

BIOSTATISTICS WITH

SUMMER WORKSHOP
(MITGEST network)

24-27 JULY 2024

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WORKSHOP SCHEDULE

- 4 days
 - 1. Intro to R and data analysis
 - 2. Statistical inference & hypothesis testing
 - 3. Modeling correlation and regression
 - 4. Examples of ML; MetaboAnalyst; Power analysis
- Each day will include:
 - Frontal class (MORNING)
 - Practical training with R about the topics discussed in the morning. (AFTERNOON)

DAY 4 – LECTURE OUTLINE

- Examples of ML
 - 1. PCA
 - 2. PLS-DA
- MetaboAnalyst
- Power analysis

Principal Component Analysis (PCA)

A type of unsupervised learning algorithm for
dimensionality reduction

Purpose of PCA

- The goal of PCA is to transform a high-dimensional dataset into a lower-dimensional dataset while retaining as much of the variance in the data as possible.
- Common use cases of PCA:
 1. to reduce the dimensionality of high-dimensional datasets
 2. to visualize the structure of the data
 3. to remove noise and redundant information from the data
 4. as a preprocessing step for other machine learning algorithms

Covariance

Population mean is unknown

$$var(x) = \frac{\sum_i^n (x_i - \bar{x})^2}{N - 1}$$

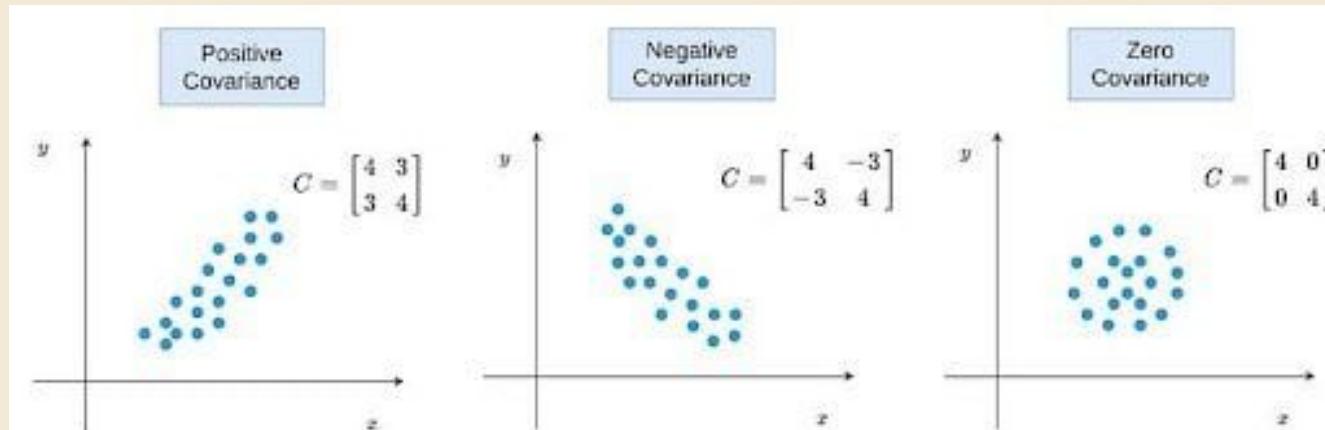
Population mean is unknown

$$cov(x, y) = \frac{\sum_i^n (x_i - \bar{x}) \cdot (y_i - \bar{y})}{N - 1}$$

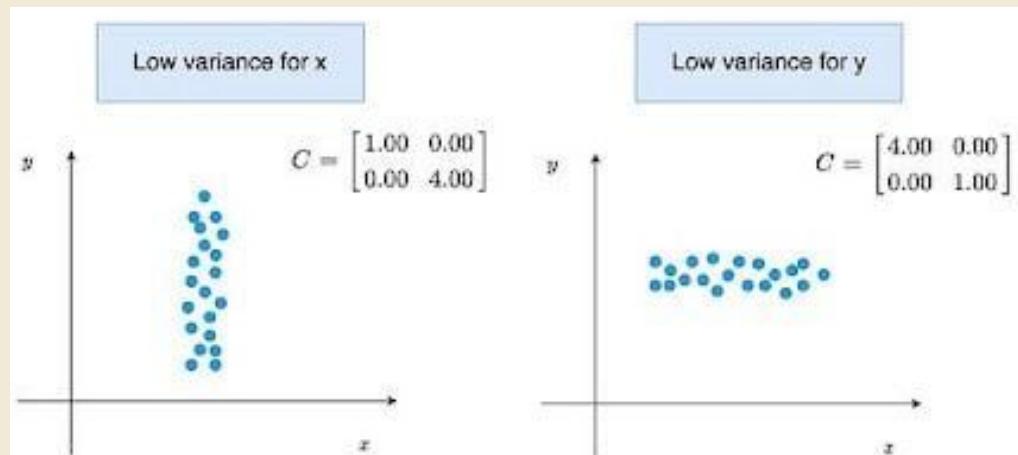
$$\begin{matrix} & x & y & z \\ x & var(x) & cov(x, y) & cov(x, z) \\ y & cov(x, y) & var(y) & cov(y, z) \\ z & cov(x, z) & cov(y, z) & var(z) \end{matrix}$$

Variance measures how the values vary in a variable.
Covariance measures how changes in one variable are associated with changes in a second variable.

Covariance



Positive, negative and zero covariance.



Different variances and zero covariance.

Source: <https://builtin.com/data-science/covariance-matrix>

PCA

PCA originally hails from the field of [linear algebra](#).

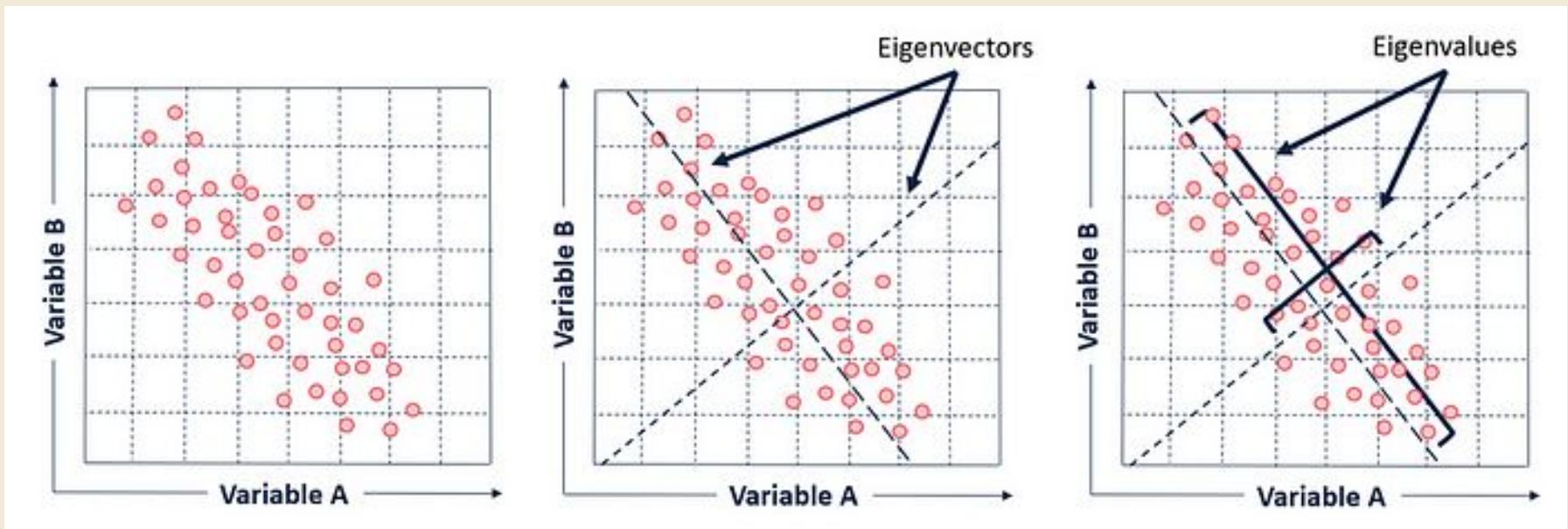
It is a transformation method that creates (weighted [linear](#)) combinations of the original variables in a data set, with the intent that the new combinations will capture as much [variance](#) in the dataset as possible while eliminating correlations (i.e., redundancy).

PCA creates the new variables using the eigenvectors and eigenvalues calculated from the [covariance matrix](#) of your original variables.

Eigenvectors & Eigenvalues

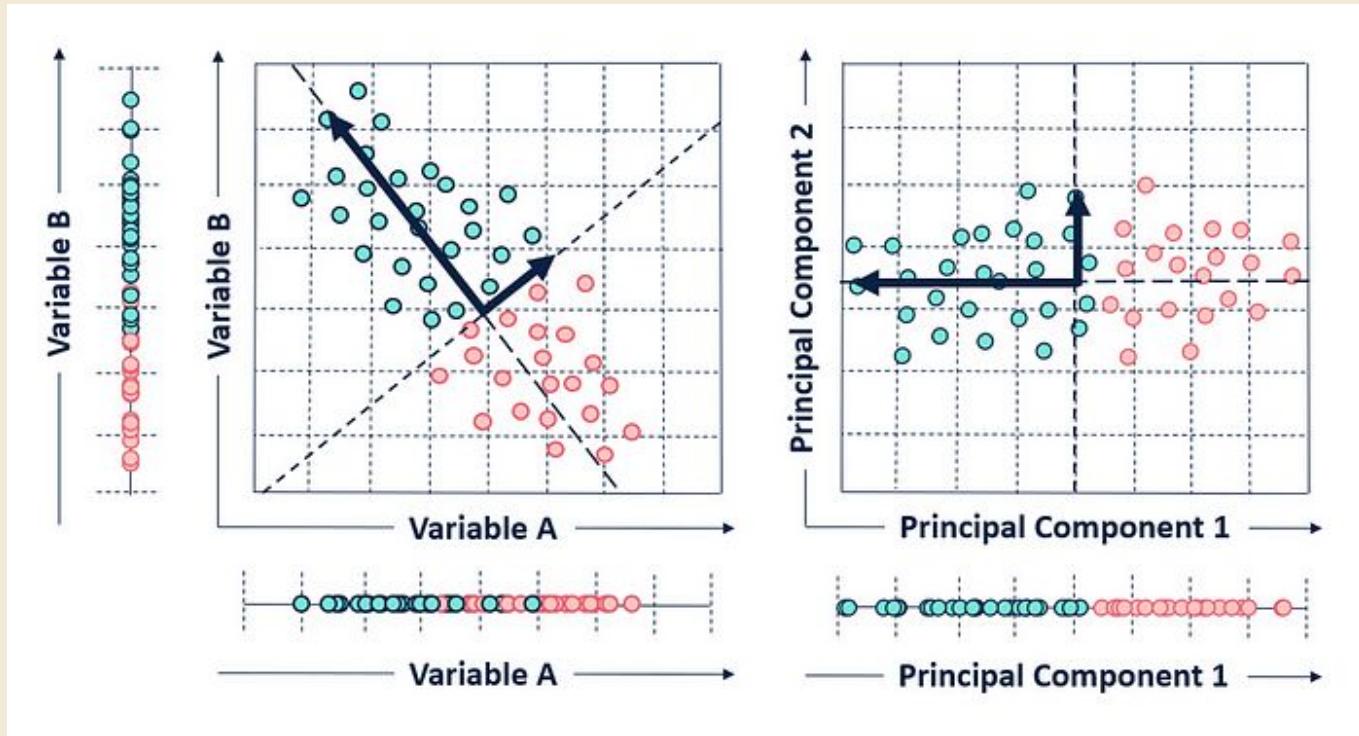
In the context of PCA

- The **eigenvectors** of the covariance matrix define the directions of the principal components calculated by PCA.
- The **eigenvalues** associated with the eigenvectors describe the variance along the new axis.



Source: <https://towardsdatascience.com/tidying-up-with-pca-an-introduction-to-principal-components-analysis-f876599af383>

Principal component

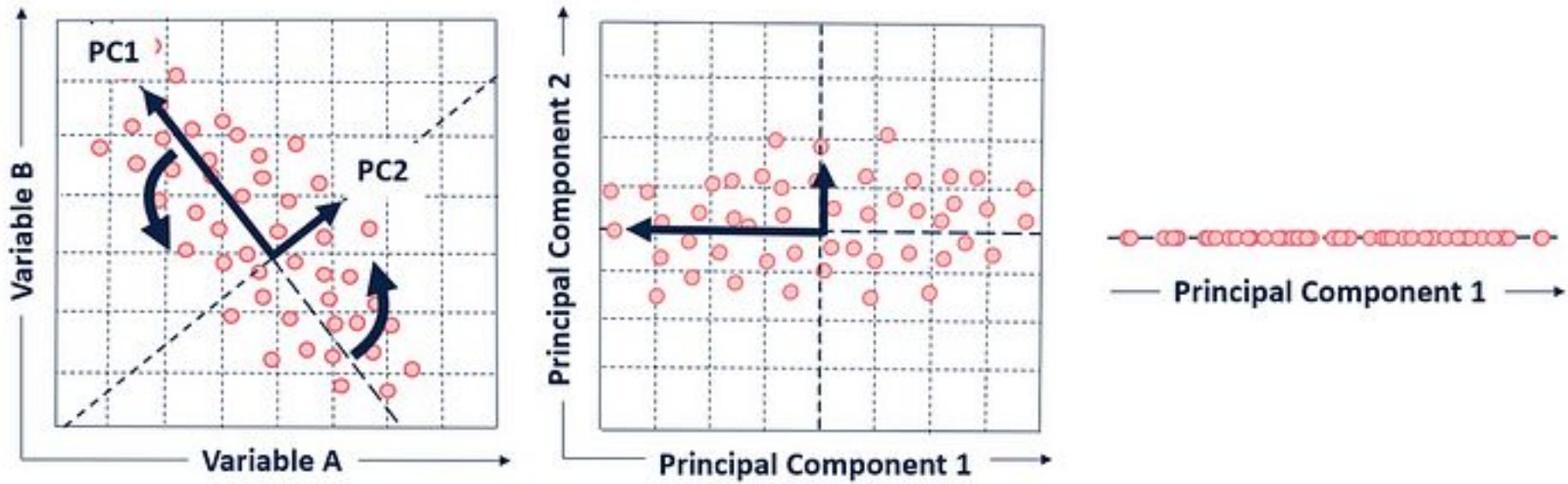


Principal Component 1 accounts for variance from both variables A and B. (dimension reduction)

The principal components (eigenvectors) are sorted by descending eigenvalue. The principal components with the highest eigenvalues are “picked first” as principal components because they account for the most variance in the data.

Source: <https://towardsdatascience.com/tidying-up-with-pca-an-introduction-to-principal-components-analysis-f876599af383>

Principal component



To convert our original points, we create a projection matrix. This projection matrix is just the selected eigenvectors concatenated to a matrix. We can then multiply the matrix of our original observations and variables by our projection matrix. The output of this process is a transformed data set, projected into our new data space — made up of our principal components!

Source: <https://towardsdatascience.com/tidying-up-with-pca-an-introduction-to-principal-components-analysis-f876599af383>

PLS Discriminant Analysis (PLS-DA)

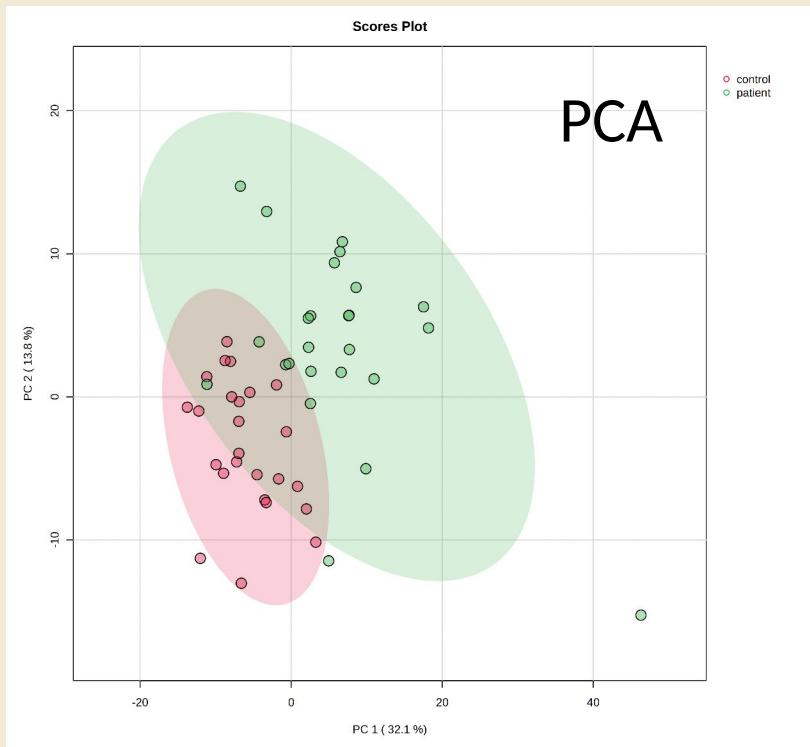
(A *supervised* alternative to PCA)

Performing simultaneous dimensionality reduction and classification

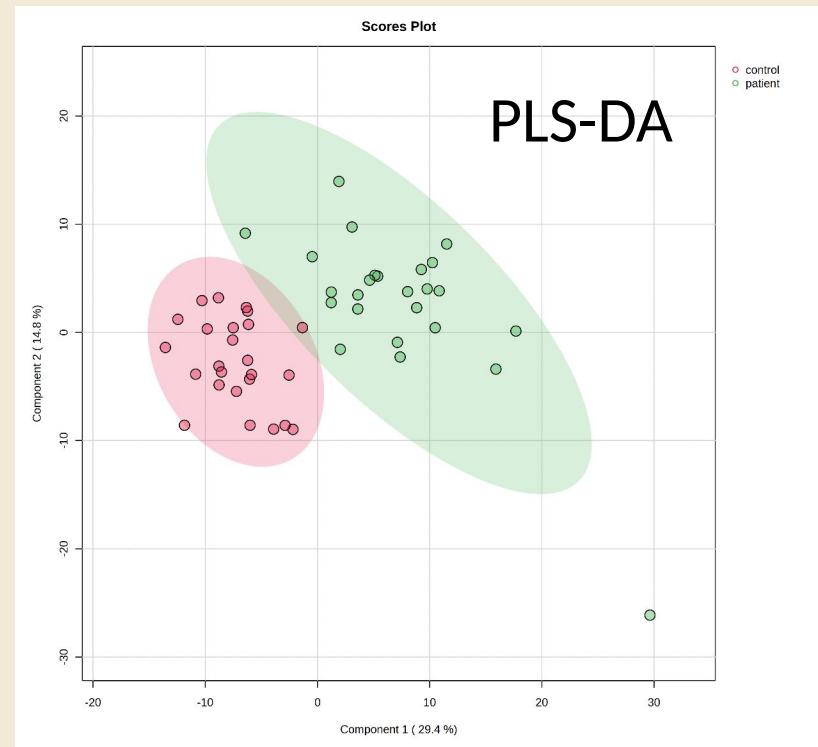
Purpose: PLS-DA vs PCA

- PCA is completely unsupervised (i.e. you don't know in advance if there are classes in your dataset)
- In PLS-DA you know how your dataset is divided in classes from the response vector Y. The goal here is then to project the predictors into a space, while maximizing the
- Common scenarios for using PLS-DA :
Metabolomics, proteomics.

Scores plot: PCA vs PLS-DA



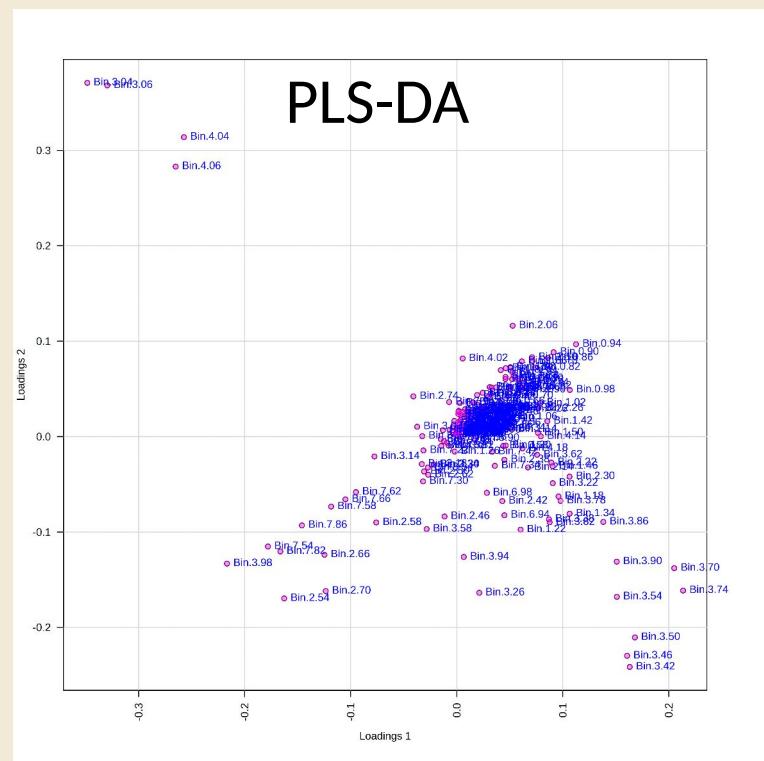
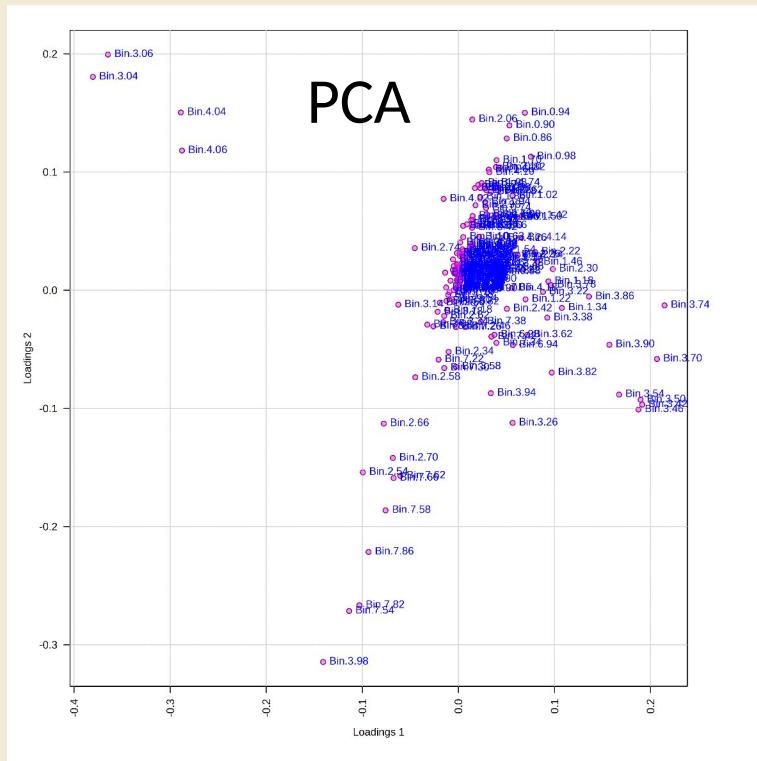
Samples projected in the space of Principal Components



Samples projected in the space of latent variables (components) that maximize the separation between groups

Source: test data ([NMR spectral bins](#)) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

Loadings plot: PCA vs PLS-DA

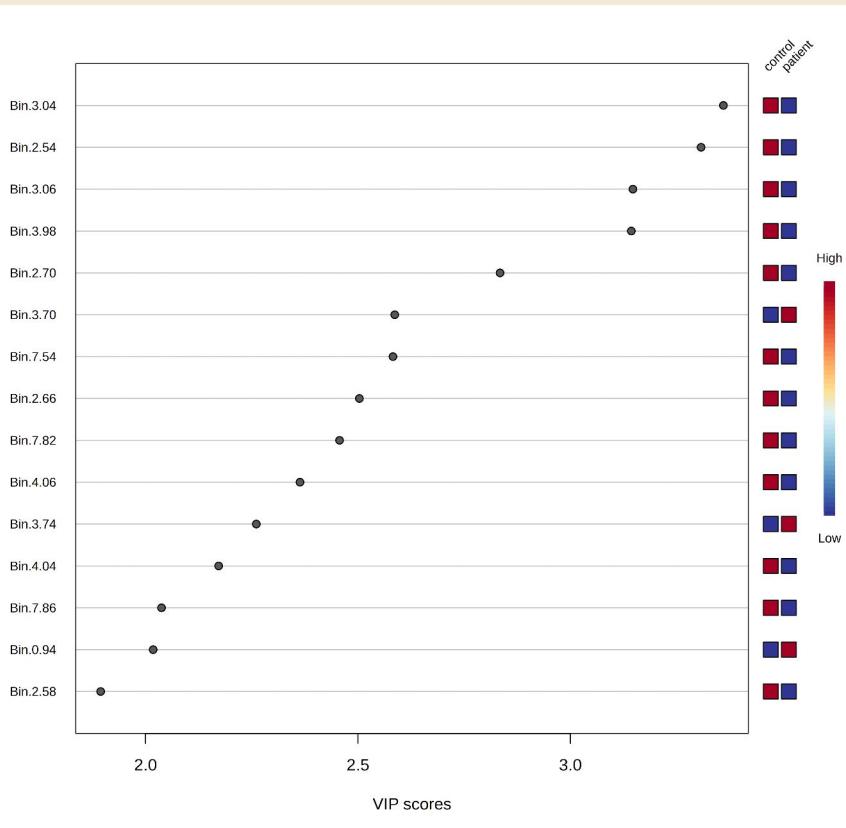


The loading vectors (here shown as points) represent the original variables in the space PCs.

The loading vectors (here shown as points) represent the original variables in the space of latent components retrieved by PLS-DA.

Source: test data ([NMR spectral bins](#)) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

Feature Importance in PLS-DA

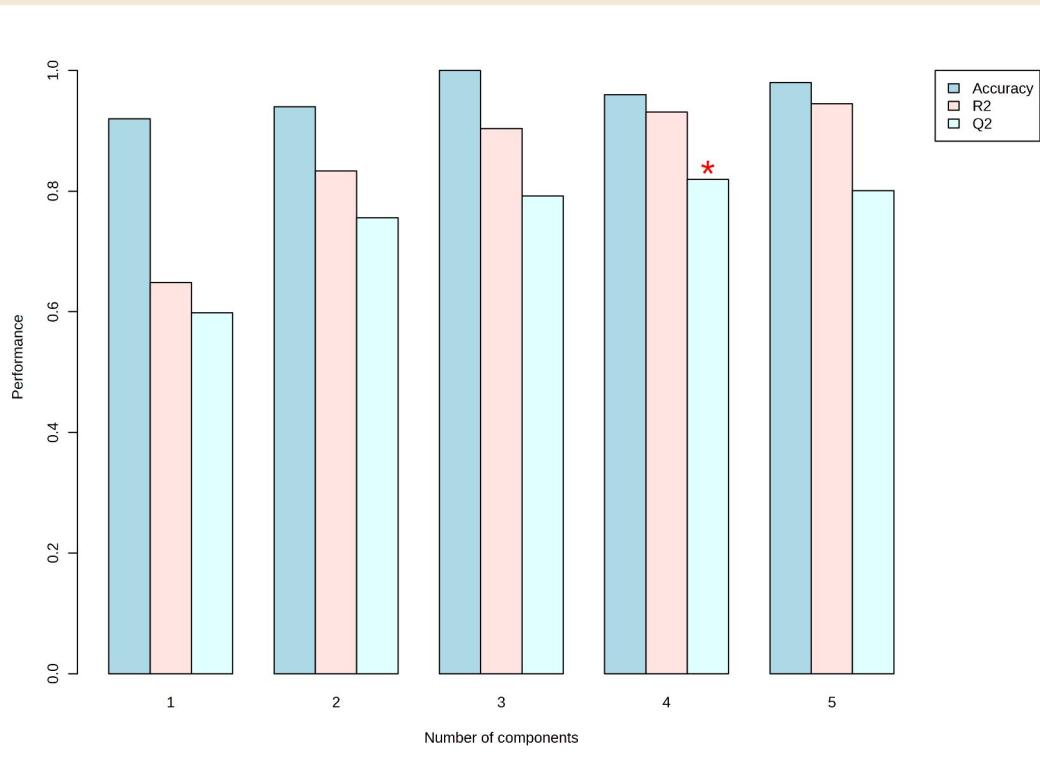


VIP (Variable Importance in Projection) scores, ranking the variables based on their significance in the PLS-DA **model of classification**.

...very useful to select potential biomarkers!

Source: test data ([NMR spectral bins](#)) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

Cross validation in PLS-DA



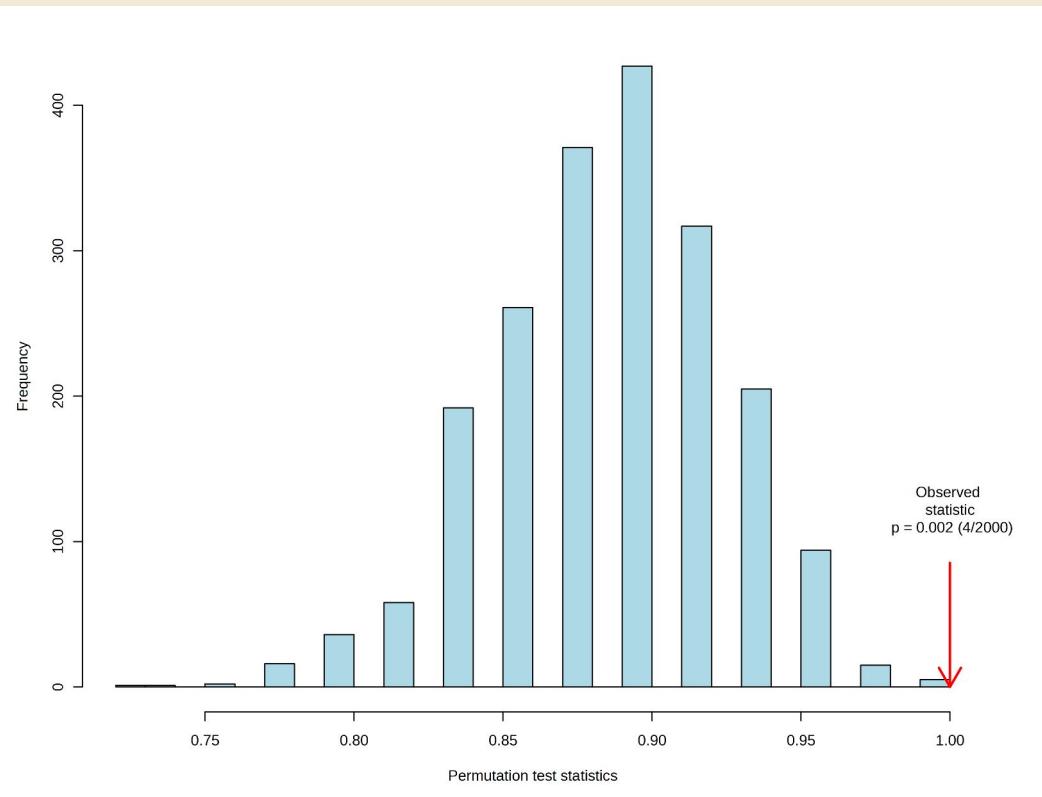
PLS-DA generate a model of classification.

By partitioning the dataset and iteratively testing the model, cross validation estimate the predictive ability of the model.

Q^2 is an analogous of R^2 in regression: the higher the better!

Source: test data ([NMR spectral bins](#)) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

Permutation in PLS-DA



Permutation testing is a non-parametric approach to assess the significance of a model's results.

In the context of PLS-DA, this test helps verify whether the observed classification accuracy is better than what would be expected by chance.

Source: test data ([NMR spectral bins](#)) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

MetaboAnalyst

An R-driven Software

Introduction to MetaboAnalyst



<https://www.metaboanalyst.ca>

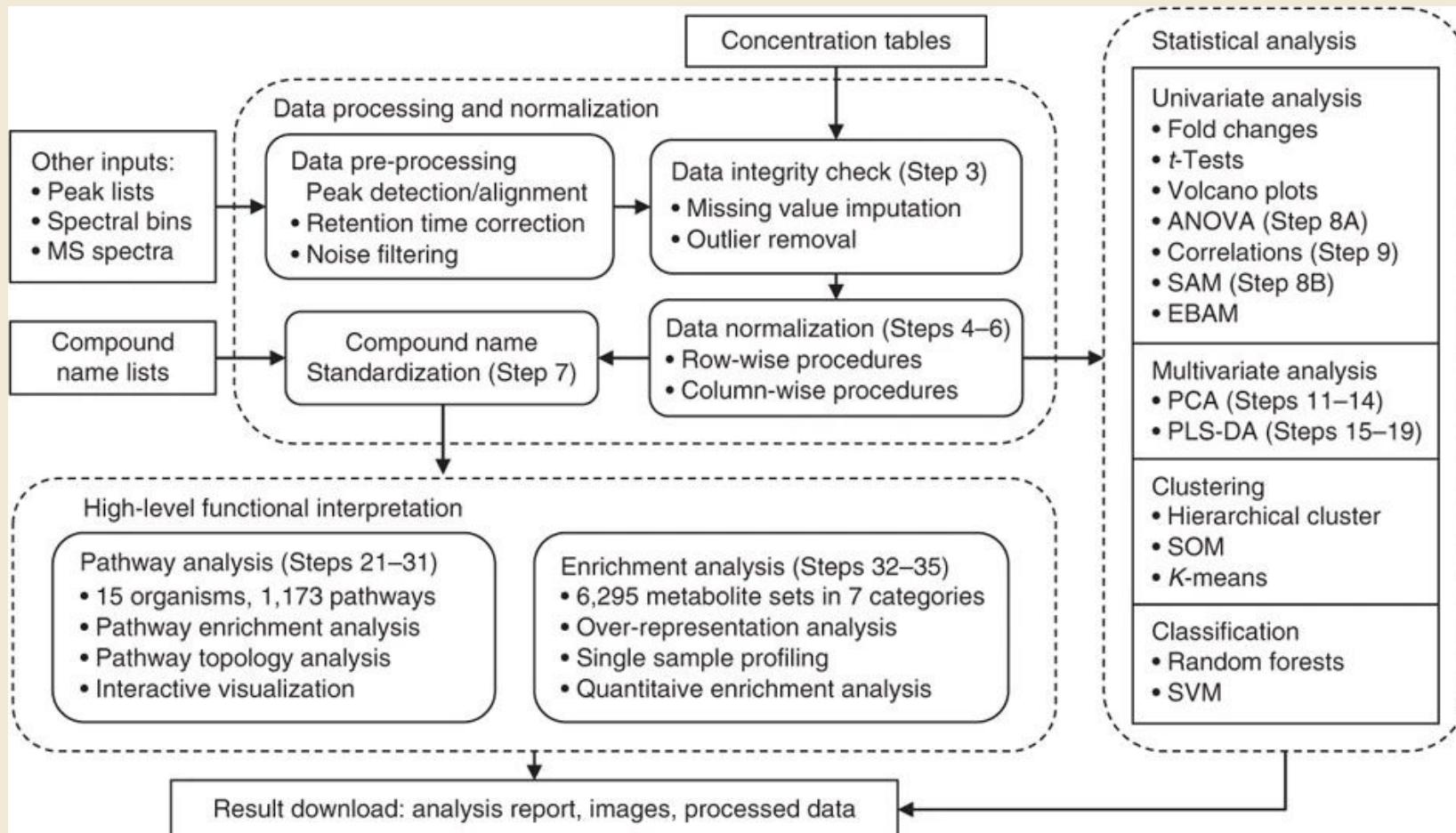
From raw spectra to biomarkers, patterns, functions and systems biology

- it is a **free** web-based platform
- it works with **R** but it has a **friendlier GUI**: anyone can make metabolomics data analysis, interpretation and integration with other omics data
- the whole metabolomics community uses it!!!

...but

- you need a statistical background to interpret the **MetaboAnalyst** outputs and to get the most of it!

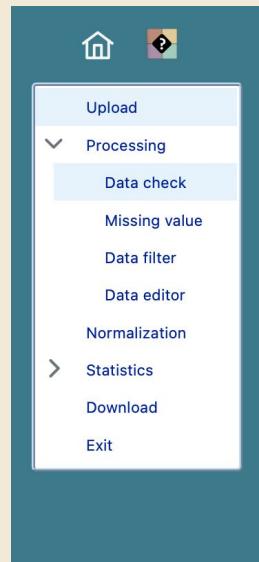
MetaboAnalyst overview



Source: Xia, J., Wishart, D. *Nat Protoc* 6, 743–760 (2011).

MetaboAnalyst workflow

1) data upload



Data Integrity Check:

- Checking sample names - spaces will be replaced with underscore, and special characters will be removed;
- Checking the class labels - at least three replicates are required in each class.
- The data (except class labels) must not contain non-numeric values.
- If the samples are paired, the pair labels must conform to the specified format.
- The presence of missing values or features with constant values (i.e. all zeros).

Data processing information:

Checking data content ...passed.
Samples are in rows and features in columns
The uploaded file is in comma separated values (.csv) format.
The uploaded data file contains 50 (samples) by 200 (spectra bins) data matrix.
Samples are not paired.
2 groups were detected in samples.
Only English letters, numbers, underscore, hyphen and forward slash (/) are allowed.
Other special characters or punctuations (if any) will be stripped off.
All data values are numeric.
A total of 0 (0%) missing values were detected.
By default, missing values will be replaced by 1/5 of min positive values of their corresponding variables
Click the **Proceed** button if you accept the default practice;
Or click the **Missing Values** button to use other methods.

Edit Groups

Missing Values

▷ Proceed

Test data 1:

Binned 1H NMR spectra of 50 urine samples using 0.04 ppm constant width ([Psihogios NG, et al.](#))

Group 1- control;

Group 2 - severe kidney disease.

MetaboAnalyst workflow

2) data filtering

The purpose of the data filtering is to identify and remove variables that are unlikely to be of use when modeling the data. No phenotype information are used in the filtering process, so the result can be used with any downstream analysis. This step is strongly recommended for untargeted metabolomics datasets (i.e. spectral binning data, peak lists) with large number of variables, many of them are from baseline noises. Filtering can usually improve the results. For details, please refer to the paper by [Hackstadt, et al.](#).

Non-informative variables can be characterized in three groups: 1) variables that show **low repeatability** - this can be measured using QC samples using the relative standard deviation($RSD = SD/\text{mean}$). Features with high percent RSD should be removed from the subsequent analysis (the suggested threshold is 20% for LC-MS and 30% for GC-MS); 2) variables that are **near-constant** throughout the experiment conditions - these variables can be detected using standard deviation (SD); or the robust estimate such as interquartile range (IQR); and 3) variables of **very small values** (close to baseline or detection limit) - these variables can be detected using mean or median.

For data filtering based on the last two categories, the default parameters follow the empirical rules: 1) Less than 250 variables: 5% will be filtered; 2) Between 250 - 500 variables: 10% will be filtered; 3) Between 500 - 1000 variables: 25% will be filtered; and 4) Over 1000 variables: 40% will be filtered. You can turn off data filtering by dragging the slider to adjust the percentage to filter out to be 0, when your data contain less than 5000 features (or 2500 for power analysis) to control computing time on our server.

Reliability filter:	<input type="checkbox"/> Filtering features based on technical repeatability QC samples	RSDs greater than:  25%
Variance filter:	<input checked="" type="radio"/> Interquartile range (IQR) <input type="radio"/> Standard deviation (SD) <input type="radio"/> Median absolute deviation (MAD) <input type="radio"/> Relative standard deviation ($RSD = SD/\text{mean}$) <input type="radio"/> Non-parametric relative standard deviation (MAD/median)	Percentage to filter out:  5%
Abundance filter:	<input checked="" type="radio"/> Mean intensity value <input type="radio"/> Median intensity value	Percentage to filter out:  0%

Submit **Proceed**

MetaboAnalyst workflow

3) data normalization

Normalization Overview:

The normalization procedures are grouped into three categories. You can use one or combine them to achieve better results.

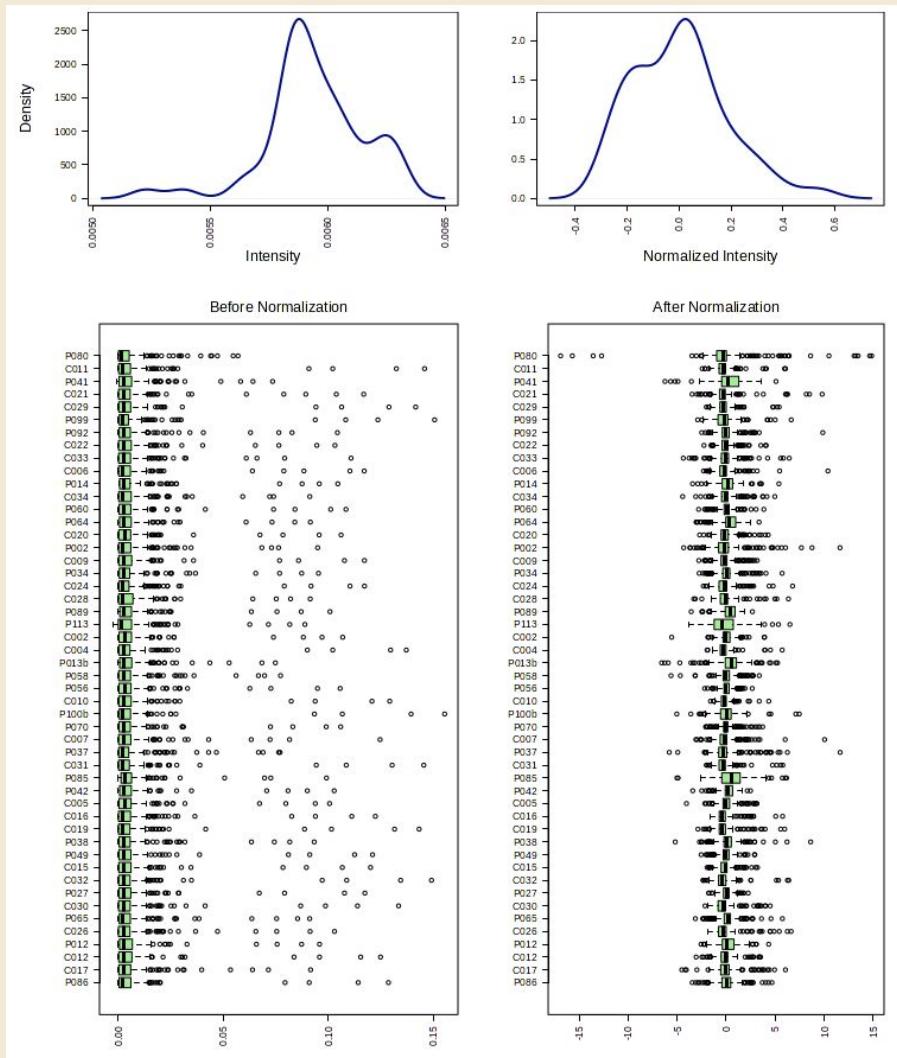
- Sample normalization
 - None
 - Sample-specific normalization (i.e. weight, volume)
 - Normalization by sum
 - Normalization by median
 - Normalization by a reference sample (PQN)
 - Normalization by a pooled sample from group (group PQN)
 - Normalization by reference feature
 - Quantile normalization (suggested only for > 1000 features)
- Data transformation
 - None
 - Log transformation (base 10)
 - Square root transformation (square root of data values)
 - Cube root transformation (cube root of data values)
- Data scaling
 - None
 - Mean centering (mean-centered only)
 - Auto scaling (mean-centered and divided by the standard deviation of each variable)
 - Pareto scaling (mean-centered and divided by the square root of the standard deviation of each variable)
 - Range scaling (mean-centered and divided by the range of each variable)

Autoscaling $\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{s_i}$

Pareto scaling $\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{\sqrt{s_i}}$

MetaboAnalyst workflow

3) data normalization

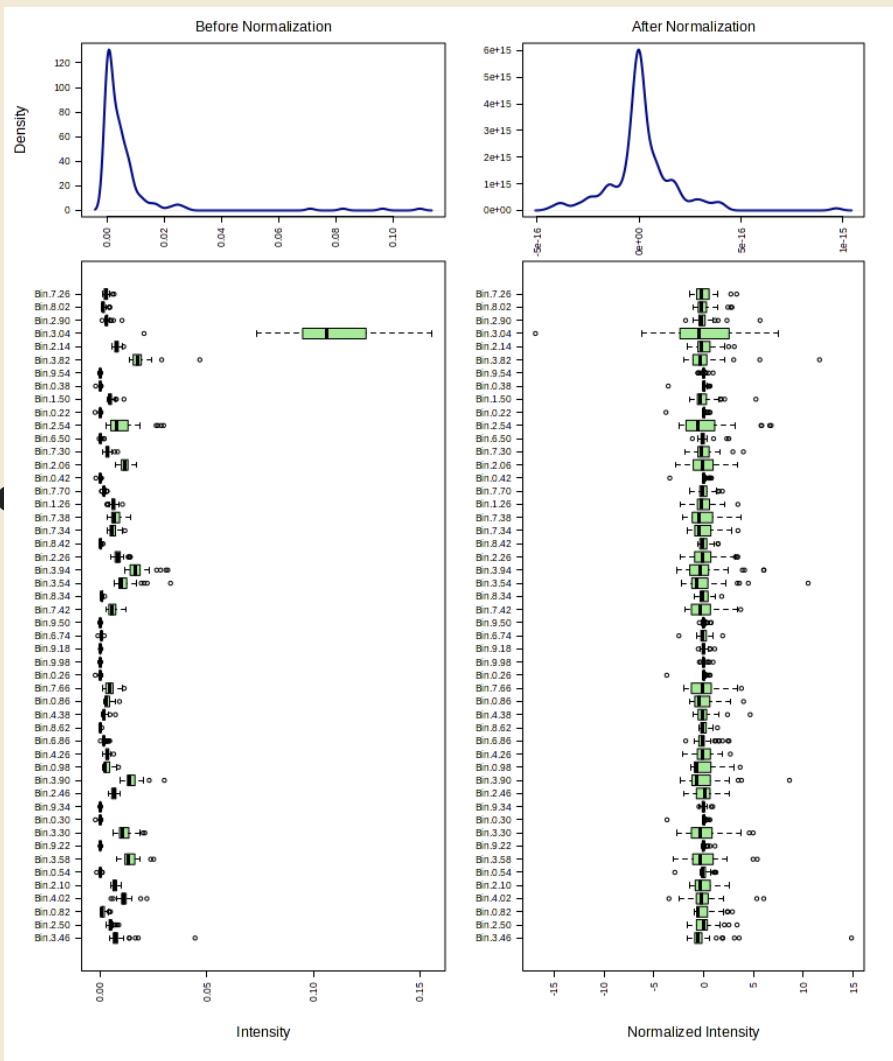


Effect of normalization over sample

MetaboAnalyst workflow

3) data normalization

Effect of features/metabolites scaling



MetaboAnalyst workflow

4) statistical analysis

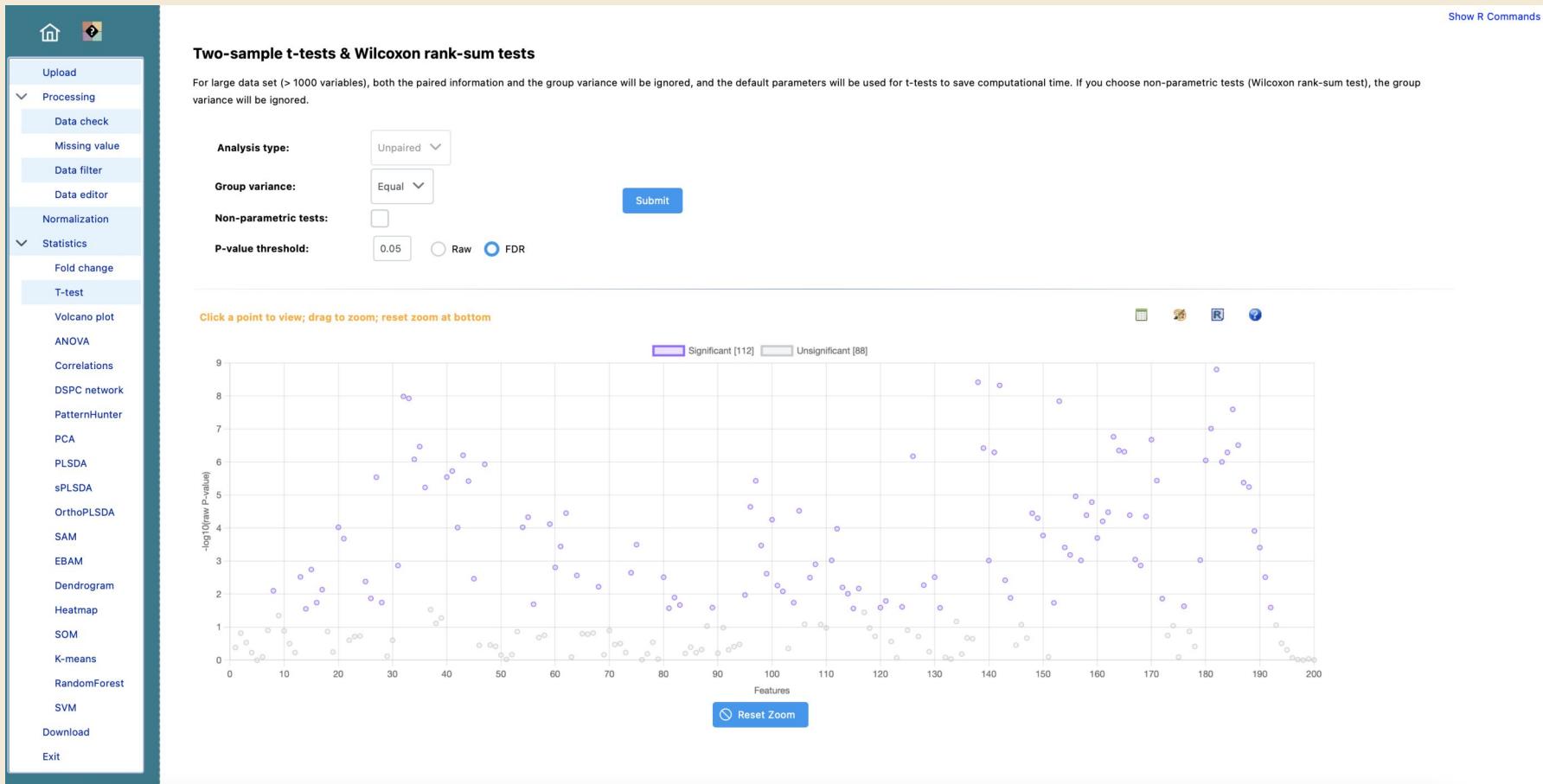
The screenshot shows the MetaboAnalyst software interface. On the left is a vertical sidebar with a dark blue background containing various menu items: Upload, Processing (with sub-options Data check, Missing value), Data filter, Data editor, Normalization, Statistics (highlighted with a blue background), Download, and Exit. At the top of the main area is a header with a house icon and a question mark icon. Below the header, the text "Select an analysis path to explore :" is displayed. A large white rectangular box contains several sections of analysis paths:

- Univariate Analysis**:
 - [Fold Change Analysis](#)
 - [T-tests](#)
 - [Volcano plot](#)
 - [One-way Analysis of Variance \(ANOVA\)](#)
 - [Correlation Heatmaps](#)
 - [Pattern Search](#)
 - [Correlation Networks \(DSPC\)](#)
- Advanced Significance Analysis**:
 - [Significance Analysis of Microarray \(and Metabolites\) \(SAM\)](#)
 - [Empirical Bayesian Analysis of Microarray \(and Metabolites\) \(EBAM\)](#)
- Chemometrics Analysis**:
 - [Principal Component Analysis \(PCA\)](#)
 - [Partial Least Squares - Discriminant Analysis \(PLS-DA\)](#)
 - [Sparse Partial Least Squares - Discriminant Analysis \(sPLS-DA\)](#)
 - [Orthogonal Partial Least Squares - Discriminant Analysis \(orthoPLS-DA\)](#)
- Cluster Analysis**:
 - Hierarchical Clustering: [Dendrogram](#) [Heatmaps](#)
 - Partitional Clustering: [K-means](#) [Self Organizing Map \(SOM\)](#)
- Classification & Feature Selection**:
 - [Random Forest](#)
 - [Support Vector Machine \(SVM\)](#)

Two red curly braces are used to group the analysis paths. One brace groups the "Univariate Analysis", "Advanced Significance Analysis", and "Chemometrics Analysis" sections, which are labeled in red text as "«Classical» analysis of variance among groups". The other brace groups the "Cluster Analysis" and "Classification & Feature Selection" sections, which are labeled in red text as "Machine learning algorithms".

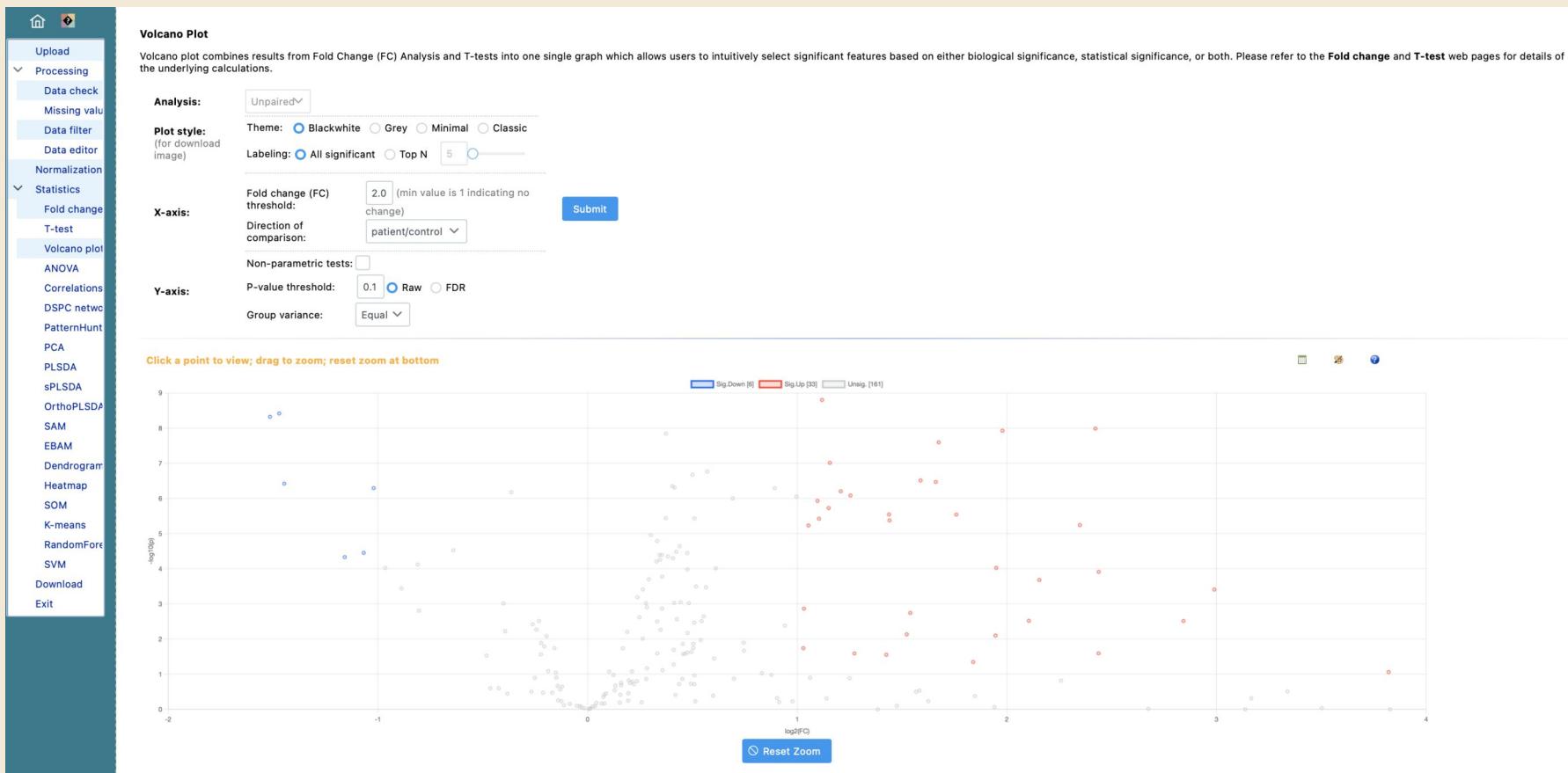
MetaboAnalyst workflow

4) univariate analysis



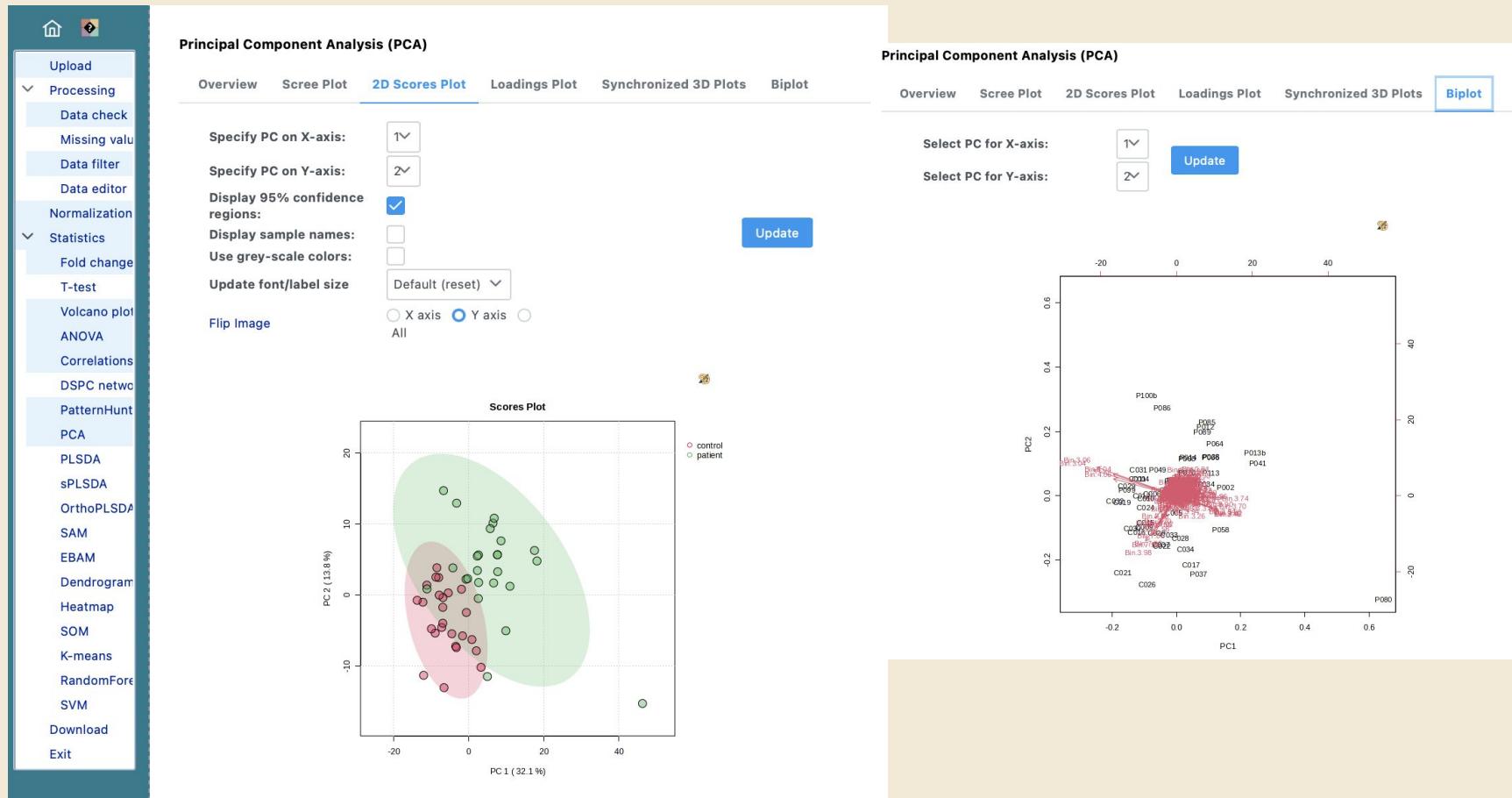
MetaboAnalyst workflow

4) univariate analysis



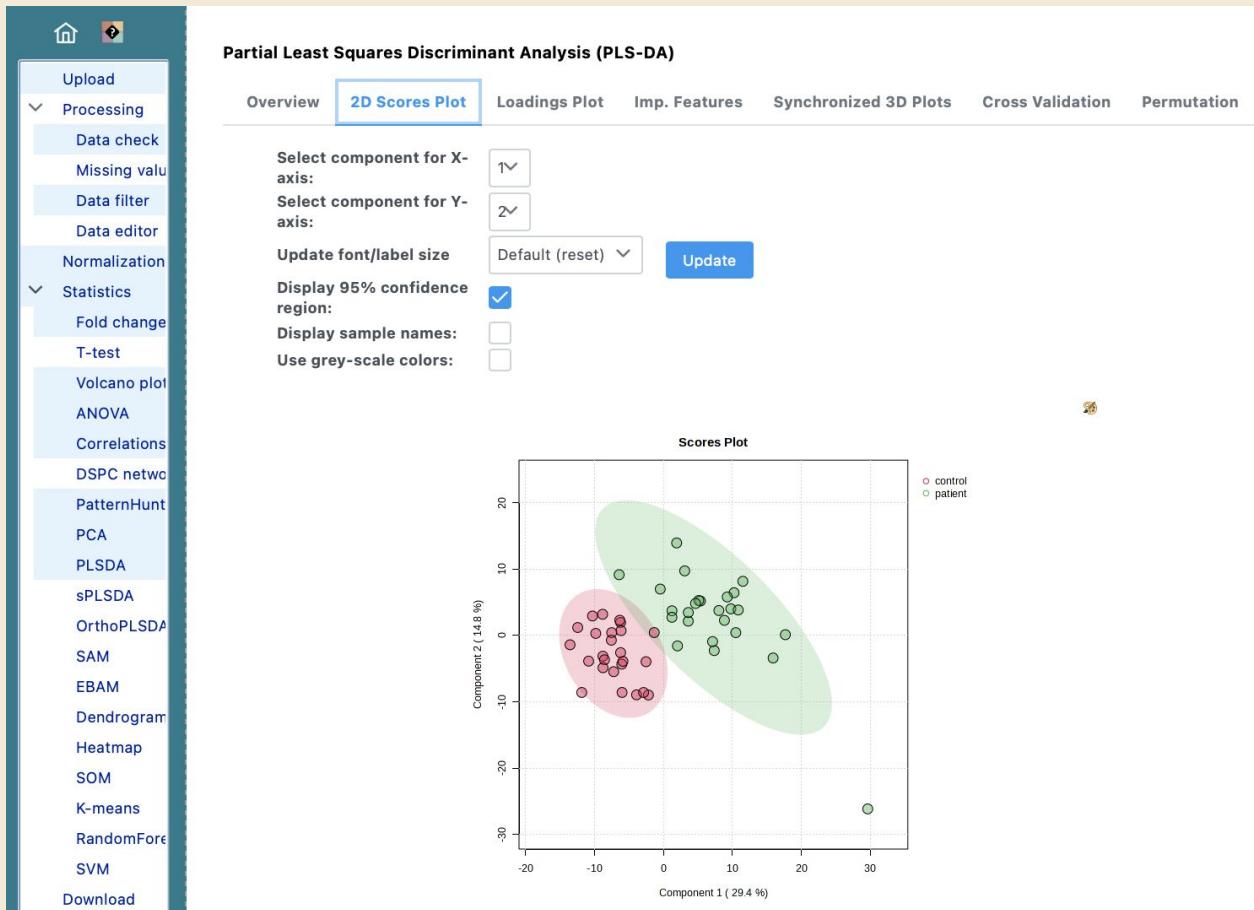
MetaboAnalyst workflow

5) chemometric analysis



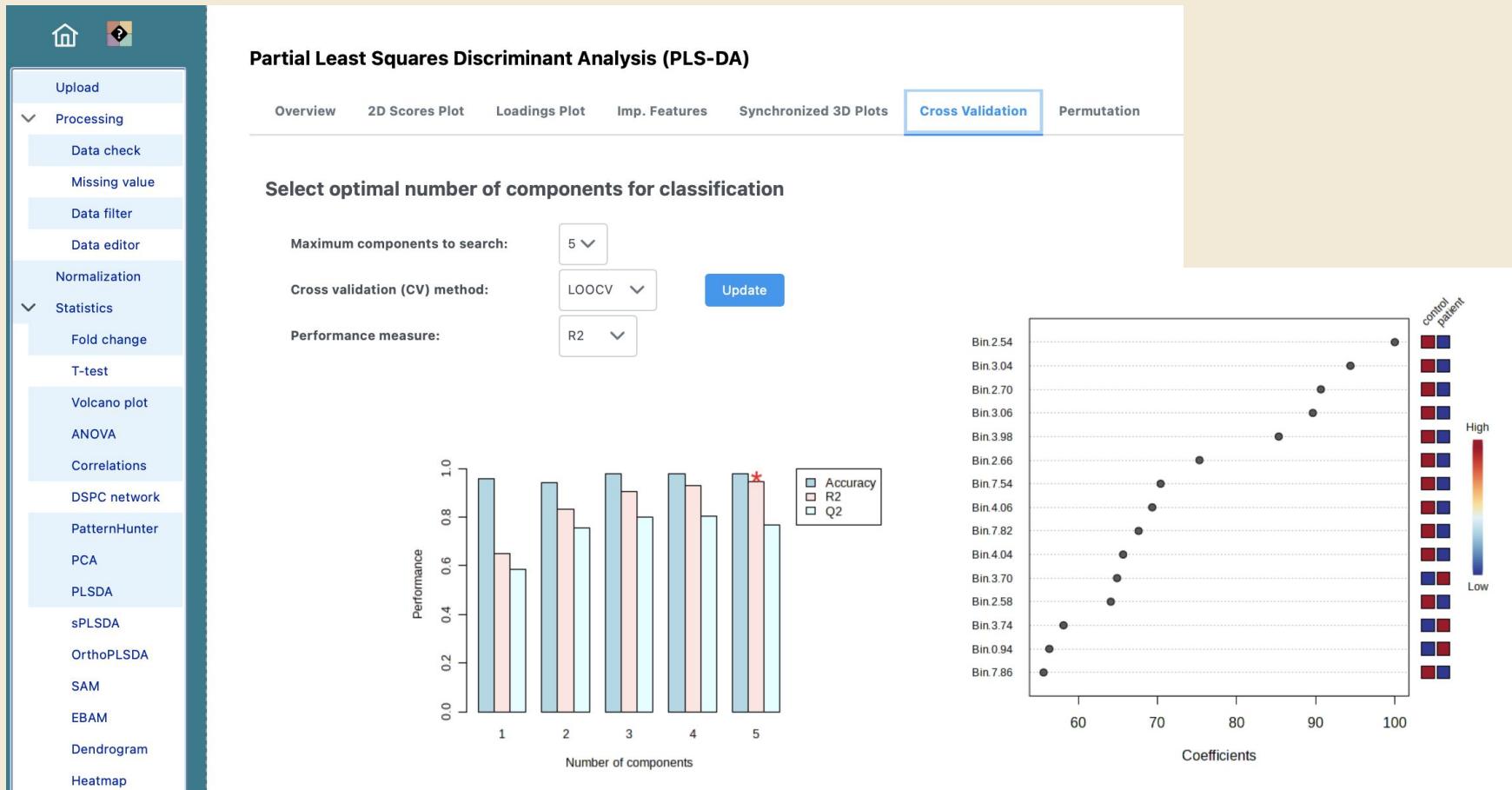
MetaboAnalyst workflow

5) chemometric analysis



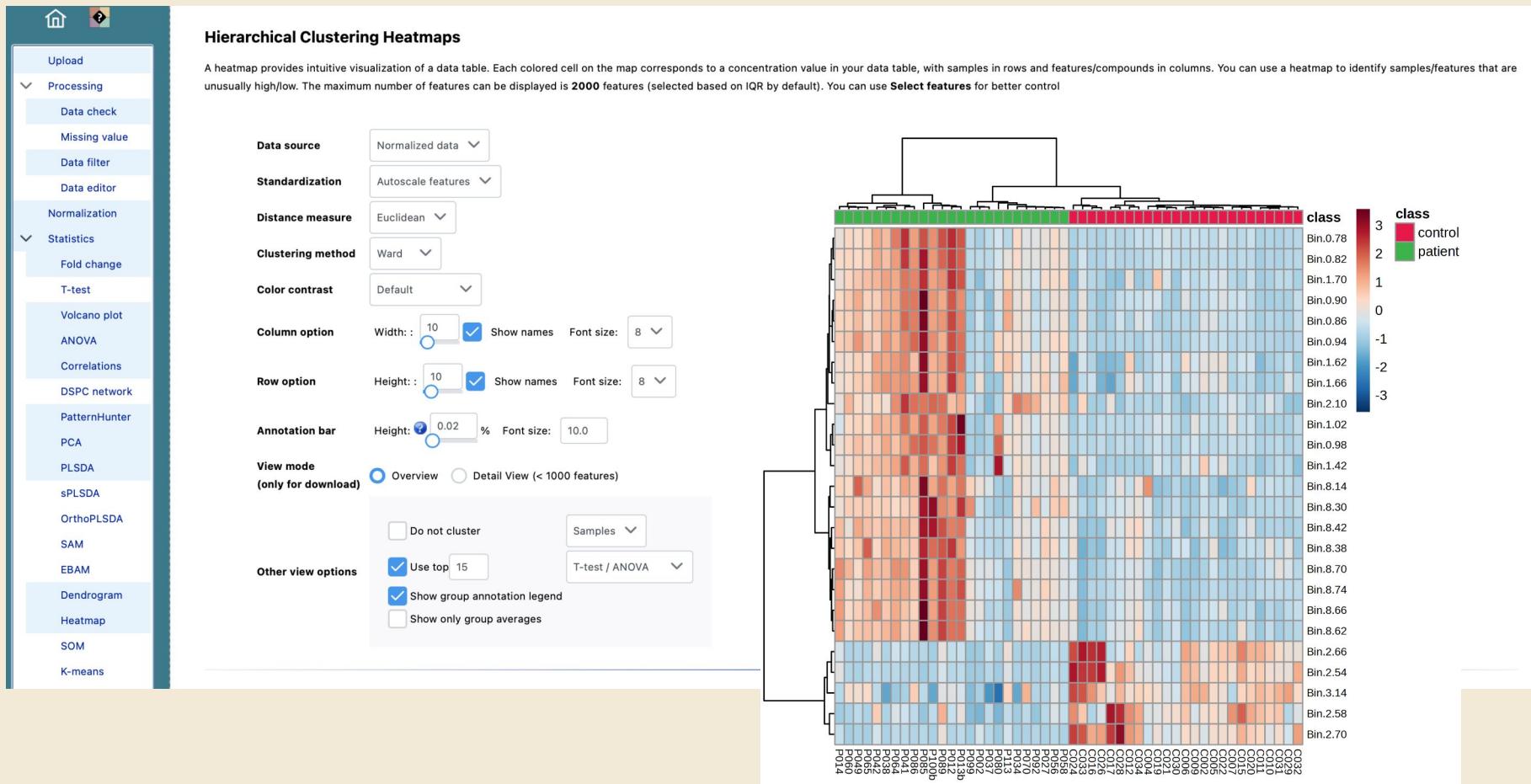
MetaboAnalyst workflow

5) chemometric analysis

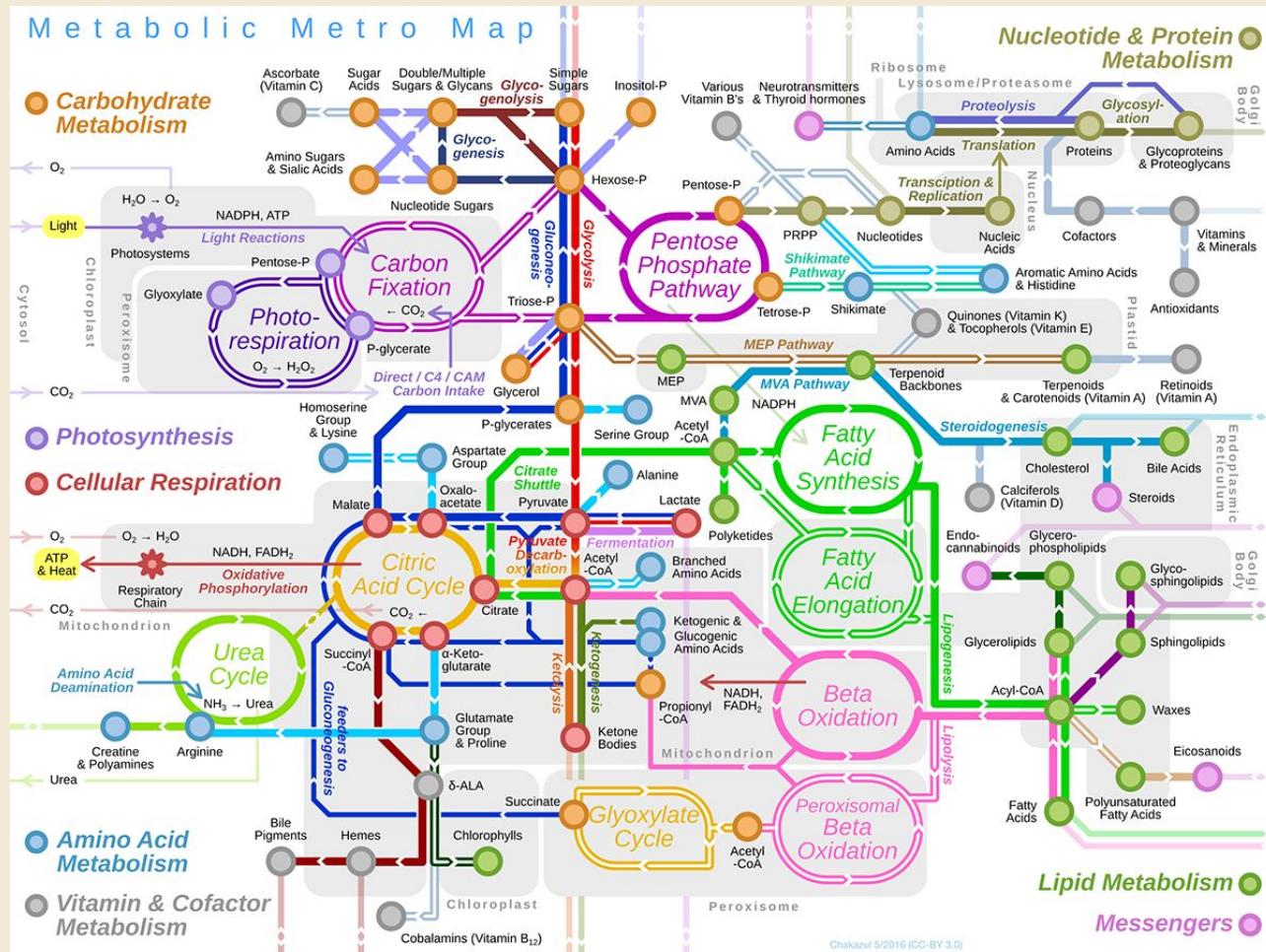


MetaboAnalyst workflow

5) chemometric analysis



Identifying the metabolic pathways deregulated by a pathology is finding a target for pharmacological therapy!



Source: <https://www.behance.net/gallery/38270165/Metro-Map-of-Metabolism-The-Overview>

MetaboAnalyst workflow

6) enrichment analysis

Data Integrity Check:

- Checking sample names - spaces will replaced with underscore, and special characters will be removed;
- Checking the class labels - at least three replicates are required in each class.
- The data (except class labels) must not contain non-numeric values.
- If the samples are paired, the pair labels must conform to the specified format.
- The presence of missing values or features with constant values (i.e. all zeros).

Data processing information:

Checking data content ...passed.
Samples are in rows and features in columns
The uploaded file is in comma separated values (.csv) format.
The uploaded data file contains 77 (samples) by 63 (compounds) data matrix.
Samples are not paired.
2 groups were detected in samples.
Only English letters, numbers, underscore, hyphen and forward slash (/) are allowed.
Other special characters or punctuations (if any) will be stripped off.
All data values are numeric.
A total of 0 (0%) missing values were detected.
By default, missing values will be replaced by 1/5 of min positive values of their corresponding variables
Click the **Proceed** button if you accept the default practice;
Or click the **Missing Values** button to use other methods.

Edit Groups **Missing Values** **▷ Proceed**

Test data 2:
Urinary metabolite concentrations from 77 cancer patients measured by ^1H NMR.
Phenotype:
N - cancer cachexic;
Y - control

MetaboAnalyst workflow

6) enrichment analysis

The screenshot shows the MetaboAnalyst interface with a red box highlighting the 'Name/ID Standardization' section and an enrichment analysis tool window.

Name/ID Standardization:

- For enrichment analysis, only well-annotated HMDB compounds (i.e. the tool in **Other Utilities** module);
- Greek alphabets are not recognized, they should be replaced by English;
- Query names in normal white indicate exact match - marked by "1" in the table;
- Query names highlighted indicate **no exact or unique match** - marked by "0";
- For **compound name**, you should click the **View** link to perform appropriate standardization;
- For **KEGG ID**, it is possible to have multiple hits, you should click the **View** link to perform appropriate standardization;

Query	Hit
1,6-Anhydro-beta-D-glucose	Levoglucosan
1-Methylnicotinamide	1-Methylnicotinamide
2-Aminobutyrate	L-alpha-Aminobutyrate
2-Hydroxyisobutyrate	2-Hydroxyisobutyrate
2-Oxoglutarate	Oxoglutaric acid
3-Aminoisobutyrate	3-Aminoisobanoid acid
3-Hydroxybutyrate	
3-Hydroxyisovalerate	3-Hydroxyisovaleric acid
3-Indoxylsulfate	Indoxyl sulfate
4-Hydroxyphenylacetate	p-Hydroxyphenylacetic acid
Acetate	Acetic acid
Acetone	Acetone
Adipate	Adipic acid
Alanine	Alanine

Name match

Matched Name	HMDB	PubChem	KEGG
3-Hydroxyisovaleric acid	HMDB0000754	69362	C20827
<input checked="" type="checkbox"/> 3-Hydroxybutyric acid	HMDB0000011	441	C01089
<input type="checkbox"/> (S)-3-Hydroxybutyric acid	HMDB0000442	94318	C03197
<input type="checkbox"/> Ethyl (±)-3-hydroxybutyrate	HMDB0040409	62572	NA
<input type="checkbox"/> Methyl 3-hydroxybutyrate	HMDB0041603	15146	NA
<input type="checkbox"/> L-Threonine	HMDB0000167	6288	C00188
<input type="checkbox"/> 4-Amino-3-hydroxybutyrate	HMDB0061877	2149	C03678
<input type="checkbox"/> 2-Methyl-3-hydroxybutyric acid	HMDB0000354	160471	NA
<input type="checkbox"/> None of the above			

OK Cancel

MetaboAnalyst workflow

6) enrichment analysis

The screenshot shows the 'Parameter Setting' page of the MetaboAnalyst interface. The left sidebar lists various workflow steps: Upload, Processing, Data check, Name check, Missing value, Data filter, Data editor, Normalization, Enrichment (which is currently selected), Set paramet, View result, Download, and Exit. The main content area is titled 'Please select a metabolite set library'. It contains a table with five rows, each representing a category of metabolite sets:

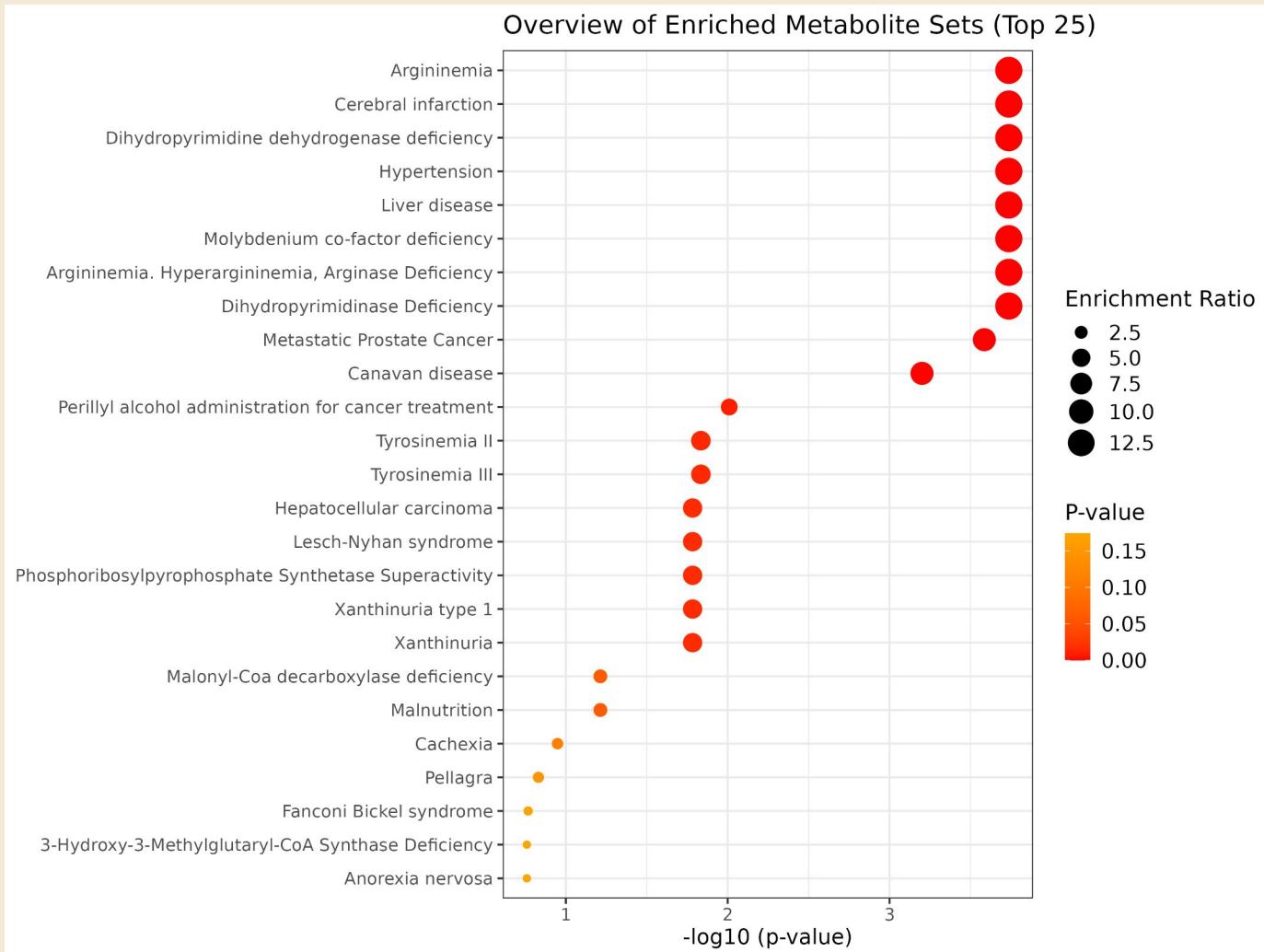
Pathway based	<input type="radio"/> SMPDB <input type="radio"/> KEGG <input checked="" type="radio"/> Drug related <input type="radio"/> RaMP-DB	99 metabolite sets based on normal human metabolic pathways. 80 metabolite sets based on KEGG human metabolic pathways (Dec. 2023). 461 metabolite sets based on drug pathways from SMPDB. 3694 metabolite and lipid pathways from RaMP-DB (integrating KEGG via HMDB, Reactome, WikiPathways).
Disease signatures	<input type="radio"/> Blood <input checked="" type="radio"/> Urine <input type="radio"/> CSF <input type="radio"/> Feces	480 metabolite sets reported in human blood. 385 metabolite sets reported in human urine. 174 metabolite sets reported in human cerebral spinal fluid (CSF). 67 metabolite sets reported in human feces.
Chemical structures	<input type="radio"/> Super-class <input type="radio"/> Main-class <input type="radio"/> Sub-class	39 super chemical class metabolite sets or lipid sets 617 main chemical class metabolite sets or lipid sets 1250 sub chemical class metabolite sets or lipid sets
Other types	<input type="radio"/> SNPs <input type="radio"/> Predicted <input type="radio"/> Locations <input type="radio"/> Exposure	4,598 metabolite sets based on their associations with SNPs loci. 912 metabolic sets predicted to change in the case of dysfunctional enzymes. 78 metabolite and lipid sets based on organ, tissue, and subcellular localizations. 62 metabolite sets based on dietary and chemical exposures.
Self defined	<input type="radio"/> Upload here	define your own customized metabolite sets

Below the table, there is a checkbox: Only use metabolite sets containing at least 2 entries. A note below says 'Please specify a reference metabolome'. Two radio button options are shown: Use all the compounds in the selected library and Upload a reference metabolome based on your analytical platform. A 'Submit' button is at the bottom.

Enrichment analysis, based on the globaltest, tests associations between metabolite sets and the outcome. The algorithm uses a generalized linear model to compute a 'Q-stat' for each metabolite set.

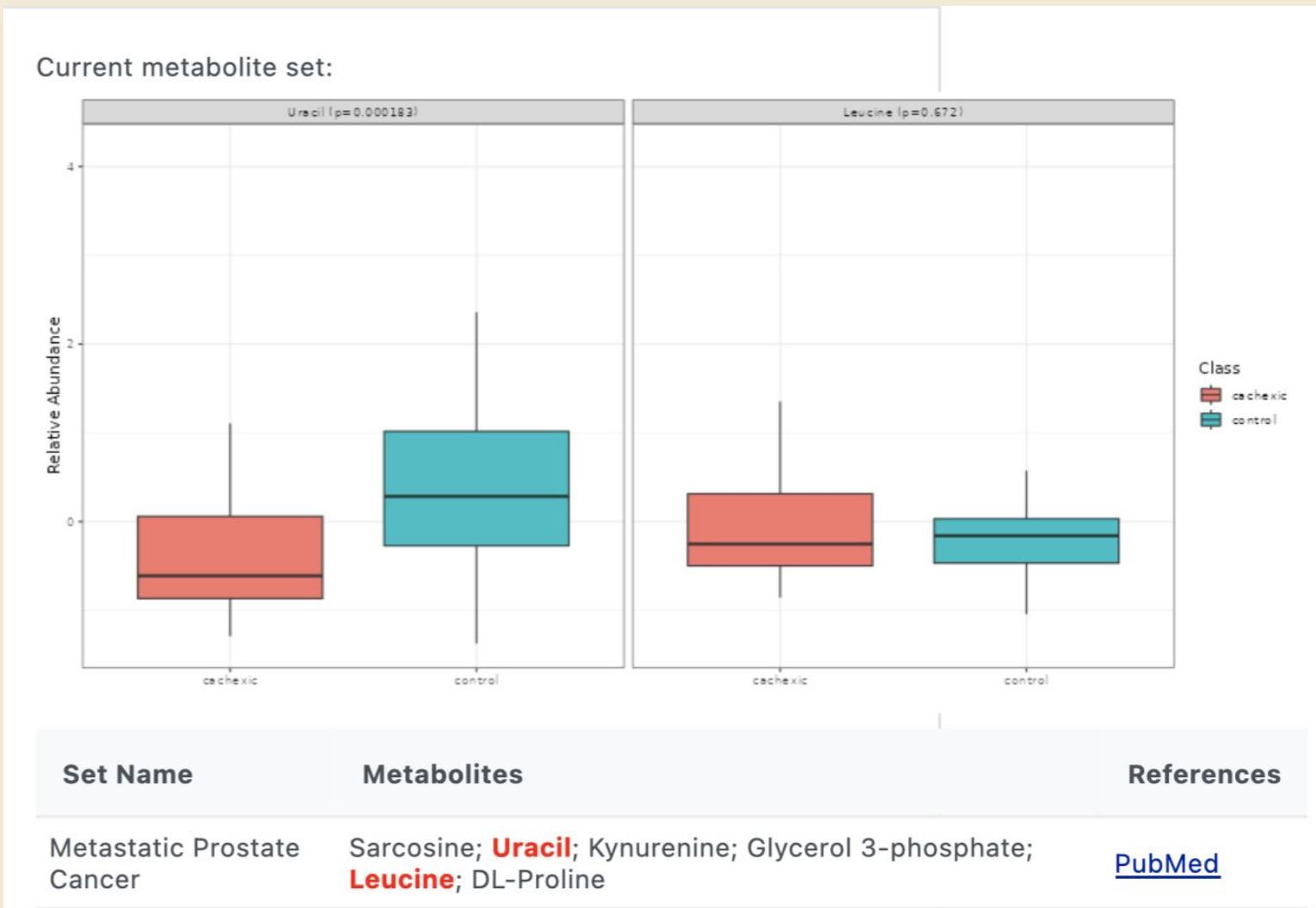
MetaboAnalyst workflow

6) enrichment analysis



MetaboAnalyst workflow

6) functional interpretation



MetaboAnalyst workflow

Metabolic pathway analysis and visualization

Result

The **metabolome view** on the left shows all matched pathways according to p values from pathway enrichment analysis and pathway impact values from pathway topology analysis. Place mouse over each pathway node will reveal its pathway name. Click each node will launch the **pathway view** on the right panel.

The pathway can be launched either by clicking the corresponding node on the left image or by clicking the pathway name from the table below. Please note, each node (compound) is clickable. You can zoom in and out using the control buttons below, and then drag the image to the locations of your interest. Place mouse over each metabolite node will reveal its common name. Click the node will trigger **compound view** of the selected compound.

Overview of Pathway Analysis

a

-log(p)

Pathway Impact

Alanine, aspartate and glutamate metabolism

b

c

L-Glutamine
Importance : 0.20703
P value : 0.03233106 [Close](#)

DB Links :

C00041

C00064

C00169

C00232

Fit

Navigation icons: magnifying glass, double arrows, up, down, left, right.

Source: Xia, J., Wishart, D. *Nat Protoc* 6, 743–760 (2011).

POWER ANALYSIS

Hypothesis testing steps

1. State the hypotheses (the null hypothesis and an alternative hypothesis)
2. Formulate an analysis plan (e.g. the significance level is 0.05, the test method one-sample z-test)
3. Analyze sample data
4. Interpret result and make decision

What are the Null and Alternative hypotheses?

Null Hypothesis	Alternative Hypothesis or
<ul style="list-style-type: none">• is the hypothesis that a sample data statistic occurs purely from chance<ul style="list-style-type: none">• e.g. there is no difference between the mean pulse rate for people doing physical exercise and the normal pulse rate• Must contain condition of equality ,• Test the Null Hypothesis directly: reject or fail to reject	<ul style="list-style-type: none">• is the hypothesis that a sample data statistic is influenced by some non-random cause<ul style="list-style-type: none">• e.g. the mean pulse rate for persons doing the physical exercise is higher than the normal• Must be true if is false (corresponding to , conditions)• 'opposite' of Null Hypothesis

Decision Errors

Two types of errors can result from a hypothesis test.

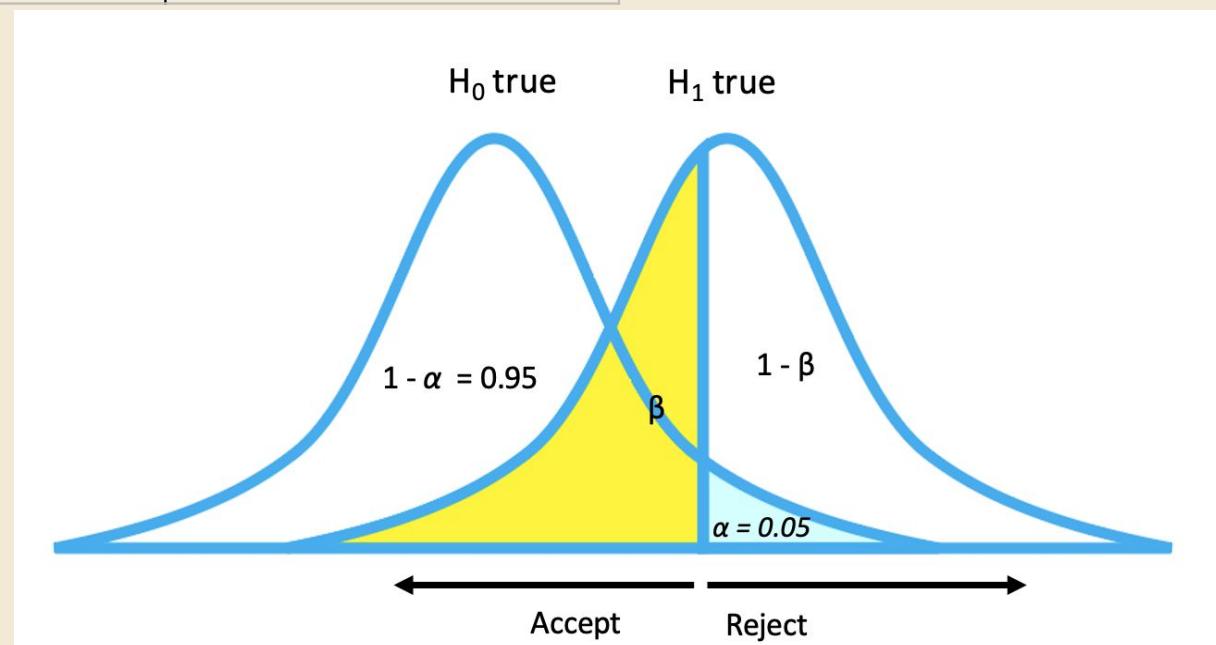
- Type I error occurs when the researcher rejects a null hypothesis when it is true. The probability of committing a Type I error is called the **significance level**. This probability is also called alpha, and is often denoted by α .
- Type II error occurs when the researcher fails to reject a null hypothesis that is false. The probability of committing a Type II error is called Beta, and is often denoted by β . The probability of not committing a Type II error is called the **Power of the test**.

Summarizing Type I and Type II Errors

	Fail to reject H0	Reject H0
H0 is true	Correct action	Type I error FALSE POSITIVE
probability	$1-\alpha$	α
H1 is true	Type II error FALSE NEGATIVE	Correct action
probability	β	power = $1-\beta$

$$\alpha = P(H_1 | H_0)$$

$$\beta = P(H_0 | H_1)$$



Which is worse: false-positive or false-negative?

	Chose H0	Chose H1
H0 is true	TRUE NEGATIVE	FALSE POSITIVE
probability	$1-\alpha$	α
H1 is true	FALSE NEGATIVE	TRUE POSITIVE
probability	β	power = $1-\beta$

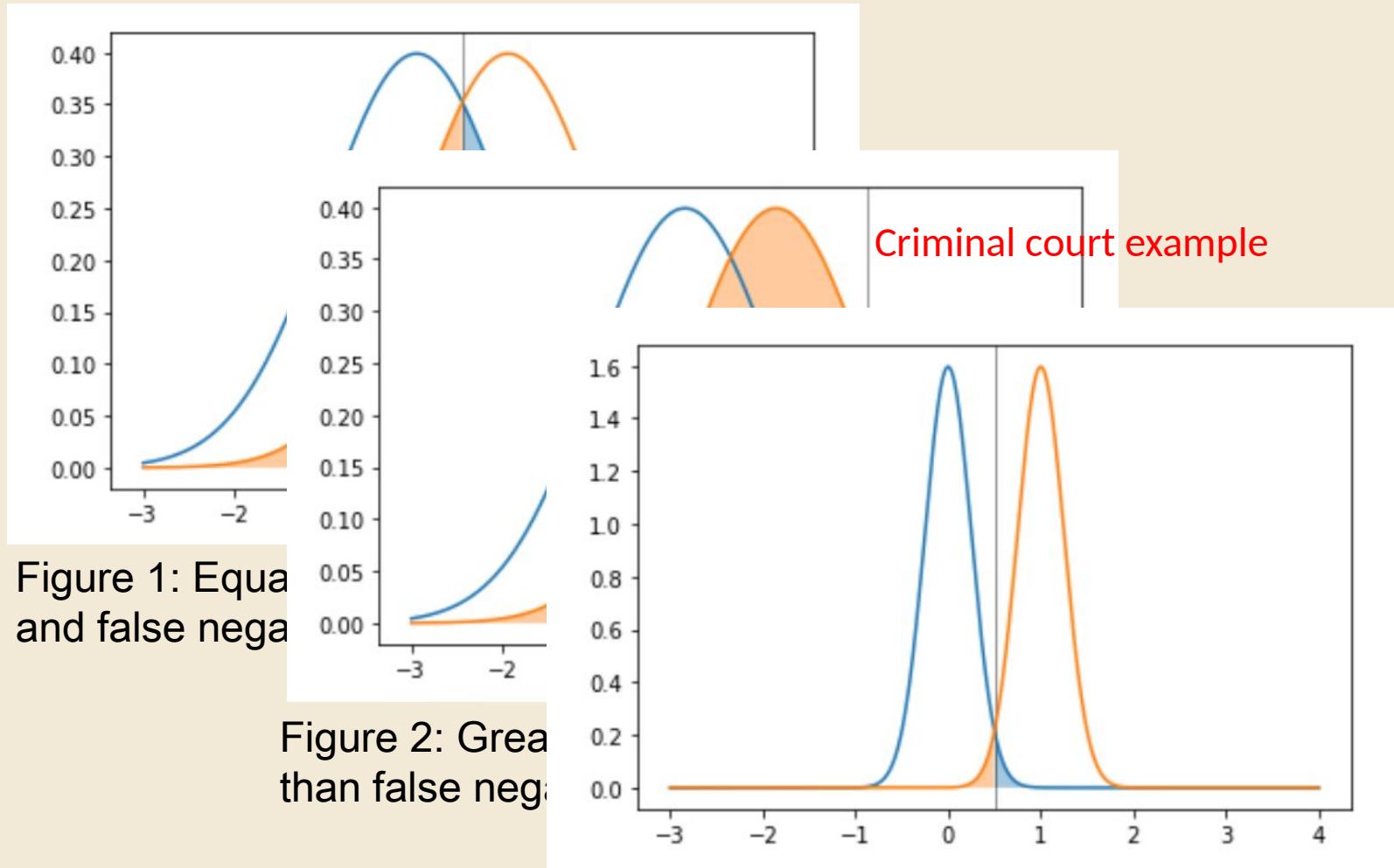
Example 1. Covid-19 test:

- False positive: although the quality control has been centred at 100%, we still have some false positives.
 - False negative: as a result, we have some false negatives.
- Example 2. Quality control in a pharma production company
- False positive: an innocent citizen is found guilty and is sent to prison or receives the death penalty
 - False negative: a criminal is declared innocent and escapes punishment
- Example 3. Disease diagnosis
- False positive: a healthy person is diagnosed with a disease.
 - False negative: a person with a disease is not diagnosed.
- Example 3. Criminal court

Controlling Type I and Type II Errors

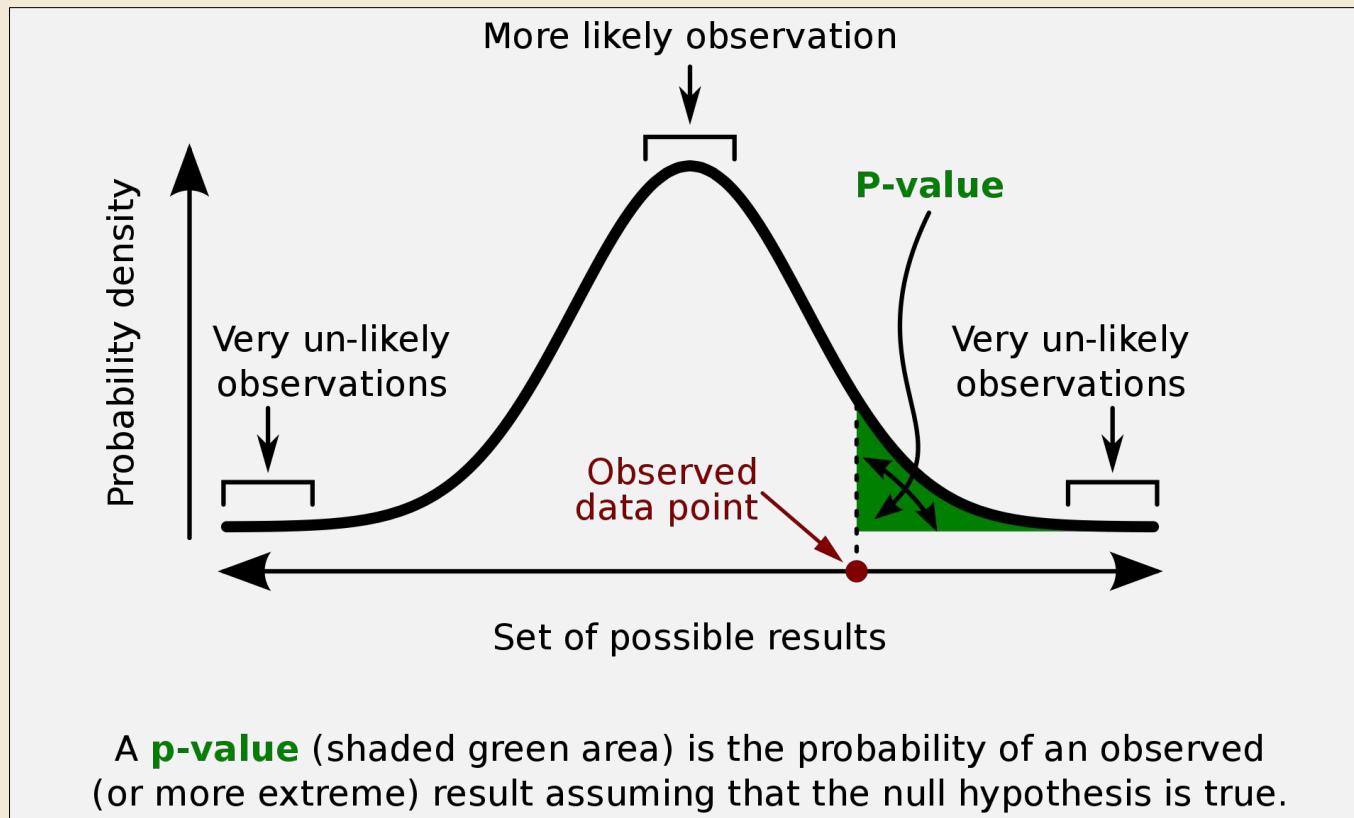
- α , β , and n are related
- when two of the three are chosen, the third is determined
- α and n are usually chosen
- try to use the largest α you can tolerate
- if Type I error (false positive) is serious, select a smaller α value and a larger n value

Controlling Type I and Type II error

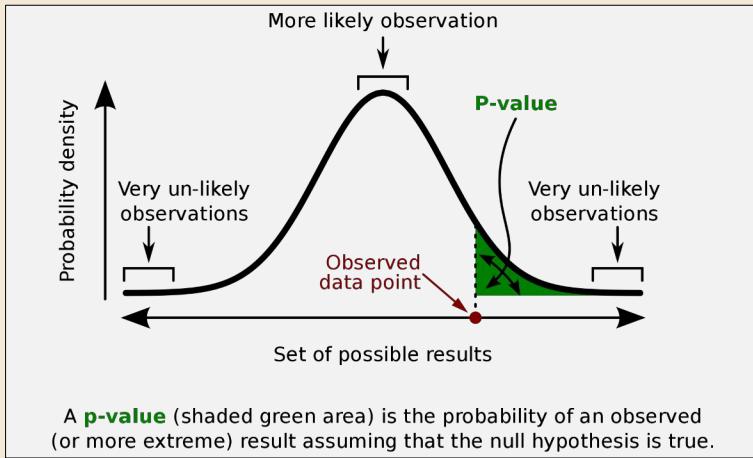


p-value

The p-value corresponds to the answer the question: what is the probability of the observed test statistic or one more extreme when H_0 is true?



p-value interpretation



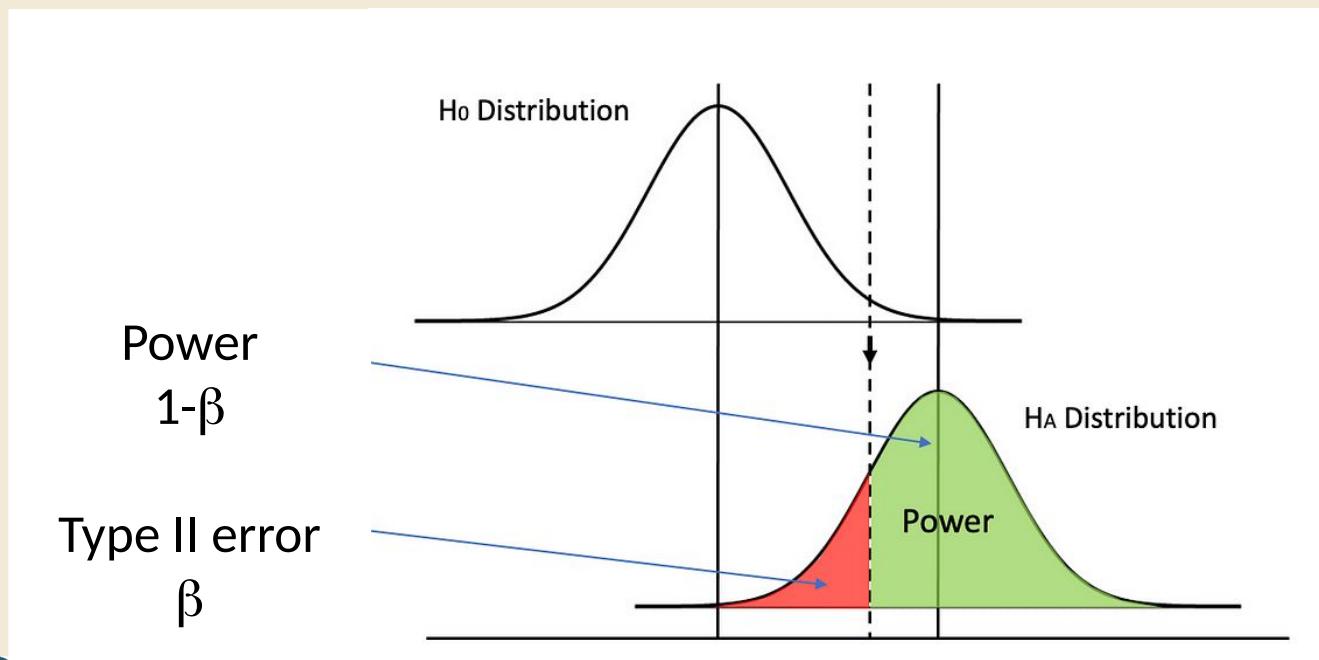
- A very small p-value means that such an extreme observed outcome would be very unlikely under the null hypothesis.
- Usually the researcher fix the type I error (a) he can tolerate before experiment and then compare the p value and takes a decision.

Conventions

$P > 0.10$	\Rightarrow	<i>non-significant evidence against H_0</i>
$0.05 < P \leq 0.10$	\Rightarrow	<i>marginally significant evidence against H_0</i>
$0.01 < P \leq 0.05$	\Rightarrow	<i>significant evidence against H_0</i>
$P \leq 0.01$	\Rightarrow	<i>highly significant evidence against H_0</i>

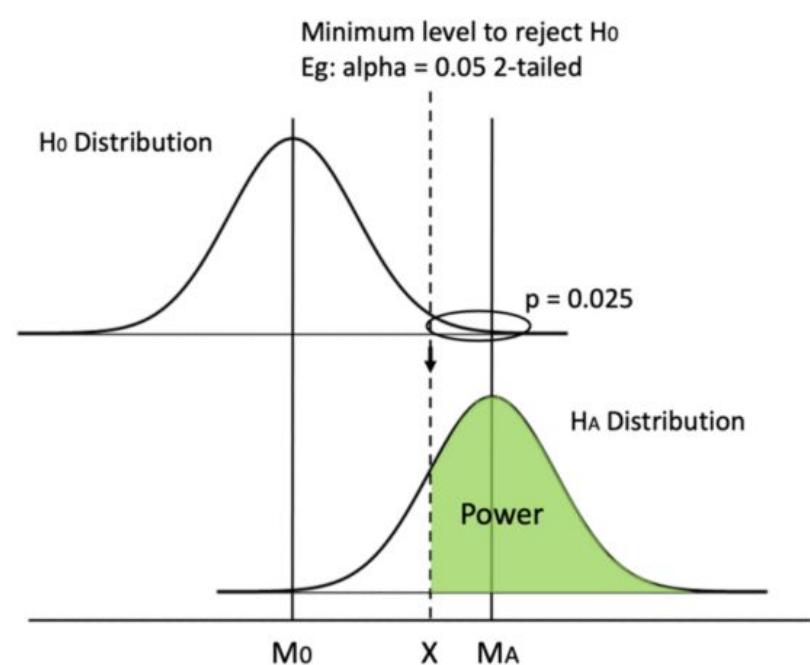
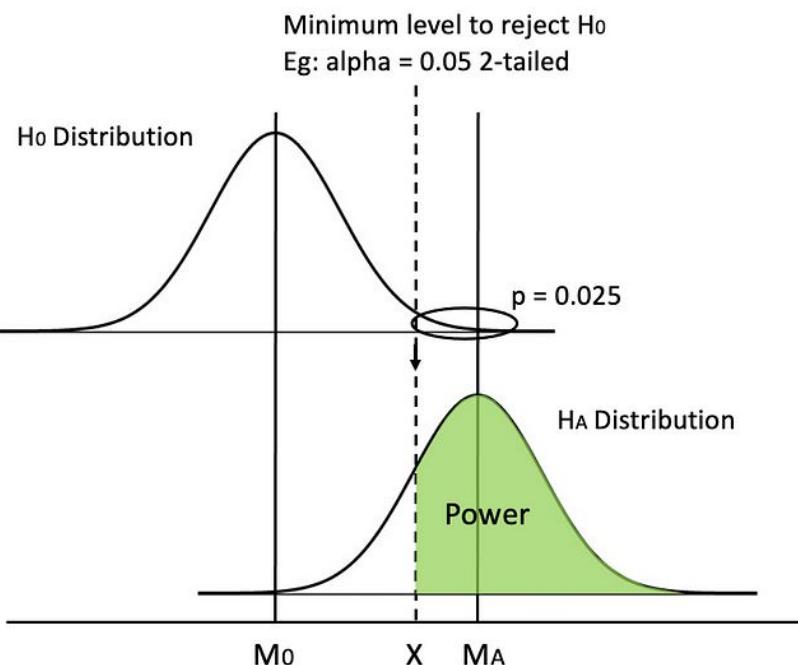
How to increase statistical power

	Fail to reject H0	Reject H0
H0 is true	Correct action	Type I error FALSE POSITIVE
probability	$1-\alpha$	α
H1 is true	Type II error FALSE NEGATIVE	Correct action
probability	β	$power = 1-\beta$



How to increase statistical power

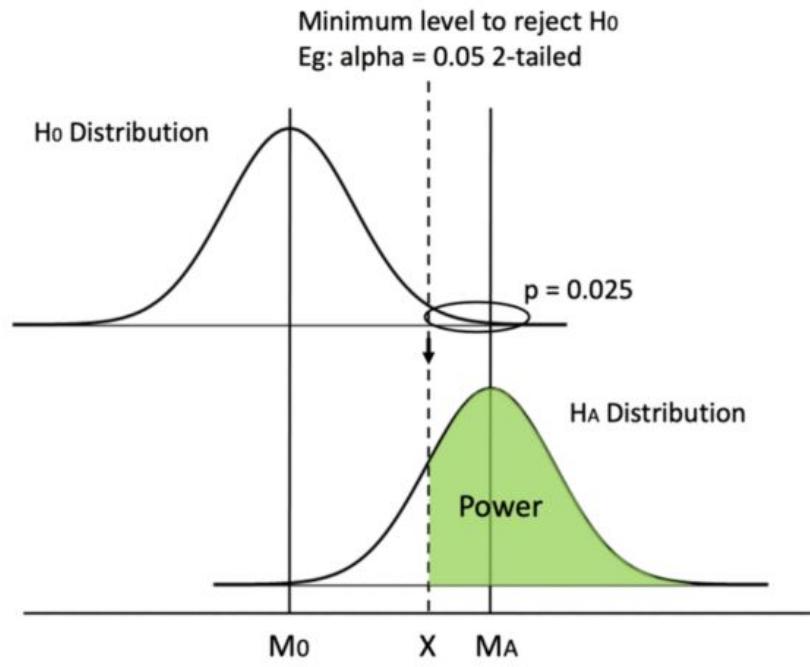
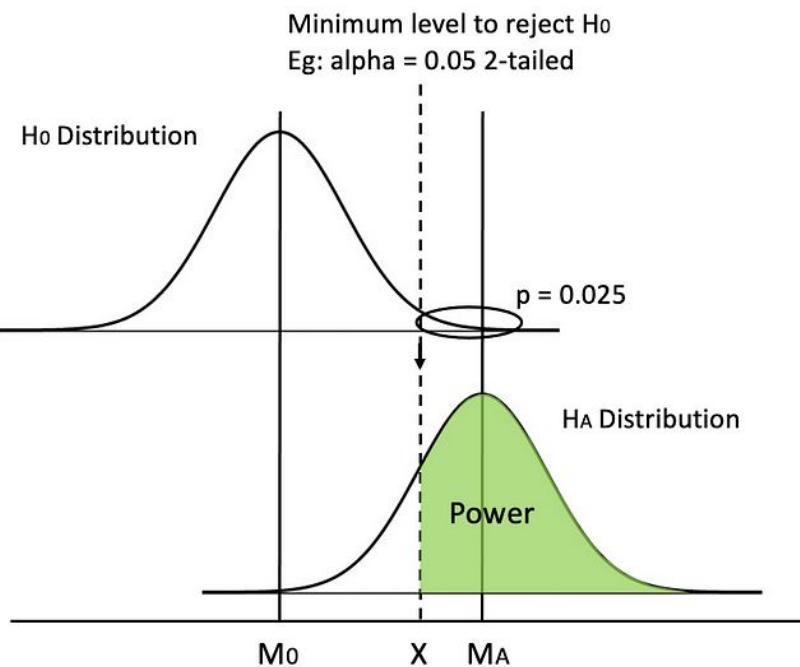
1) Raise significance level alpha (the **WRONG** way)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

How to increase statistical power

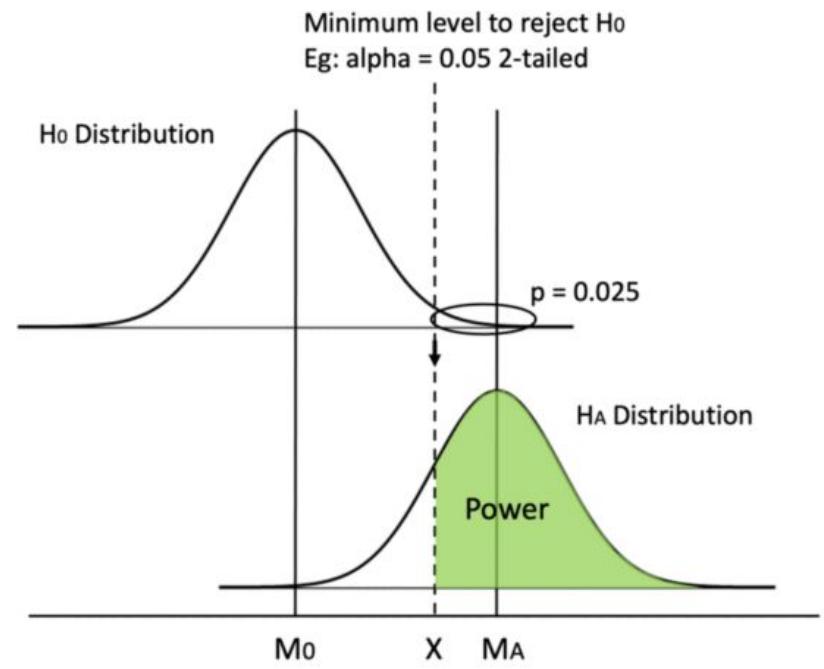
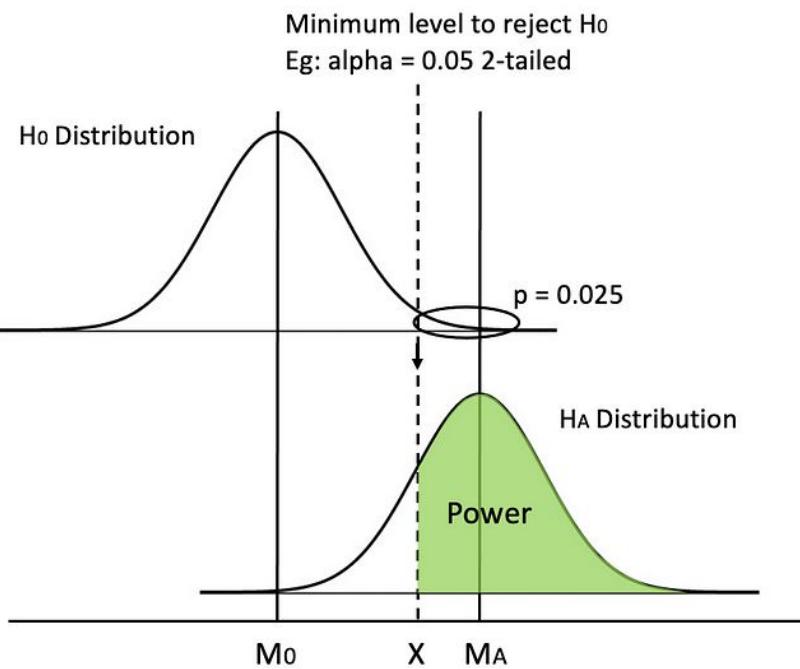
2) Switch from a 2-tailed test to a 1-tailed test **CORRECT** if possible)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

How to increase statistical power

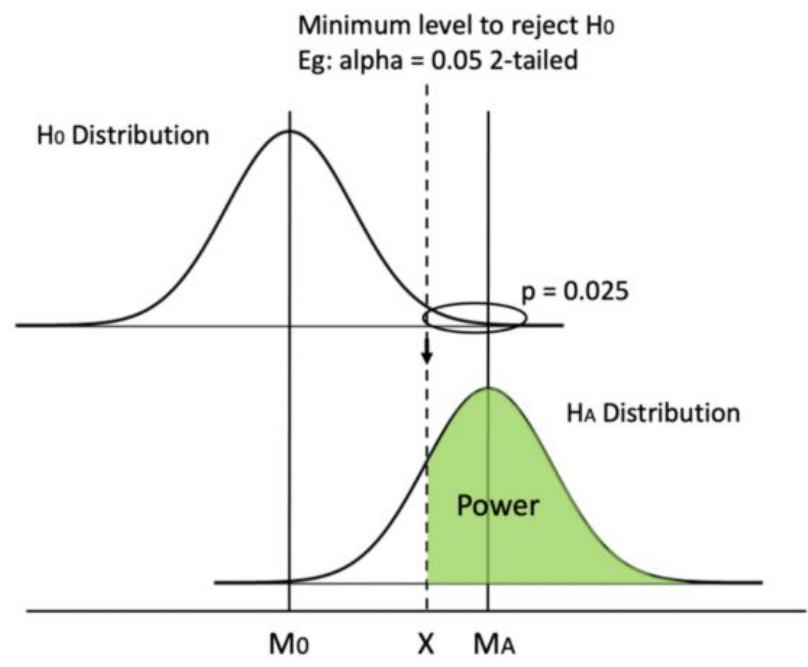
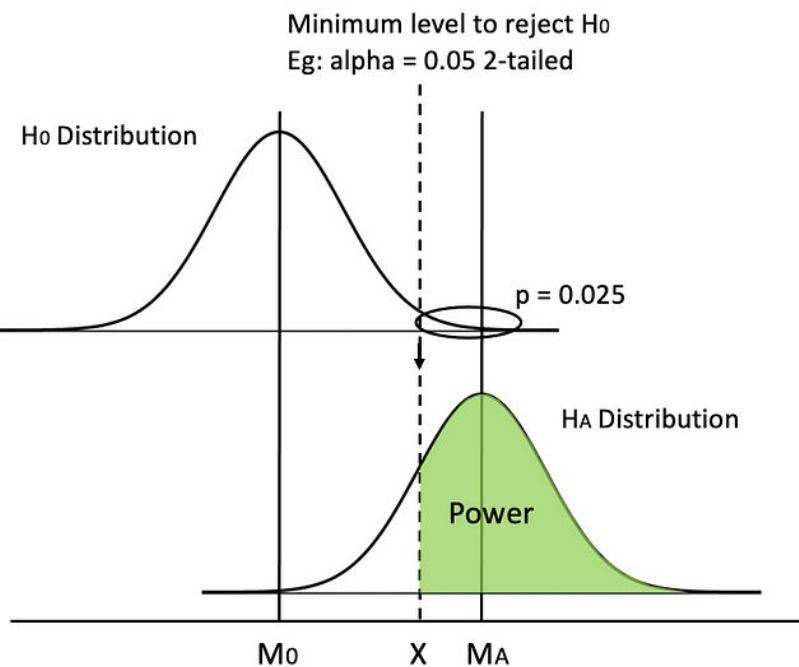
3) Increase mean difference (or increase the effect size)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

How to increase statistical power

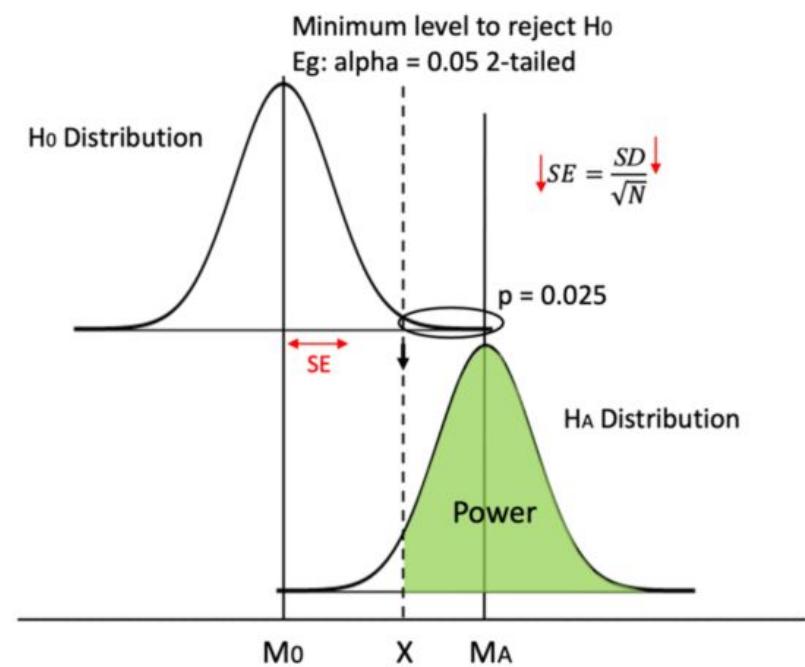
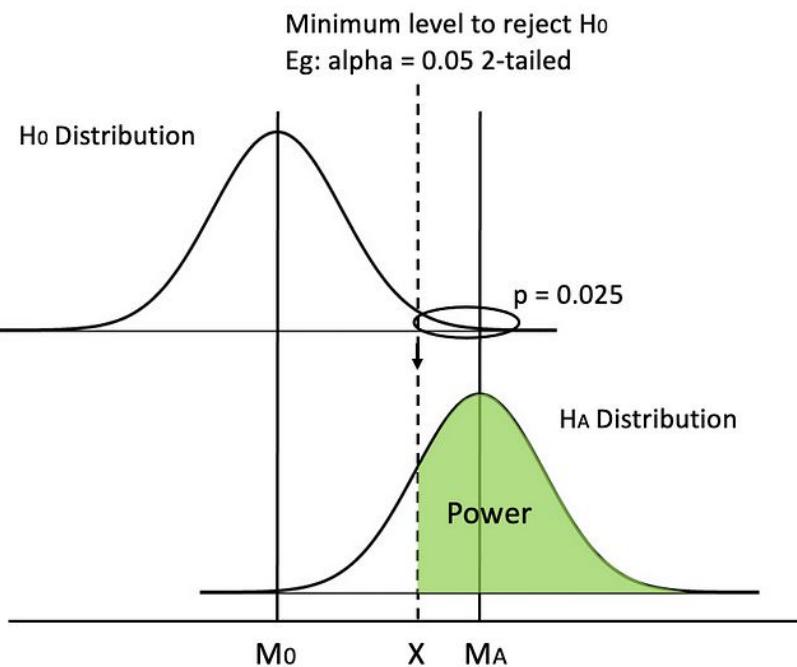
4) Use z distribution instead of t distribution (appropriate when we know the population mean.)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

How to increase statistical power

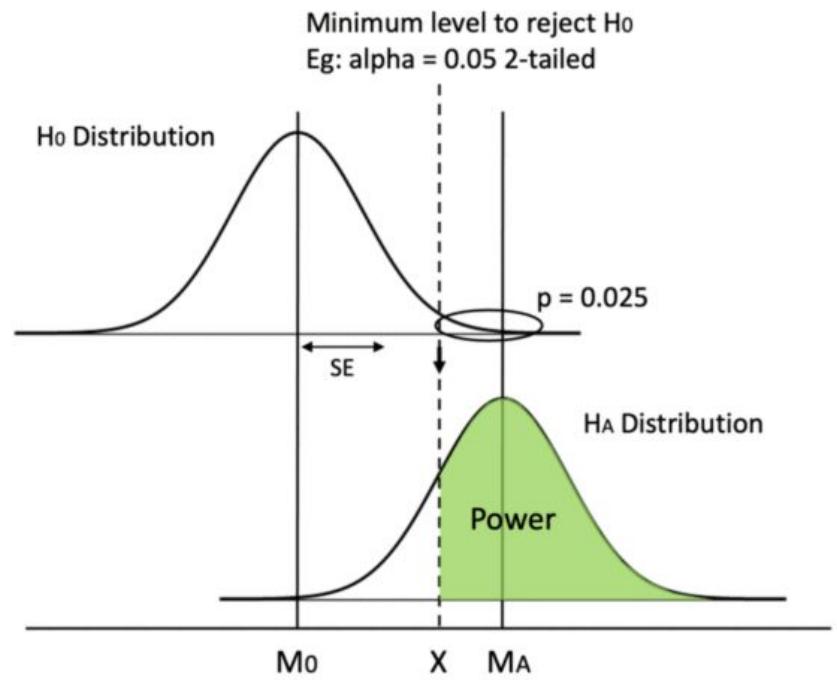
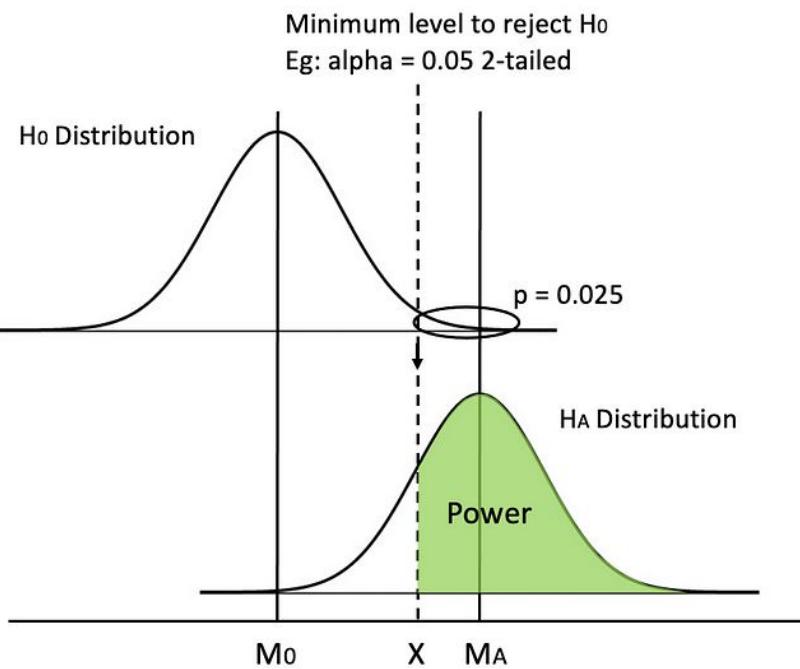
5) Decrease standard deviation (using more precise measurements to have less error and less noise)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

How to increase statistical power

6) Increase sample size (the most practical way)



Source: <https://towardsdatascience.com/5-ways-to-increase-statistical-power-377c00dd0214>

Effect size

An **effect size** is a way to quantify the difference between two or more groups.

The measurement of the effect size depends on the type of analysis you are doing.

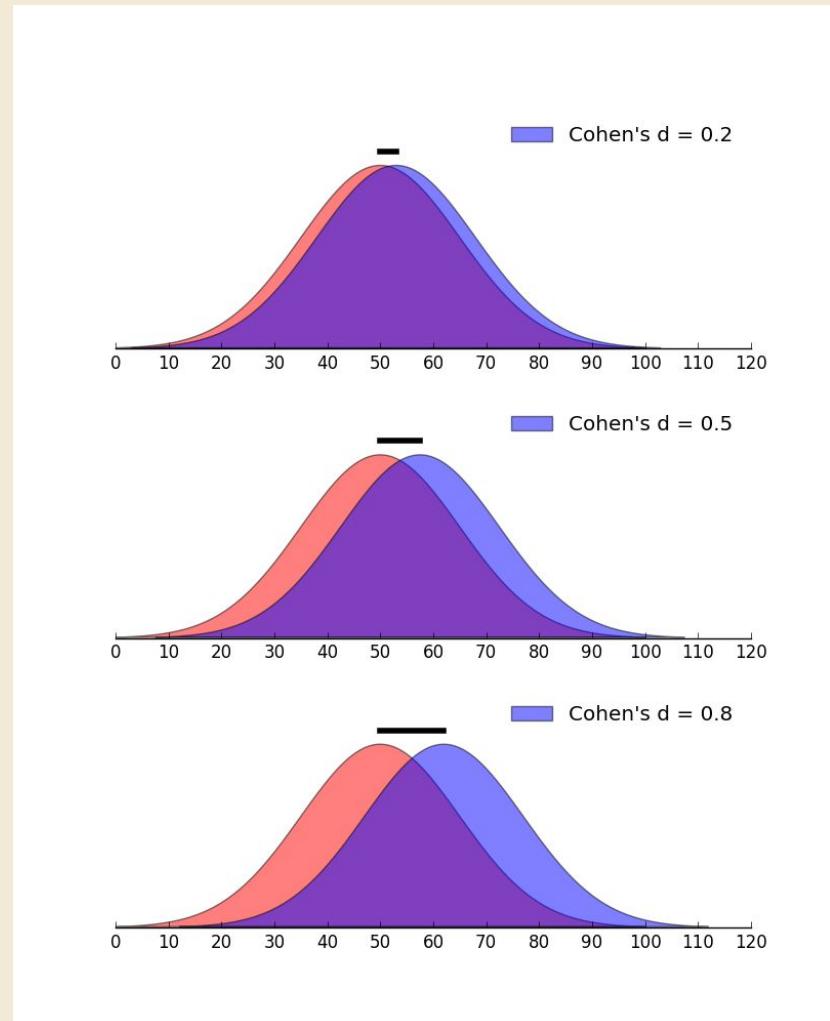
1. Studying the mean difference between two groups

In this case you use a standardized mean difference (Cohen's d)

Effect size

$$\text{Cohen's } d = (\text{mean}_1 - \text{mean}_2) / s$$

Cohen's d	Effect size
0.20	Small
0.5	Medium
0.8	Strong



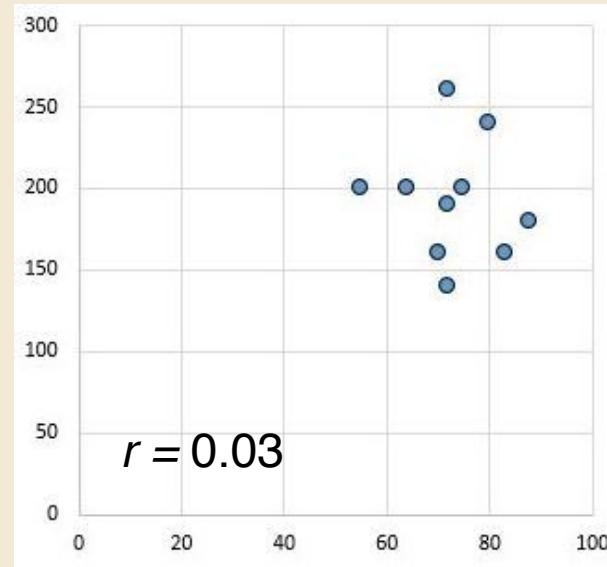
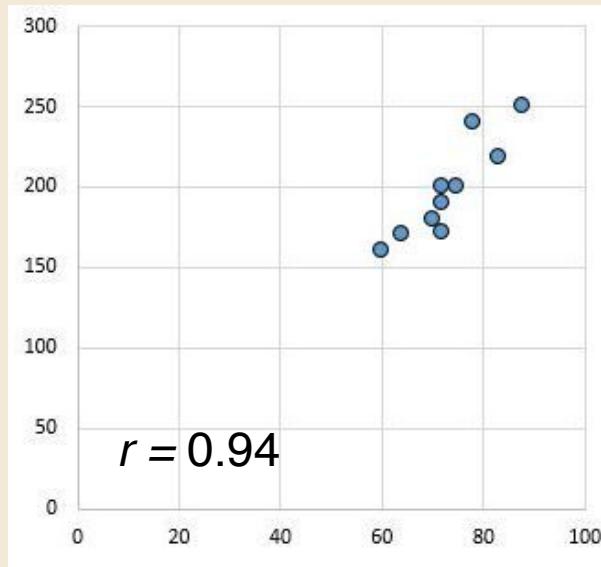
Effect size

2) Pearson Correlation Coefficient: measuring the linear association between two variables X and Y.

-1 = perfectly negative linear correlation between two variables

0 = no linear correlation between two variables

1 = perfectly positive linear correlation between two variables



Source: <https://www.statology.org/effect-size/>

Effect size

Pearson Correlation Coefficient

r	Effect size
0.1	small
0.3	medium
>0.5	large

Effect size: Cohen's guidelines

Test	Effect Size	Small	Medium	Large
All t-tests: • one-sample t-test • independent samples t-test • paired samples t-test	Cohen's d	0.20	0.50	0.80
Difference between many means (ANOVA)	Cohen's f	0.10	0.25	0.40
Chi-squared test	Cohen's w	0.10	0.30	0.50
Pearson's correlation coefficient	Pearson's	0.10	0.30	0.50
Linear Regression (entire model)	Cohen's	0.02	0.15	0.35