

STATISTICS WITH

INTENSIVE WORKSHOP

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. Machine Learning; 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 Machine Learning
 - PCA
 - PLS-DA
- MetaboAnalyst
 - Overview
 - Workflow
- Power analysis
 - Hypothesis testing
 - Decision errors
 - Statistical power
 - Effect size

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

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

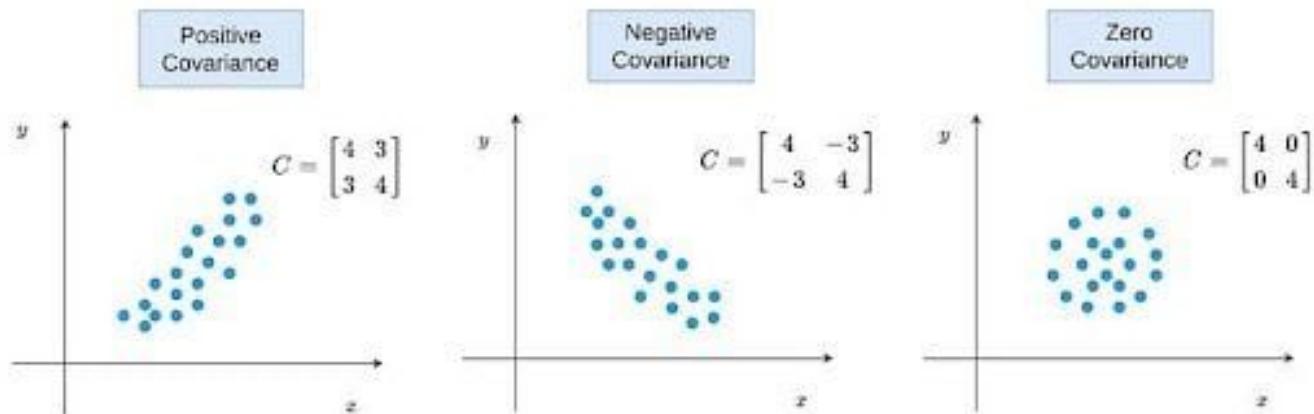
Population mean is unknown

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

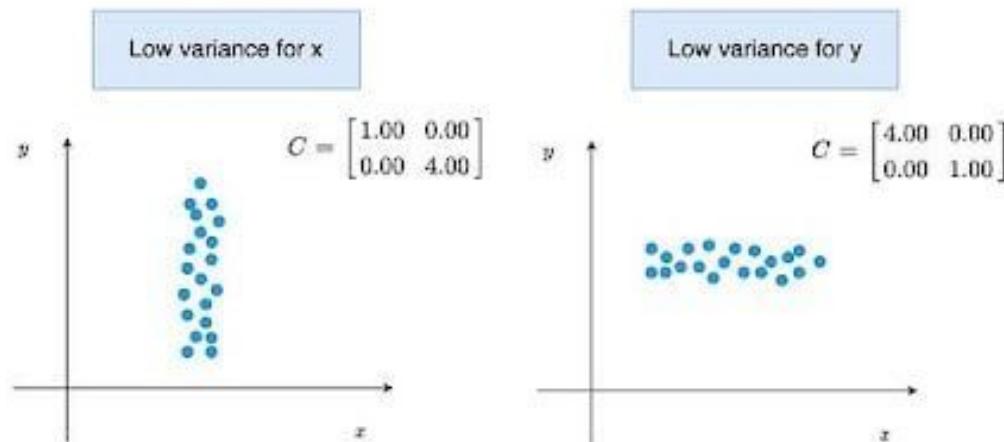
$$\begin{matrix} & \begin{matrix} x & y & z \end{matrix} \\ \begin{matrix} x \\ y \\ z \end{matrix} & \begin{bmatrix} var(x) & cov(x, y) & cov(x, z) \\ cov(x, y) & var(y) & cov(y, z) \\ cov(x, z) & cov(y, z) & var(z) \end{bmatrix} \end{matrix}$$

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 is a linear algebra operation.

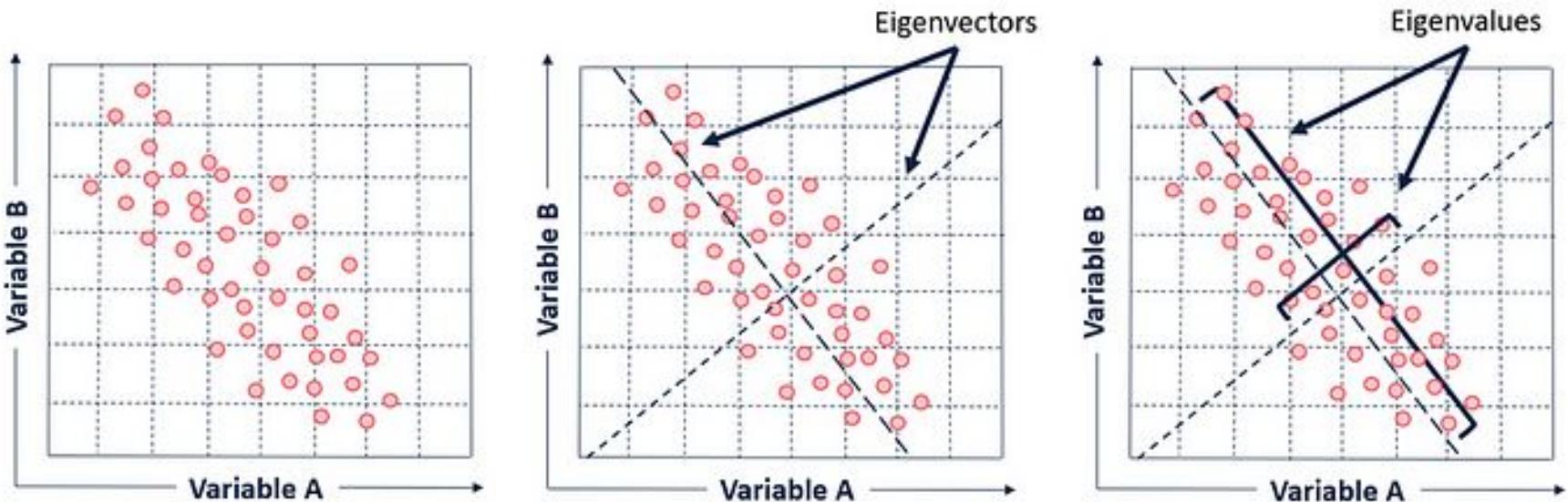
It is a transformation method that creates (weighted linear) combinations of the original variables in a data set, with the intent that the new combinations will capture as much variance in the dataset as possible while eliminating correlations (i.e., redundancy).

PCA creates the new variables using the eigenvectors and eigenvalues calculated from the covariance matrix of your original variables.

Eigenvectors & Eigenvalues

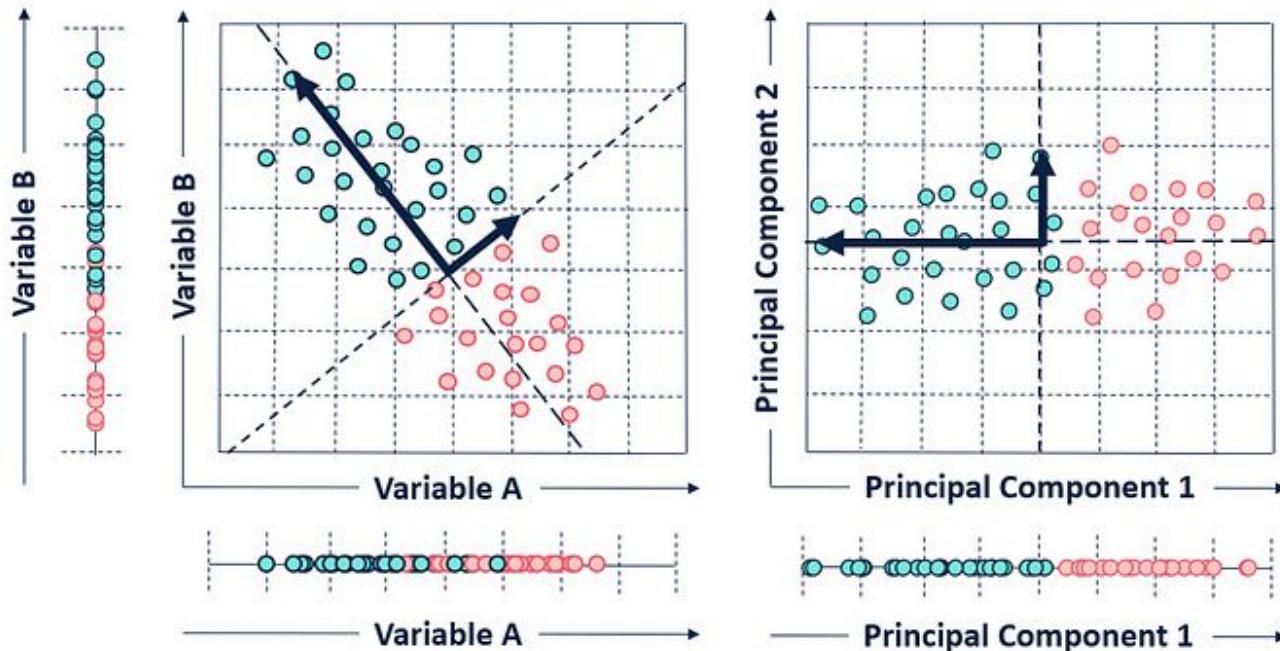
In the context of PCA

- 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 components

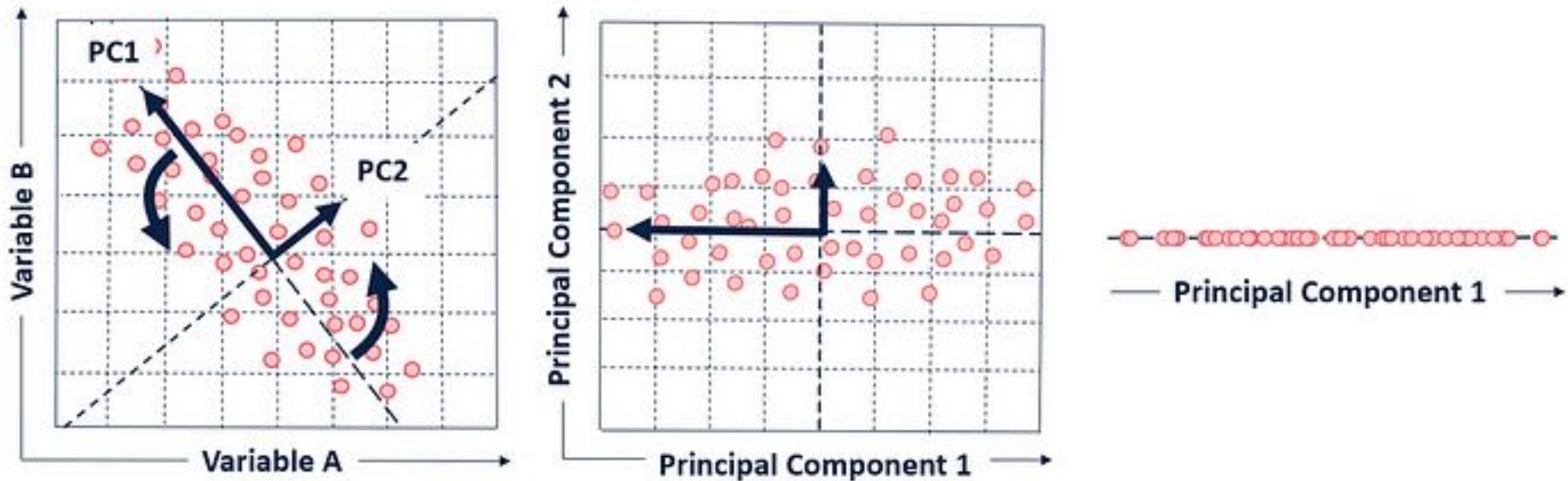


Principal Component 1 accounts for variance from both variables A and B. (dimension reduction)

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 components



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>

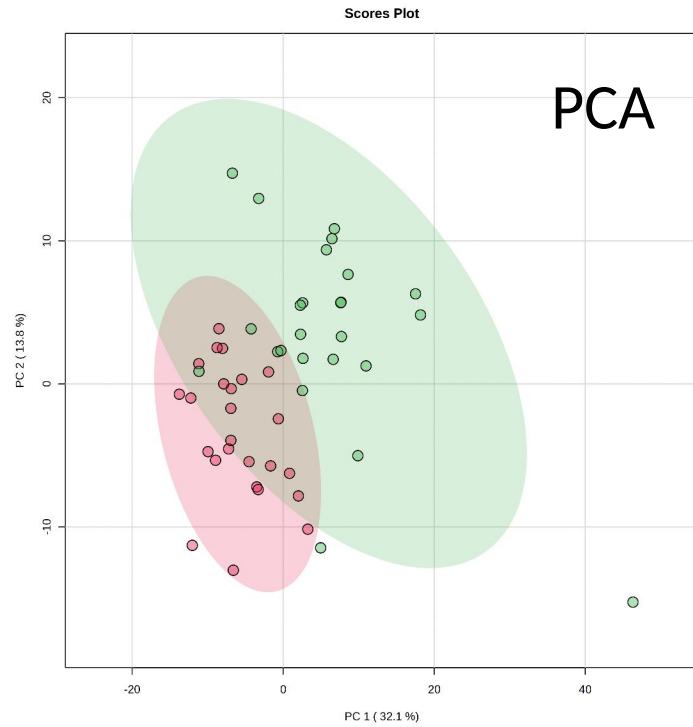
PLS Discriminant Analysis (PLS-DA)

A *supervised* alternative to PCA
performing simultaneous dimensionality
reduction and classification

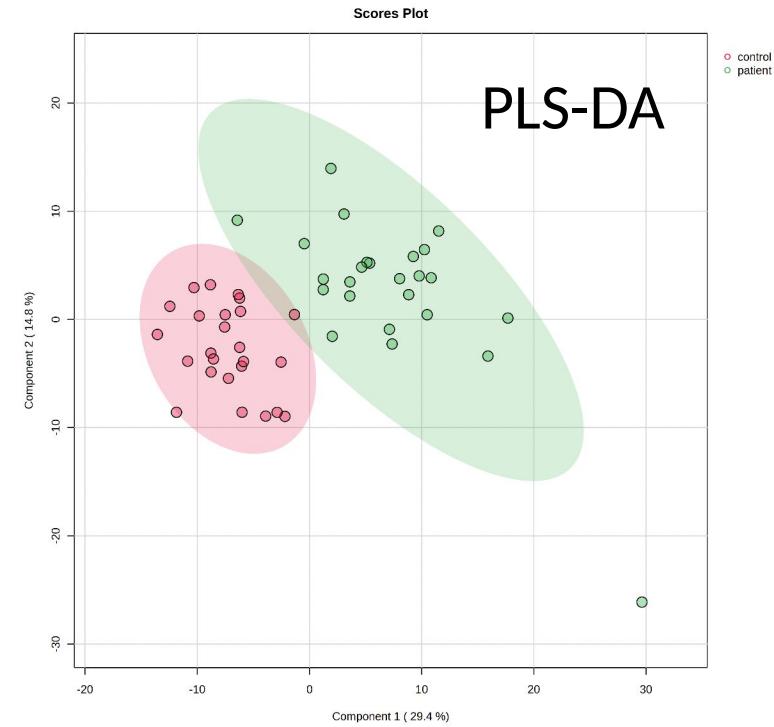
Purpose: PLS-DA vs PCA

- 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: omics sciences.

Scores plot: PCA vs PLS-DA



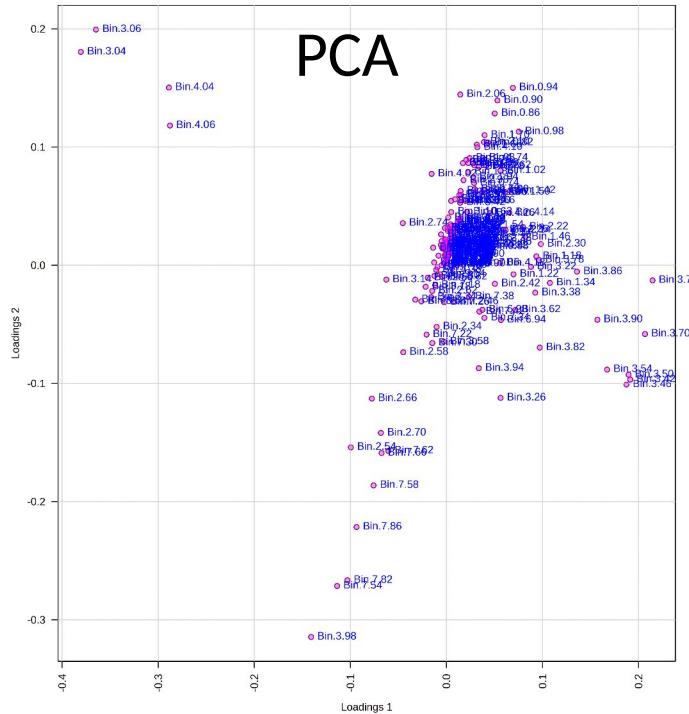
Samples projected in the space of Principal Components



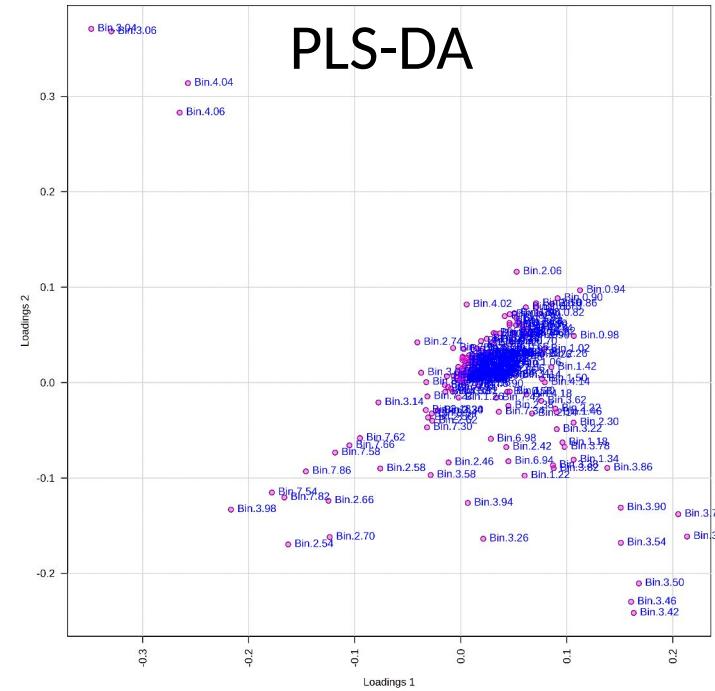
Samples projected in the space of latent variables (components) that maximize the separation between groups

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

Loadings plot: PCA vs PLS-DA



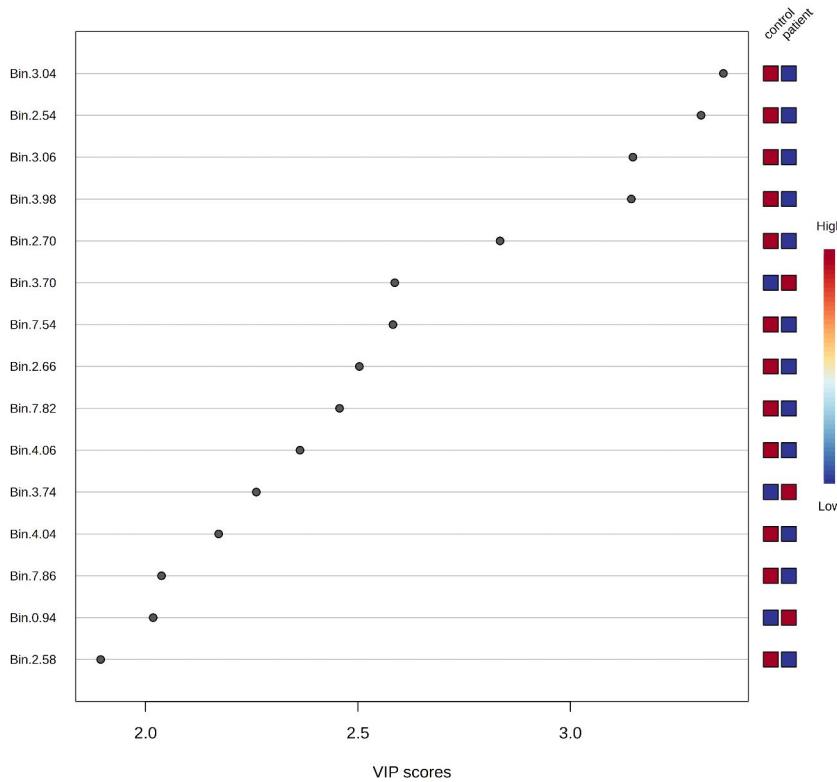
The loading vectors (here shown as points) represent the original variables in the space PCs.



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**.

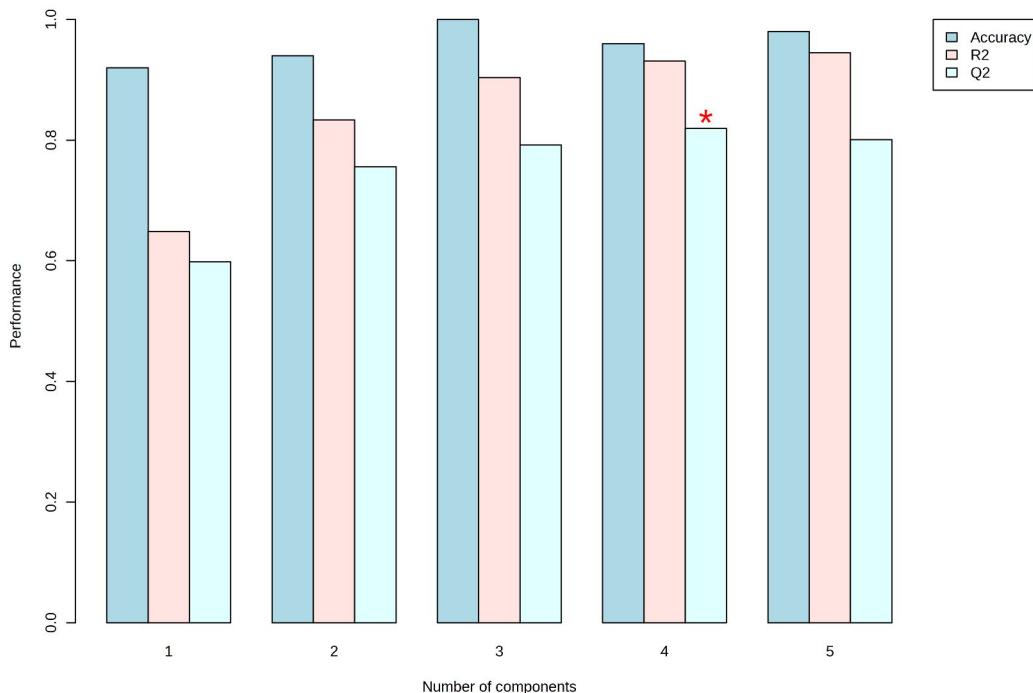
...very useful to select potential biomarkers!

Source: Test data (**NMR spectral bins**) provided by METABOANALYST platform:

<https://www.metaboanalyst.ca>
19/09/2024

<https://lulliter.github.io/R4stats/>

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.

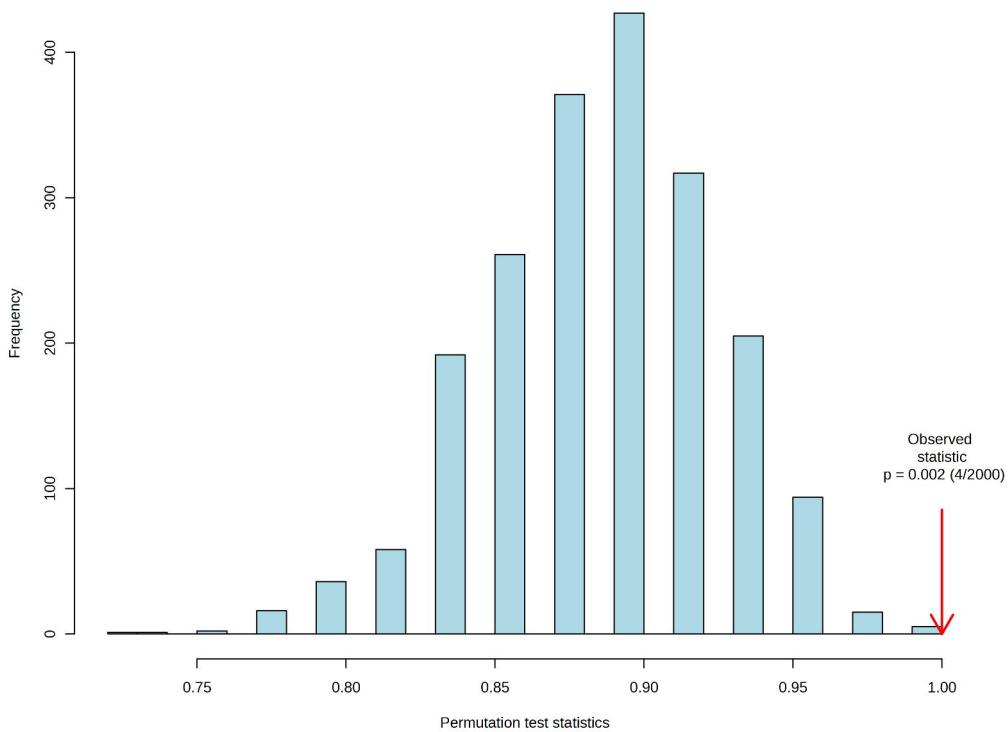
Q^2 is an analogous of R^2 in regression: the higher the better!

Source: Test data (**NMR spectral bins**) provided by METABOANALYST platform:

<https://www.metaboanalyst.ca>
19/09/2024

<https://lulliter.github.io/R4stats/>

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.

Test data (**NMR spectral bins**) provided by METABOANALYST platform: <https://www.metaboanalyst.ca>

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- MetaboAnalyst

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1. Hypothesis testing
2. Decision errors
3. Statistical power
4. Effect size

MetaboAnalyst

An R-driven Software

Introduction to MetaboAnalyst



<https://www.metaboanalyst.ca>

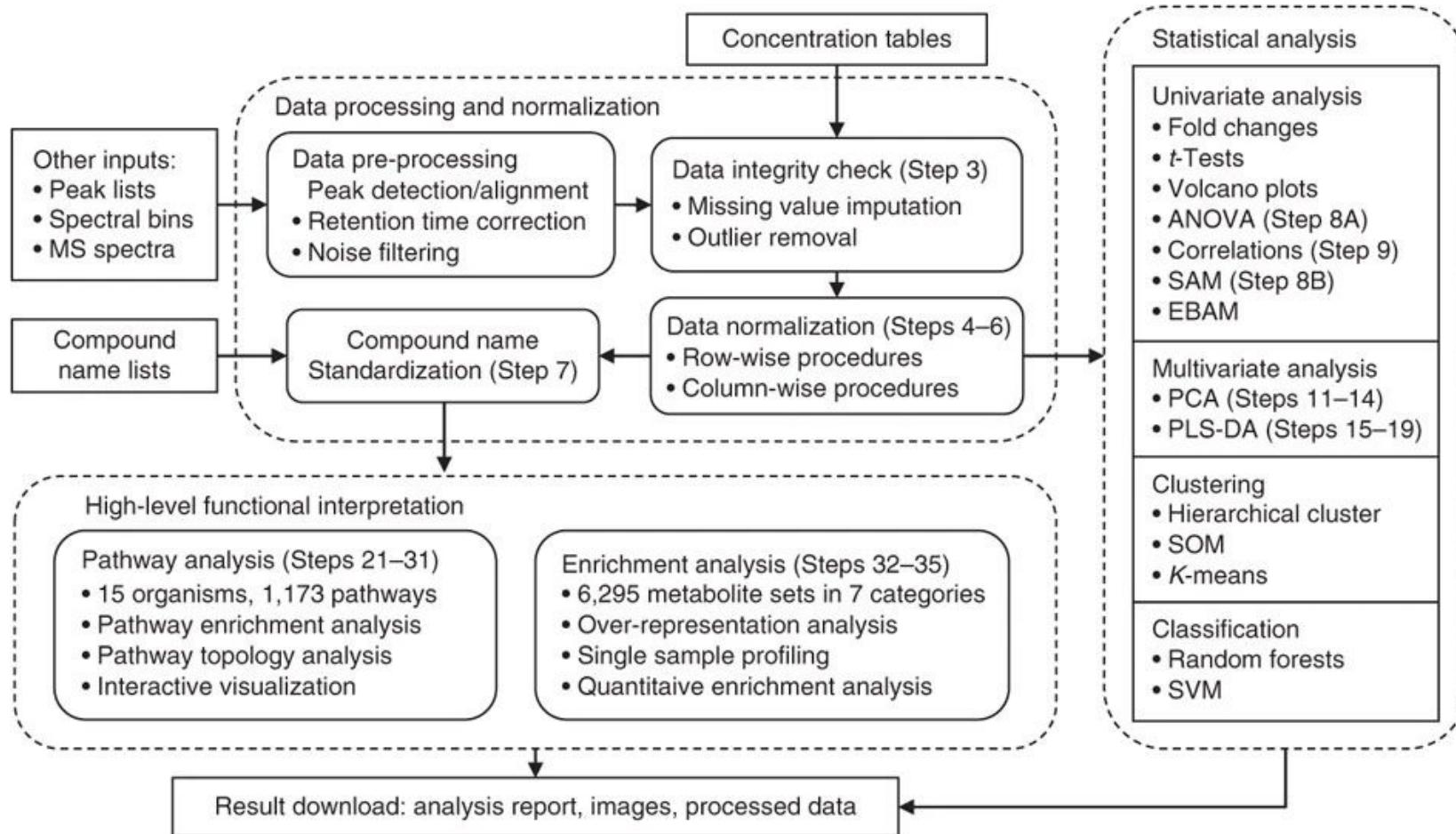
From raw spectra to biomarkers, patterns, functions and systems biology

- 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

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.



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

MetaboAnalyst workflow

2) data filtering

Upload

Processing

Data check

Missing value

Data filter

Data editor

Normalization

Statistics

Download

Exit

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($RSD = SD/mean$). 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: <input type="range" value="25"/> 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 ($RSD = SD/mean$) <input type="radio"/> Non-parametric relative standard deviation (MAD/median) | Percentage to filter out: <input type="range" value="5"/> 5% |
| Abundance filter: | <input checked="" type="radio"/> Mean intensity value <input type="radio"/> Median intensity value | Percentage to filter out: <input type="range" value="0"/> 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 is for general-purpose adjustment for systematic differences among samples;
- Data transformation applies a mathematical transformation on individual values themselves. A simple mathematical approach is used to deal with negative values in log and square root Please search OmicsForum using "normalization #metaboanalyst" to find more information.
- Data scaling adjusts each variable/feature by a scaling factor computed based on the dispersion of the variable.

Sample normalization

- None
- Sample-specific normalization (i.e. weight, volume) Specify
- Normalization by sum
- Normalization by median
- Normalization by a reference sample (PQN) Specify
- Normalization by a pooled sample from group (group PQN) Specify
- Normalization by reference feature Specify
- 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

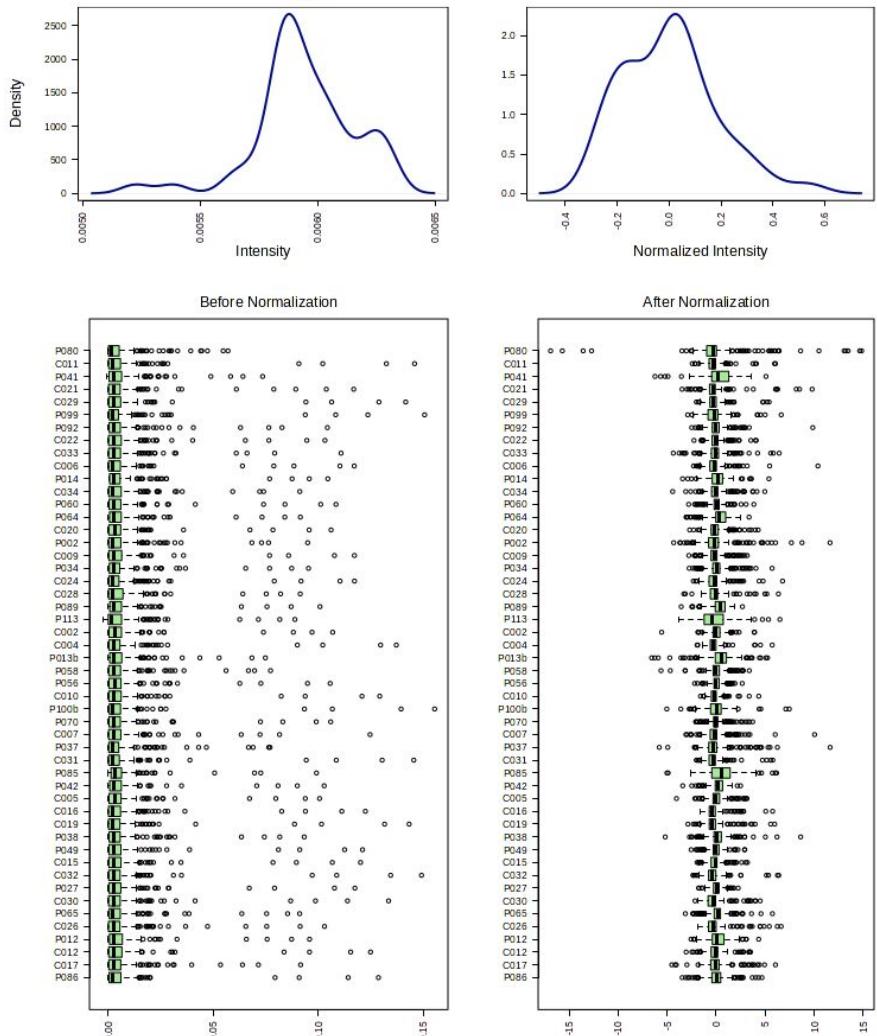
- None
- Mean centering (mean-centered only)
- Auto scaling (mean-centered and divided by the standard deviation of each variable)
- Pareto scaling (mean-centered and divided by the square root of the standard deviation of each variable)
- Range scaling (mean-centered and divided by the range of each 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

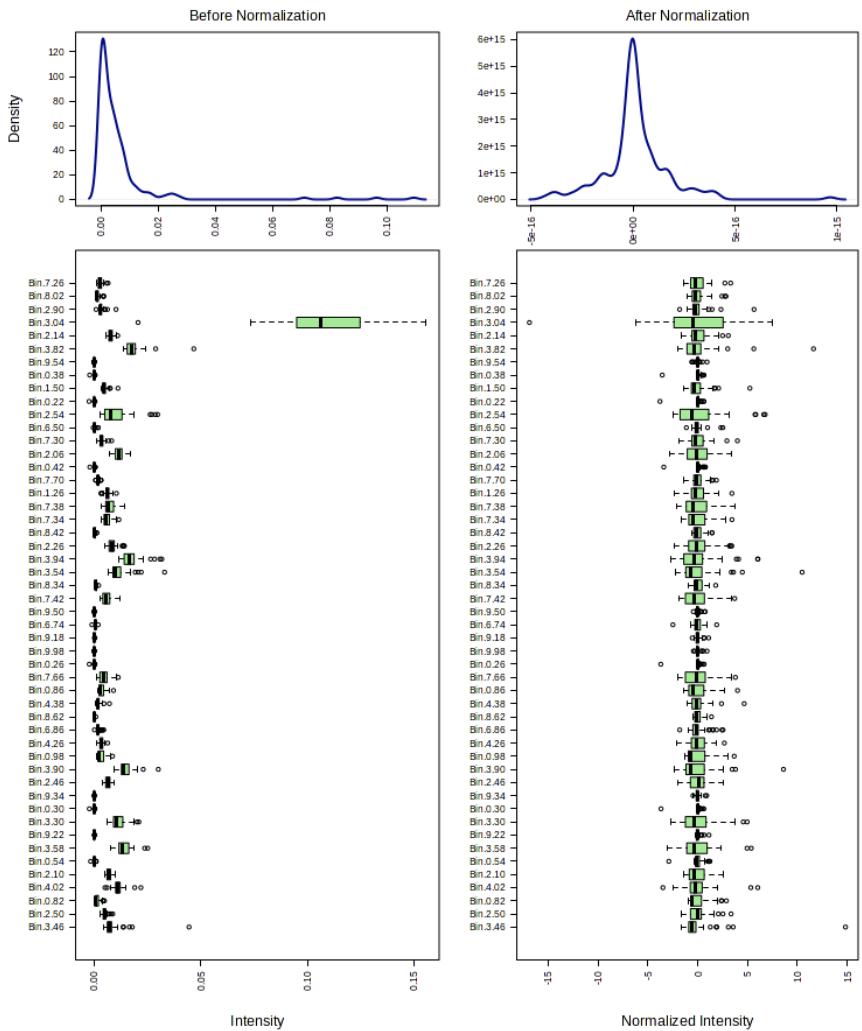


Effect of normalization over sample

MetaboAnalyst workflow

3) data normalization

Effect of features/metabolites scaling



MetaboAnalyst workflow

4) statistical analysis

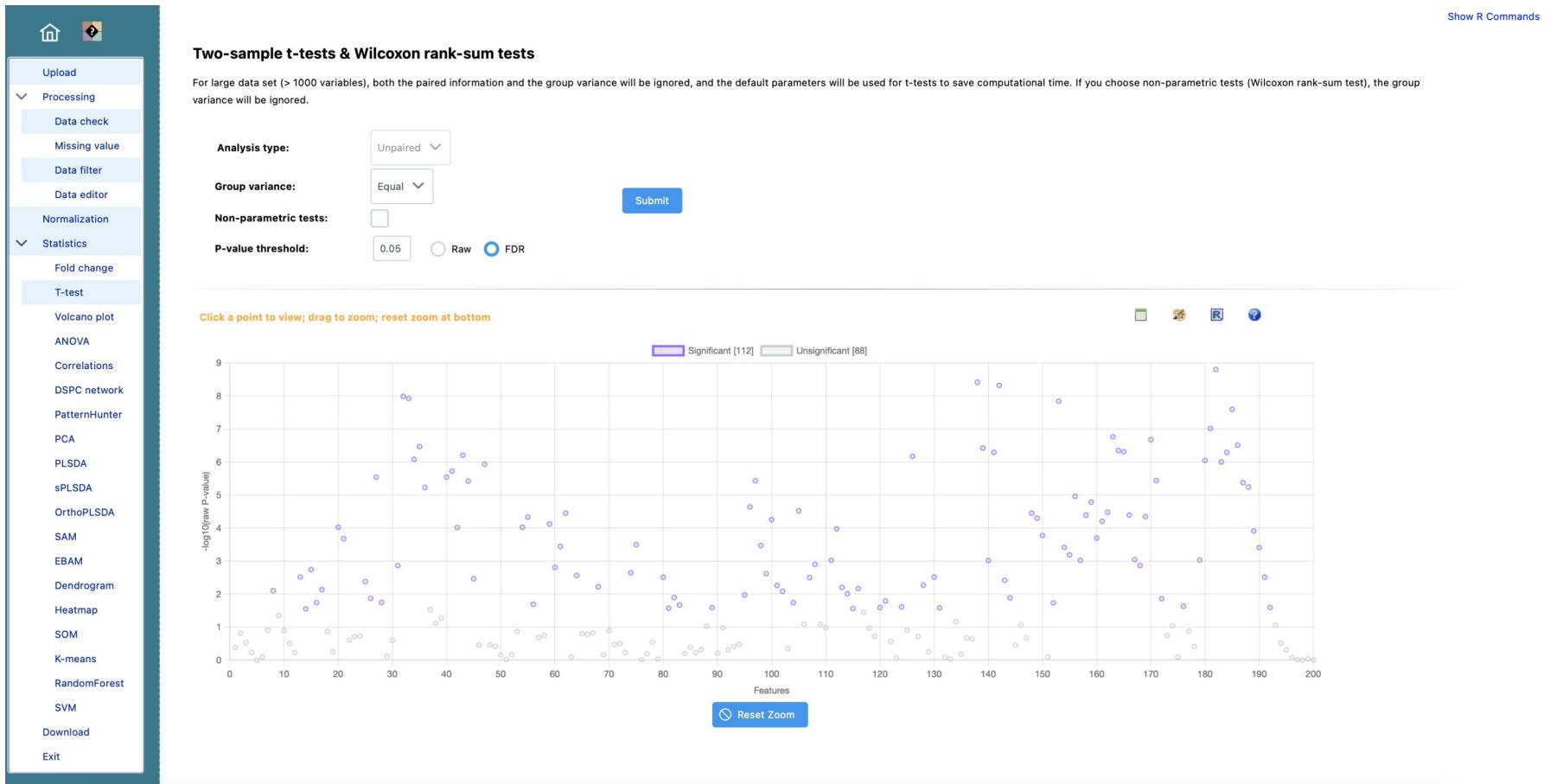
The screenshot shows the MetaboAnalyst software interface. On the left, a sidebar menu lists various analysis paths: Upload, Processing (Data check, Missing value), Data filter, Data editor, Normalization, Statistics (selected), Download, and Exit. The main content area is titled "Select an analysis path to explore:" and contains several sections:

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

A large red bracket on the right side of the content area groups the first two sections (Univariate Analysis and Advanced Significance Analysis) under the heading "«Classical» analysis of variance among groups". Another large red bracket groups the last three sections (Chemometrics Analysis, Cluster Analysis, and Classification & Feature Selection) under the heading "Machine learning algorithms".

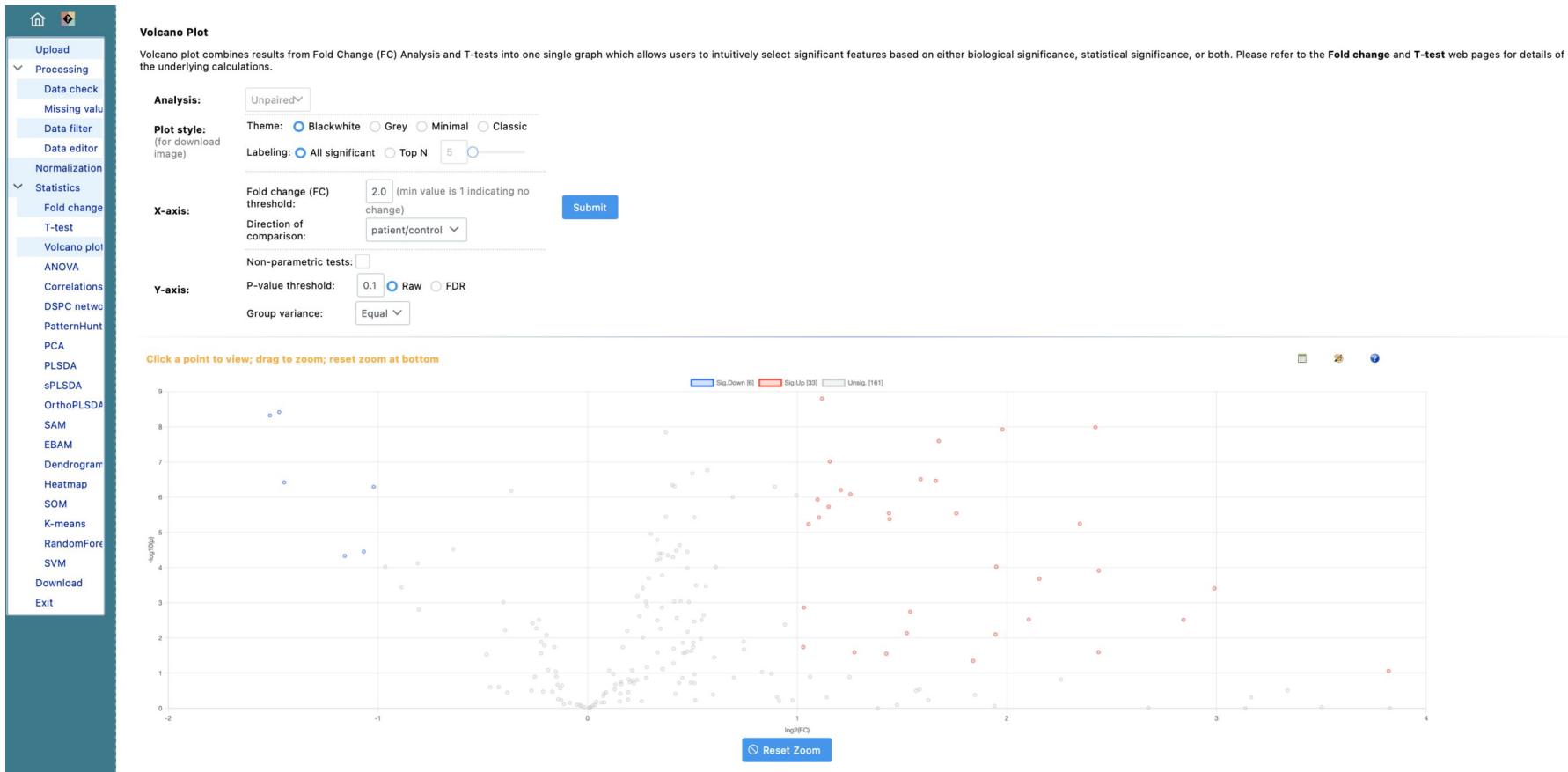
MetaboAnalyst workflow

4) univariate analysis



MetaboAnalyst workflow

4) univariate analysis



MetaboAnalyst workflow

5) chemometric analysis



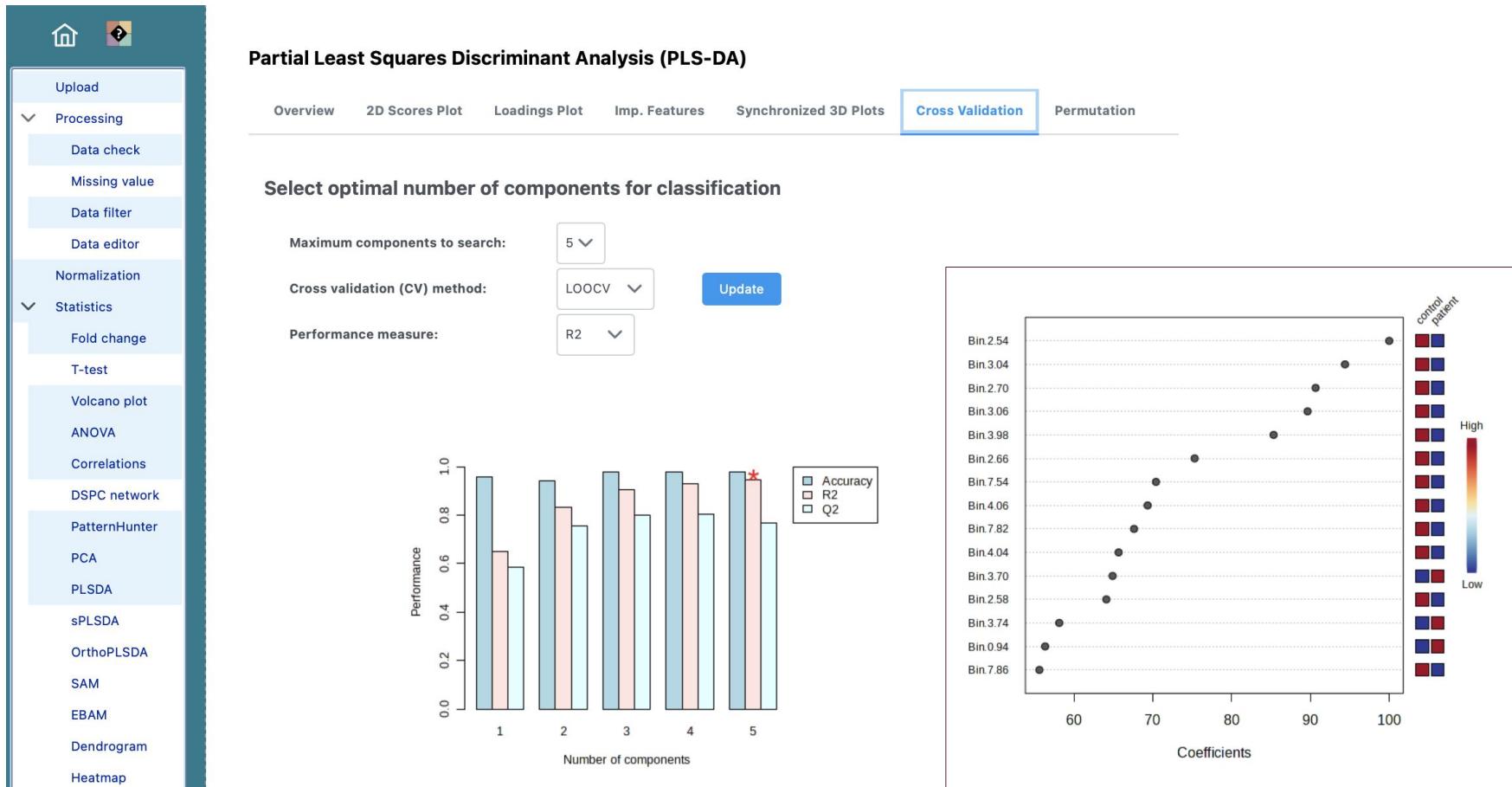
MetaboAnalyst workflow

5) chemometric analysis



MetaboAnalyst workflow

5) chemometric analysis



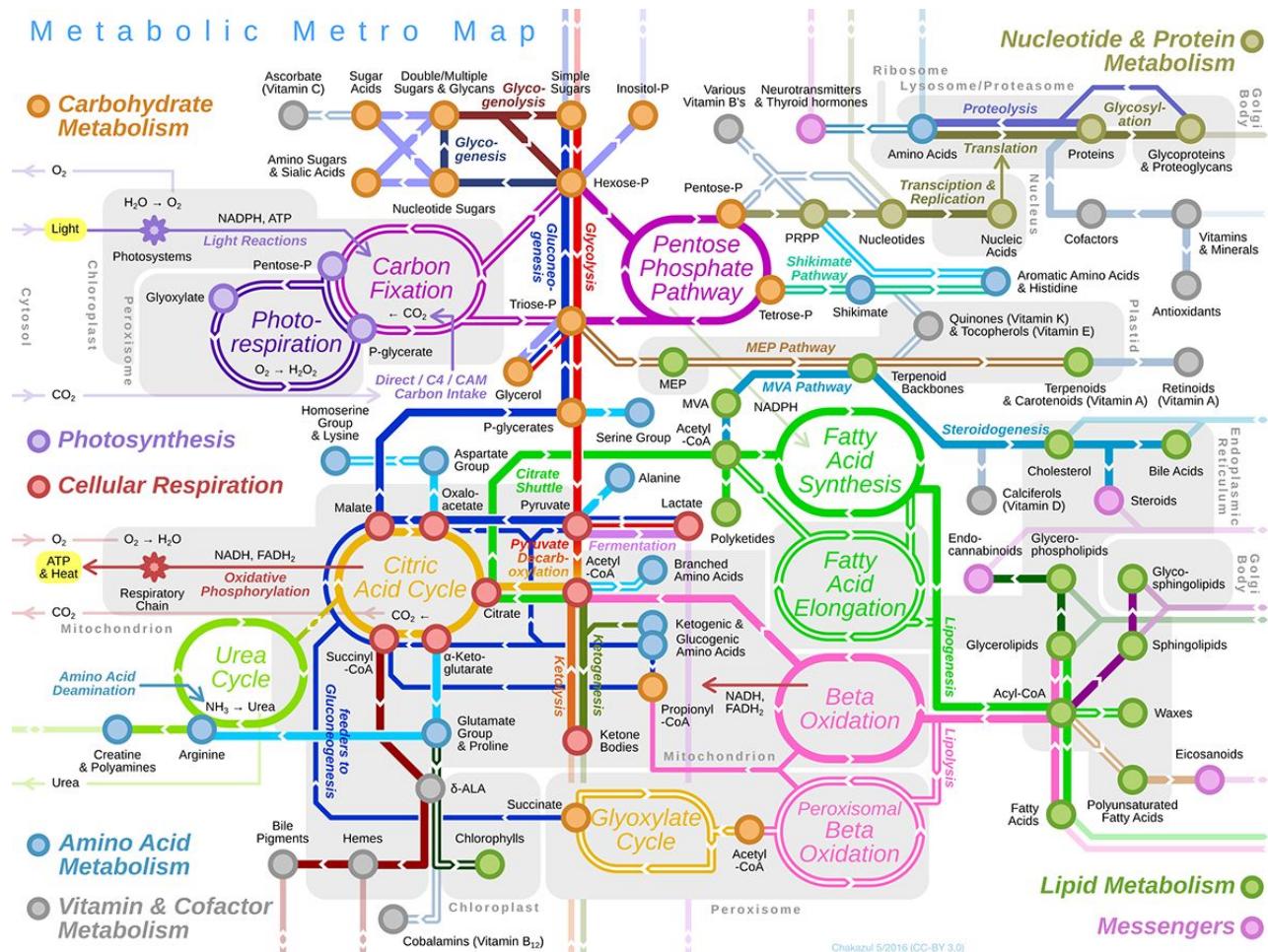
MetaboAnalyst workflow

5) chemometric analysis



Heatmap of the top 25 T-test features

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 check' step in the left sidebar and the 'Name match' modal.

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 list;
- 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

Parameter Setting

Enrichment tests are based on the well-established **globaltest** to test associations between metabolite sets and the outcome. The algorithm uses a generalized linear model to compute a 'Q-stat' for each metabolite set. The Q-stat is calculated as the average of the Q values calculated for each single metabolites; while the Q value is the squared covariance between the metabolite and the outcome. The globaltest has been shown to exhibit similar or superior performance when tested against several other popular methods.

Metabolite sets: Unlike transcriptomics which allows comprehensive gene expression profiling, targeted metabolomics usually covers only a small percentage of metabolome (the actual coverage is platform/protocol specific). This means that metabolites (defined in our current pathways or metabolite sets) do not have equal probabilities of being measured in your studies, and the enriched functions are the results from both platform/protocol specific effects and biological perturbations. Since the primary interest is to detect the latter, we highly recommend **uploading a reference metabolome** containing all measurable metabolites from your platform to eliminate the former effects.

Please select a metabolite set library

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

Only use metabolite sets containing at least 2 entries

Please specify a reference metabolome

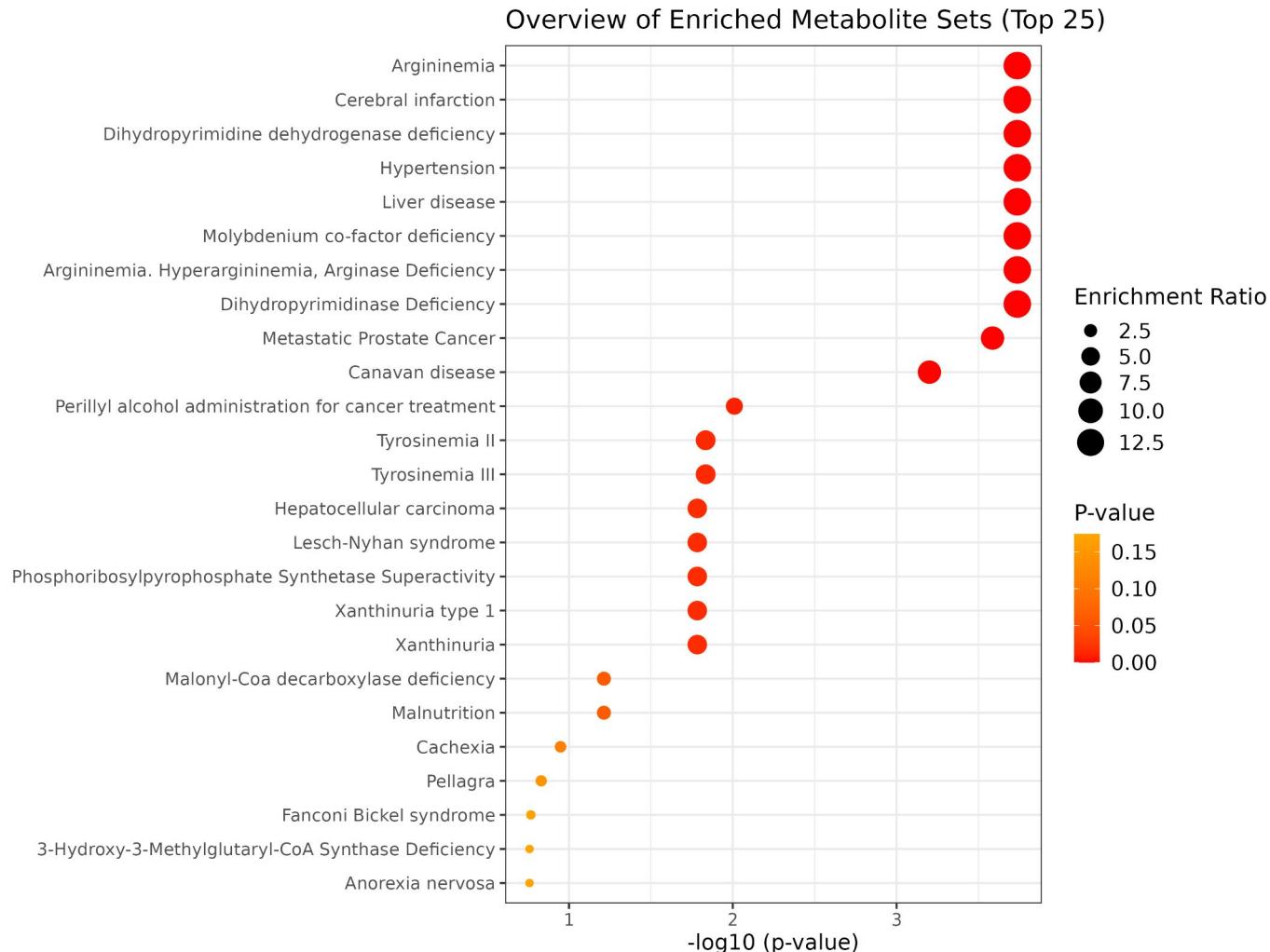
Use all the compounds in the selected library
 Upload a reference metabolome based on your analytical platform

Submit

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

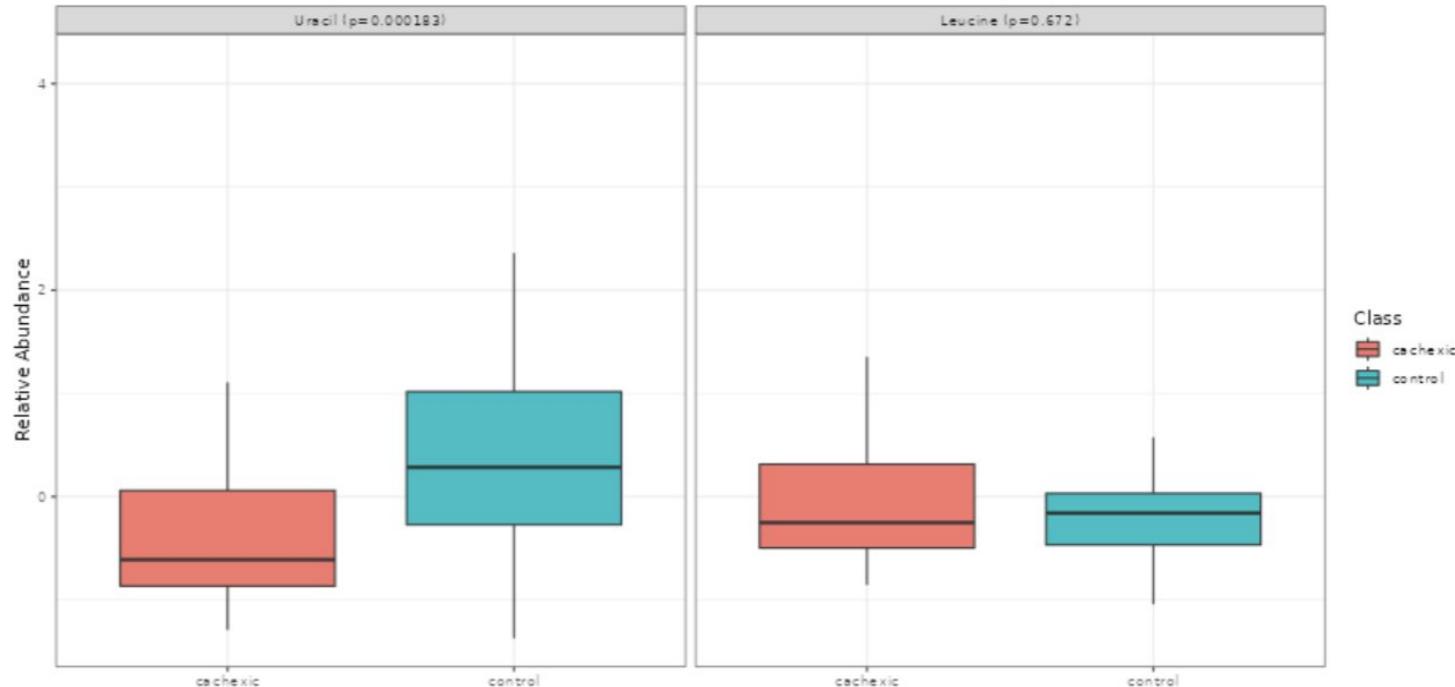
6) enrichment analysis



MetaboAnalyst workflow

6) functional interpretation

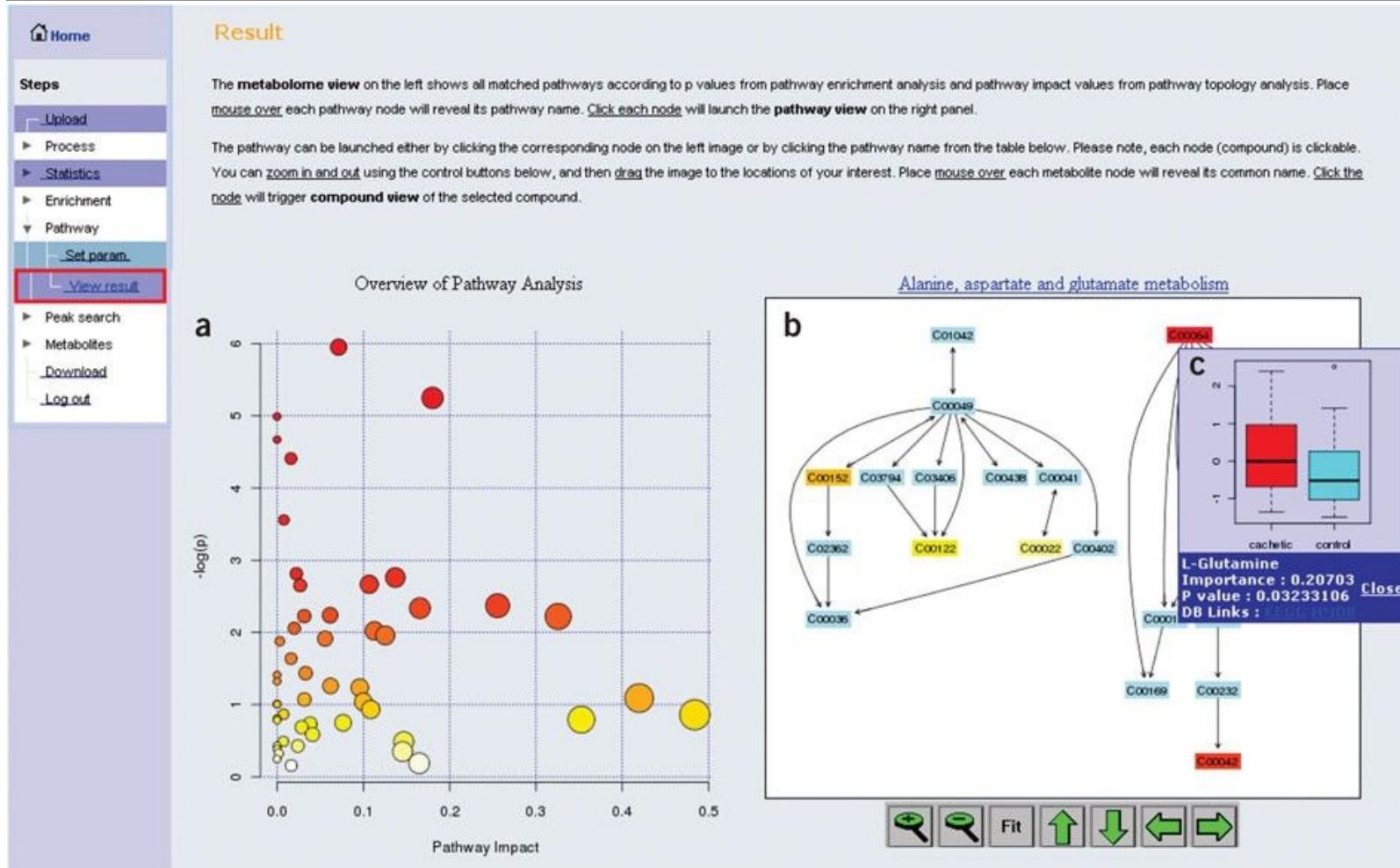
Current metabolite set:



| Set Name | Metabolites | References |
|----------------------------|--|------------------------|
| Metastatic Prostate Cancer | Sarcosine; Uracil ; Kynurenone; Glycerol 3-phosphate; Leucine ; DL-Proline | PubMed |

MetaboAnalyst workflow

Metabolic pathway analysis and visualization



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

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Hypothesis testing steps

1. State the hypotheses (the **null hypothesis** and an **alternative hypothesis**)
2. Design the analysis (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.

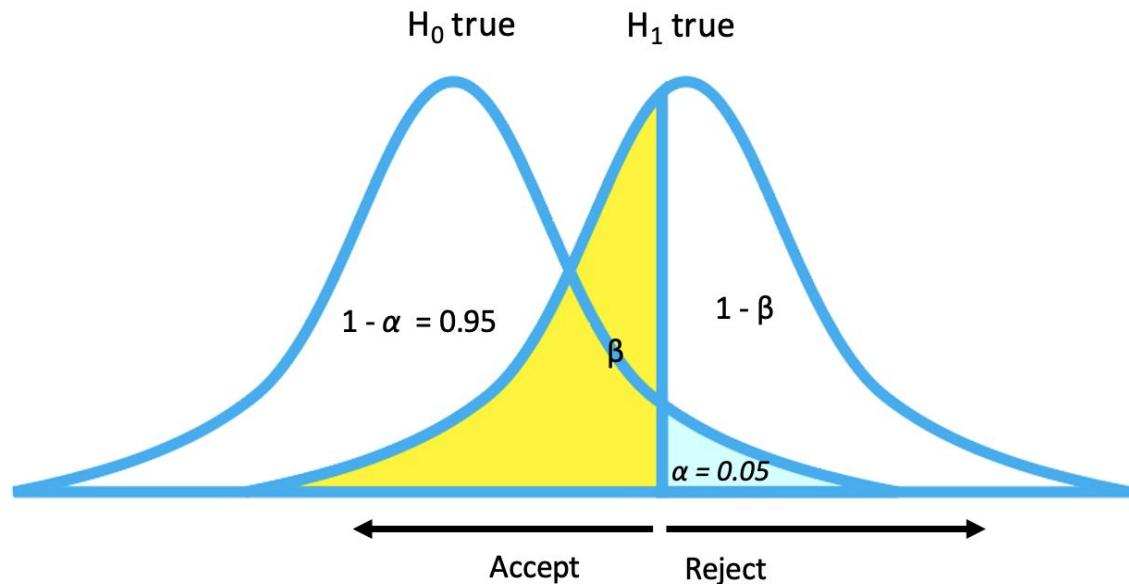
- Type I error occurs when the researcher rejects a null hypothesis when it is true. The probability of committing a Type I error is called the **significance level**. This probability is also called alpha, and is often denoted by α .
- Type II error occurs when the researcher fails to reject a null 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 H_0 | Reject H_0 |
|---------------|--|---------------------------------------|
| H_0 is true | Correct action | Type I error FALSE POSITIVE |
| probability | $1-\alpha$ | α |
| H_1 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?

| | Fail to reject H0 | Reject H0 |
|-------------|-------------------|-------------------|
| 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, we still have some outliers.
 - False negative: as a defendant, the prosecution failed to prove the fatal evidence.
- 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
- 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
- usually the researcher fix the type I error (α) he can tolerate **before** experiment and then compare the **p-value** and takes a decision

Controlling Type I and Type II error

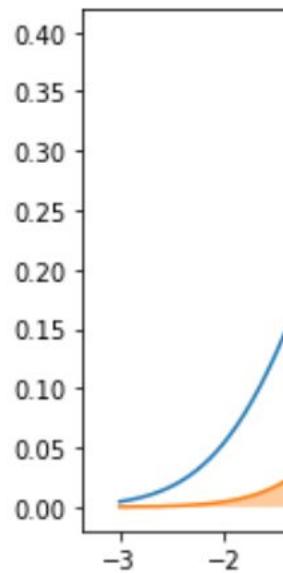


Figure 1: Equal
and false nega

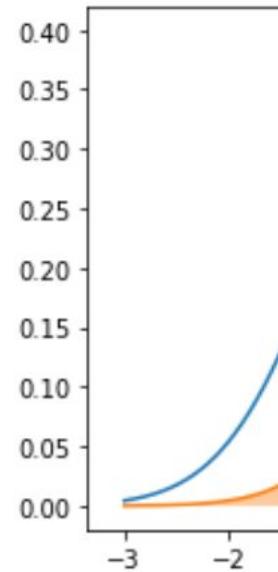


Figure 2: Great
than false nega

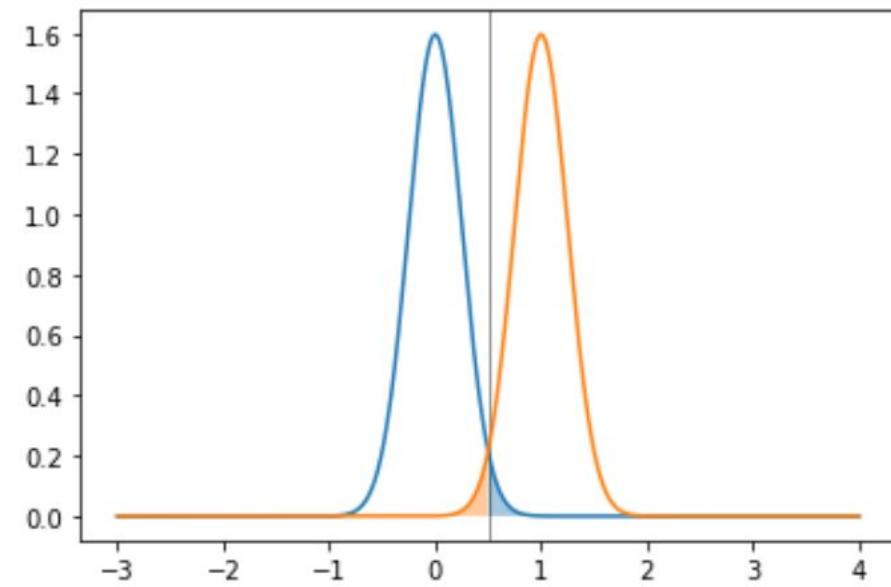
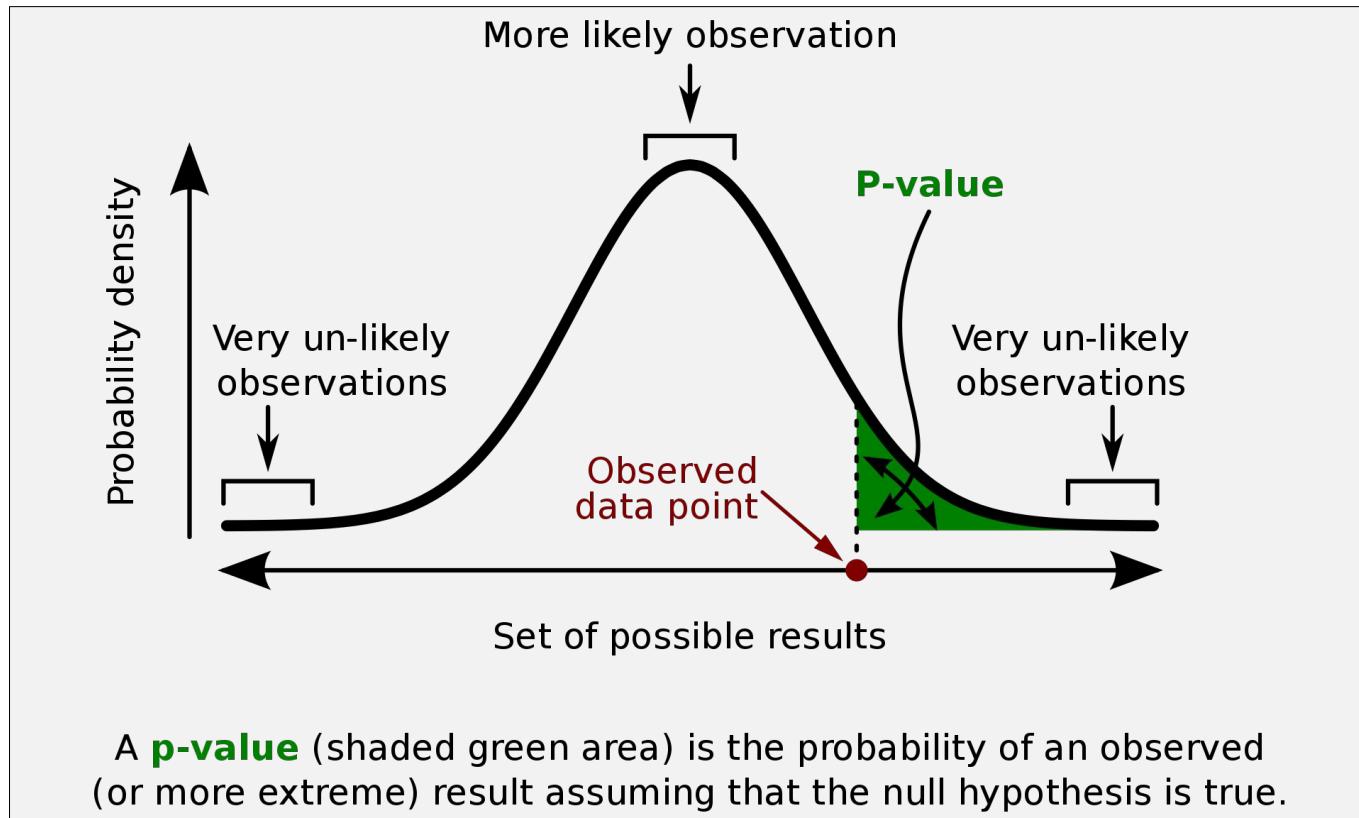


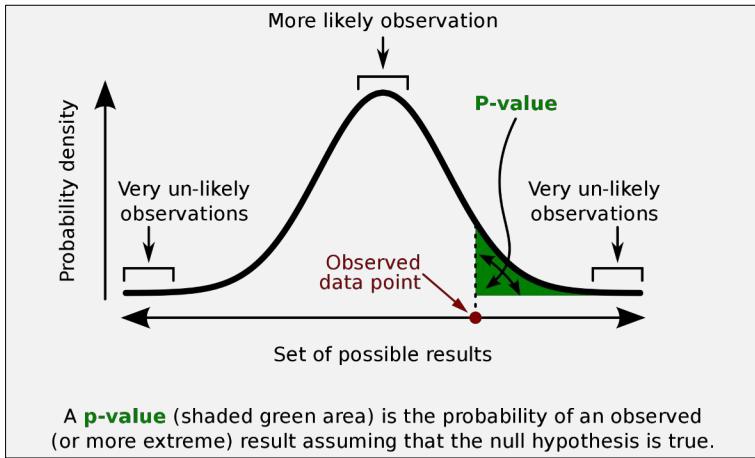
Figure 3: Lowered uncertainty through
more informative features.

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 H₀ is true?



p-value interpretation



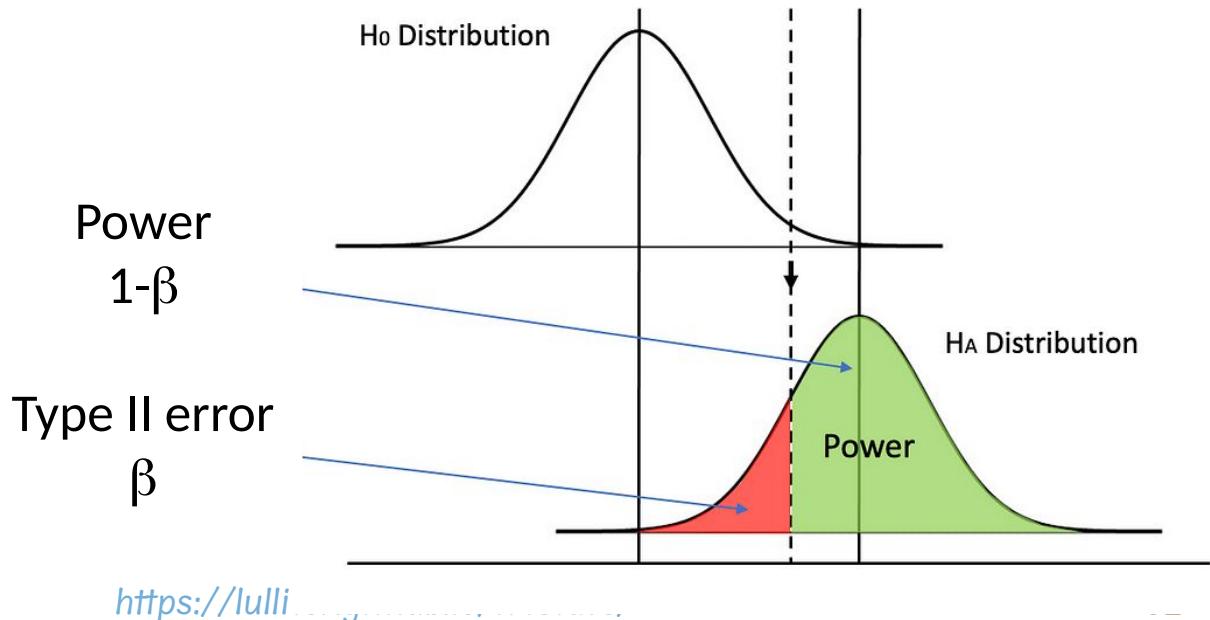
- A very small p-value means that such an extreme observed outcome would be very unlikely under the null hypothesis.
- Usually the researcher fix α before experiment and then compare the p-value and takes a decision.

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> |

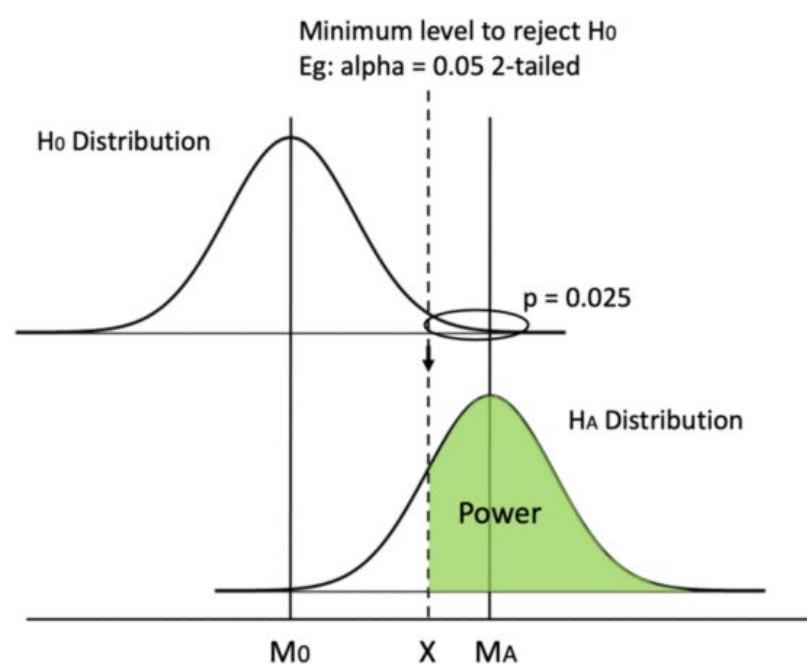
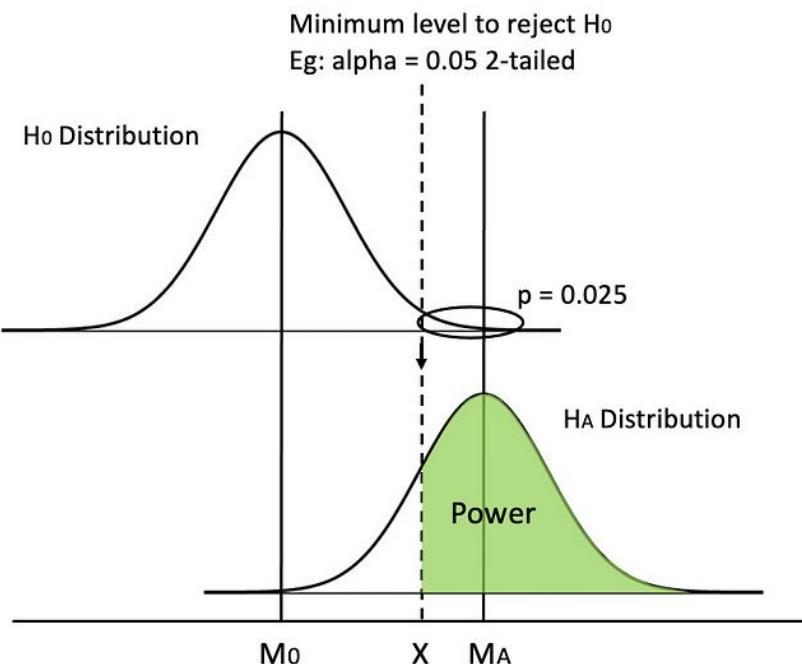
How to increase statistical power

| | 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

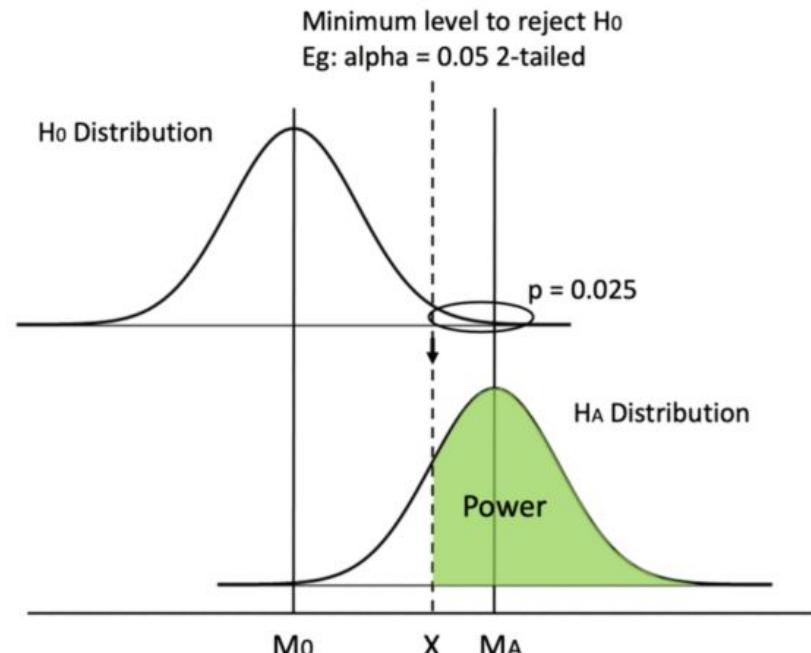
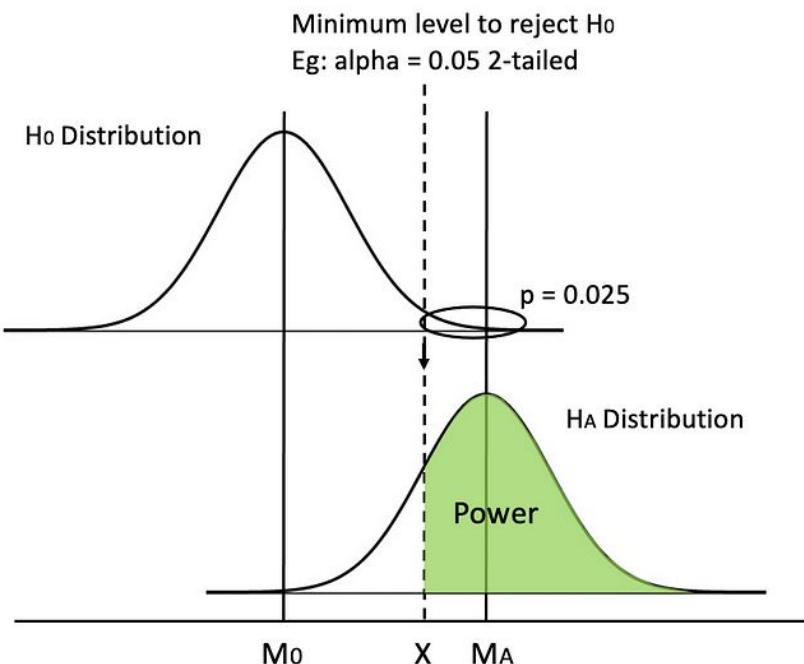
1) Raise significance level alpha (the **WRONG** way)



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

How to increase statistical power

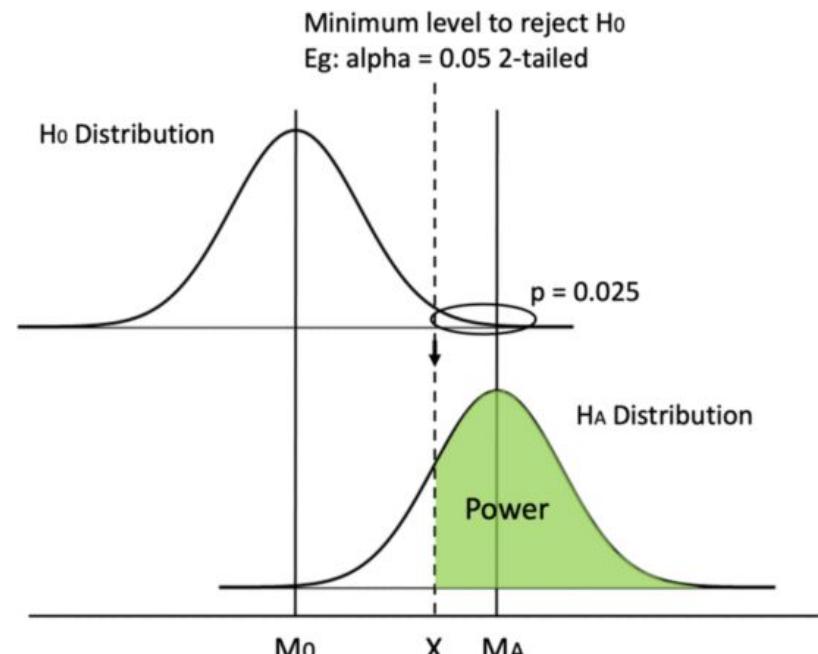
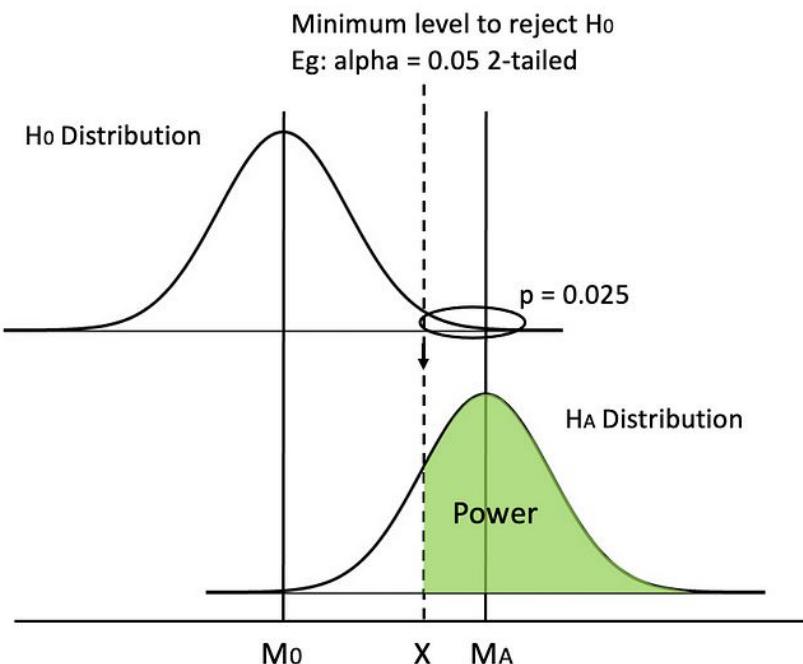
2) Switch from a 2-tailed test to a 1-tailed test (**CORRECT** if possible)



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

How to increase statistical power

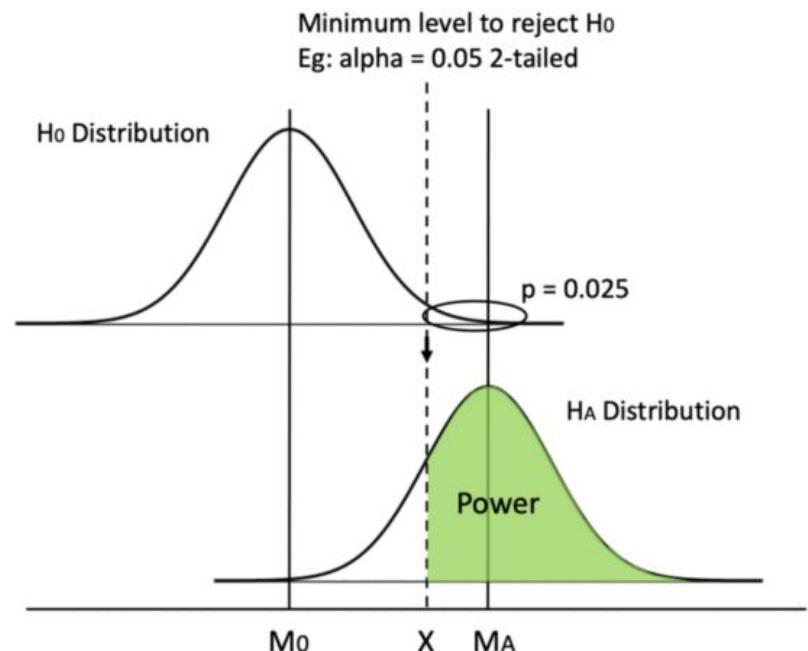
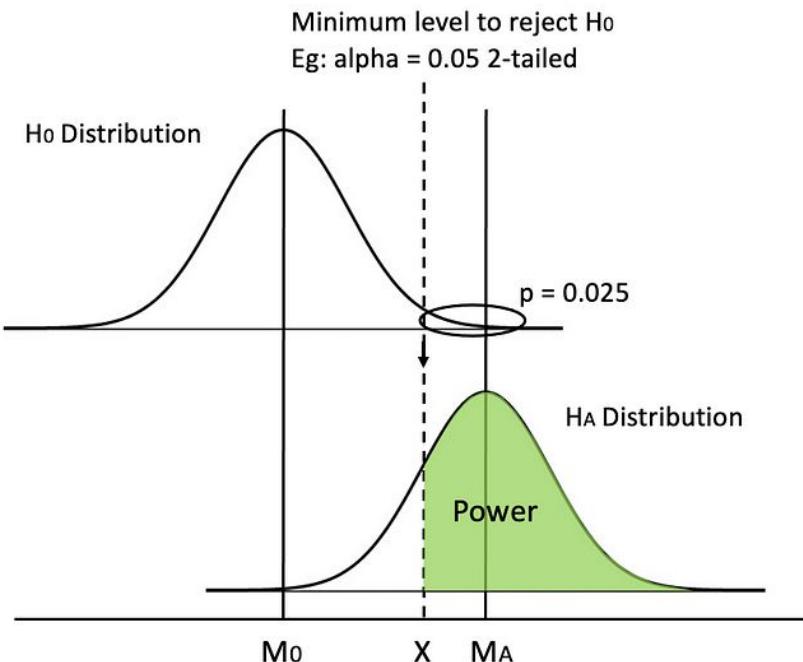
3) Increase mean difference (or increase the effect size)



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

How to increase statistical power

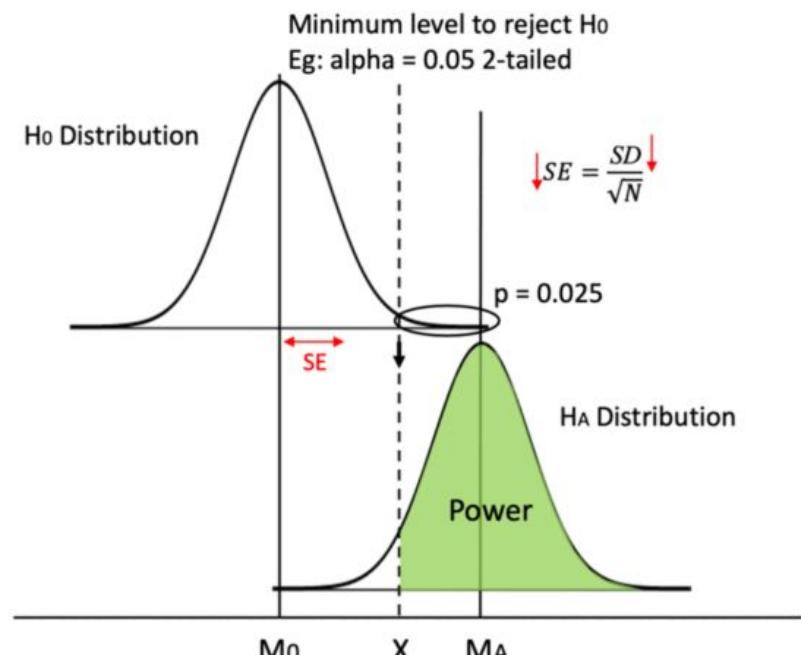
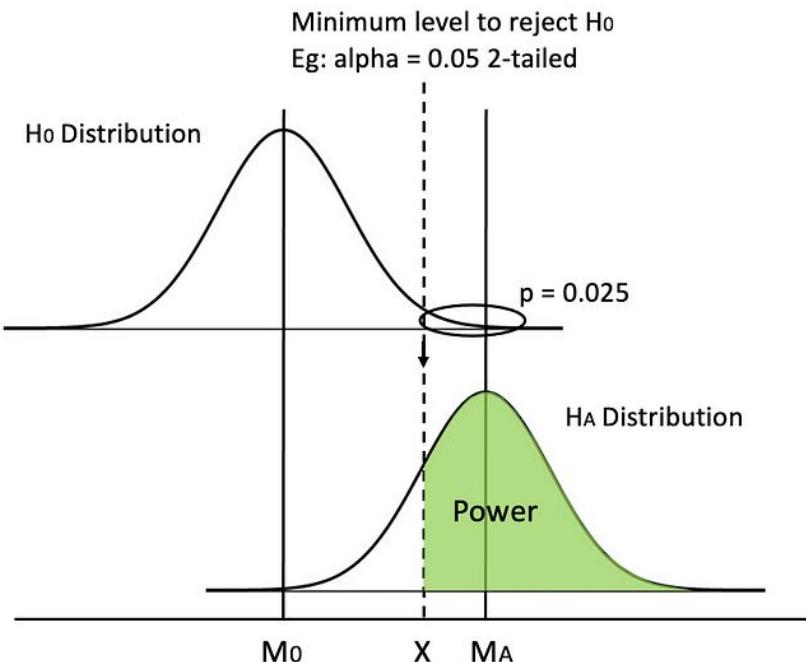
4) Use z distribution instead of t distribution (appropriate when we know the population mean)



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

How to increase statistical power

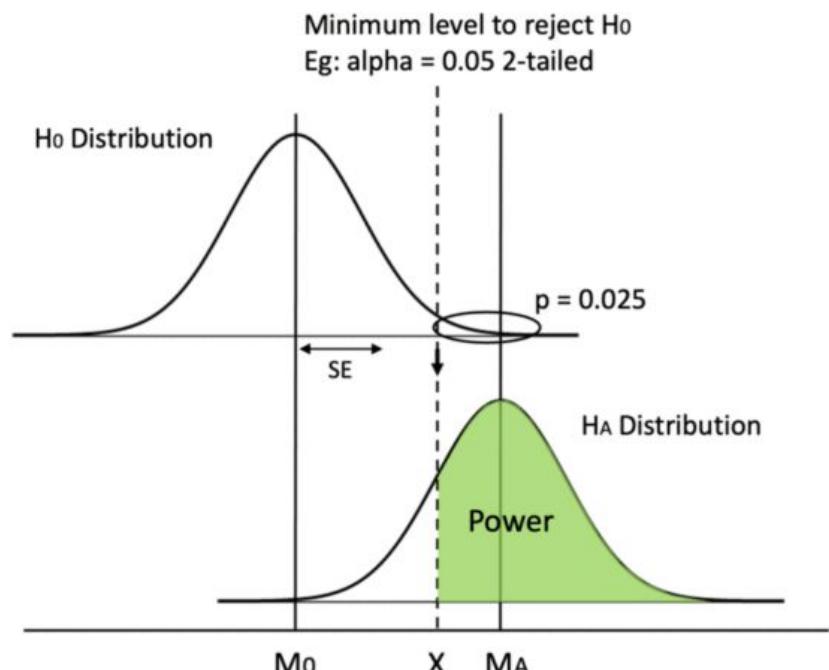
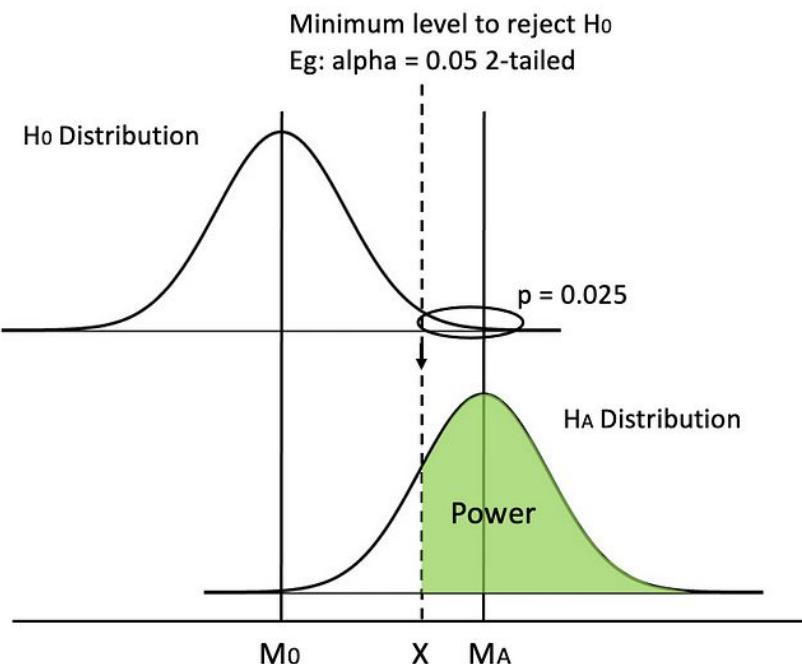
5) Decrease standard deviation (using more precise measurements to have less error and less noise)



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

The **effect size** is an estimate of 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

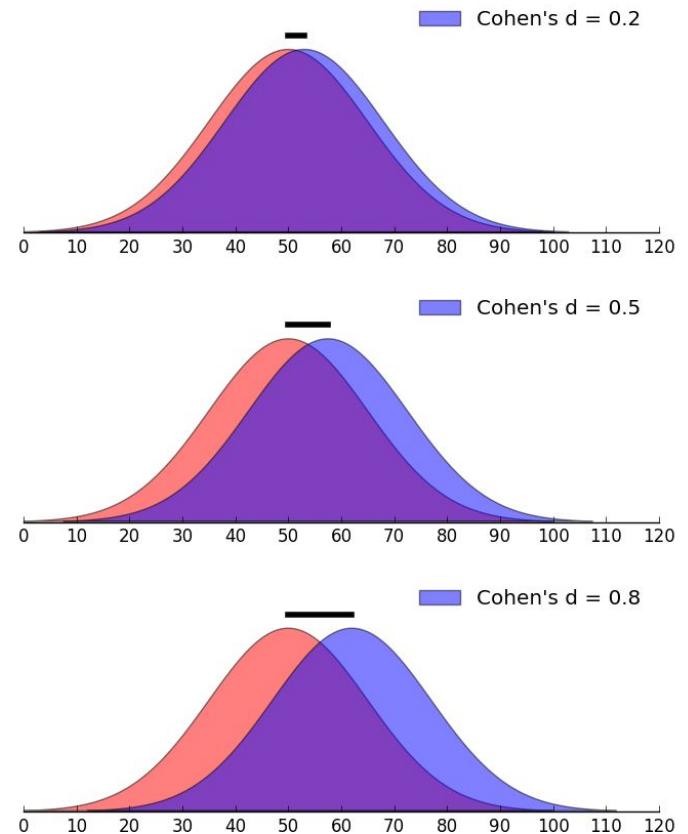
$$Cohen's\ d = \frac{\mu_1 - \mu_2}{\sigma}$$

Mean value of the population 1 Mean value of the population 2
Standard deviation of the population

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\hat{\sigma}}$$

Mean value of sample 1 Mean value of sample 2
Estimated standard deviation of the population from the sample

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



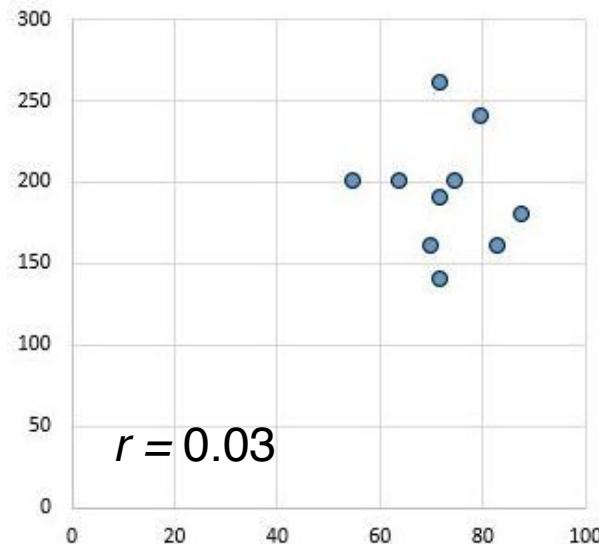
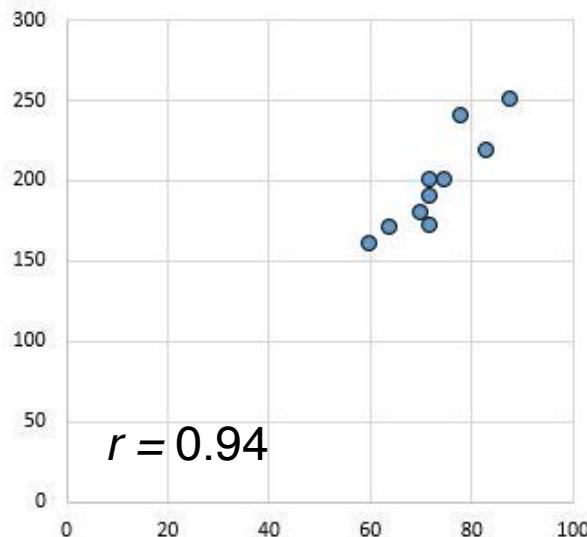
Effect size

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 in different scenarios

| Test | Effect Size | Small | Medium | Large |
|--|--------------------------------|-------|--------|-------|
| All t-tests: • one-sample t-test • independent samples t-test • paired samples t-test | Cohen's d $d =$ | 0.20 | 0.50 | 0.80 |
| Difference between many means (ANOVA) | Cohen's f $f =$ | 0.10 | 0.25 | 0.40 |
| Chi-squared test | Cohen's ω $\omega =$ | 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 |

Source: https://en.wikipedia.org/wiki/Effect_size#Overview