Targeted Metabolomics Data Analysis: Unlocking Insights with Machine Learning, AI and Statistics

Day 3 – Lecture 2

June 11-14, 2024
Barcelona, Spain

Outline

Introduction and motivation

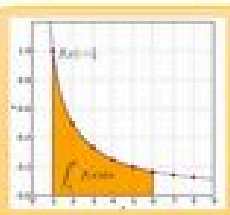
Omics data are high dimensional

- So far, our analyses are dealing with a single variable (i.e. univariate analysis)
  - T-tests: one variable, two groups
  - ANOVA: one variable, > 2 groups
- Even when we analyze many variables, we proceed *one at a time*
  - Determine how to analyze one single variable separatedly
  - Apply the procedure to all variables,
  - Perform multiple test adjustment
- This is not wrong, but, at least it can be criticized because interactions (correlations) are missed.
- Even if we attempt to account for interactions, visualization are limited to three dimensions.
- So the question arises of ***How can we analyze & visualize high-dimensional data in a global ("holistic") way, that is considering all data together?***

The analysis of high dimensional data

- The classical approach to analyzing high dimensional data is Multivariate Statistics which orginates in the early 20th century.
    - Pearson 1901, Hottelling 1933: Principal Components Analysis
    - TW Anderson (1958): First Multivariate Statistics (as we know today) book
- Although classical MV Statistics considered Exploratory Analysis it was strongly rooted in Statistical Modelling and Distribution theory.
- By the end of the 20th century, as computing power became available and data did not stop growing many aspects of multivariate statistics were re-casted from the side of computer science
    - Their approach was not so much in modelling multivariate distributions but on the algorithms.
- The underlying idea of this *Machine Learning* approach is that instead of focussing on a model, we focus on howw to build an algorithm to solve a certain problem given a set of of examples.
- This lead to the idea of *Learning from Data*

Machine learning and Statistics



Statistics +  **Machine Learning** = $\sum_{i=1}^{n}(y_i - \sum_j x_{ij}\beta_j)^2 + \lambda \sum_{j=1}^{p} |\beta_j|$ **Statistical Learning**

	Statistics	Machine Learning	Statistical Learning
Subfield of...	Mathematics	Computer Science (AI)	Statistics & Machine Learning
Focus on...	Building models with explicitly programmed instructions	Creating systems that learn from data	Sets of tools for modeling and understanding complex data
Purpose	Inferences; Relationships between variables	Optimization; Prediction accuracy	Building statistical models for prediction; understanding data
Prior assumptions about data	Some knowledge about population usually required	None	Some knowledge about population may be required
Dimensionality of data	Usually applied to low-dimensional data	Usually applied to high dimensional data; ML learns from data	Usually applied to high dimensional data
Knowledge overlap	No ML knowledge required	Some stats knowledge usually needed: stats is basis for algorithms	Knowledge of statistics and ML required

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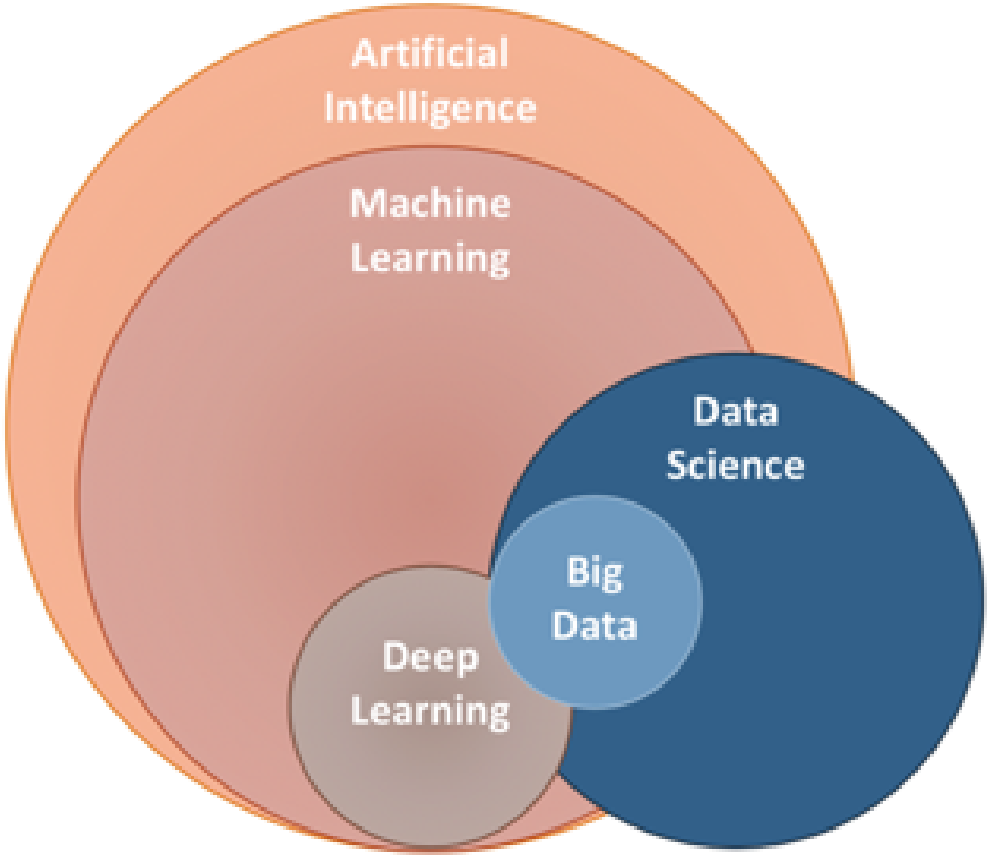
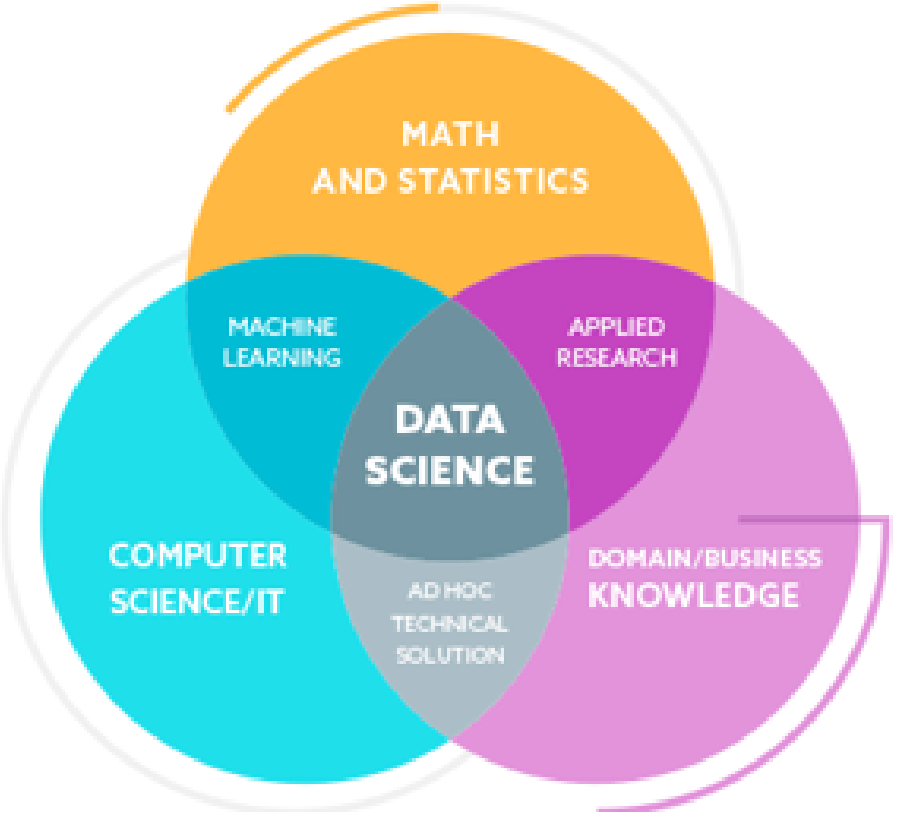
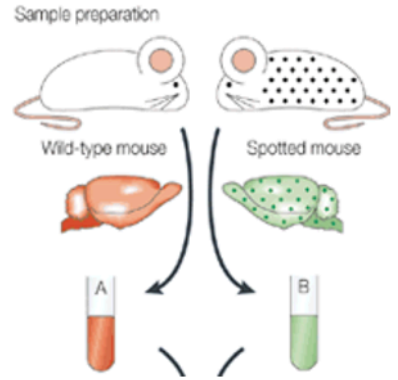
More actors in the field



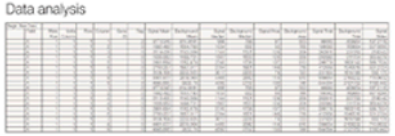


In any case, what do we need it for?

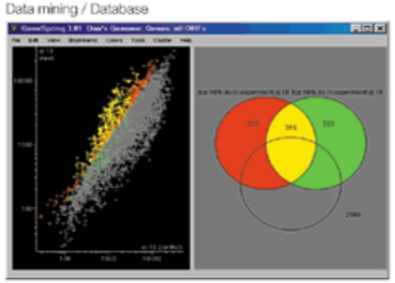
Class Comparison



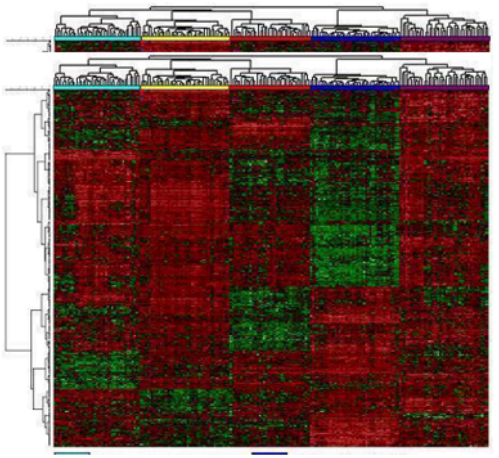
Data analysis

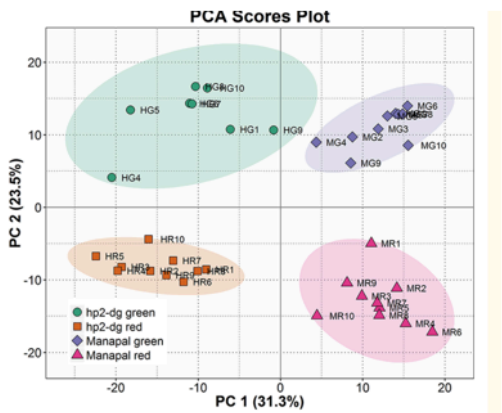


Data mining / Database

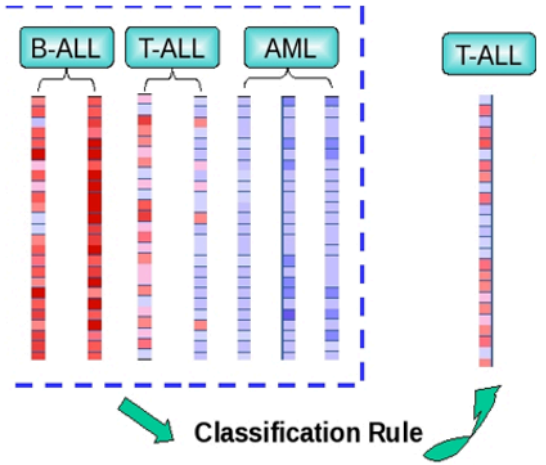


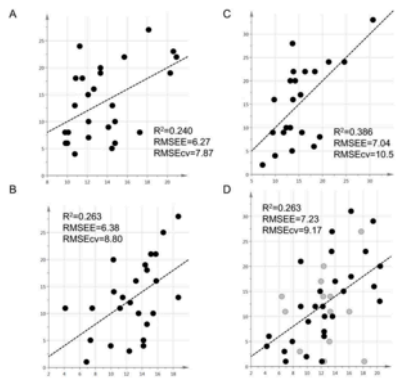
Class Discovery and Data Visualization





Class Prediction and Predictive modelling





Types of (multivariate) problems

-**Class discovery (Unsupervised learning)**

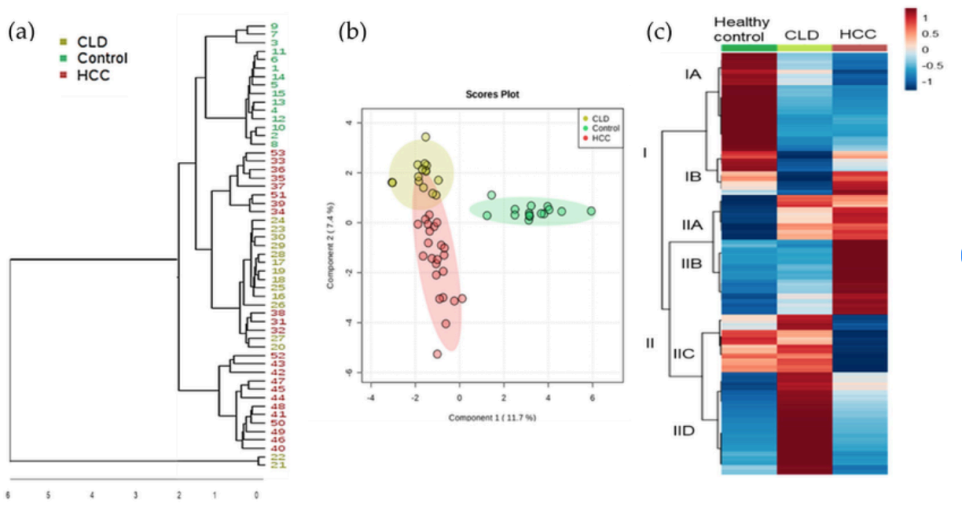
- Goal: explore the data to find some intrinsic structures in them
  - Disregard whether they are related to the class labels or not)
  - These patterns can be used to understand the key information within the data itself
- Methods: Clustering / Principal Components

- **Class prediction / Predictive modelling**
  - Goal: discover patterns in the data that relate data attributes with related to a target (categorical or numerical) attribute.
  - These patterns can be utilized to predict the values of the target attribute in future data instances.
  - Methods: Regresion(s), Classification

Unsupervised methods (1) : Clustering

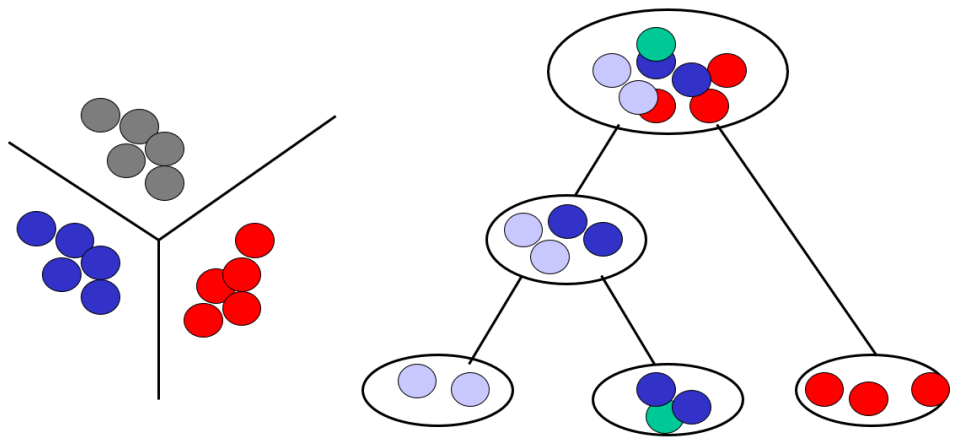
Discovering Groups in Data

- Clustering techniques allow for the identification of patterns and structures in data.
- They work by partition the data space in regions where the data are more similar within each region than among regions.
- Many methods and approaches. Here we only consider:
  - Hierarchichal clustering
  - K-Means, a partiive algorith



Partitioning

Hierarchical

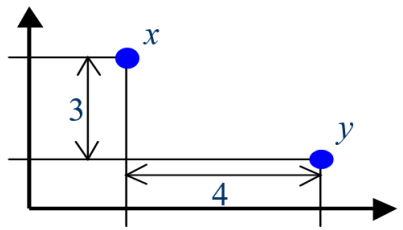


The components of clustering

- There are many clustering methods and algorithms but they all have a common goal: *Try to put togeher objects that are logically similar in characteristics*
- Cluster analysis is based on two main ingredients:
  - A *Distance measure*: to quantify the (dis)similarity of objects.
    - Notice we need to be able to measure the distance between individuals, but also between clusters.
  - *Cluster algorithm*: A procedure to group objects. Aim: small within-cluster distances, large between-cluster distances.

What distance to use?

- Euclidean : $d_E(x,y) = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$
- Manhattan : $d_M(x,y) = \sum_{i=1}^{n}|x_i - y_i|.$
- Correlation $d_C(x,y) = 1 - \frac{\sum_{i=1}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}(x_i - \bar{x})^2 \sum_{i=1}(y_i - \bar{y})^2}}.$



1, Euclidean distance : $\sqrt[2]{4^2+3^2} = 5.$

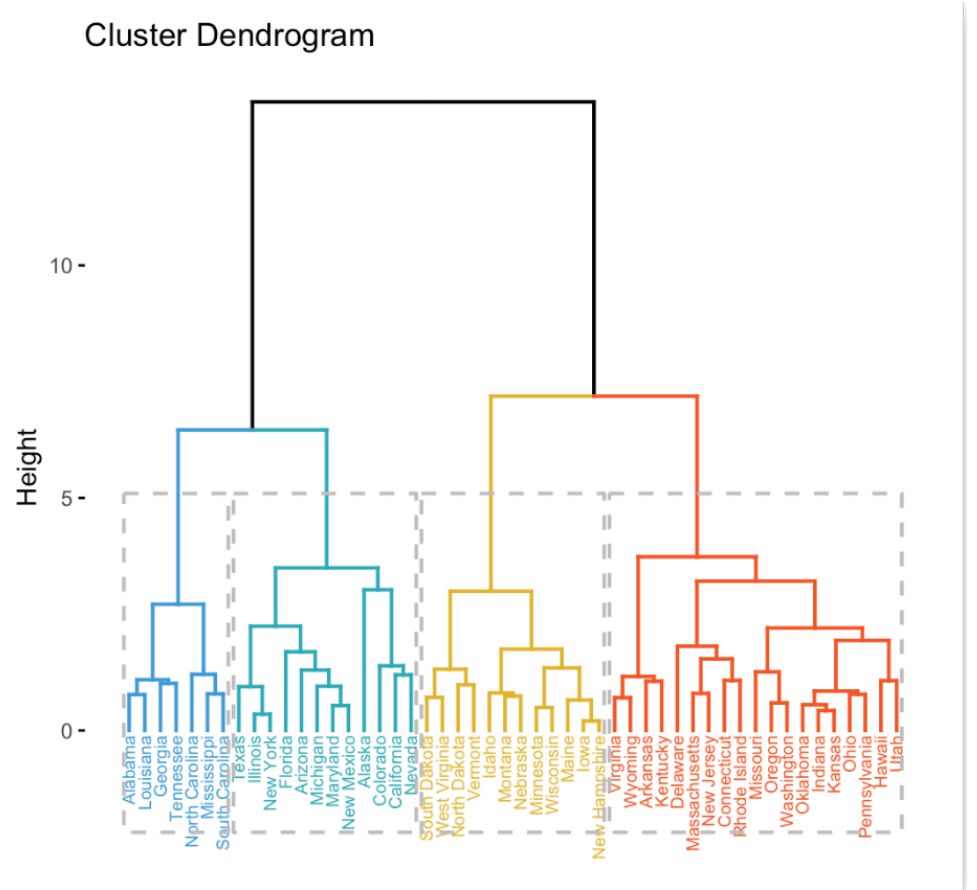
2, Manhattan distance : $4+3=7.$

3, "sup" distance : $\max\{4,3\} = 4.$

- Base the choice of distance in:
  - the application area
  - your understanding of what sort of similarities you wish to detect?
- Correlation distance dc measures trends or relative differences.
- Euclidean and Manhattan distance both measure absolute differences between vectors.
- Manhattan distance is more robust against outliers.
- After standardization, Euclidean and correlation distance are equivalent

Hierarchichal clustering

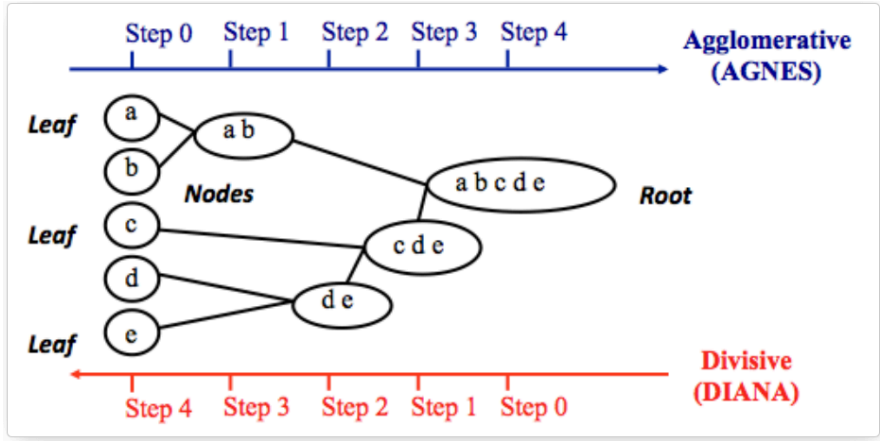
- Hierarchical Clustring Analysis (HCA) seeks to build a hierarchy of clusters by iteratively grouping/splitting groups by their similarity.
- The result of HCA is a tree-based representation of the objects, which is also known as *dendrogram*.
- Observations can be subdivided into groups by *cutting the dendrogram at a desired similarity level*.
- HCA avoid specifying the number of clusters by providing a partition for each k obtained from cutting the tree at some level.

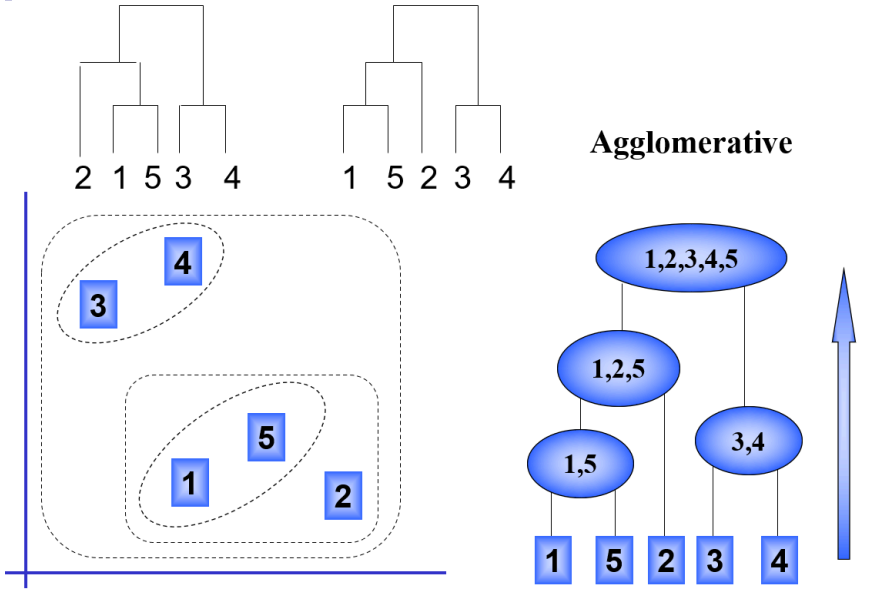


Strategies for Hierarchical clustering

They generally fall into two categories:

- *Agglomerative*
  - 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*:
  - This is a "top-down" approach:
  - All observations start in one cluster, and
  - Splits are performed recursively as one moves down the hierarchy.





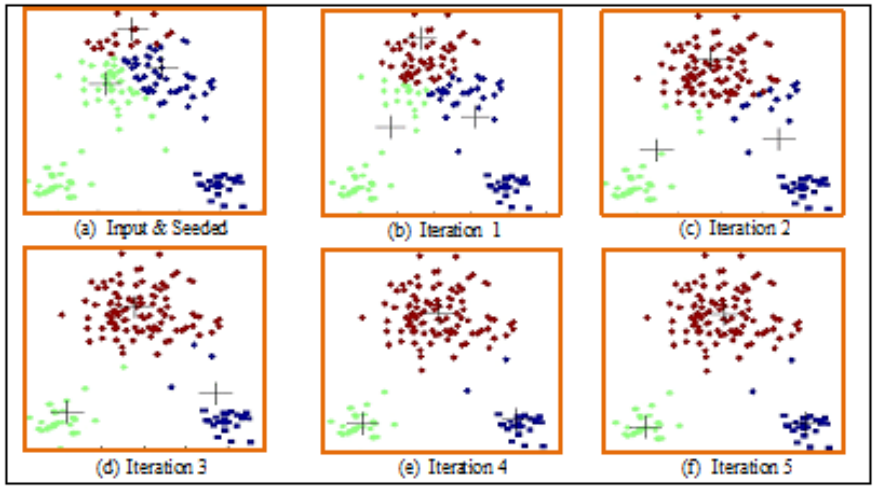
Source: Hierarchical clustering in R

Partitioning Methods

- These algorithms partition the data into a **pre-specified** number, k, of mutually exclusive and exhaustive groups.
- They work by iteratively reallocating the observations to clusters until some criterion is met,
    - For example until "within cluster sums of squares" is minimized.
- Some Partitioning methods are:
    - k-means,
    - Partitioning Around Medoids, PAM.
    - Self-organizing maps (SOM)
    - Fuzzy clustering which needs to have un underlying stochastic model, e.g. Gaussian mixtures.

The K-means algorithm

1. Randomly chooses k observations from the dataset and uses these as the initial means.
2. For the next object calculate the similarity to each existing centroid.
3. If the similarity is greater than a threshold add the object to the existing cluster and re-compute the centroid, else use the object to start new cluster
4. Return to step 2 and repeat until done



Other clustering related issues

- Selecting the number of clusters
  - Often an important issue. Needs to devote some time
  - There exist criteria such as "maximize average silhouette"
- Determining the validity of clusters
  - All cluster algorithms will yield clusters
  - Need some way to determine if *they are real*
  - Typical approximation: Use resampling to build multiple clusterings and check consistency.

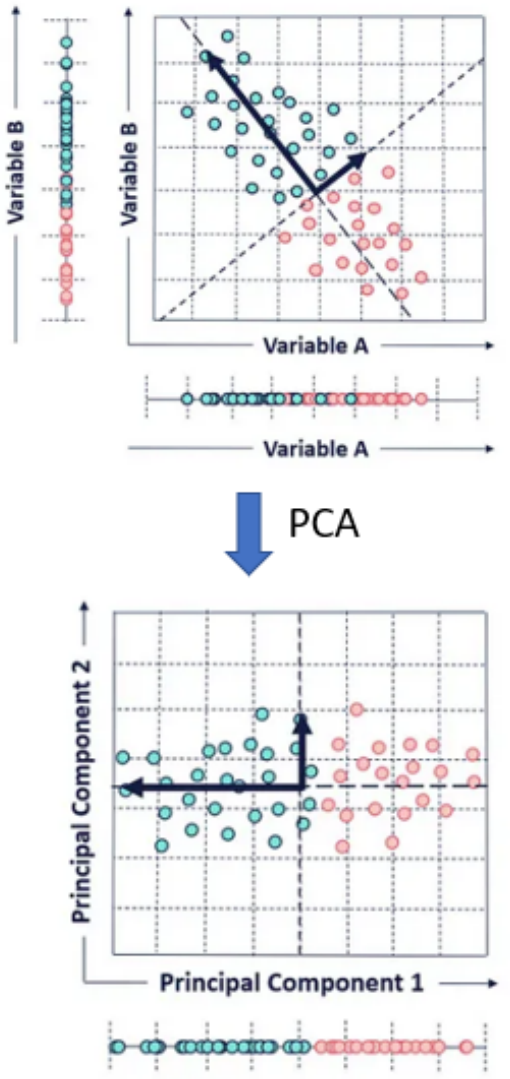
Dimension Reduction

Motivation for PCA

- High dimensional data consist of dataset with
  - many observations (samples, individuals ...)
  - many measurements (variables, features) performed on each sample.
- Although a priori it seems they must be informative this is not immediate because
  - The data may have structures that we don't see at first sight
  - The data may contain noise
  - Many variables may be correlated: they do not contribute as much as one might expect.

Principal Component Analysis

- Given a KxN data matrix containing
  - K variables (probably correlated measurements) in
  - N samples (objects/individuals...)
- Assuming $K < N$, PCA transforms the variables into K new components that:
  - Reflect the different sources of variability in the data, but
  - Are not correlated, i.e., *each component represents a different source of variability,*



Designed to Improve

- These new components are constructed in such a way that:
  - They are orthogonal (perpendicular) to each other
  - *They have decreasing explanatory power*: each component explains more (variance) than the next one.
- I most cases, a few components (2 or 3) suffice to summarize most of the information contained in the original variables.
- That is, one can use PCA values to obtain a decent representation in *reduced dimension*.

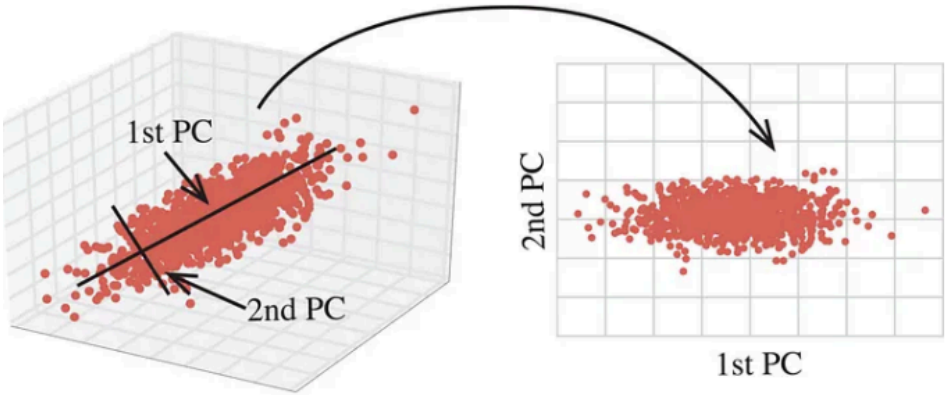
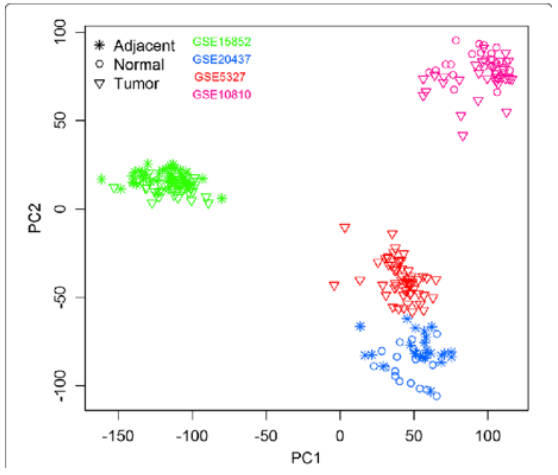


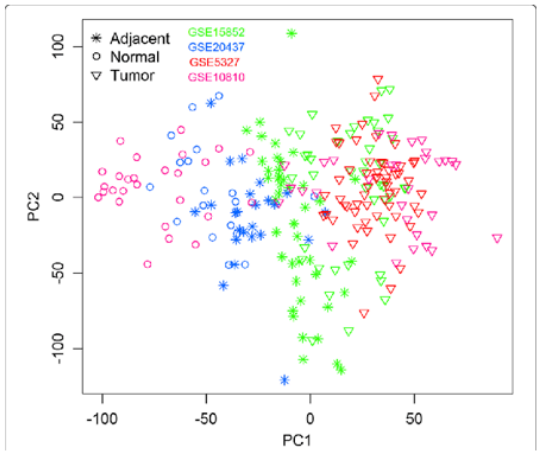
image source- google

This picture depicts how, after performing PCA and retining the two first components, almost no information is lost.

PCA highlights latent information

- It is generally assumed that PCA can be the basis to
  - Highlight dominant latent structures in the data,
  - As well as revealing natural groups, like genotypes or metabotypes,
  - And alos non natural unexpected groupings, due to batch effect.
- The image on the right shows the aspect of a PCA plot of a dataset before and after performing a batch adjustment.





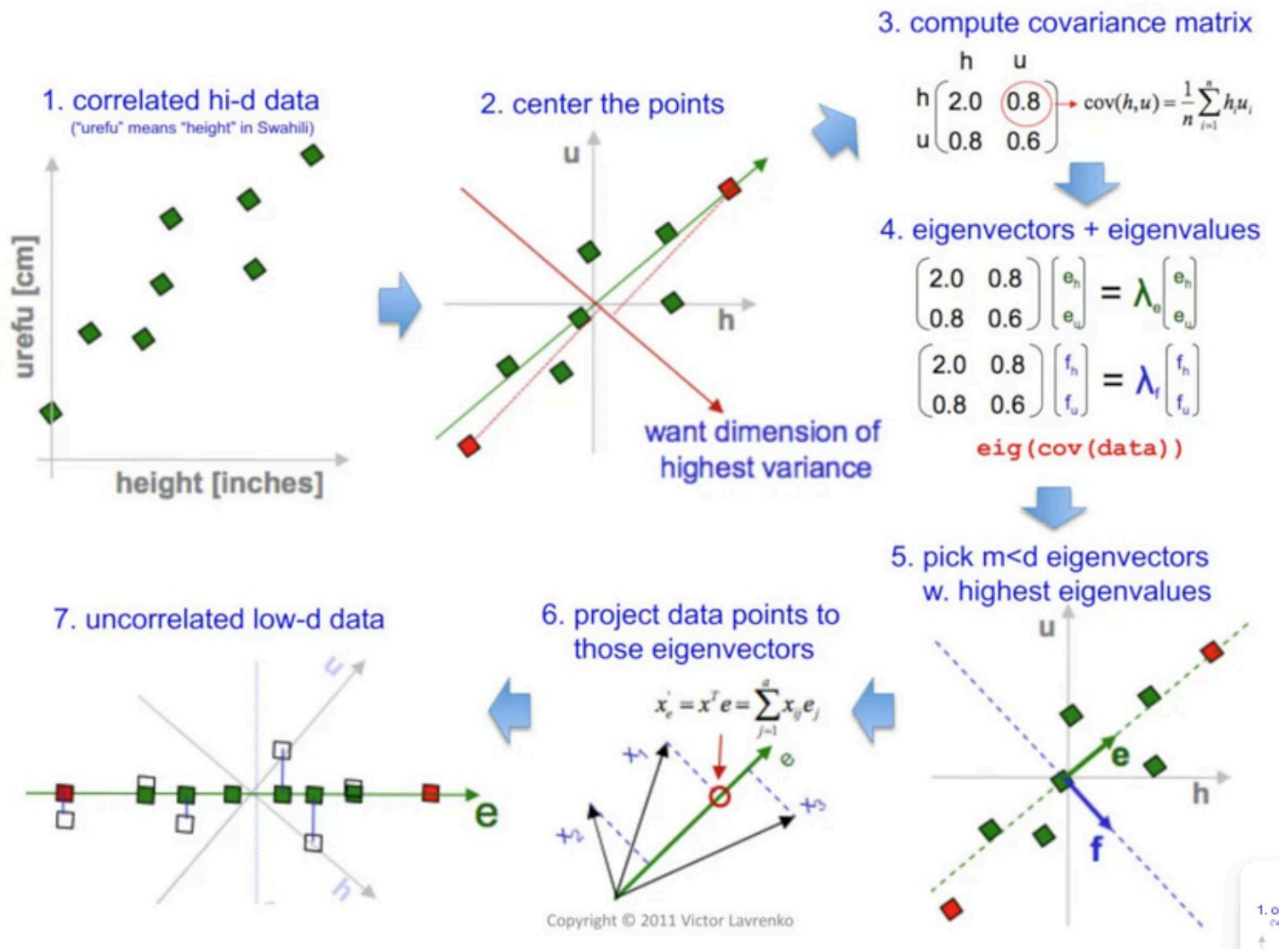
How does PCA work?

- Let's assume a KxN data matrix of two correlated variables.
- Because the data is correlated, it is difficult to separate each source of variability
  - To obtain a new system of coordinates the following optimization problem is considered:
  - Searching, sequentially, for a transformation of the original matrix
  - in which the resulting vectors are orthogonal two to two, that is, The new variables (new coordinate axes) are independent of each other.
- Notice that, what we are asking is that our variance-covariance matrix becomes diagonal, since the covariance of independent variables is zero.

Obtaining the PCs

- Under general conditions, the way to obtain a new coordinate system where the covariance matrix is diagonal can be obtained by *diagonalizing the variance-covariance matrix of the centered data*
    - Diagonalization is a calculation performed on a matrix that results in:
    - A vector of eigenvalues (VAPs) proportional to the variance of each variable in the new coordinates
    - A matrix of eigenvectors (VEPs) that are, precisely, the scores or scores of the observations in these new coordinates.
- This operation yields a new system of coordinates where
    - the covariances of the new variables become 0, that is, independent variables are obtained.
    - the eigenvalues (the diagonal of the new matrix) are ordered from highest to smallest, which corresponds to the idea that each one has a greater variability than the next ones.

PCA in a nutshell



Interpreting Principal Components

- The first main component is
  - A linear combination (LC) of the original variables
  - That goes in the direction of greater variability in the data
  - Explains the *maximum amount of variation* in the data
- The 2nd and successive PCs are also a LC of all the original variables, although with other coefficients calculated in such a way that:
  - It aligns with the next direction of greatest variability, orthogonally to the previous PCs.
  - Explain the maximum amount of *remaining variation*
- Sometimes interpretation of the new components may be clear, corresponding to real biological dimensions or, sometimes, not at all.
- To facilitate this, the correlation between components and the original variables is computed.

Data visualization in the PCA space

- Scores are the new coordinates in the orthogonal system defined by the PCs
- They have been built so that
  - the first PC explains the highest quantity of variability,
  - the second PC explains explains the highest quantity of remaining variability, and so on ...
- This means that it is not necessary to use all PCs to visualize the data in this new coordinate system, so a visualization can be made in a lower dimension.]
  - Often, takin only the first 2 or 3 first PCs is enough,
  - It should always be verified that this is the case
  - The "scree plot" graph together with a criterion of "change of slope" or elbow, are usually useful.

Examples

- PCA example in MetaboAnalystR
- FactoMineR Decathlon Tutorial
- R tutorial: Principal Component Analysis with Metabolomics Data

Predictive Modeling: regression and Relatives

Why predictive modeling

- We are interested in Y, an informative quantity but difficult (or expensive, or slow) to measure,
- If Y is related to others, say $X_1, X_2, \dots X_n$ that may be easier (faster, cheaper) to obtain, we may attempt

    1. To model the relation

    $$Y = f(X_1, \dots, X_n) + \epsilon$$

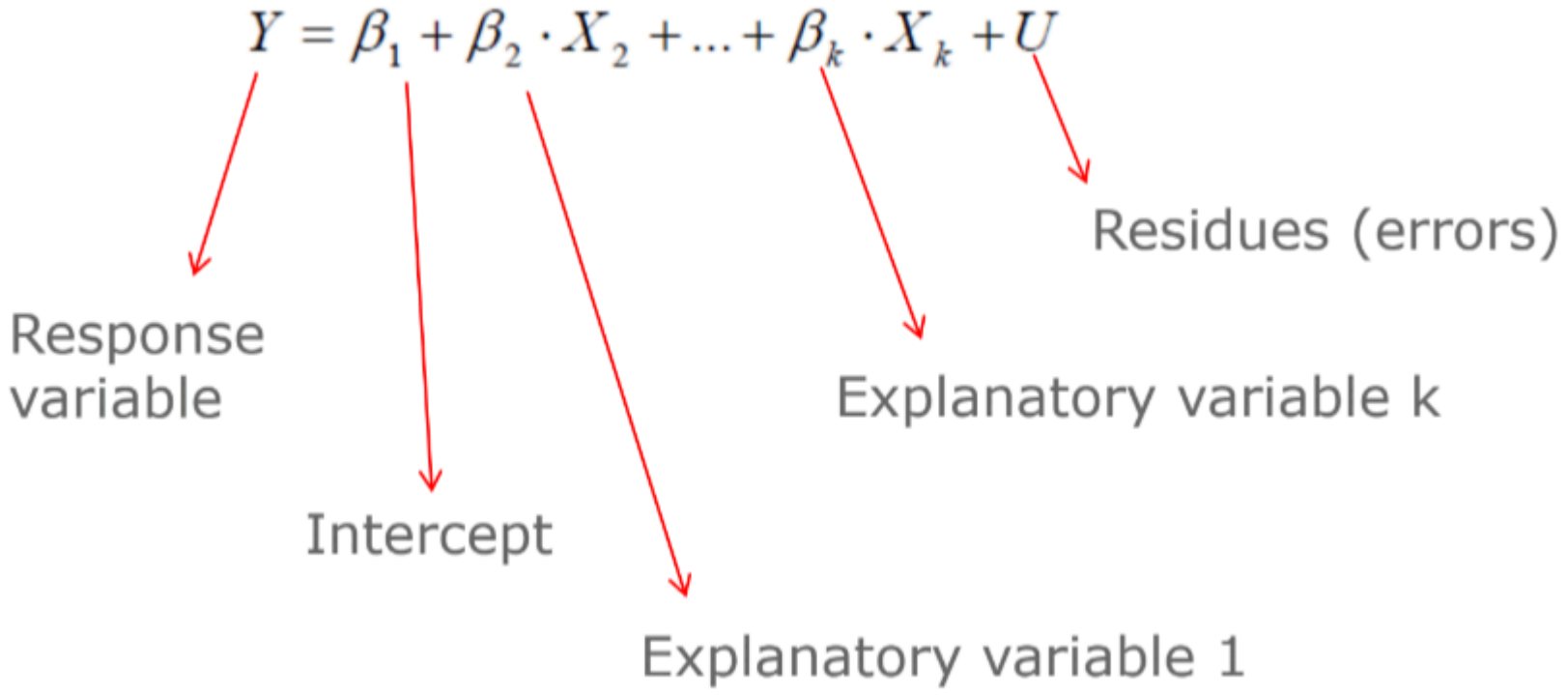
    2. Fit the model to obtain an approximation, $\hat{f}$, to f or its parameters

    3. Use that approximation to predict the values of Y for a set given certain values $x_1, \dots, x_n$.

    $$\hat{y} = \hat{f}(x_1, \dots, x_n)$$

- The most common approach to model such relations is using *Regression models*

The (multiple) linear regression model



- The relation between the response and the independent variable is assumed to be linear.
- Restrictive and very simple, but powerful when assumptions hold and the model is valid.

Logistic regression

- If the response variable is dichotomous (yes/no) the MLR is not adequate.
- Logistic regression models the probabilities of a sample being a member of either of two groups for a set of predictors.

$$\ln \frac{p(1|X)}{p(2|X)} = \beta_0 + \beta_1 X_1 + \ldots + \beta_p X_p$$

- Notice that it is also a linear regression model where the response variable is the log odds.

High dimensional data issues

Working with high dimensional data requires to account for a series of issues that may not be apparent in lower dimensions

- **Curse of Dimensionality:** High-dimensional spaces can dilute the signal, making it hard to distinguish between true signal and noise.
- **Computational Complexity:** Processing and analyzing high-dimensional data requires substantial computational resources.
- **Scalability:** Classical methods struggle to scale efficiently with increasing dimensionality.
- **Sparse Data:** High-dimensional data can lead to sparsity, making it challenging to detect meaningful patterns.

Limitations of classical linear models

The high dimensionality of omics data usually determines that these models are not adequate due to a variety of reasons

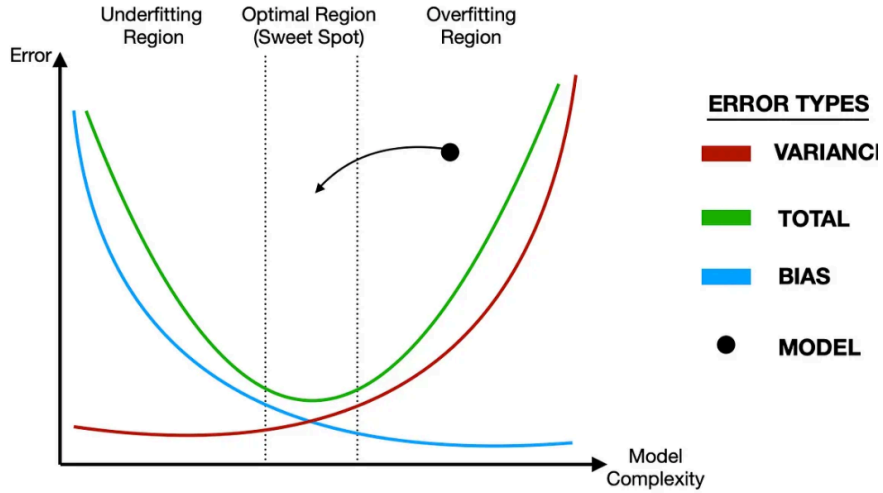
- **Overfitting:** High-dimensional data can lead to overfitting, where the model captures noise instead of the underlying pattern.
- **Multicollinearity:** Metabolomics data often have highly correlated variables, causing instability in coefficient estimates.
- **Feature Selection:** Requires manual or algorithmic feature selection, which can be computationally intensive and prone to bias.
- **Interpretability:** Coefficients become harder to interpret with a large number of features.
- **Class Imbalance:** Performance can be significantly affected if there is an imbalance in the class distribution.

Alternatives for predictive models

- The fields of Statistics and Machine Learning have developed a long collection of alternatives.
- Each type of solution may be appropriate for one or more of the problems highlighted above.
    - Regularization Techniques
    - Dimension Reduction Techniques
    - Non linear methods
    - Ensemble methods
    - Machine learning methods
    - Sparse models
    - Neural networks

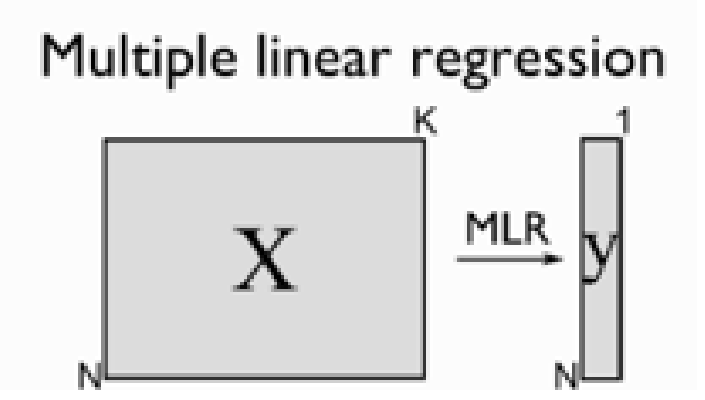
Regularization Techniques

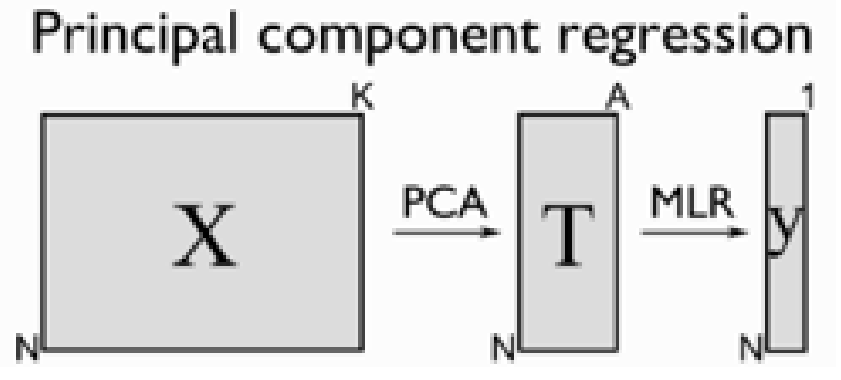
- **Lasso Regression (L1):** Adds a penalty for the absolute value of coefficients to induce sparsity.
- **Ridge Regression (L2):** Adds a penalty for the square of coefficients to handle multicollinearity.
- **Elastic Net:** Combines L1 and L2 penalties to balance sparsity and multicollinearity handling.



Dimension Reduction Techniques

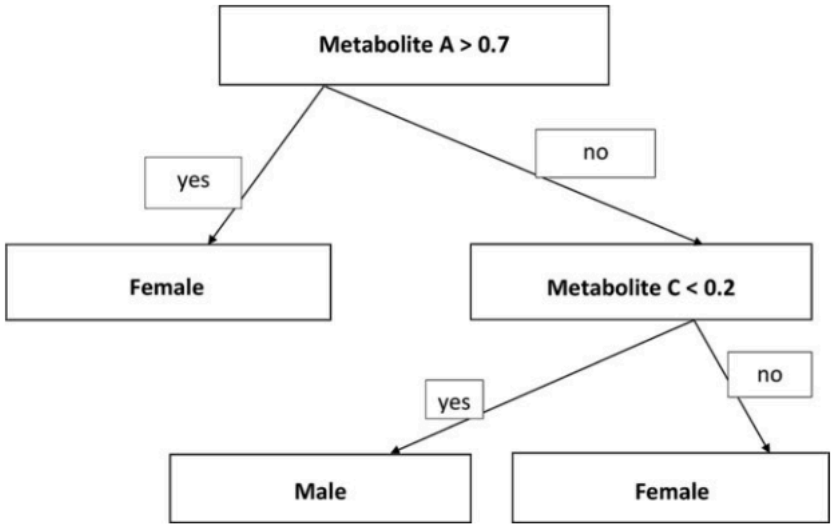
- **Principal Component Analysis (PCA):** Reduces dimensionality by projecting data onto principal components.
- **Principal Component Regression (PCR):** Reduces dimensionality projecting independent variables (predictors) onto principal components and fits a regression model to PCs.
- **Partial Least Squares (PLS):** Projects predictors and response variables onto a new space to maximize covariance.





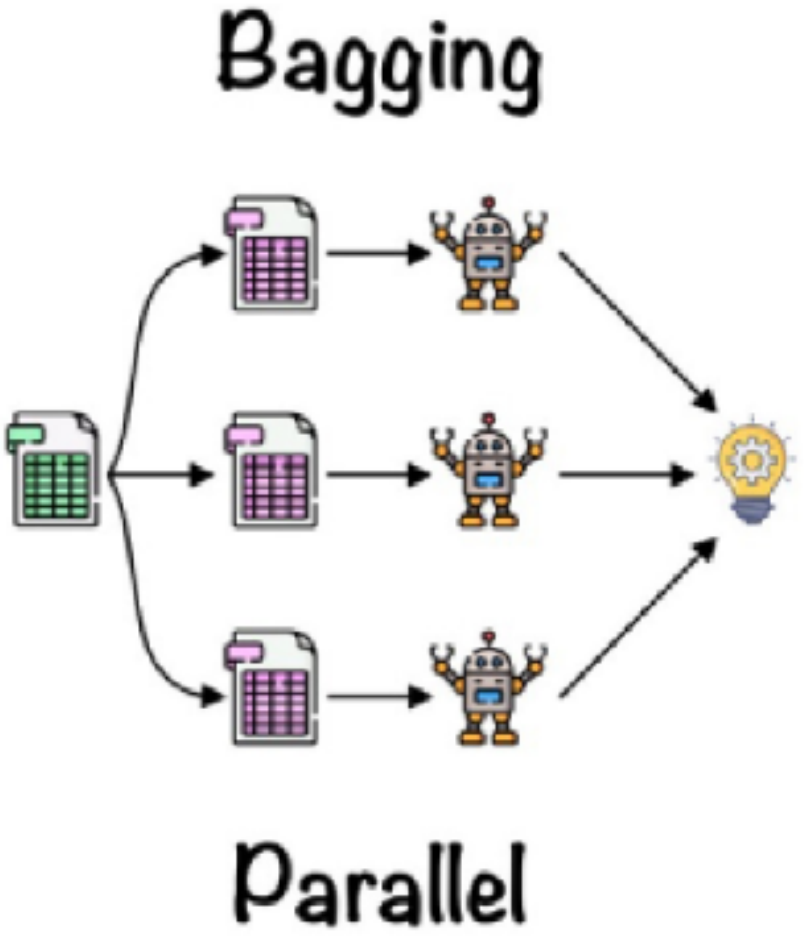
Nonlinear Methods

- **Kernel Methods:** Extend linear methods to nonlinear relationships using kernel functions.
- **Decision Trees:** Nonlinear models that split data based on feature values, leading to simple and interpretable predictive models.



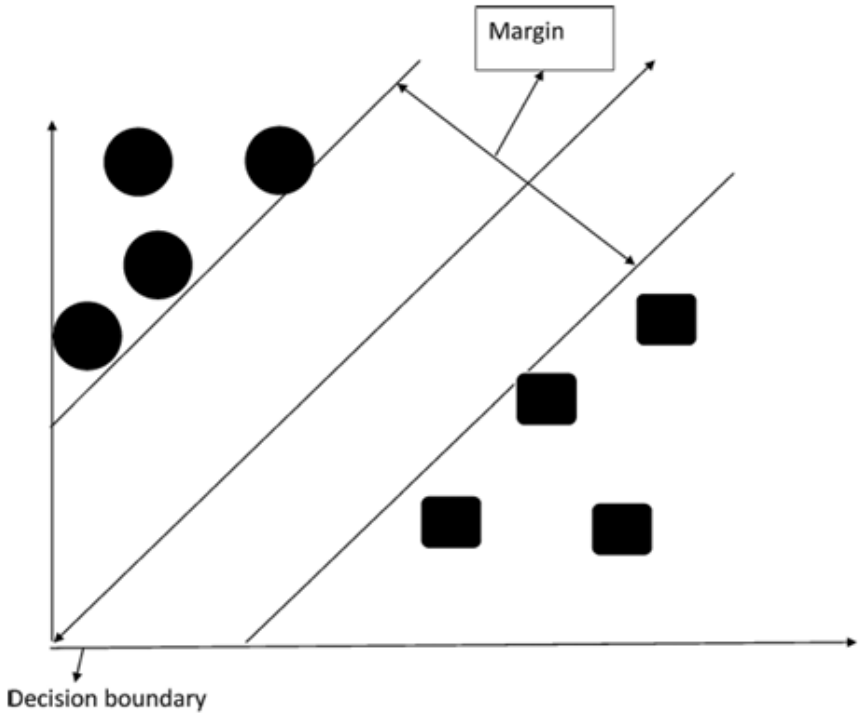
Ensemble methods

- **Bagging:** Ensemble method that handles high-dimensional data by averaging multiple decision trees built on bootstrap resamples of the original sample.
- **Random Forest:** Ensemble method that handles high-dimensional data by averaging multiple decision trees built on resamples, but also using random sets of features at each node.
- **Gradient Boosting Machines (GBM):** Builds an ensemble of weak learners to improve predictive performance.



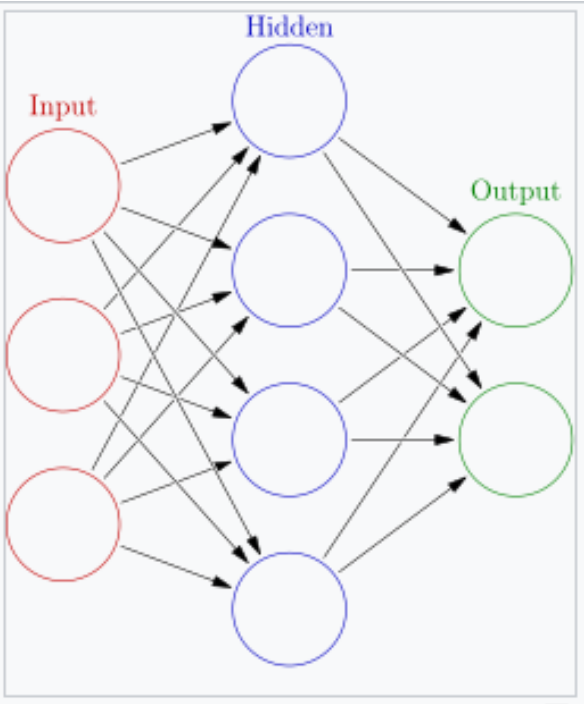
Machine Learning Methods (SVMs)

- **Support Vector Machine (SVM):**
  - Find the hyperplane that best separates data points of different classes by maximizing the margin between them.
  - They are effective in high-dimensional spaces and can handle nonlinear data using kernel functions. SVMs are robust to overfitting, especially in high-dimensional datasets.



Neural Networks

- **Neural Networks** Deals with non-linear realtions in a black-box automatic approach.
- **Deep Learning:** Handles high-dimensional data with multiple layers of abstraction, especially useful with large datasets.
- **Autoencoders:** Unsupervised neural networks that learn efficient codings of input data for dimensionality reduction.



An artificial neural network is an interconnected group of nodes, inspired by a simplification of neurons in a brain. Here, each circular node represents an artificial neuron and an arrow represents a connection from the output of one artificial neuron to the input of another.

Other approaches

Sparse models

- **Sparse Partial Least Squares (sPLS):** Combines PLS with sparsity to handle high-dimensional data.
- **Sparse Logistic Regression:** Applies sparsity constraints to logistic regression for feature selection.

Bayesian Methods

- **Bayesian Regression:** Incorporates prior information and deals with uncertainty in high-dimensional settings.
- **Bayesian Networks:** Models complex relationships between variables and handles high-dimensional data efficiently.

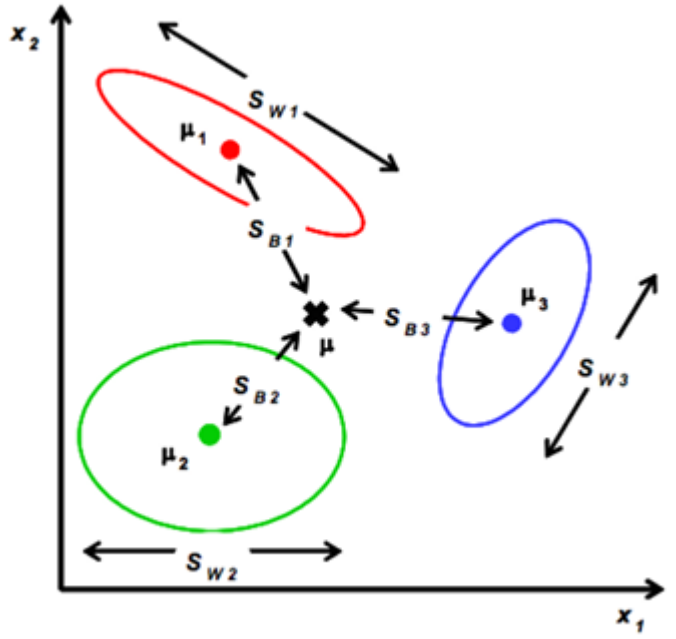
PLS and PLS-LDA

Partial Least Squares

- From all the presented alternatives, probably the best known, most used in chemometrics and metabolomics is PLS and PLS-LDA
- PLS appears as a natural alternative to MLR and PCR
  - **Multiple Linear Regression (MLR)**: MLR directly models the relationship between predictors and response variables but can suffer from multicollinearity and overfitting in high-dimensional data.
  - **Principal Component Regression (PCR)**: PCR first reduces the dimensionality of predictors using PCA and then applies MLR. However, PCA does not consider the response variable in the dimension reduction step.
  - **PLS vs. PCR**: PLS, unlike PCR, takes the response variable into account when determining the components, leading to better predictive performance when predictors are highly correlated.

Interlude: Linear Discriminant Analysis

- LDA is a classification technique, developed by R.A. Fisher used to find a linear combination of features that best separates two or more classes.
- It works by maximizing the ratio of between-class variance to within-class variance, ensuring that the classes are as distinct as possible.



Key Points of LDA

- LDA projects data onto a lower-dimensional space where the classes are well separated.
- It assumes that the features follow a Gaussian distribution and that each class has the same covariance matrix.
- LDA is *particularly useful* when the class separability is linear and the dimensionality of the data needs to be reduced for efficient computation and improved classification performance.

Combining PLS and LDA

- PLS-LDA is a classification technique that combines PLS for dimensionality reduction with LDA for classification.
- PLS-LDA uses PLS to reduce the dimensionality of the predictor variables while considering the response variable (class labels).
- After reducing the dimensionality, LDA is applied to classify the samples based on the new PLS components.
- PLS-LDA is effective for high-dimensional classification tasks, leveraging the strengths of both PLS in handling multicollinearity and LDA in classification.

Performance assessment in class prediction

Performance assessment

- The process of building and validating classifiers involves more than simply fitting a predictive model and performing the classification.
- Before using a classifier for prediction or prognostic one needs some performance measures
  - For instance the accuracy of a predictor is usually measured by the *missclassification rate*: The % of individuals belonging to a class which are erroneously assigned to another class by the predictor.
- This is however more complicated than simply computing error rates because *we are not interested in the ability of the predictor for classifying current samples*
  - One needs to estimate *future performance* based on what is available.

Error rate estimation

- Using the same dataset on which we have built the predictor to estimate the missclassification rate may lead to erroneously low values due to overfitting.
    - This erroneous estimator is known as the *resubstitution estimator*.
- Ideally, we should use a *completely independent* dataset to evaluate the classifier, but it is rarely available.
- Usually alternatives approaches such as
    - Test set estimators
    - Cross-validation estimators

Performance assesment (1)

- **Resubstitution estimator**
  - Computes the error rate on the learning set.
  - Problem: downward bias
  - Should never be used to provide final error estimates!
- **Test set estimation**
  - Proceeds in two steps
  - Divide learning set into two sub-sets, Test and Train
    - Build the classifier on the train set
    - Compute error rate on the test set
  - This is a better approach, but not free from problems
    - Test and Train sets should be independent and i.d.
    - This approach reduces effective sample size.

Performance assesment (2)

- If spliting the learning set can yield to too small sets, cross-validation can be an alternative.
- It works by splitting the data into multiple subsets as follows:
    - 1. **Data Splitting:** The original dataset is divided into (k) subsets, or folds, of approximately equal size.
    - 1. **Training and Validation:** The model is trained (k) times, each time using (k-1) folds for training and the remaining fold for validation.
    - 1. **Performance Evaluation:** Performance metrics such as accuracy, precision, recall, and F1 score are computed for each validation fold.
    - 1. **Aggregation:** The performance metrics from all folds are averaged to obtain a final assessment of the classifier's performance.

Key Benefits of CV

- **Reduced Bias:** Cross-validation provides a more robust estimate of model performance compared to a single train-test split, reducing the risk of bias from a particular data split.
- **Maximized Data Utility:** Every data point is used for both training and validation, maximizing the use of available data and ensuring thorough evaluation.
- **Parameter Tuning:** Cross-validation aids in hyperparameter tuning by evaluating multiple parameter configurations across different folds, helping to find optimal model settings.

Cross-validation is essential for accurately assessing a classifier's performance, providing insights into its generalization capabilities across different data subsets and ensuring reliable predictions in real-world applications.

Types of Cross-Validation

- **K-Fold Cross-Validation:** The dataset is divided into (k) folds, and each fold is used as the validation set once while the remaining (k-1) folds are used for training.
- **Stratified K-Fold Cross-Validation:** Ensures each fold preserves the proportion of classes, useful for imbalanced datasets.
- **Leave-One-Out Cross-Validation (LOOCV):** Each data point is sequentially used as a single data point validation set while the remaining (n-1) points are used for training, which is computationally expensive but useful for smaller datasets.

References and Resources

Resources

- MetaboAnalystR Statistical Tutorials
- Multivariate Data Analysis Course. Carlos O. Sanchez Course Slides
- Principal Component Analysis Overview PCA Overview
- Hierarchical Clustering
  - Hierarchical Clustering Overview
  - Hierarchichal clustering in R
- Iris Data PCA Iris Data PCA Code