8379

Spectral clustering result (k=13)

Best solution according to NMI



True labels



Bladder
Breast
Cervical squamous cell
Esophageal
Head and Neck squamous cell
Kidney clear cell
Kidney papillary
Liver
Ovarian
Skin Cutaneous Melanoma
Stomach
Thyroid
Uterine Corpus Endometrial

Take home message: clustering

- The choice of the clustering method should be advised by data structure
  - Visualize the data first
  - E.g, ellipsoids => GMM; sophisticated connected components => structural clustering
- Choosing the number of clusters can be done using:
  - Elbow method
  - Silhouette method
  - BIC or AIC
- Different methods for the estimation of the number of clusters provide different results
  - E.g., BIC is more conservative than AIC

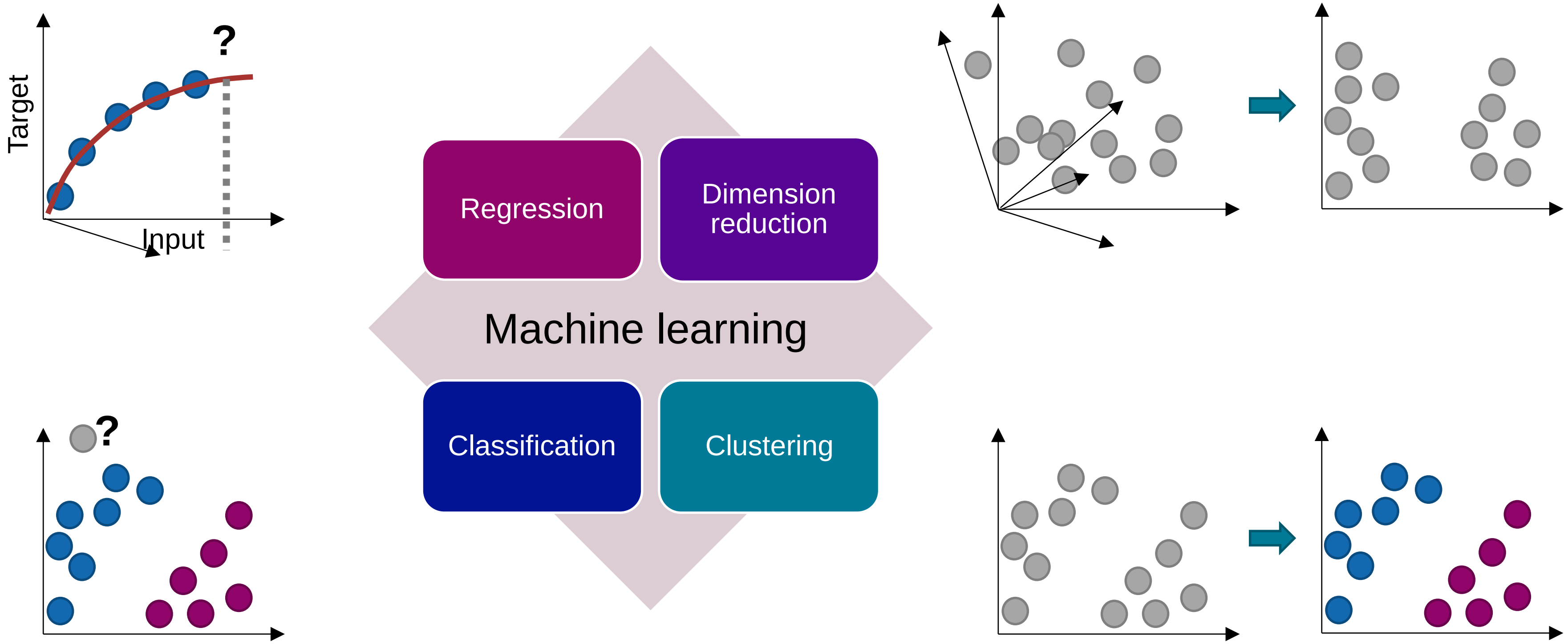
What we did not cover today

- Graph-based clustering methods (Leiden and Louvain algorithms, commonly used on scRNA-seq data)
- Topic models/LDA
- Autoencoder-based methods for dimensionality reduction
- Integration of different data types (e.g., clusters of clusters)

Take home message

- Dimensionality reduction can be used as a step prior to clustering
- One usually tries different dimensionality reduction techniques to choose the one that fits the expectations
- The choice of clustering method should match the data structure

Map of classical machine learning methods



Regression

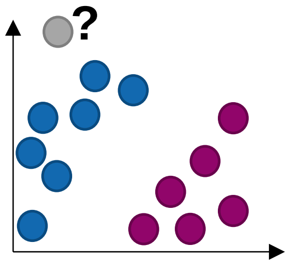
Dimension reduction

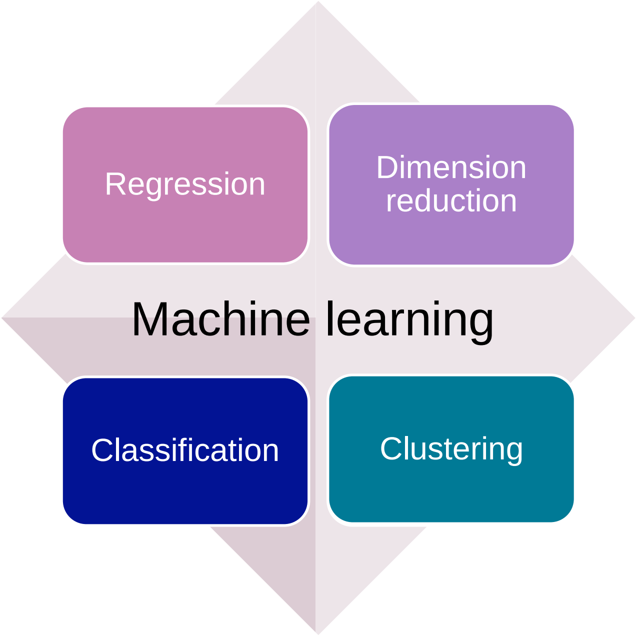
Machine learning

Classification

Clustering

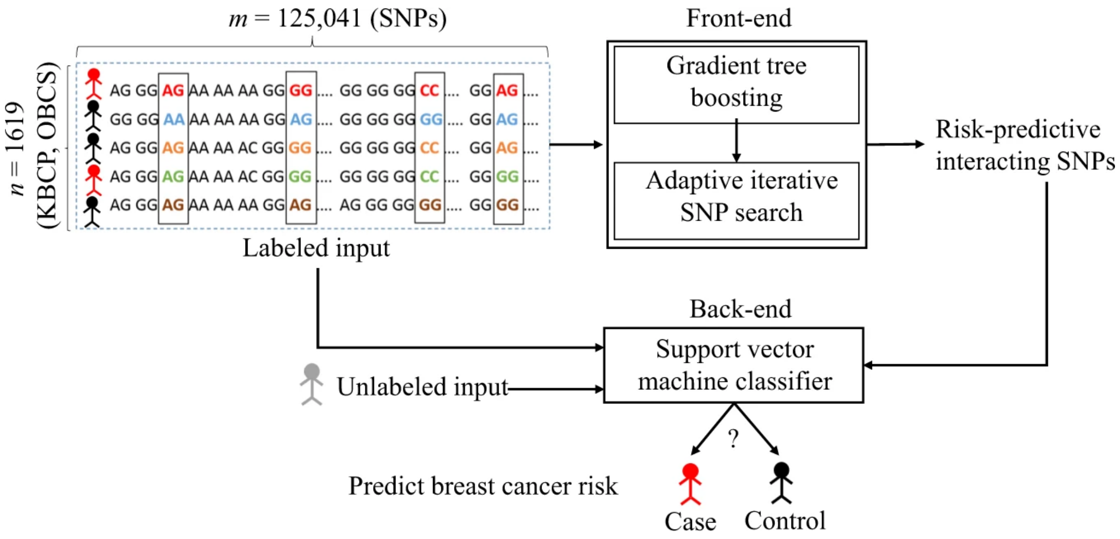
Map of classical machine learning methods





Classification: Biological examples

- Prediction of risk groups
  - **Machine learning identifies interacting genetic variants contributing to breast cancer risk [...]** Behravan et al., *Sci Rep.* 2018
- Primary diagnosis
  - **DNA methylation-based classification of central nervous system tumours.** Capper et al. *Nature* 2018



m = 125,041 (SNPs)
n = 1619 (KBCP, OBCS)
AG GG AG AA AA AA GG GG .... GG GG GG CC .... GG AG ....
GG GG AA AA AA AA GG AG .... GG GG GG GG .... GG AG ....
AG GG AG AA AA AC GG GG .... GG GG GG CC .... GG AG ....
AG GG AG AA AA AC GG GG .... GG GG GG CC .... GG GG ....
AG GG AG AA AA AA GG AG .... AG GG GG GG .... GG GG ....
Labeled input
Front-end
Gradient tree boosting
Adaptive iterative SNP search
Risk-predictive interacting SNPs
Back-end
Support vector machine classifier
Unlabeled input
?
Predict breast cancer risk
Case
Control

Proposed breast cancer risk prediction approach using identified risk-predictive interacting SNPs

Classification: Biological examples

- Prediction of risk groups
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- Primary diagnosis
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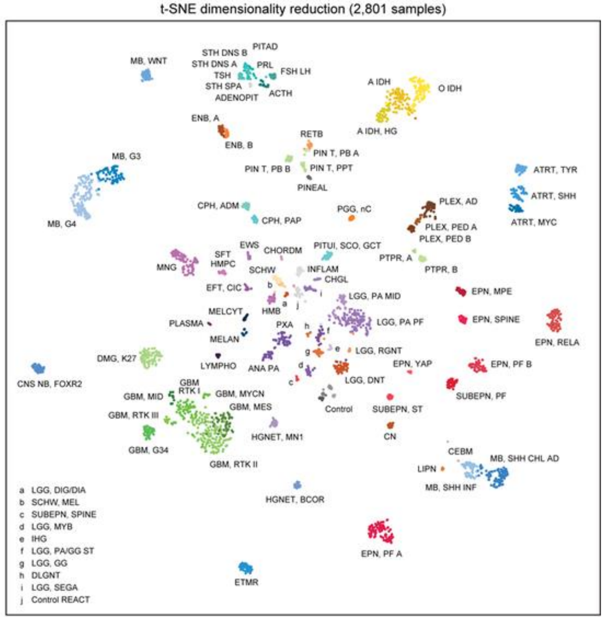
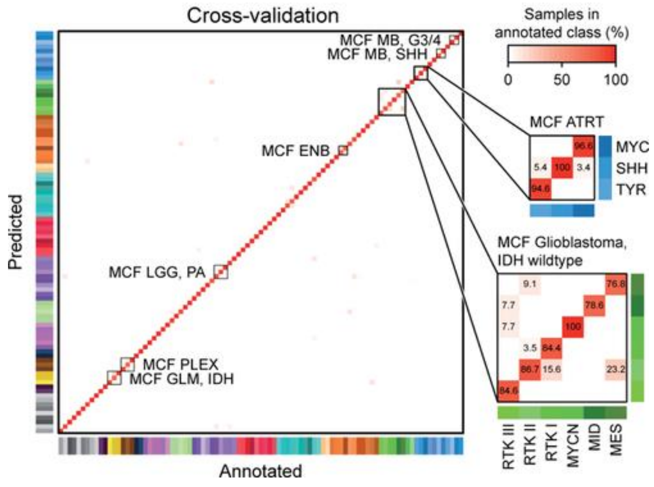
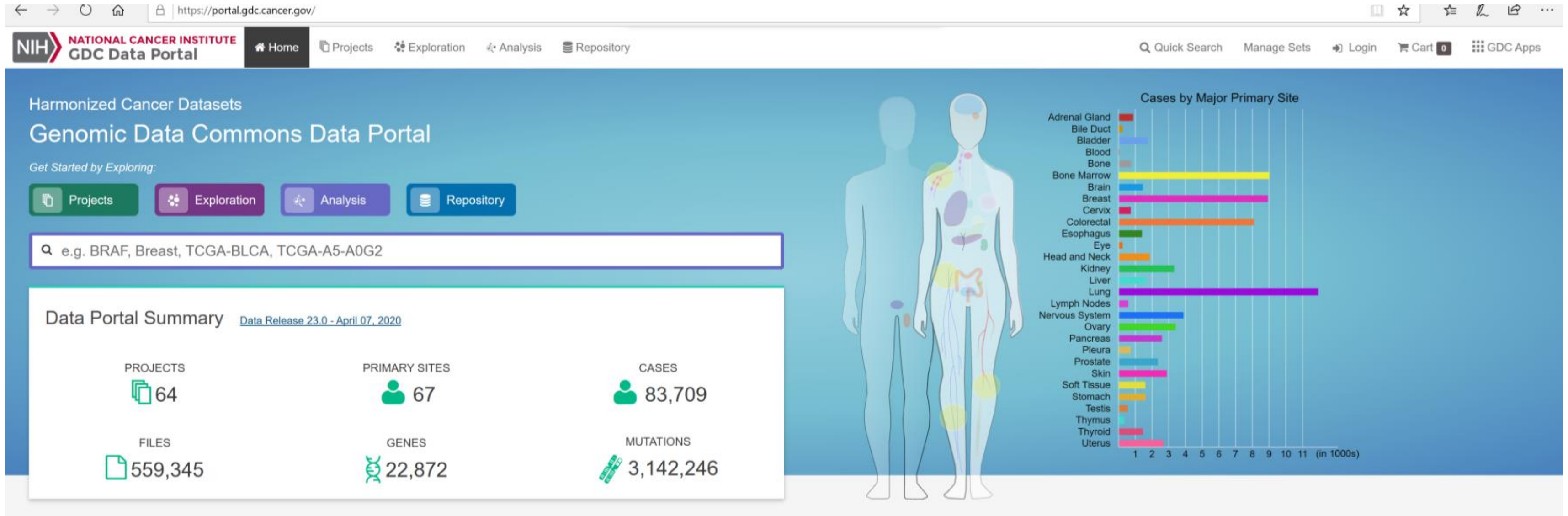




Hands-on:
1. Primary cancer diagnosis from gene expression
2. Breast cancer patients’ stratification based on gene expression

- **Input:** The Cancer Genome Atlas (TCGA) mRNA expression data



Harmonized Cancer Datasets

Genomic Data Commons Data Portal

Get Started by Exploring:

e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2

Data Portal Summary Data Release 23.0 - April 07, 2020

PROJECTS 64

PRIMARY SITES 67

CASES 83,709

FILES 559,345

GENES 22,872

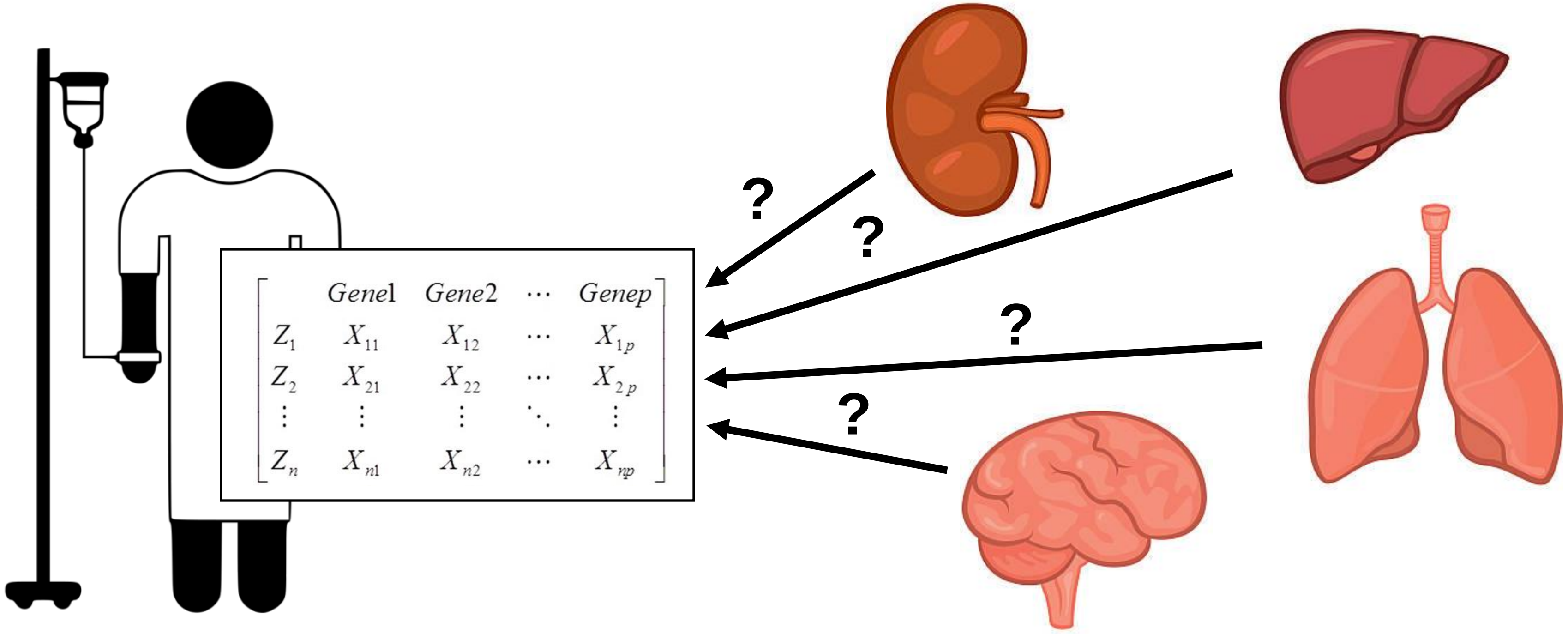
MUTATIONS 3,142,246

https://github.com/BoevaLab/Teaching or https://ml4h2023.jupyter.inf.ethz.ch/

Hands-on:
1. Primary cancer diagnosis from gene expression
2. Breast cancer patients’ stratification based on gene expression

- **TASK 1:**

  Given mRNA expression, predict cancer type

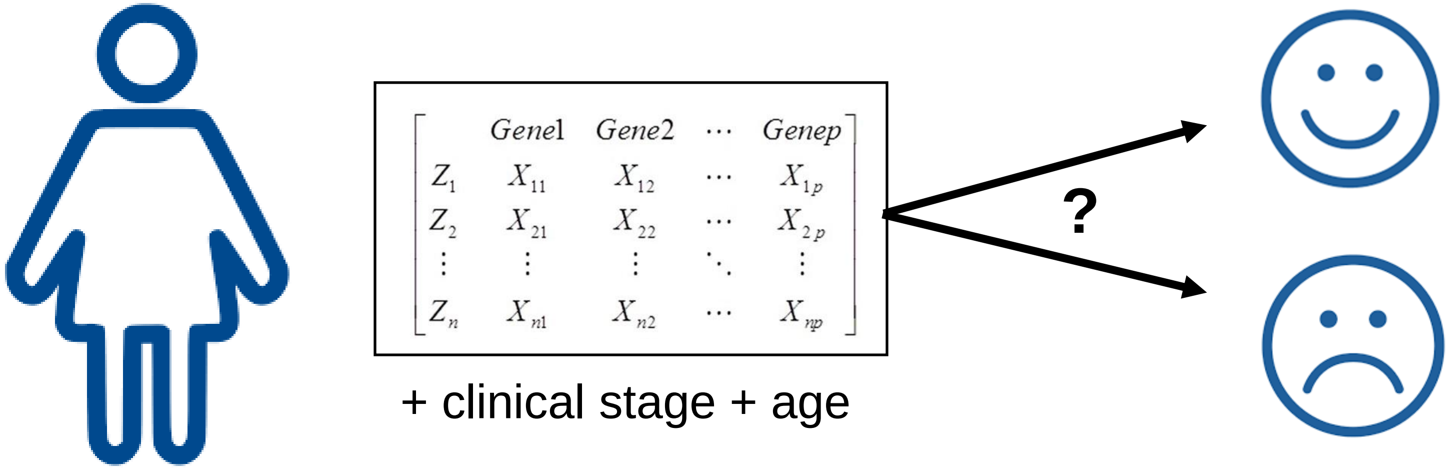


$$\begin{bmatrix} & Gene1 & Gene2 & \cdots & Genep \\ Z_1 & X_{11} & X_{12} & \cdots & X_{1p} \\ Z_2 & X_{21} & X_{22} & \cdots & X_{2p} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ Z_n & X_{n1} & X_{n2} & \cdots & X_{np} \end{bmatrix}$$

Hands-on:
1. Primary cancer diagnosis from gene expression
2. Breast cancer patients’ stratification based on gene expression

- **TASK 2:**

  Given mRNA expression (and clinical data: stage, age), stratify patients according to good and bad prognosis



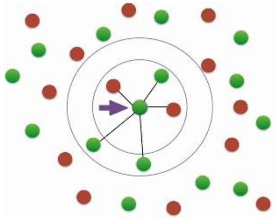
$$\begin{array}{ccccc} & Gene1 & Gene2 & \cdots & Genep \\ Z_1 & X_{11} & X_{12} & \cdots & X_{1p} \\ Z_2 & X_{21} & X_{22} & \cdots & X_{2p} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ Z_n & X_{n1} & X_{n2} & \cdots & X_{np} \end{array}$$

+ clinical stage + age

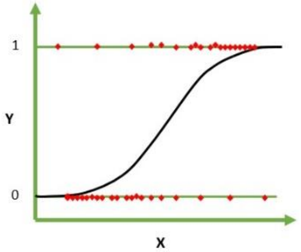
Aggressive treatment to be applied

Standard approaches for classification

- k Nearest Neighbors (k-NN)

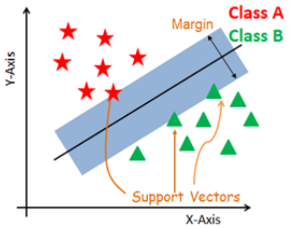


- Logistic Regression

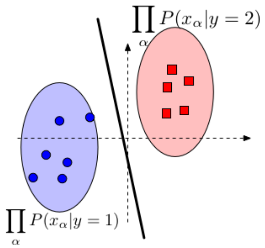


- Logistic Regression with L1+L2 (Elastic Net) penalty

- Support Vector Machines (SVM)



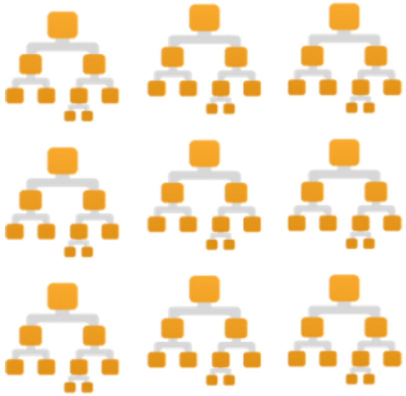
- Naive Bayes (Gaussian)


$\prod_{\alpha} P(x_\alpha|y=2)$
$\prod_{\alpha} P(x_\alpha|y=1)$

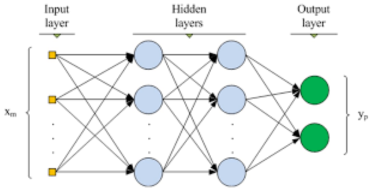
- Random Forest

- AdaBoost

- Gradient Tree Boosting (gradient boosting machine, GBM)



- Multi-layer perceptron (MLP)



Hands-on:
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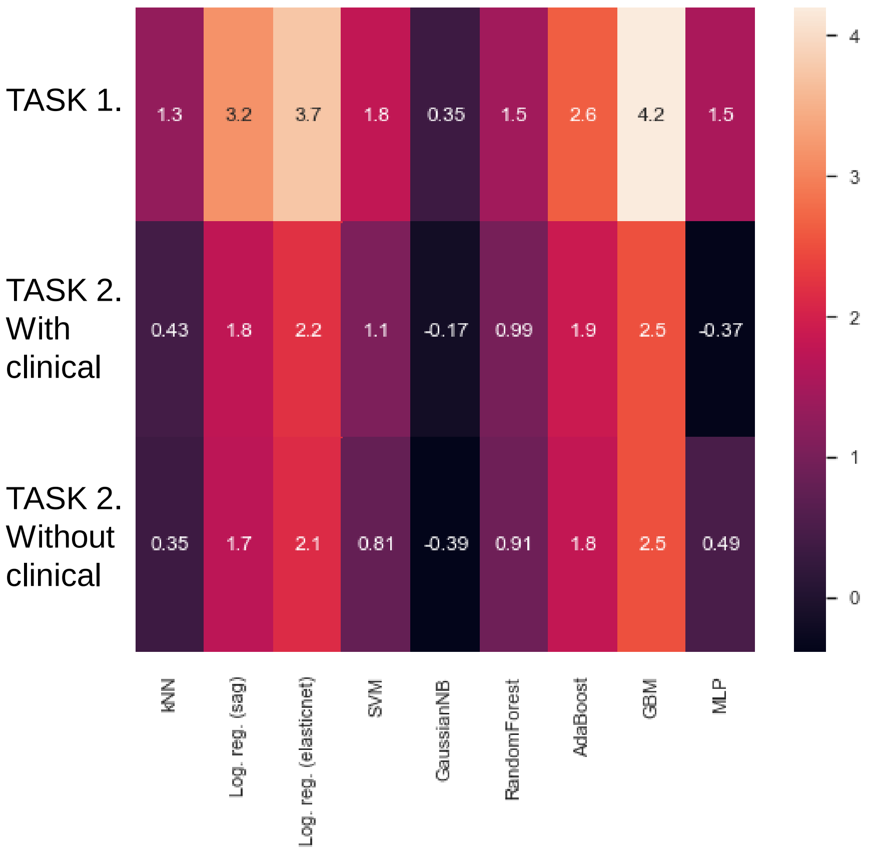
$$\text{Accuracy} = \frac{\text{TP + TN}}{\text{TP + TN + FP + FN}}$$

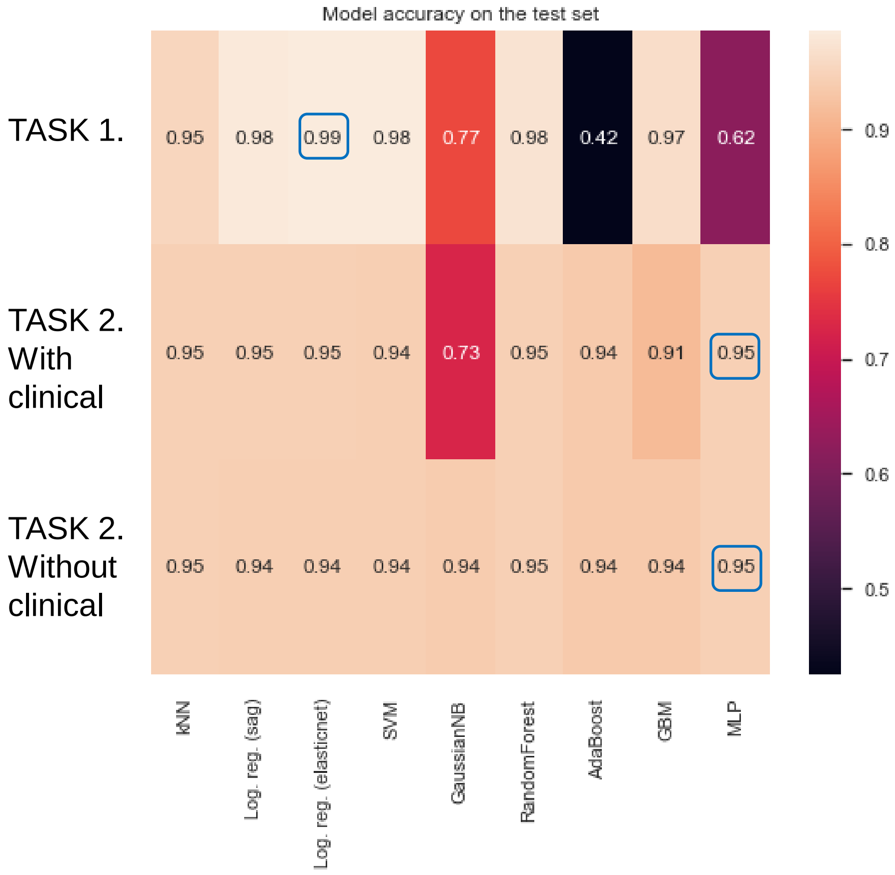
Fraction predicted correctly

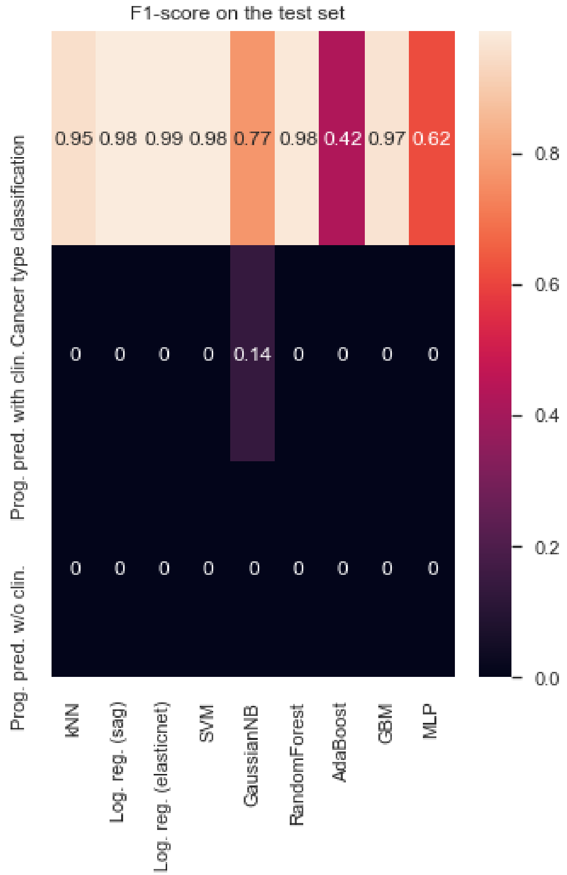
F1 score: harmonic mean of recall and precision

$$F1 = \frac{2}{\frac{1}{precision} + \frac{1}{recall}} = \frac{2 * (precision * recall)}{precision + recall}$$

Time to train the model
Log10 seconds



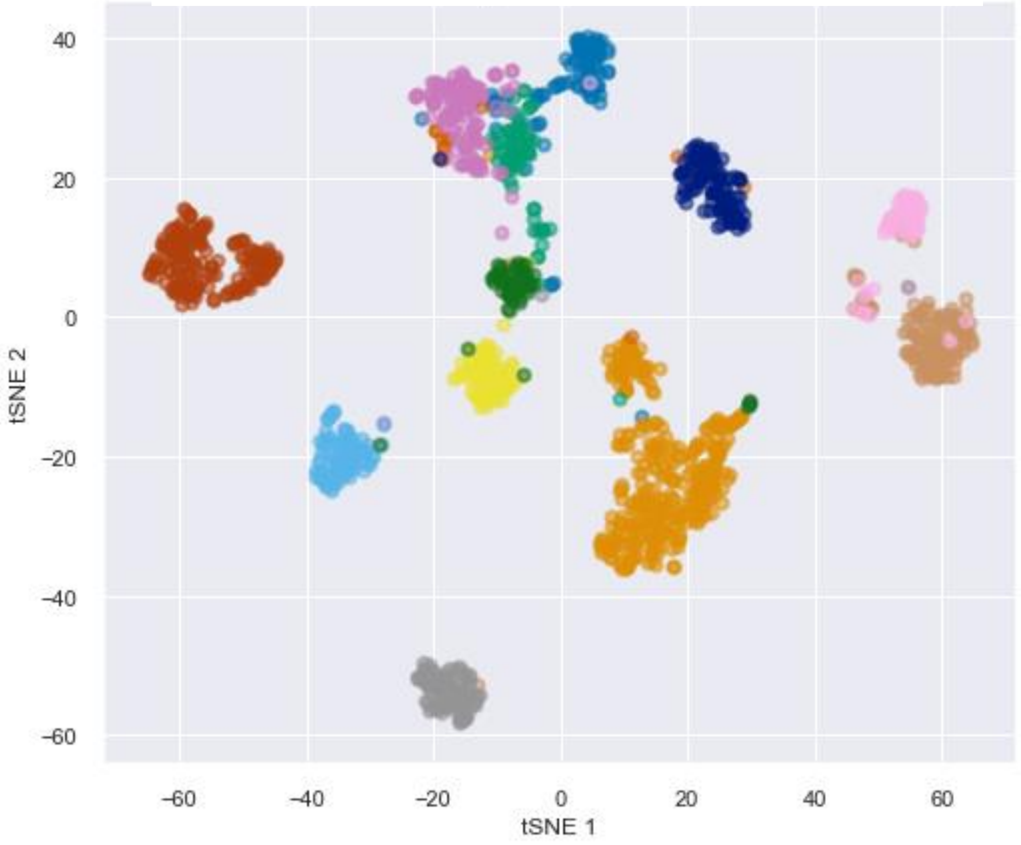




Hands-on:

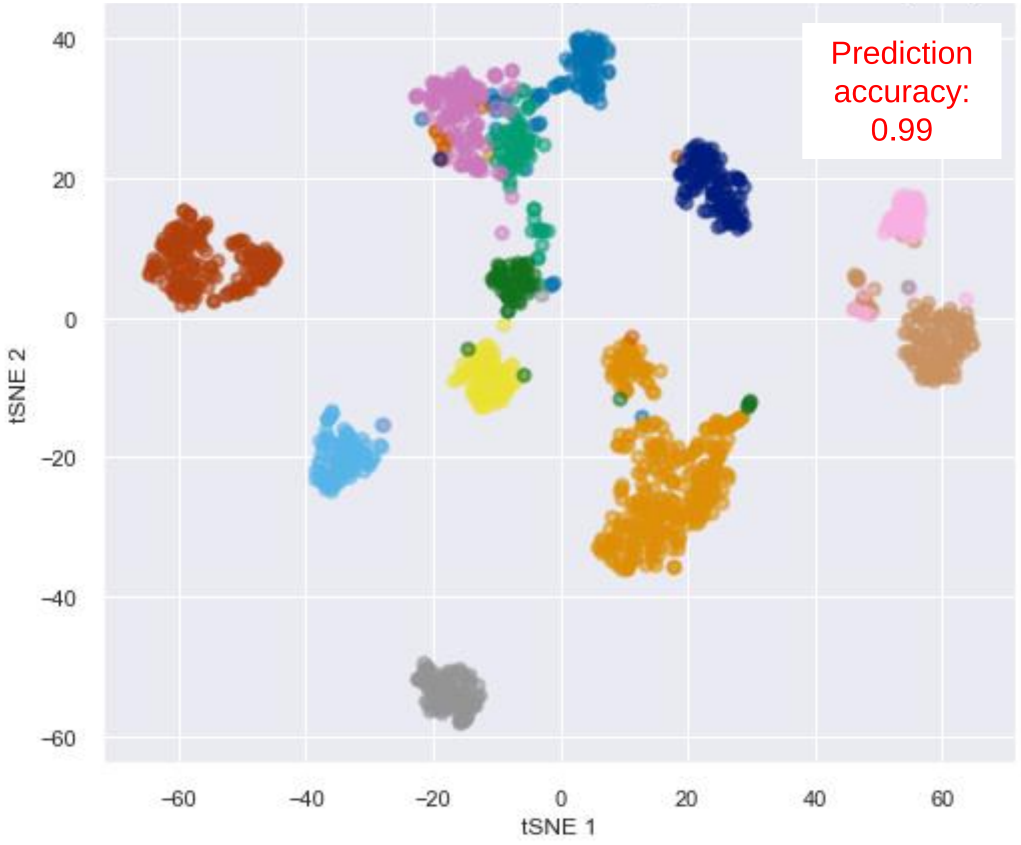
1. Primary cancer diagnosis from gene expression
2. Breast cancer patients’ stratification based on gene expression

True values, tumor classification task



Validation set (colors correspond to true cancer types)

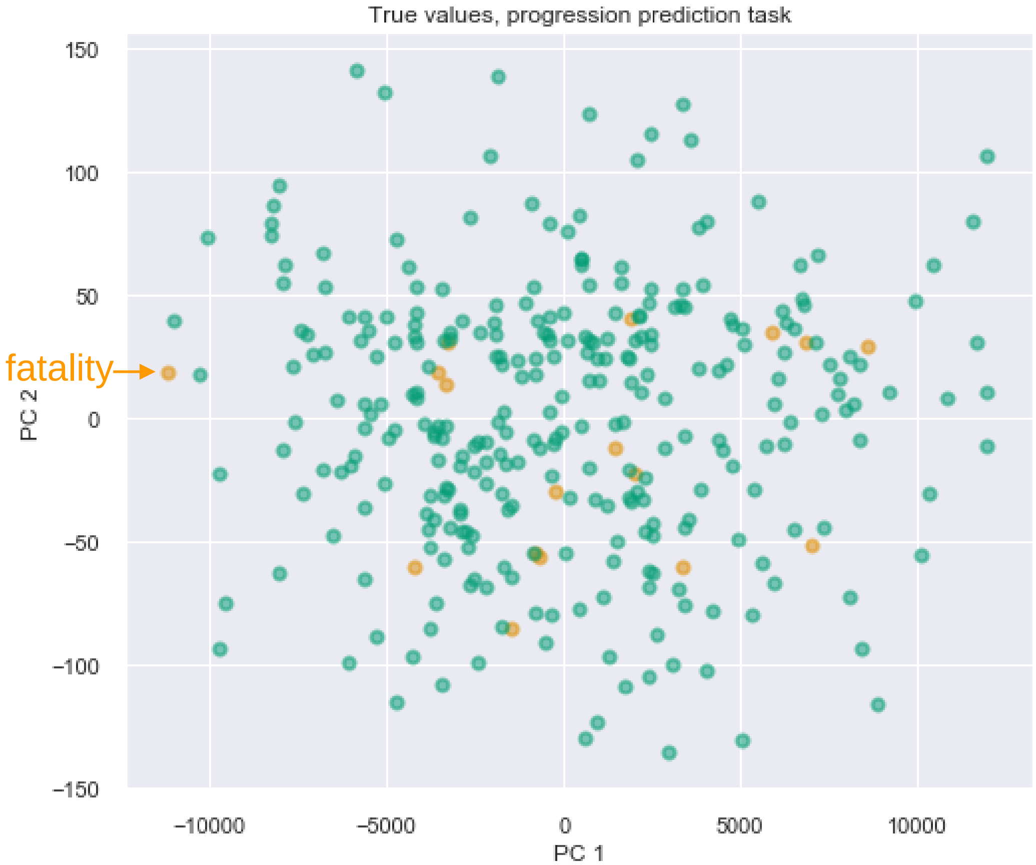
Best solution: Elastic net logistic regression



Prediction accuracy: 0.99

Elastic net on the validation set (colors correspond to predictions)

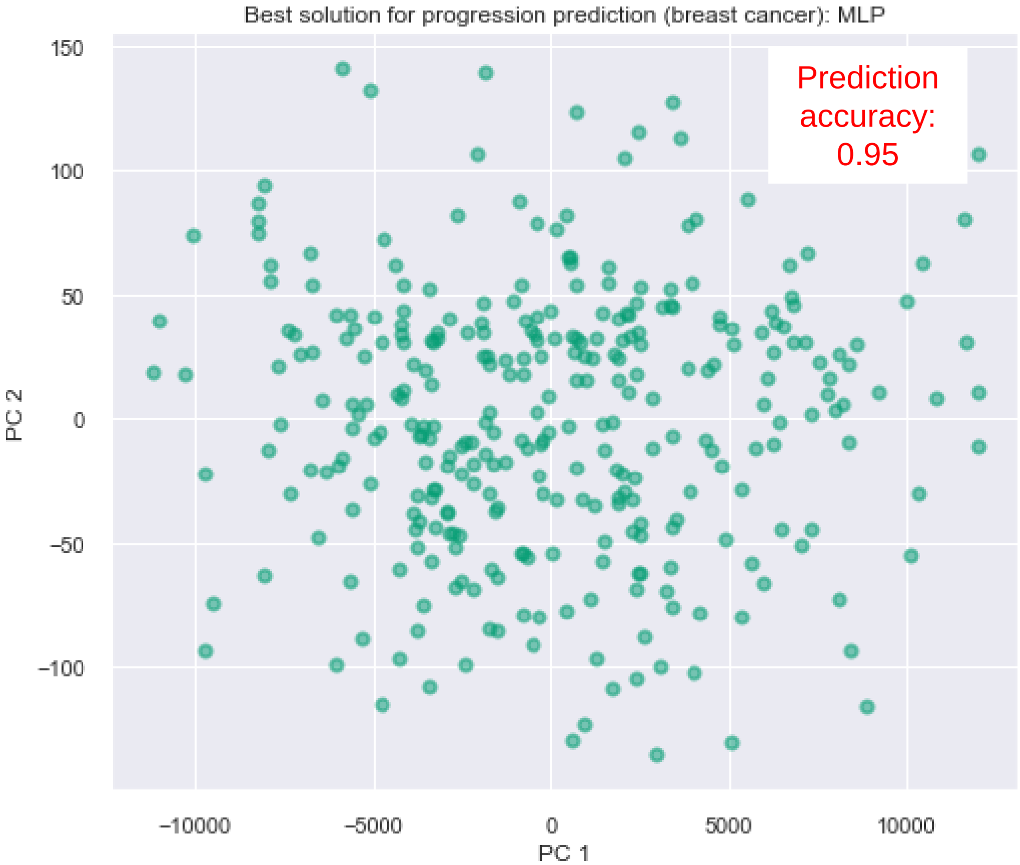
Hands-on:
1. Primary cancer diagnosis from gene expression
2. Breast cancer patients’ stratification based on gene expression



True values, progression prediction task

fatality

Validation set (true colors)



Best solution for progression prediction (breast cancer): MLP

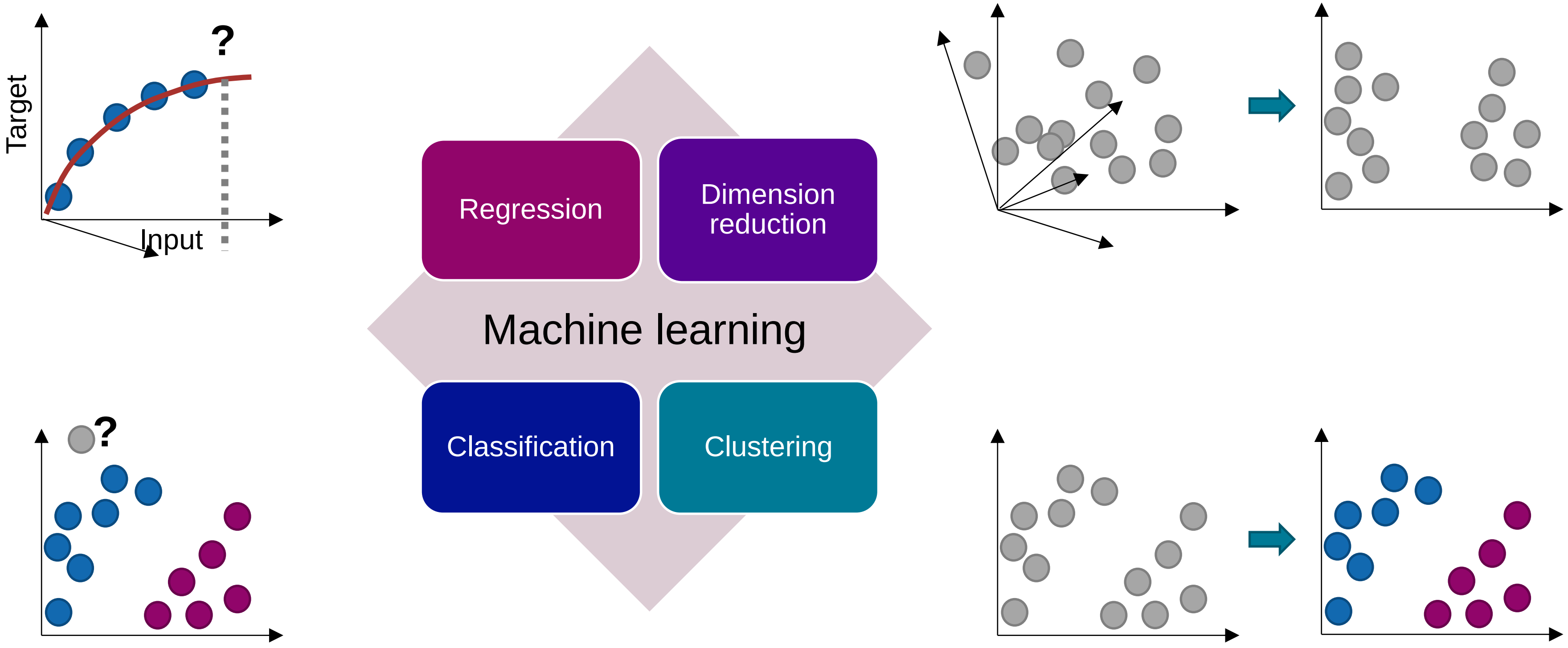
Prediction accuracy: 0.95

multi-layer perceptron on the validation set

Take home message: Classification

- Linear and non-linear models can provide similar prediction accuracy (TASK1)
- Classification on imbalanced groups with low information content may fail (TASK2)
  - Study your data first
    - Check data summary
    - Visualize your data
  - Use the right evaluation metrics (e.g., precision and recall)
  - Consider redesigning your task

Map of classical machine learning methods



Regression

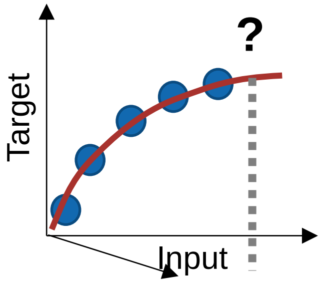
Dimension reduction

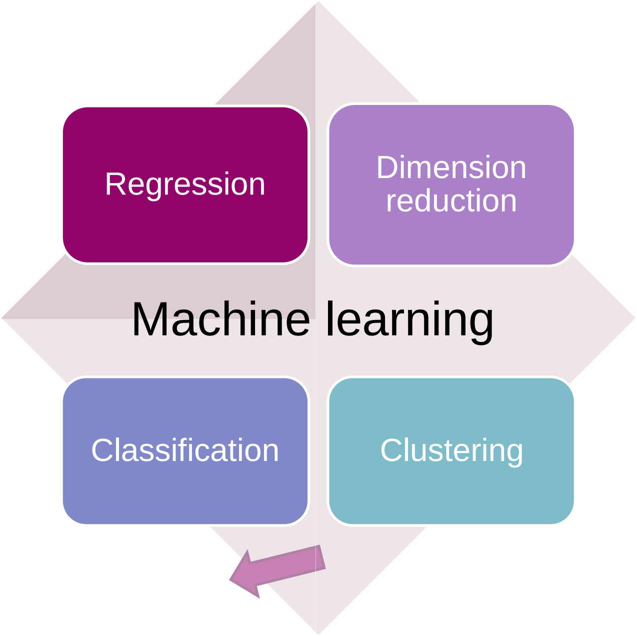
Machine learning

Classification

Clustering

Map of classical machine learning methods





Regression

Dimension reduction

Machine learning

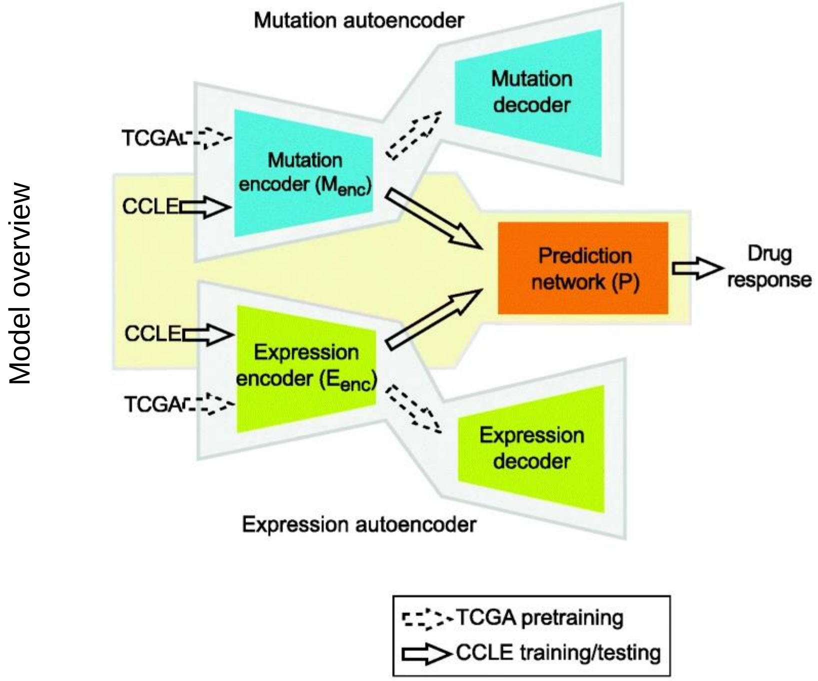
Classification

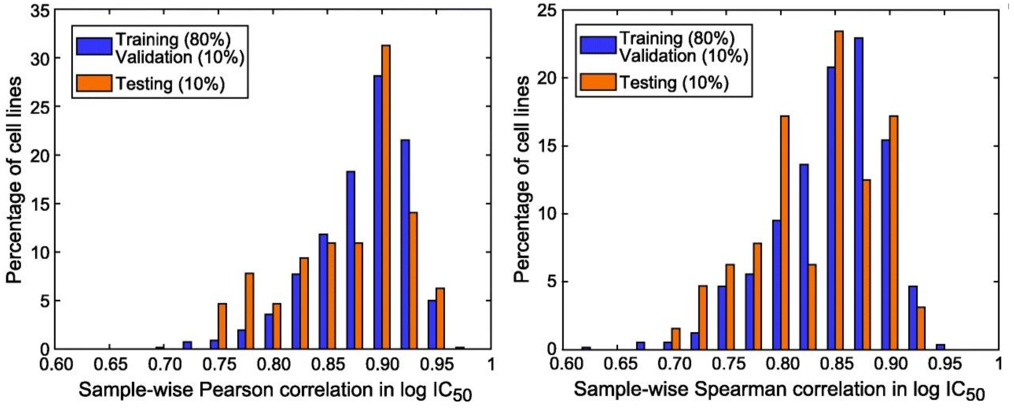
Clustering

Regression: Biological examples

- Prediction of treatment efficiency / drug response
  - **Predicting drug response of tumors from integrated genomic profiles by deep neural networks.** Chiu et al., *BMC Med. Genomic*, 2019

- Prediction of molecular/cellular properties (e.g., protein-DNA binding affinities)
  - **Predicting the sequence specificities of DNA- and RNA-binding proteins by deep learning**. Alipanahi et al., *Nature Biotech*. 2015





Sample-wise Pearson and Spearman correlation between imputed and predicted IC_{50} data of CCLE samples

Regression: Biological examples

- Prediction of treatment efficiency / drug response
  - **Predicting drug response of tumors from integrated genomic profiles by deep neural networks.** Chiu et al., *BMC Med. Genomic*, 2019

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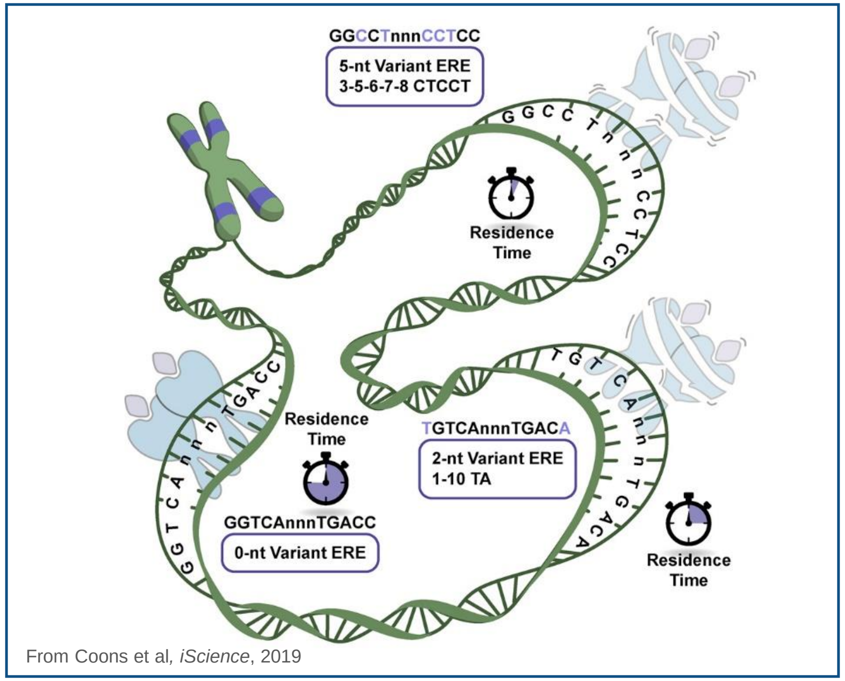
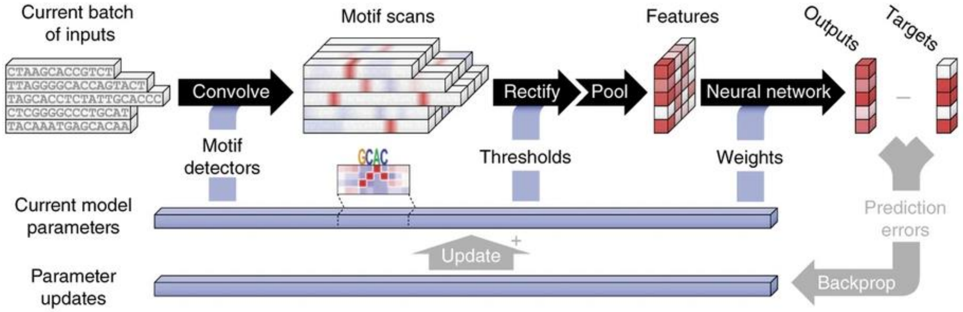


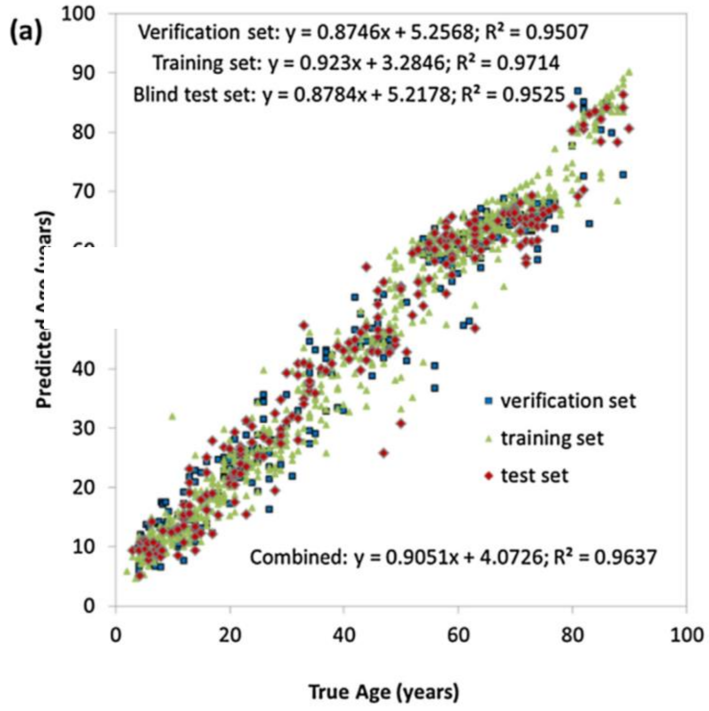
From Coons et al, *iScience*, 2019



DeepBind model

Regression: Biological examples

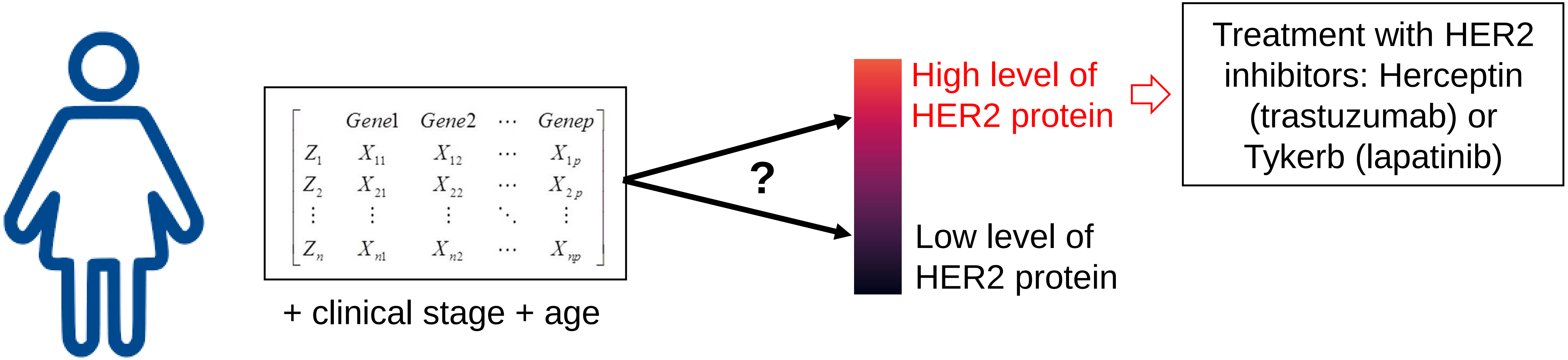
- Age prediction from DNA methylation (e.g., for forensics)
  - **DNA methylation-based forensic age prediction using artificial neural networks and next generation sequencing.** Vidaki et al. *Forensic Sci Int Genet.* 2017



Hands-on: Prediction of protein concentration based on mRNA data (Breast cancer samples)
1. Concentration of HER2 (coded by the *ERBB2* gene)
2. Concentration of p53 (coded by the *TP53* gene)

https://github.com/BoevaLab/Teaching

TASK 1:



$$\left[\begin{array}{ccccc} & Gene1 & Gene2 & \cdots & Genep \\ Z_1 & X_{11} & X_{12} & \cdots & X_{1p} \\ Z_2 & X_{21} & X_{22} & \cdots & X_{2p} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ Z_n & X_{n1} & X_{n2} & \cdots & X_{np} \end{array}\right]$$

+ clinical stage + age

?

High level of HER2 protein ⇨

Low level of HER2 protein

Treatment with HER2 inhibitors: Herceptin (trastuzumab) or Tykerb (lapatinib)

The HER2 protein is coded by the *ERBB2* gene, frequently amplified in human breast cancer

Target variable (y): Reverse Phase Protein Array (*RPPA*) value of HER2 presence in tumor cells

Hands-on: Prediction of protein concentration based on mRNA data (Breast cancer samples)

1. Concentration of HER2 (coded by the *ERBB2* gene)
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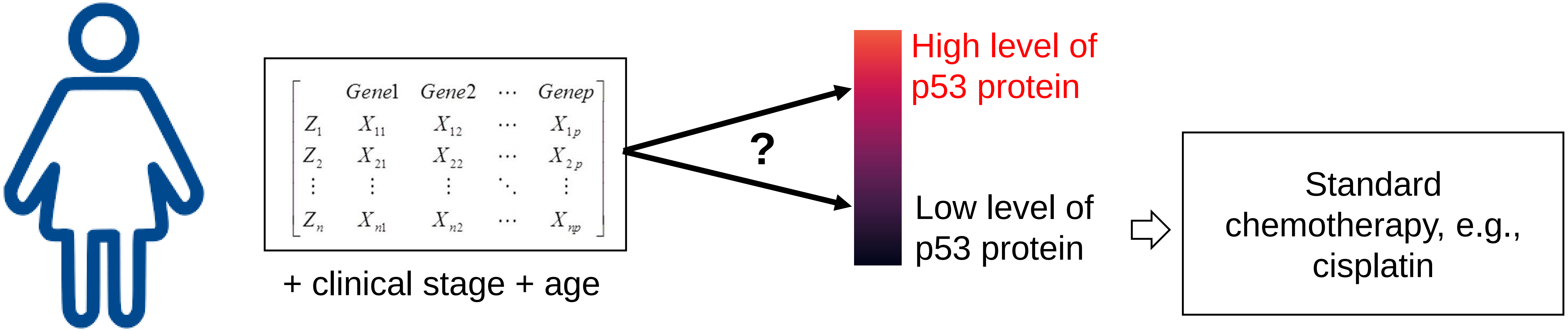
TASK 2:



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+ clinical stage + age

?

High level of p53 protein

Low level of p53 protein

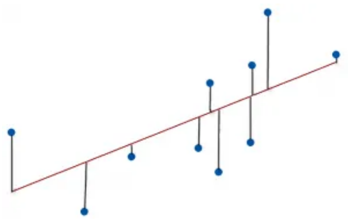
Standard chemotherapy, e.g., cisplatin

The p53 protein is coded by the *TP53* gene, frequently deleted, mutated or repressed in human cancers

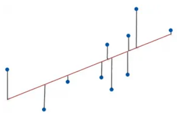
Target variable (y): Reverse Phase Protein Array (*RPPA*) value of p53 presence in tumor cells

Classic regression models

- Ordinary Least Squares

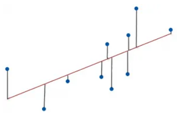


- Lasso (L1 penalty on model coefficients)



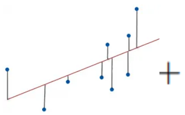
$$+\lambda\sum_{j=1}^{p}|\beta_j|$$

- Ridge (L2 penalty on model coefficients)



$$+\lambda\sum_{j=1}^{p}|\beta_j|^2$$

- Elastic Net (L1 and L2 penalty on model coefficients)

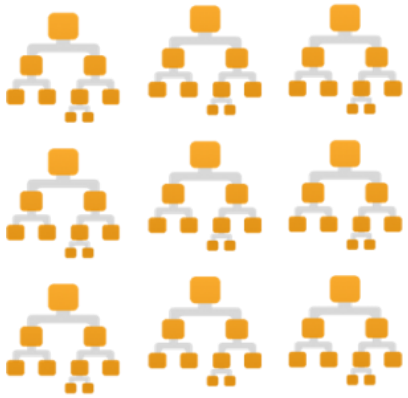


$$+\lambda_1\sum_{j=1}^{p}|\beta_j|+\lambda_2\sum_{j=1}^{p}|\beta_j|^2$$

- Random Forest

- AdaBoost

- Gradient Tree Boosting (gradient boosting machine, GBM)



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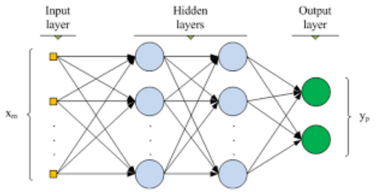

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1. Concentration of HER2 (coded by the *ERBB2* gene)
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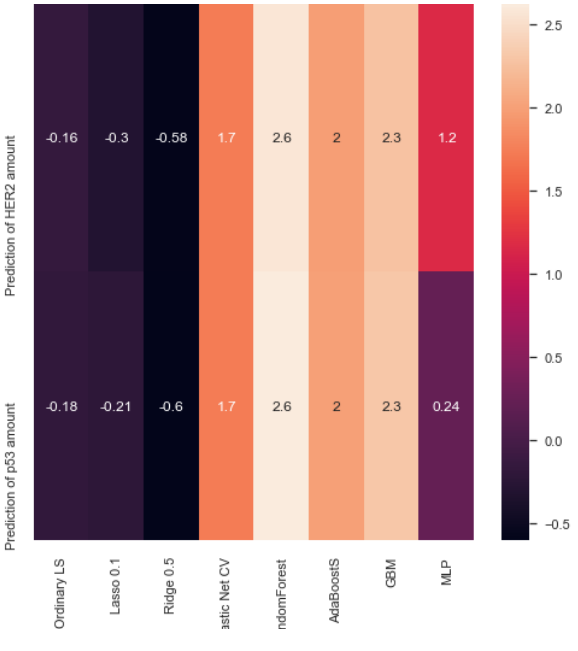
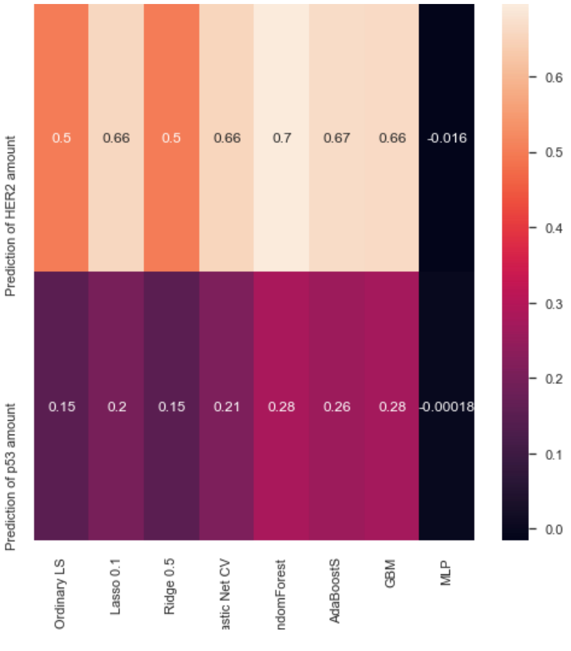
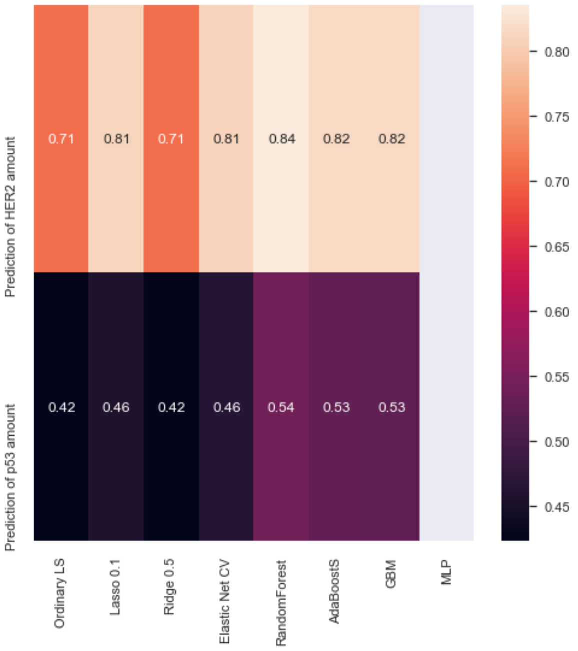
Time (log10 sec)



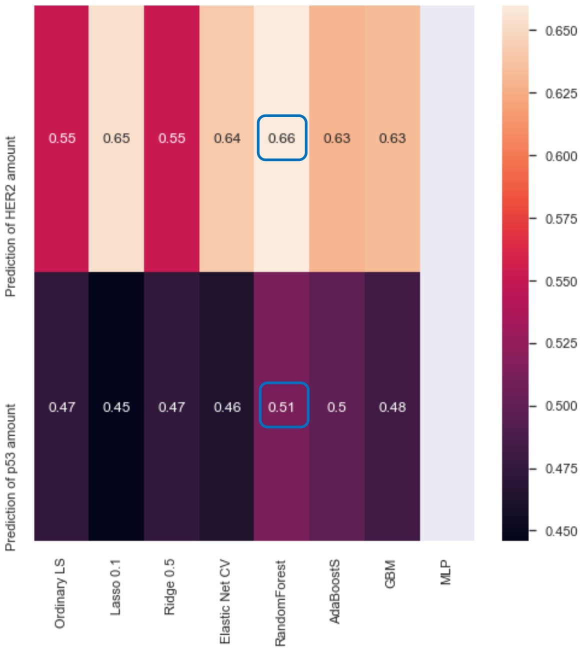
Sklearn accuracy score R^2



Pearson correlation R



Spearman correlation ρ



Coefficient of determination $R^2 = (1 - \frac{RSS}{\sum(y_i - \bar{y})^2})$

$$Spearman\ \rho\ (HER2, ERBB2) = 0.63$$

$$Spearman\ \rho\ (p53, TP53) = 0.27$$

Hands-on: Prediction of protein concentration based on mRNA data (Breast cancer samples)
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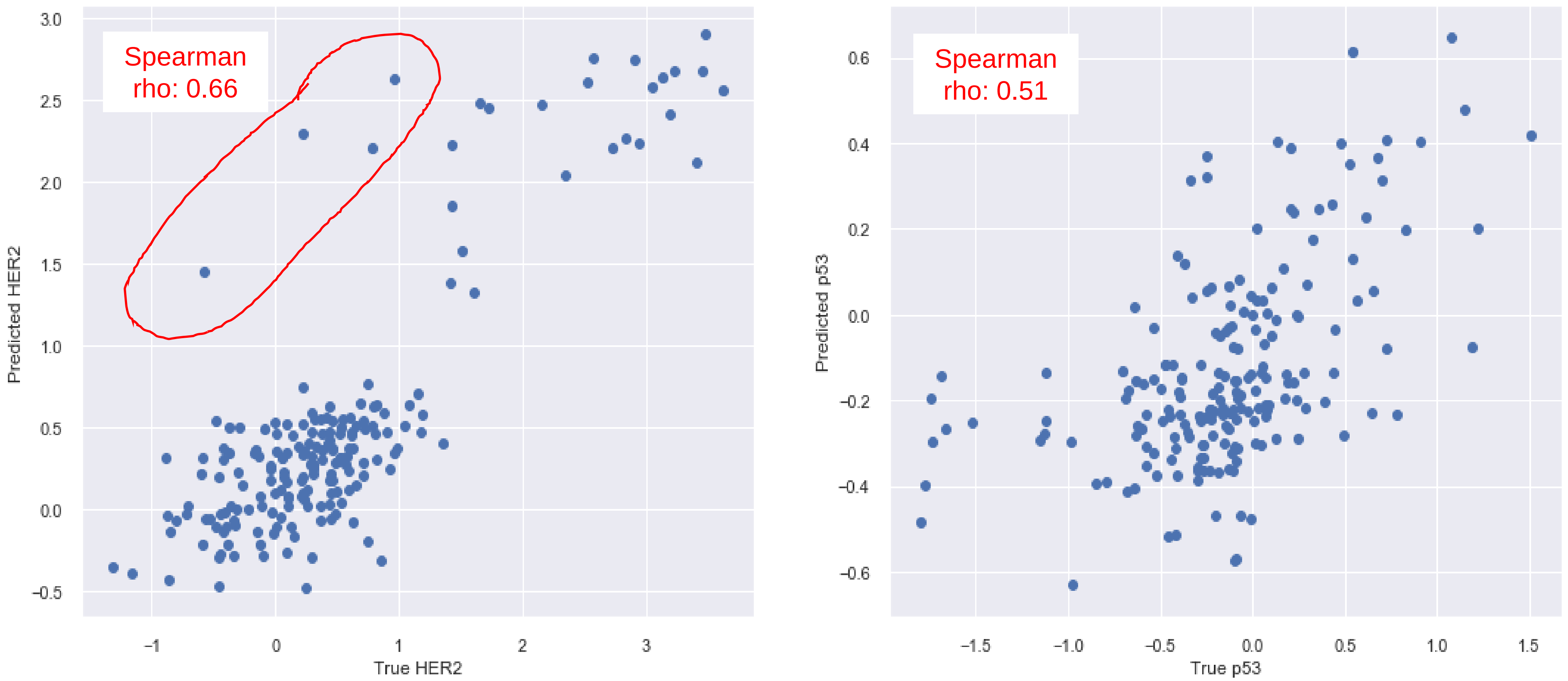

Random Forest predictions

Hands-on: Prediction of protein concentration based on mRNA data (Breast cancer samples)

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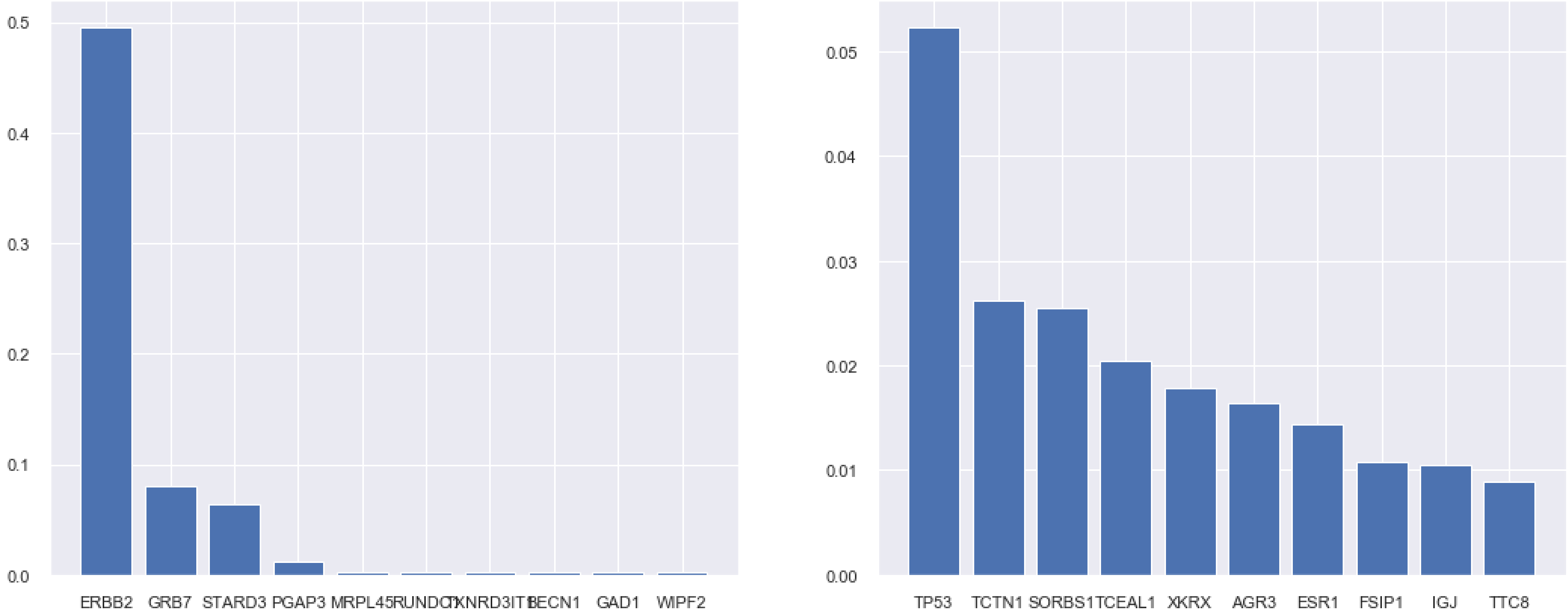
FEATURE IMPORTANCE:



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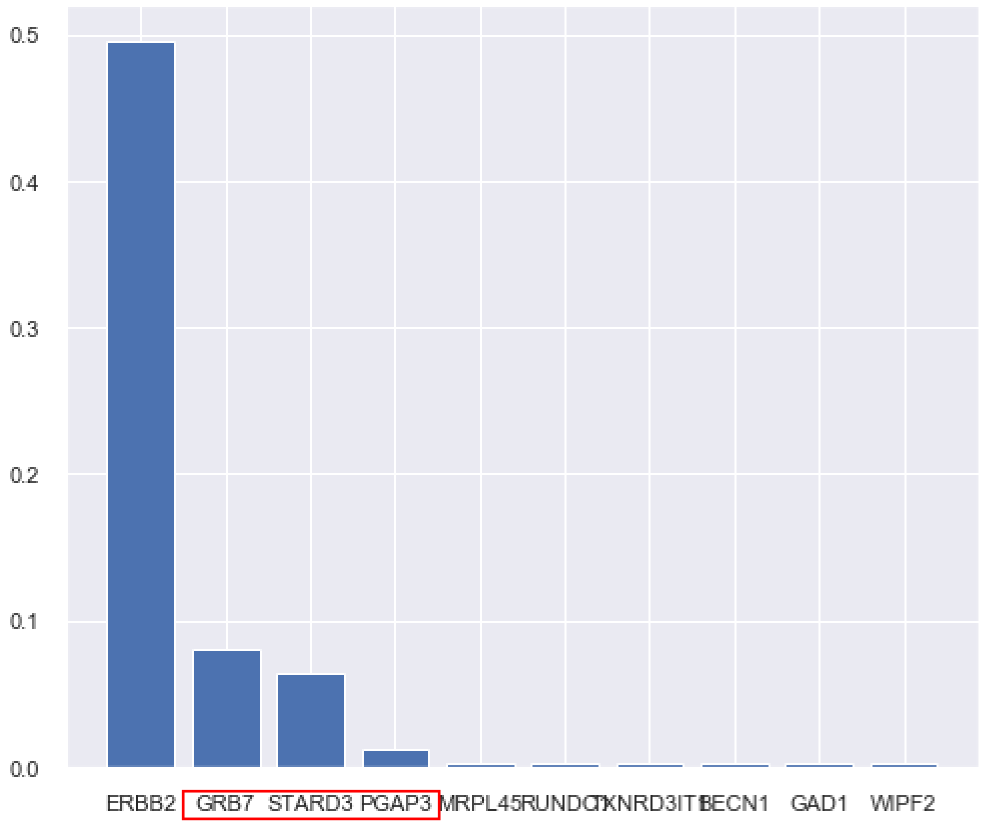

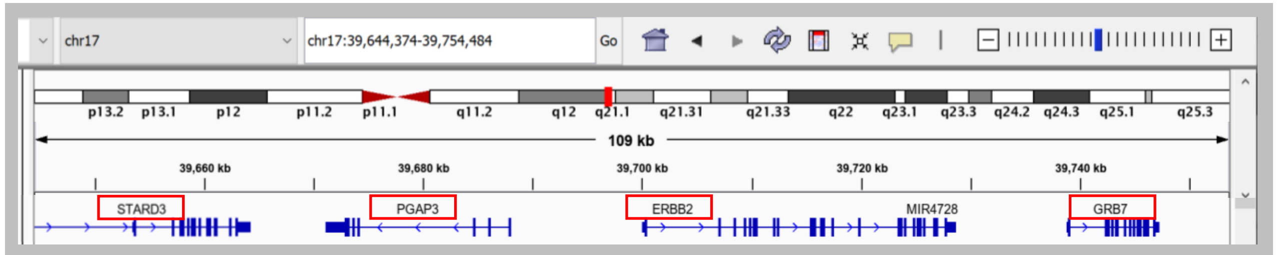

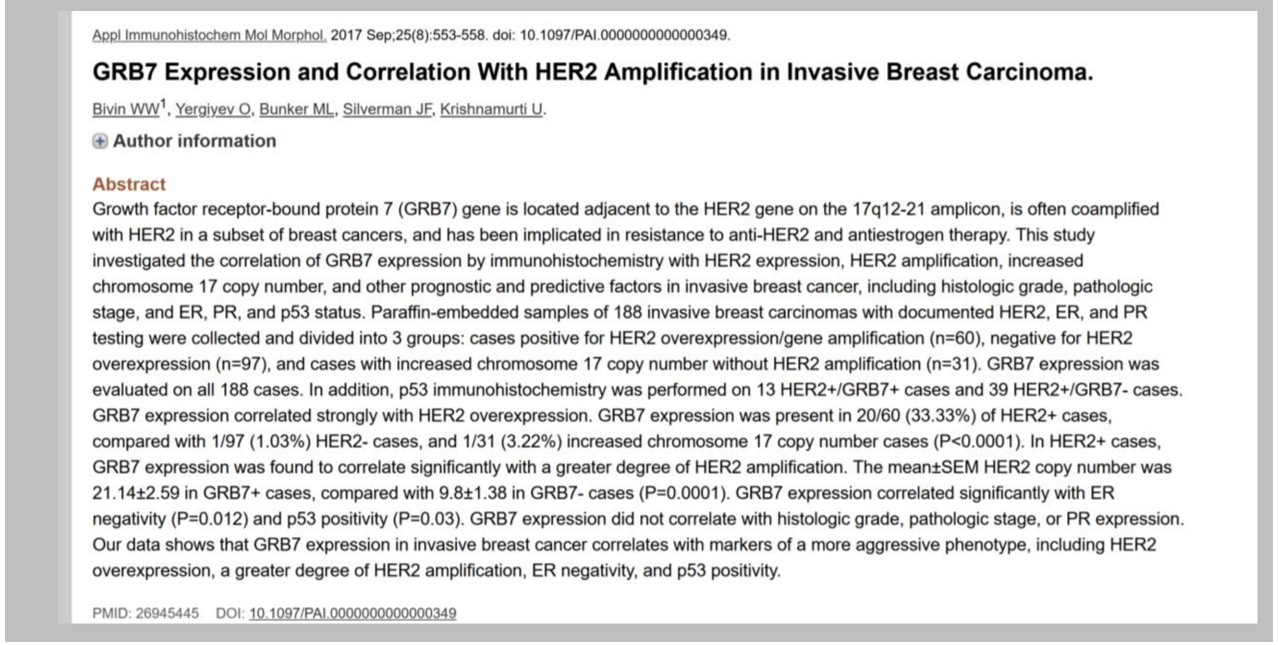

Appl Immunohistochem Mol Morphol. 2017 Sep;25(8):553-558. doi: 10.1097/PAI.0000000000000349.

GRB7 Expression and Correlation With HER2 Amplification in Invasive Breast Carcinoma.

Bivin WW[1], Yergiyev O, Bunker ML, Silverman JF, Krishnamurti U.

⊕ Author information

Abstract

Growth factor receptor-bound protein 7 (GRB7) gene is located adjacent to the HER2 gene on the 17q12-21 amplicon, is often coamplified with HER2 in a subset of breast cancers, and has been implicated in resistance to anti-HER2 and antiestrogen therapy. This study investigated the correlation of GRB7 expression by immunohistochemistry with HER2 expression, HER2 amplification, increased chromosome 17 copy number, and other prognostic and predictive factors in invasive breast cancer, including histologic grade, pathologic stage, and ER, PR, and p53 status. Paraffin-embedded samples of 188 invasive breast carcinomas with documented HER2, ER, and PR testing were collected and divided into 3 groups: cases positive for HER2 overexpression/gene amplification (n=60), negative for HER2 overexpression (n=97), and cases with increased chromosome 17 copy number without HER2 amplification (n=31). GRB7 expression was evaluated on all 188 cases. In addition, p53 immunohistochemistry was performed on 13 HER2+/GRB7+ cases and 39 HER2+/GRB7- cases. GRB7 expression correlated strongly with HER2 overexpression. GRB7 expression was present in 20/60 (33.33%) of HER2+ cases, compared with 1/97 (1.03%) HER2- cases, and 1/31 (3.22%) increased chromosome 17 copy number cases ($P<0.0001$). In HER2+ cases, GRB7 expression was found to correlate significantly with a greater degree of HER2 amplification. The mean±SEM HER2 copy number was 21.14±2.59 in GRB7+ cases, compared with 9.8±1.38 in GRB7- cases ($P=0.0001$). GRB7 expression correlated significantly with ER negativity ($P=0.012$) and p53 positivity ($P=0.03$). GRB7 expression did not correlate with histologic grade, pathologic stage, or PR expression. Our data shows that GRB7 expression in invasive breast cancer correlates with markers of a more aggressive phenotype, including HER2 overexpression, a greater degree of HER2 amplification, ER negativity, and p53 positivity.

PMID: 26945445 DOI: 10.1097/PAI.0000000000000349

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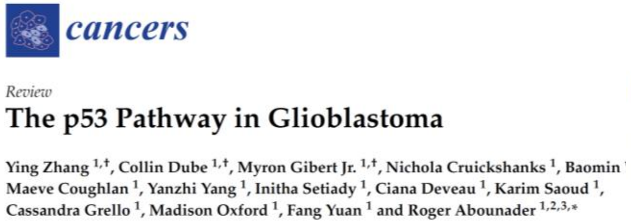
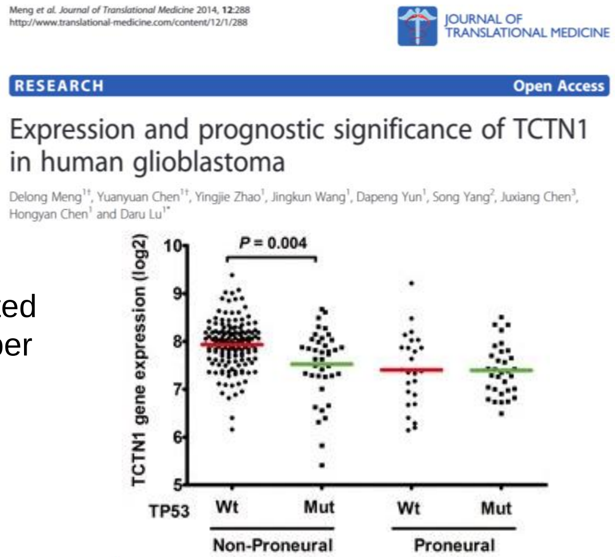


cancers

Review

The p53 Pathway in Glioblastoma

Ying Zhang [1,†], Collin Dube [1,†], Myron Gibert Jr. [1,†], Nichola Cruickshanks [1], Baomin
Maeve Coughlan [1], Yanzhi Yang [1], Initha Setiady [1], Ciana Deveau [1], Karim Saoud [1],
Cassandra Grello [1], Madison Oxford [1], Fang Yuan [1] and Roger Abounader [1,2,3,*]



Meng *et al. Journal of Translational Medicine* 2014, **12**:288
http://www.translational-medicine.com/content/12/1/288

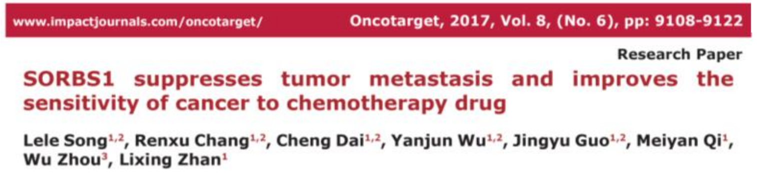
JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH **Open Access**

Expression and prognostic significance of TCTN1 in human glioblastoma

Delong Meng[1†], Yuanyuan Chen[1†], Yingjie Zhao[1], Jingkun Wang[1], Dapeng Yun[1], Song Yang[2], Juxiang Chen[3], Hongyan Chen[1] and Daru Lu[1*]

"ARF deletion [from p53 pathway] is correlated with overexpression of tectonic family member 1 (TCTN1), a protein involved in a diverse range of cellular processes, including promotion of GBM cell proliferation".



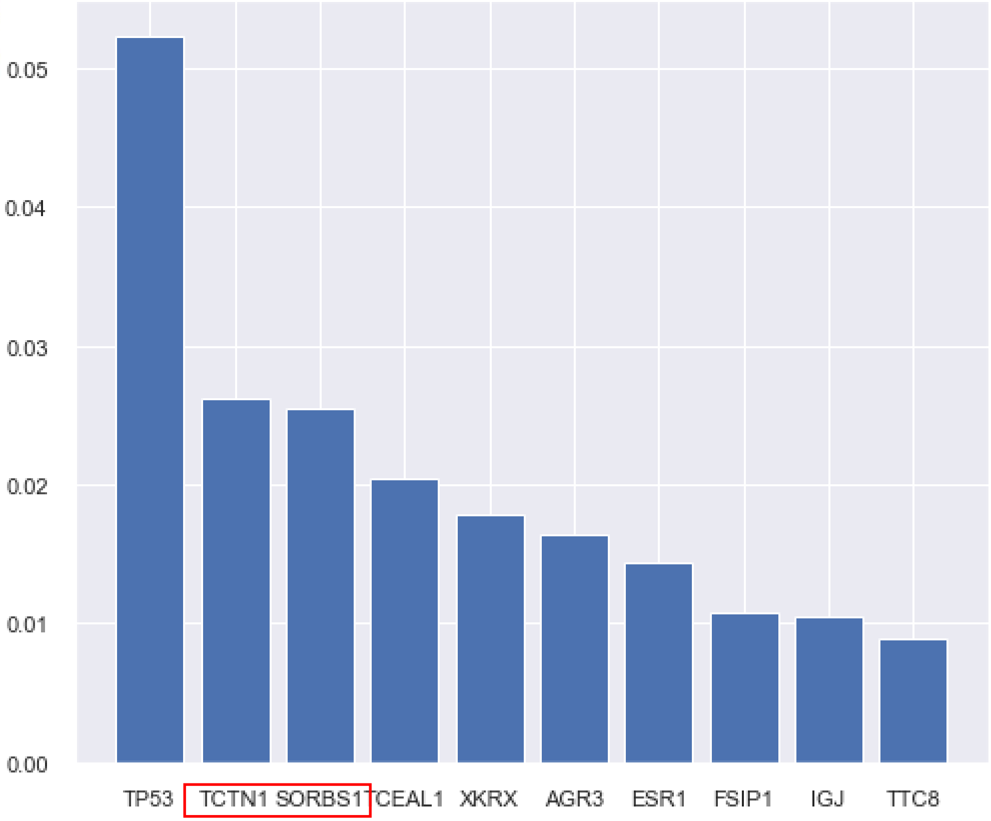
www.impactjournals.com/oncotarget/ **Oncotarget, 2017, Vol. 8, (No. 6), pp: 9108-9122**

Research Paper

SORBS1 suppresses tumor metastasis and improves the sensitivity of cancer to chemotherapy drug

Lele Song[1,2], Renxu Chang[1,2], Cheng Dai[1,2], Yanjun Wu[1,2], Jingyu Guo[1,2], Meiyan Qi[1], Wu Zhou[3], Lixing Zhan[1]

"Silencing of SORBS1 […] attenuates chemical drug sensitivity especially that to cisplatin, by inhibition of p53 in **breast cancer cells.**"

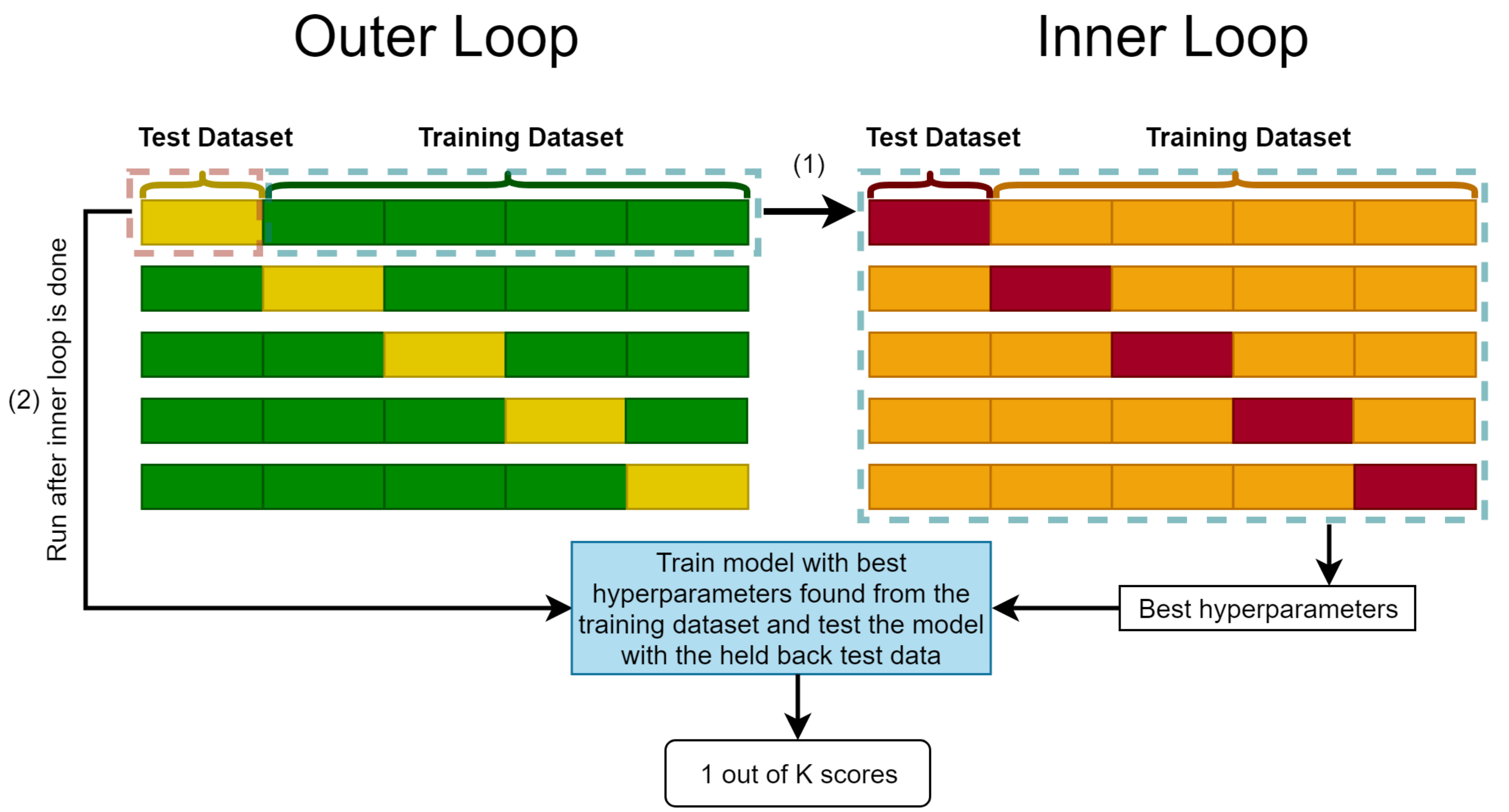


Take home message: Regression

- Regularized methods generally work better
  - Regularization may prevent over-fitting and select “important” features
- Regularized linear methods may provide accuracy similar to non-linear methods
- Neural networks do not always win
- Checking the feature importance may provide insights into biological mechanisms

Selection of hyperparameters via cross validation

What we did not do today, but in real life we should do it:



Outer Loop
Inner Loop
Test Dataset
Training Dataset
Test Dataset
Training Dataset
(1)
(2)
Run after inner loop is done
Train model with best hyperparameters found from the training dataset and test the model with the held back test data
Best hyperparameters
1 out of K scores

Nested cross validation

Take home message

- Classification and regression are extremely widely used in biology and medicine to automatize decisions of clinicians (diagnosis, choice of treatment) and predict treatment response and side effects
- The accuracy of predictions depends a lot on the information present in the data rather than on the ML method used
  - In our hands-on exercises the difference in accuracy between linear and non-linear methods varied between 0.5% and 15%
- There is no method that works the best in any situation
- Cross validation should be always applied to select the best hyperparameters

- In real life, one should compare a model built on omics data (+ clinical) with a model built using clinical variables only

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Thank you for your attention!

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