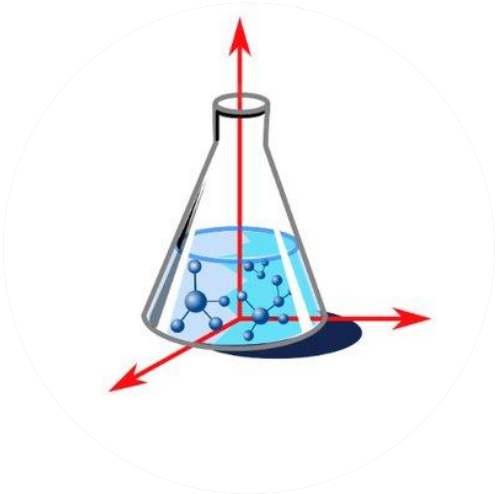
Hands on data: Chemometrics

ANTARES 7th ANNUAL WORKSHOP

27th October, Mercedes Bertotto



WAGENINGEN
UNIVERSITY & RESEARCH

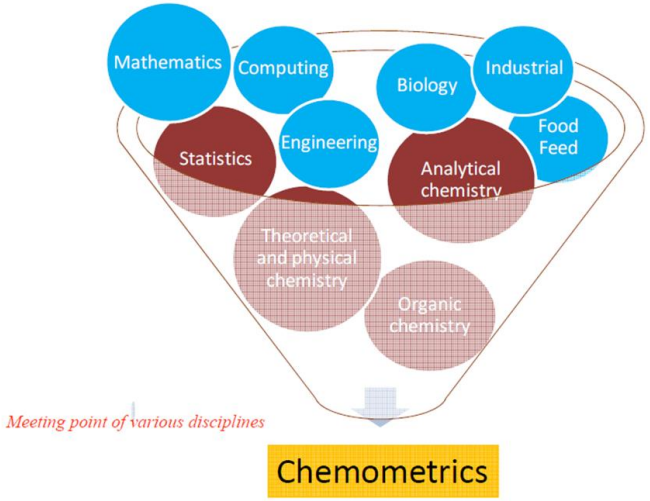
Workshop agenda: Chemometrics

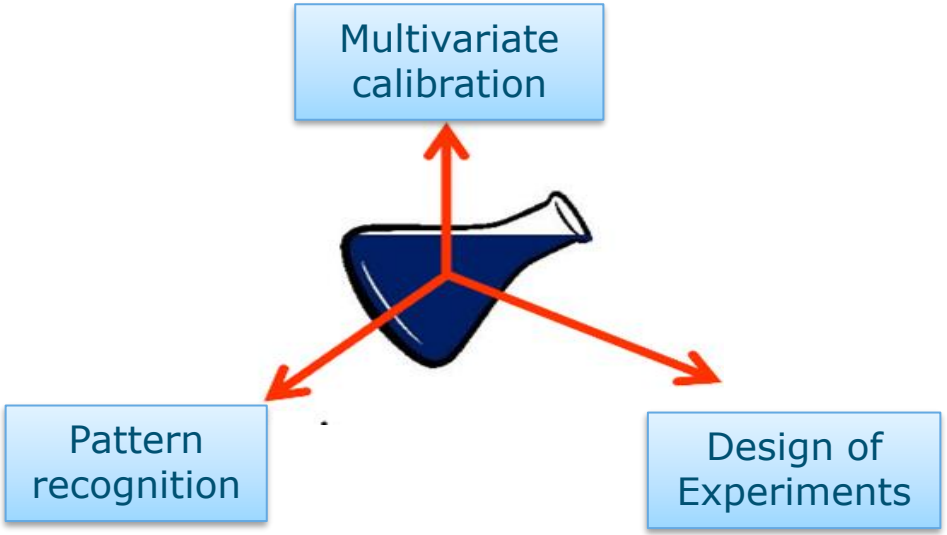
1. Introduction to Chemometrics
2. Exploratory analysis and Outlier Detection
3. Pretreatments on raw spectra
4. Feature selection
5. Cross validation or Data Split
6. Discrimination (PLSDA)
7. Hands on Data (30 min)

Part 1: Introduction

Chemometrics

"Chemometrics is the chemical discipline that uses mathematical, statistical, and other methods employing formal logic to design or select optimal measurement procedures and experiments, and to provide maximum relevant chemical information by analyzing chemical data". **D. L. Massart (1941-2005)**





WAGENINGEN
UNIVERSITY & RESEARCH

Why Chemometrics? Why Linear Algebra?

- NIR spectra are of high dimension
- The data are **highly correlated (collinearity problem)** and obscured by the presence of overlapping absorbances, harmonics, and **combination bands**
- Spectra are often complicated by light scattering and other physical effects
- Multivariate methods (chemometrics) are required to address these issues

- ***"Linear algebra is the language of chemometrics. To understand most chemometric techniques, a basic understanding of linear algebra is required."*** **(Wise and Gallagher, 1998)**

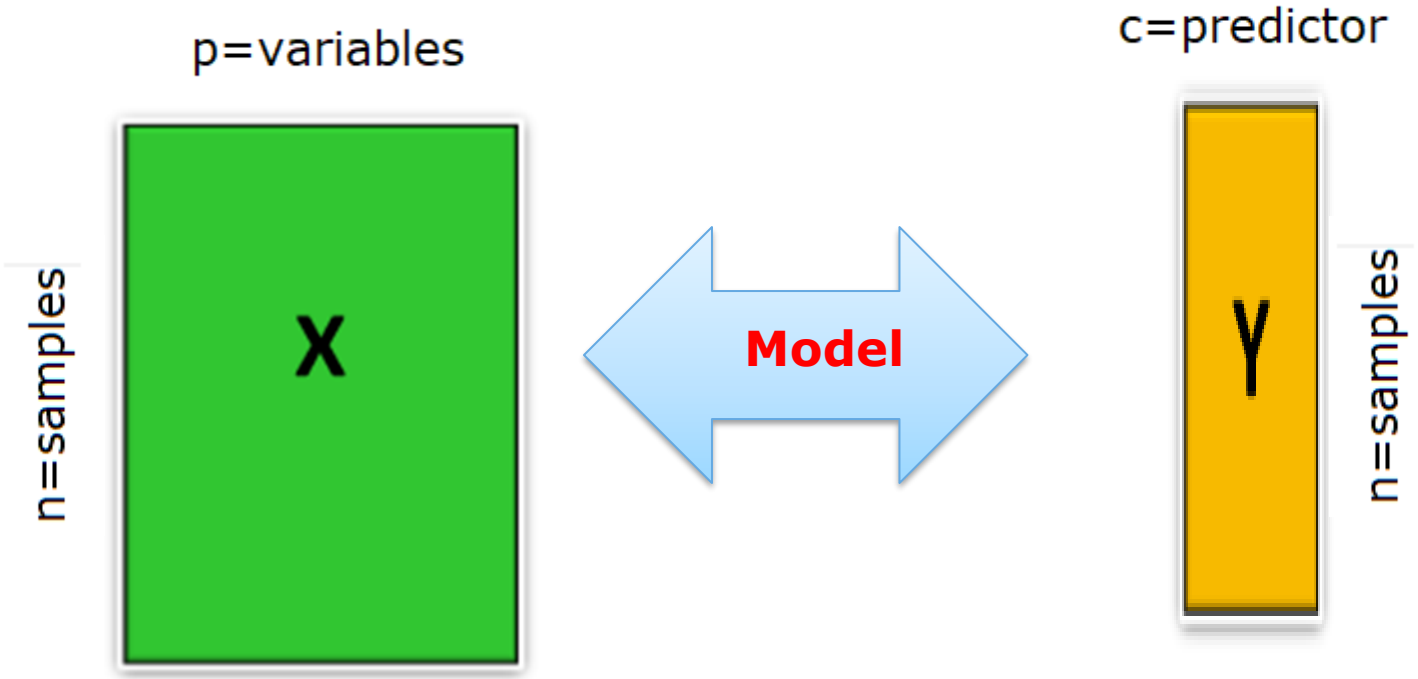
Statistics versus chemometrics

- Focus on distributions
- Inference based on hypothesis tests of parameters
- Density estimation
- Mandatory courses at every biological education

- Focus on individual samples
- Inferences based on future performance
- Clustering
- Voluntary course at only few institutions

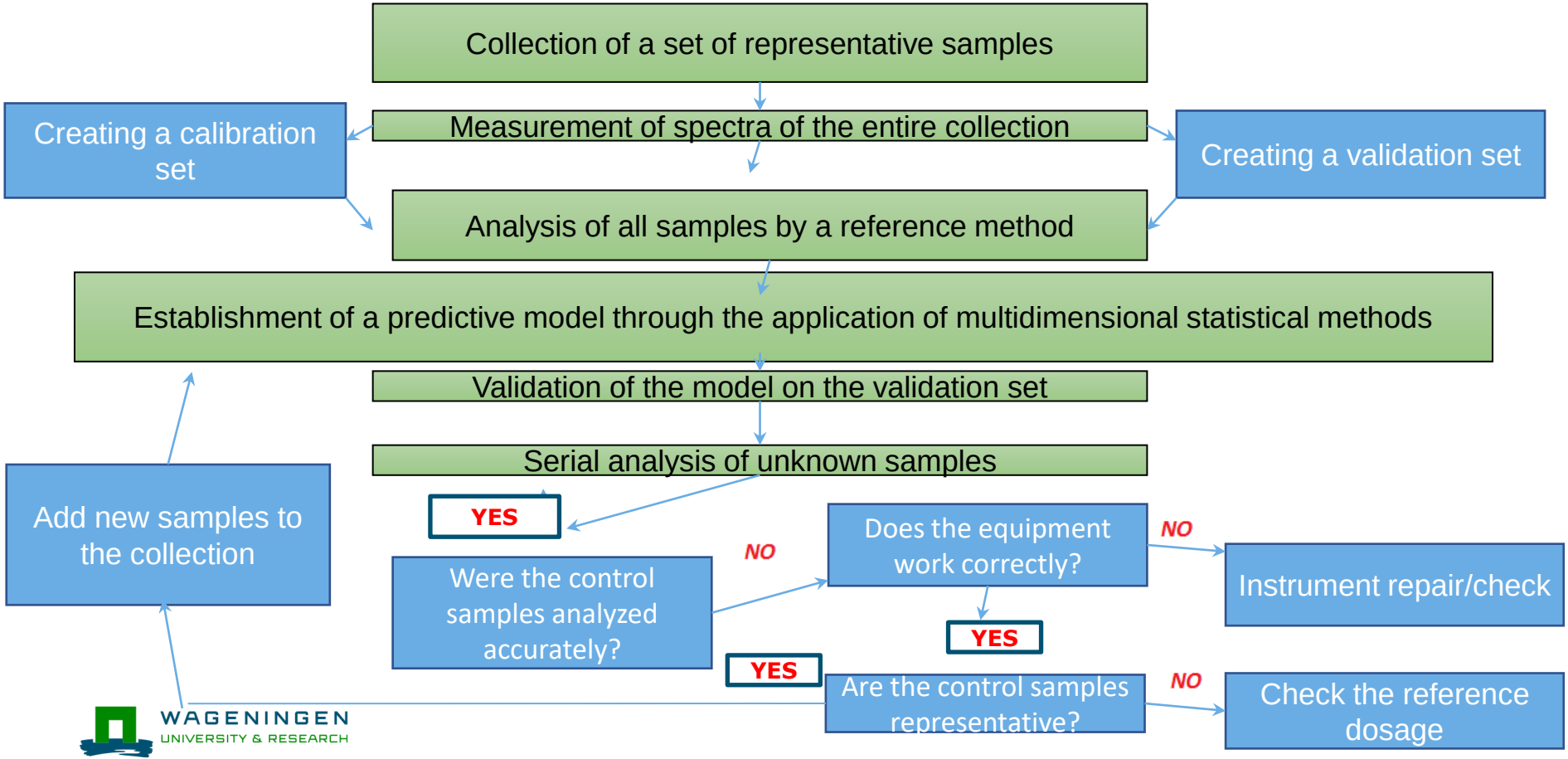
Source: International School of Chemometrics 2023
Morten Arendt Rasmussen

Multivariate analysis



The predictor variables are partially selective of the chemical properties

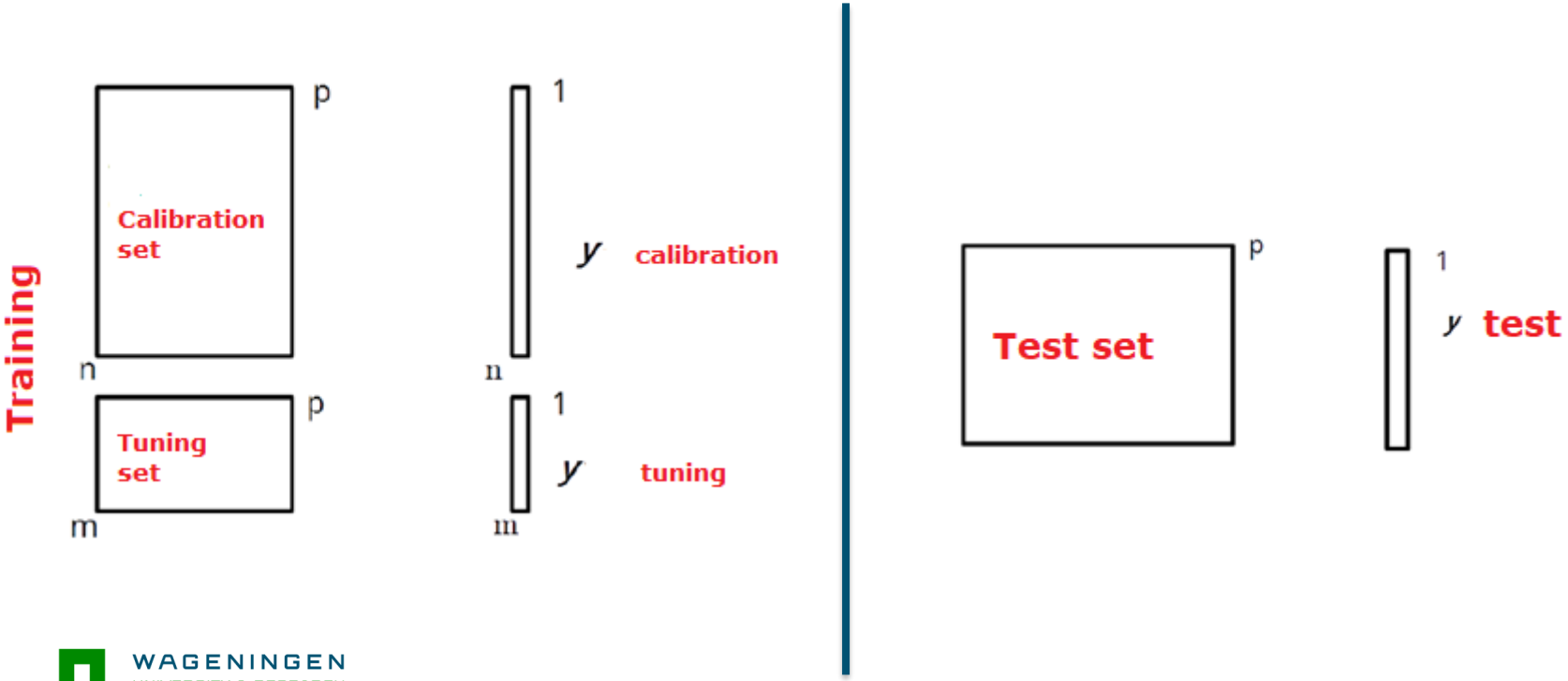
GOOD MODELING PRACTICES (GMP)



WAGENINGEN
UNIVERSITY & RESEARCH

GOOD MODELING PRACTICES (GMP)

Data Set Preparation



Part 2: Exploratory analysis and Outlier Detection

"*Delve deep enough into anything, and you will find mathematics*" -
Dean Schlicter

WAGENINGEN
UNIVERSITY & RESEARCH

Reducing dimensionality

- Identifying and Removing Irrelevant Variables to:

- Enhance Computational Performance
- Improve Model Interpretability and Result Comprehension
- Avoid overfitting and collinearity

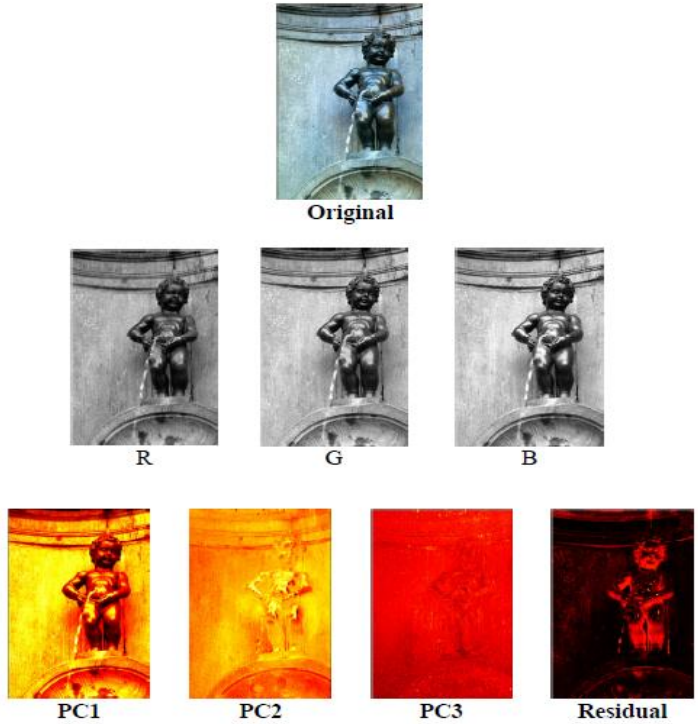
One way to solve the problem: Transforming the initial set of variables into a lower-dimensional set while retaining most of the information

Principal Component Analysis (PCA)

Objectives:

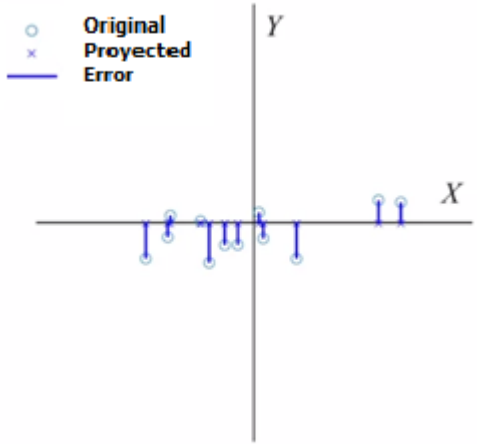
- Exploration/ Description/ **Visualization**
- Dimensionality Reduction
- Preparation and Cleaning (**Outlier Identification**, Noise)
- Discrimination of Individual Groups
- Determination of Relationships Among Individuals
- **Time evolution of scores is used to detect deviations in process monitoring**
- **Preliminary Stage for Further Chemometric Treatment**

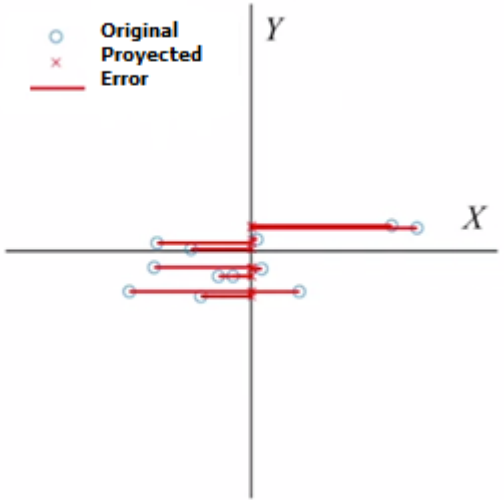
Principal Component Analysis (PCA)

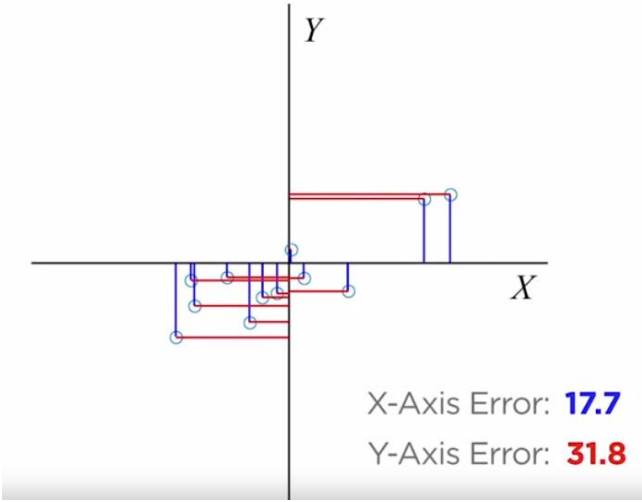


Source: ChemFlow-FUN (France Université Numerique)

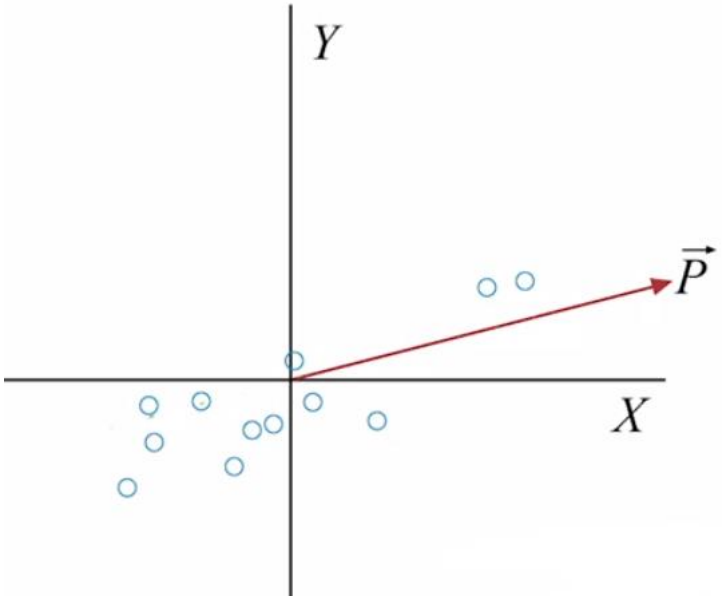
Principal Component Analysis (PCA)

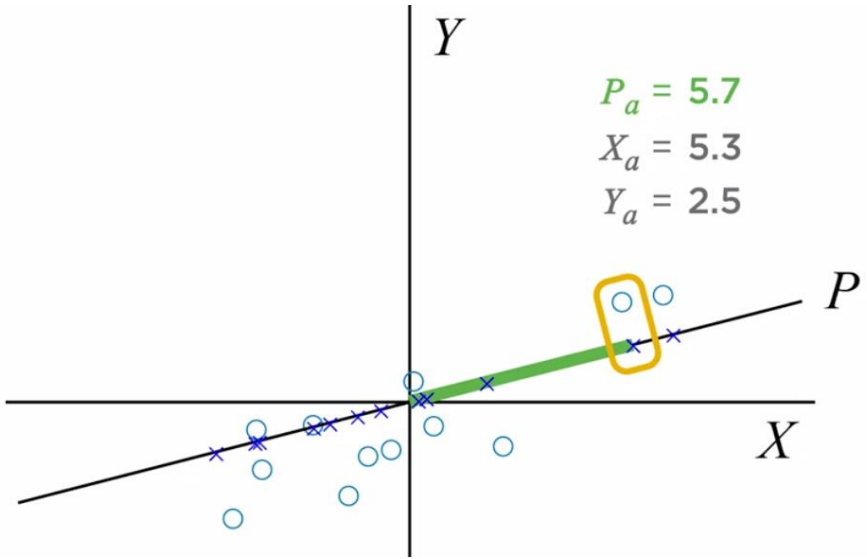




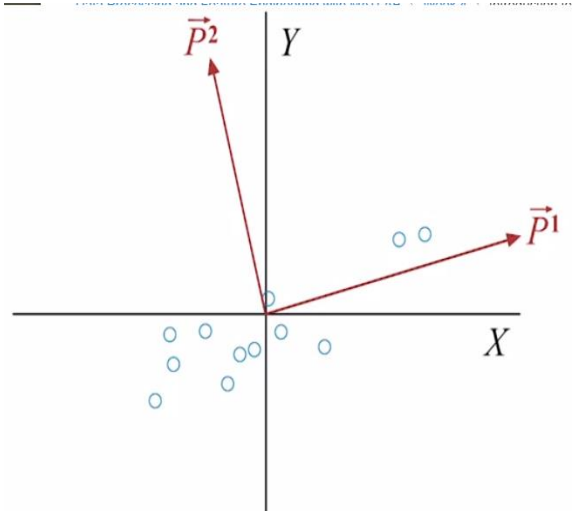


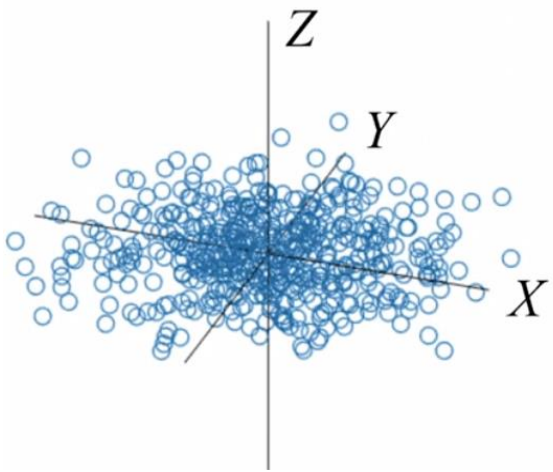
Principal Component Analysis (PCA)

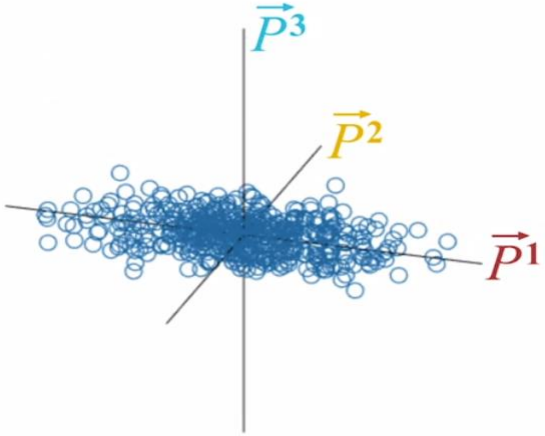




Principal Component Analysis (PCA)







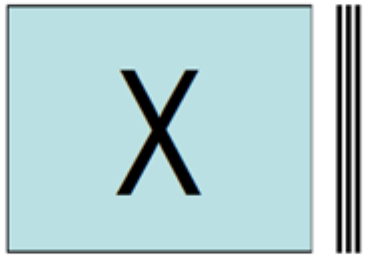
PCA: Matrix decomposition

(N x P)

$$X = T.P' + E$$

(N x A)

T: scores



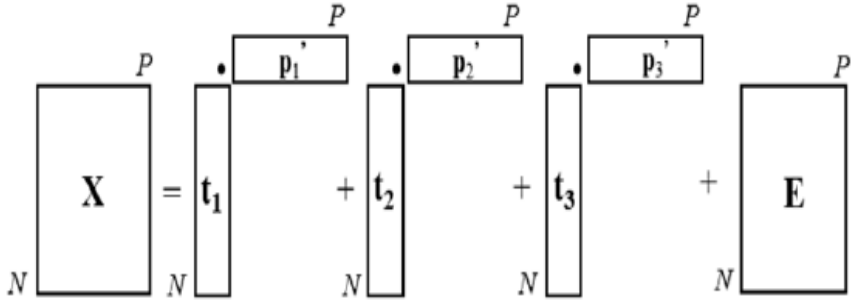
(P x A)

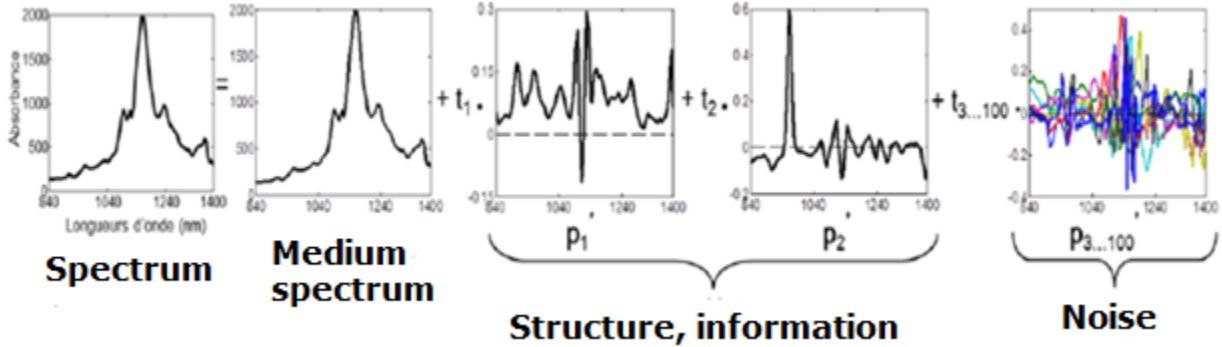
loadings P'

A: Number of PCs

$$X = TP^T + E - t_1 p_1^T + t_2 p_2^T + \ldots + E$$

PCA: Matrix decomposition





Change of basis: Linear transformation

$$\begin{bmatrix} 2 & -1 \\ 1 & 1 \end{bmatrix}$$

Vector in our "language"

$$\begin{bmatrix} -1 \\ 2 \end{bmatrix}$$

Transformation

$$\begin{bmatrix} ? \\ ? \end{bmatrix} = -1\begin{bmatrix} 2 \\ 1 \end{bmatrix} + 2\begin{bmatrix} -1 \\ 1 \end{bmatrix} = \begin{bmatrix} -4 \\ 1 \end{bmatrix}$$

Vector in another "language"

Eigenvalues and eigenvectors

- An eigenvalue of a matrix is a scalar that represents how a particular transformation, described by that matrix, stretches or compresses a vector in space
- Eigenvalues quantify how much the direction of a vector changes when it is multiplied by a matrix
- if A is a square matrix, and λ is a scalar, then λ is an eigenvalue of A if there exists a non-zero vector v (called an eigenvector) such that the following equation holds:

$$A * v = \lambda * v$$

The value λ is the eigenvalue associated with the eigenvector v

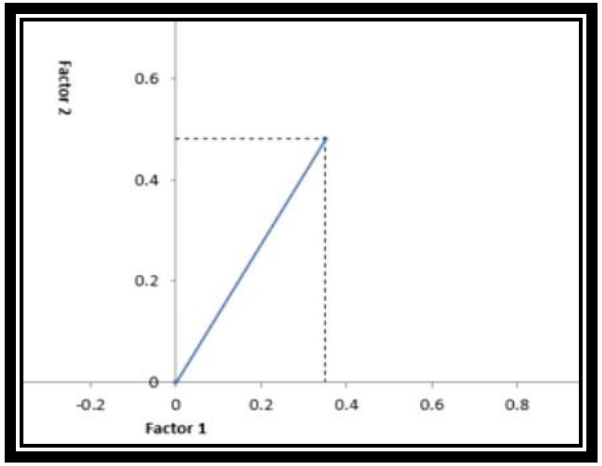
PCA explains the original variables through the strength of their relationship with the factors

Eigenvalues	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
	4.349726834	1.92281186	1.290080831	1.149885024	1.129518656	1.014206025	0.977356029	0.9318098

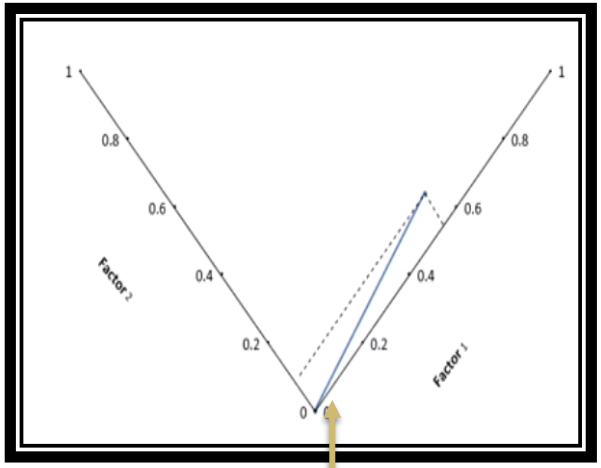
- ✓The factors are extracted from the data **sequentially**
- ✓The factor that explains the highest % of variance is extracted first
- ✓Then, the factor that explains the second highest % of variance is extracted, and so on
- ✓The factors are **orthogonal** to each other
- ✓They have zero correlation between them, each representing something **unique**

Factor rotation

Ideal



Real



The variables that are weakly related to the component are located near the center of the graph

Loadings

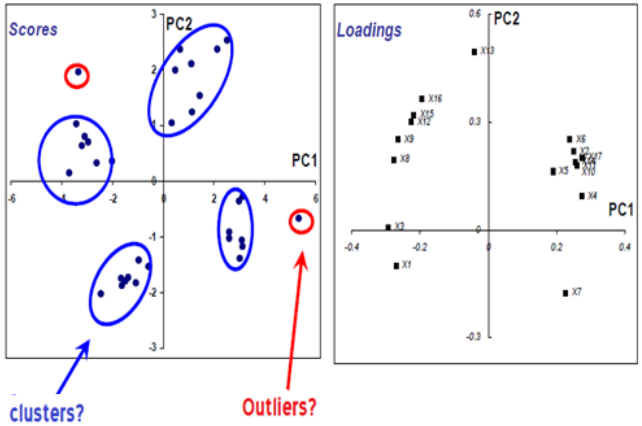
Correlation coefficients
-1.0 : Perfect negative correlation
0.0: No correlation
1.0: Perfect positive correlation

In yellow: Loadings>0.5

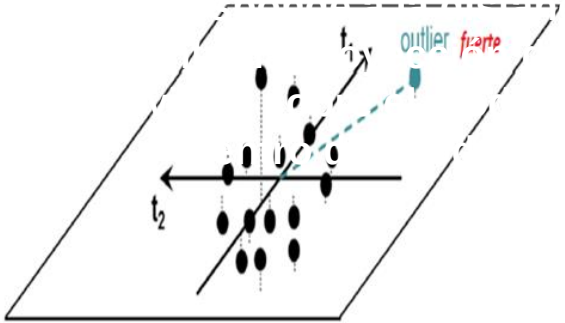
The variables that load strongly on the same factor may share something in common. For example, products C, D, J, and U load strongly on the first factor. In this example, they are antibiotics

	Factor 1	Factor 2	Factor 3
Product A	0.338392	0.359125	-0.35551
Product B	-0.03337	0.359674	-0.44017
Product C	0.709268	0.313117	-0.10372
Product D	0.866839	0.026961	-0.09743
Product E	0.037134	-0.10703	-0.40144
Product F	0.140315	0.202643	-0.10759
Product G	0.118608	0.235802	-0.35763
Product H	0.029118	0.683191	0.2143
Product I	0.027812	0.522297	-0.23995
Product J	0.915644	-0.03241	-0.04724
Product K	-0.01446	0.280687	0.000529
Product L	0.138604	0.709171	-0.05589
Product M	0.017498	0.246222	-0.16969
Product N	0.329753	0.132799	-0.40502
Product O	0.136257	0.141867	-0.5055
Product P	0.189386	0.041223	-0.52597
Product Q	0.298836	0.418047	-0.32678
Product R	0.112088	0.08413	-0.55576
Product S	0.001687	0.16686	-0.55969
Product T	0.024188	0.358303	-0.17977
Product U	0.715734	-0.03241	-0.23856

Outlier detection

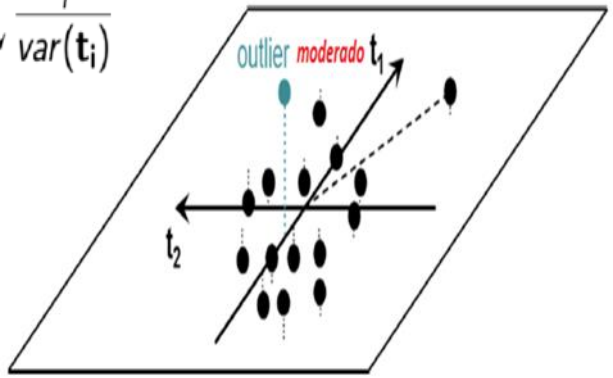


Hotelling or T2



$$T^2 = \sum_{i=1}^{A} \frac{t_i^2}{var(t_i)}$$

Q or residual



$$Q_i = e_i e_i^T = x_i (I - P_k P_k^T) x_i^T$$

*"The **test hypotheses** are:*
- ***Null hypothesis*** *(H_0): the two samples are from populations with the same multivariate mean*
- ***Alternate hypothesis*** *(H_1): the two samples are from populations with different multivariate means"*

https://www.statisticshowto.com/hotellings-t-squared/

Isolation Forest

- We pick a column at random, and we select an arbitrary threshold within its range
- We divide all the samples into two groups according to that threshold
- There is a higher chance that the outlier point would end up in the smaller group or alone
- This randomized splitting process is repeated recursively
- The more common the point is, the more splits it will take to be isolated

Isolation depth: Number of partitions that it takes to isolate a point

One tree has a lot of variability → A final score is obtained by averaging results of many trees

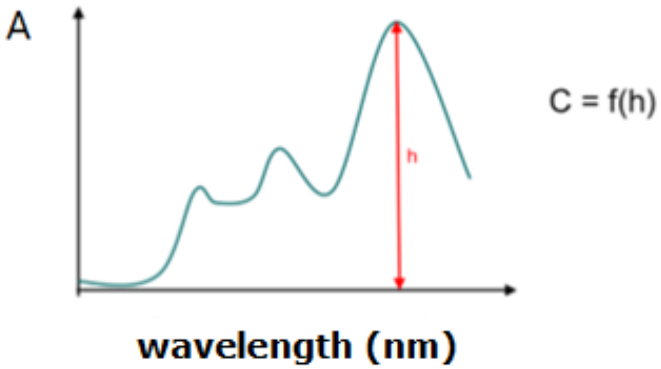
Part 3: Pretreatments on Raw Spectra

Near Infrared Spectroscopy

Ideal conditions

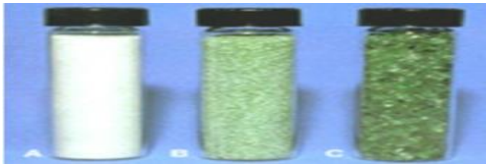
$$\mathcal{A}(\lambda) = \varepsilon(\lambda)CL$$

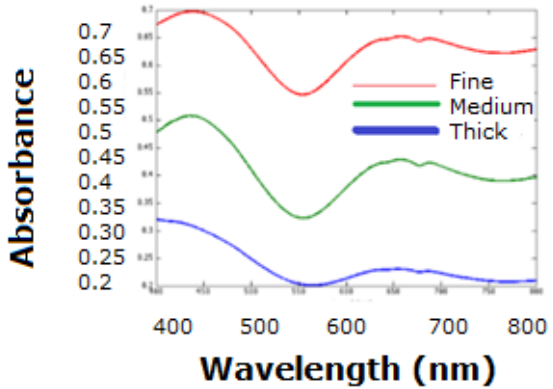
Lambert-Beer Law



C = f(h)

Real conditions





Pretreatments eliminate spectral deformations to approach the pure contribution of the studied parameter

Multiplicative and additive effects

Real spectrum may be influenced by:

Photon diffusion

1) Enlarges the medium length of the optical path by factor **k**

2) Will cause a certain number of photons to escape from the captor, creating a leak term

Measurement noise

It is due to a set of random phenomena present throughout the measurement chain

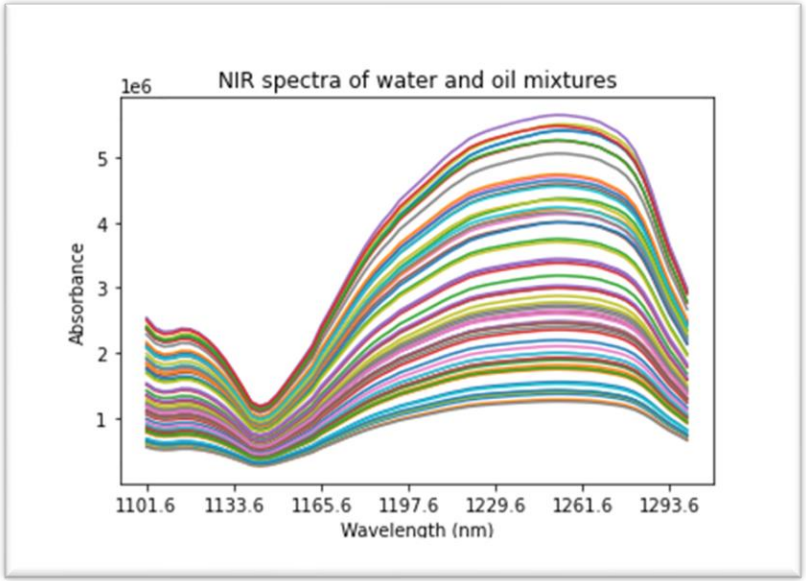
Addiive effect

$$\mathcal{A}(\lambda) = k\varepsilon(\lambda)LC + \mathcal{A}_f(\lambda) + \mathcal{A}_b(\lambda)$$

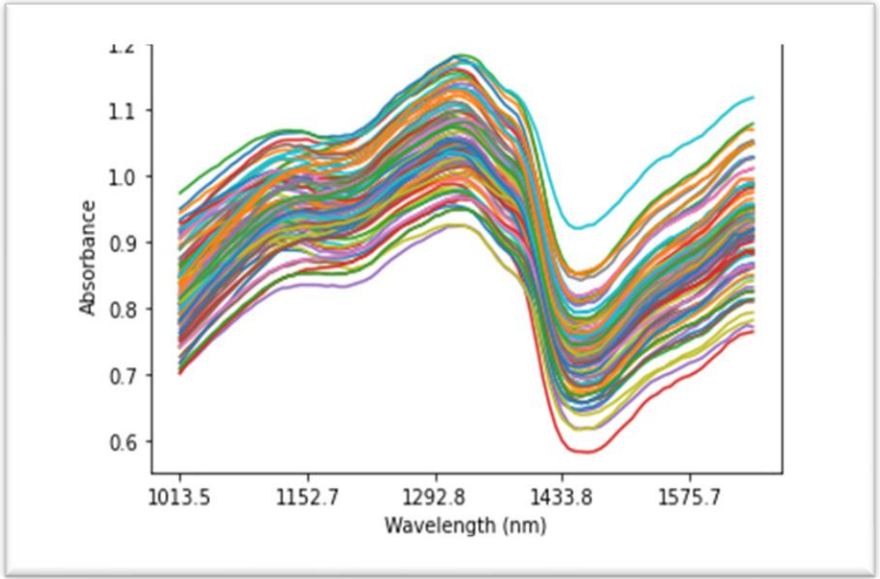
WAGENINGEN
UNIVERSITY & RESEARCH

How to distinguish additive and multiplicative effects

NIR spectra of water and oil mixtures showing a multiplicative effect. Source: WUR.

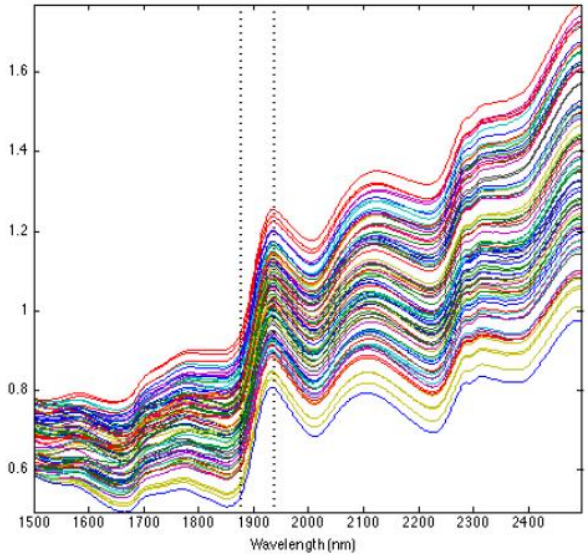


NIR spectra showing an additive effect. Source: WUR.

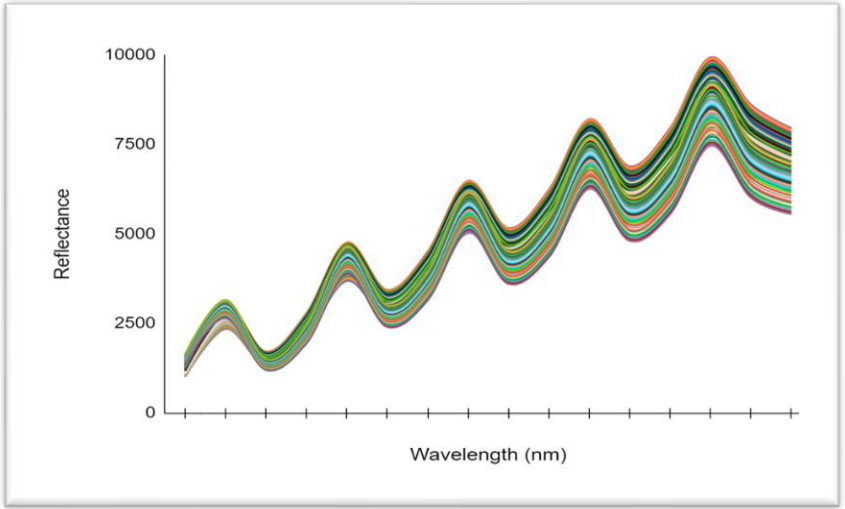


How to distinguish additive and multiplicative effects

(Fictional) example of an additive effect of a rising baseline. Source: IRSTEA Montpellier

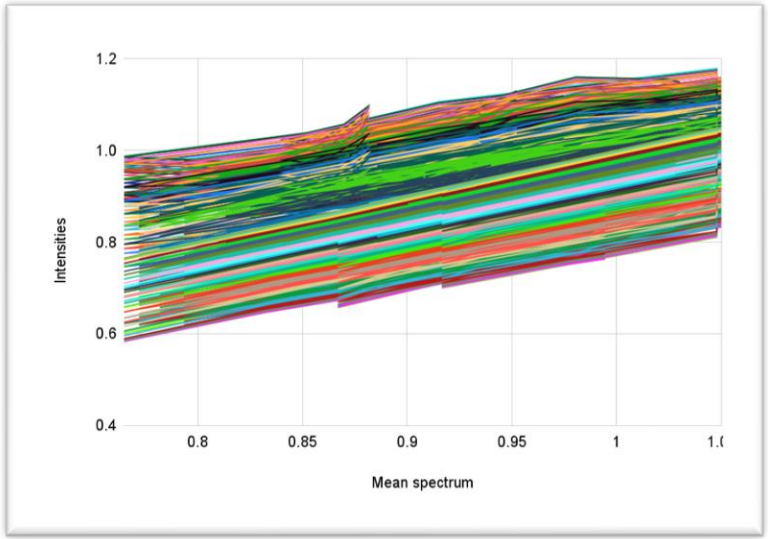


Handmade example of NIR spectra with combined effect (additive and multiplicative)
Source: WUR

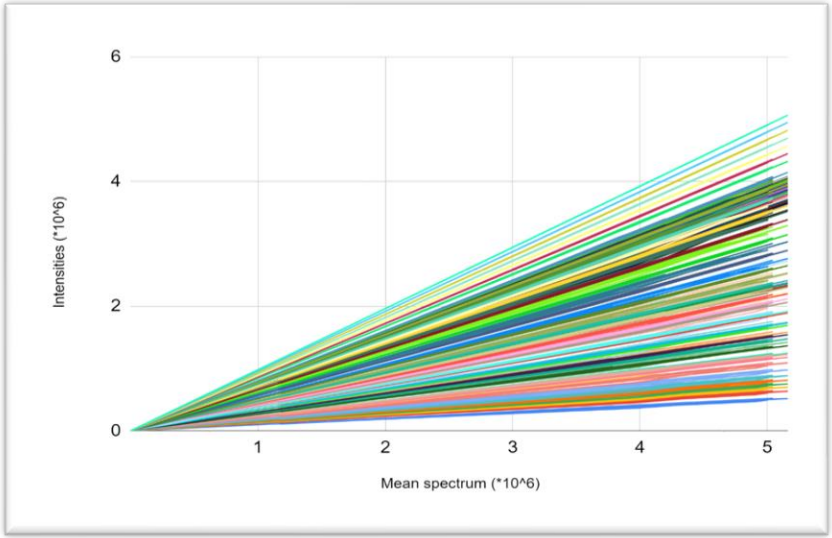


How to distinguish additive and multiplicative effects

A millefeuille shape observed when plotting NIR spectra with additive effect versus the mean spectrum. Source: WUR.

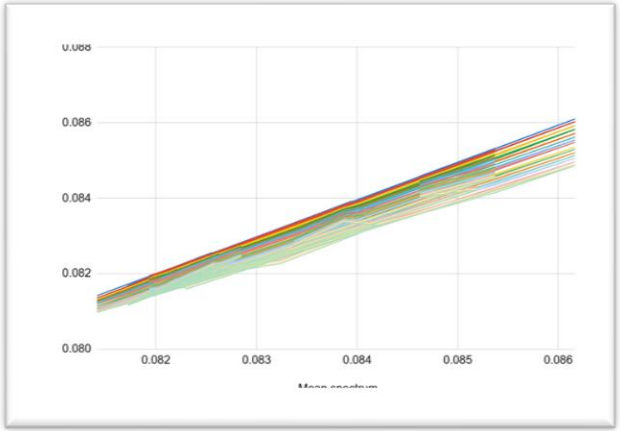


A cone shape observed when plotting spectra with multiplicative versus their mean spectrum. Source: from author

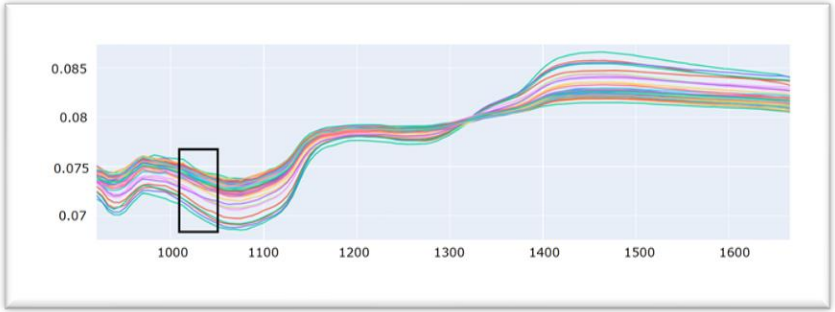


How to distinguish additive and multiplicative effects

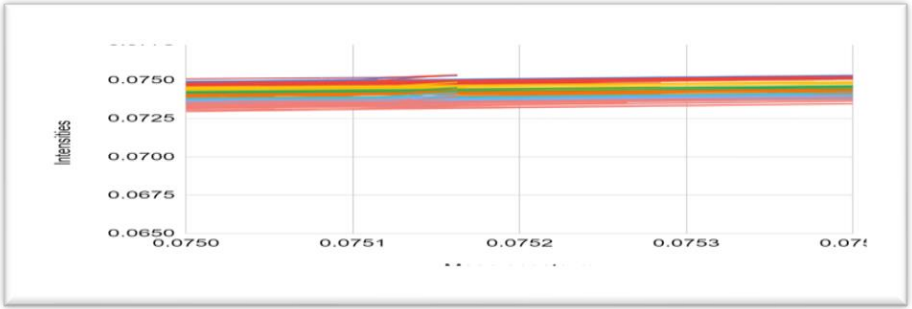
No clear cone neither millefeuille shape shown; difficult to tell if there is additive or multiplicative effect present in spectra. Source: WUR



Intensities from the black rectangle can be plotted versus their mean spectrum, to understand the type of effect present. Source: WUR



This shows an additive effect in that spectral range. Source: WUR.

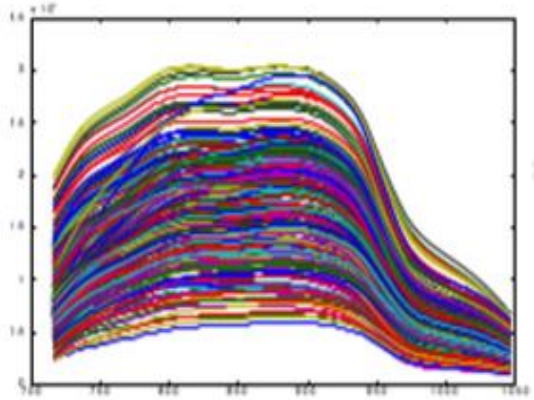


WAGENINGEN
UNIVERSITY & RESEARCH

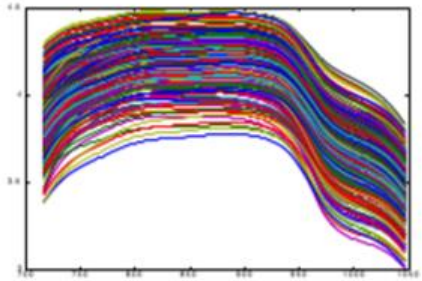
How to reduce a multiplicative effect (*k*)?

$$log(ab) = log(a) + log(b).$$

$$(kx) \quad (log(k)\mathbf{1} + log(\mathbf{x})).$$

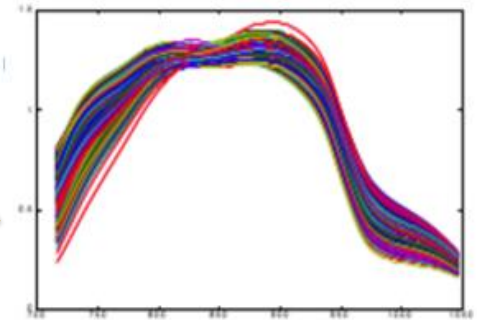


Logarithm



+ Detrend

Normalization



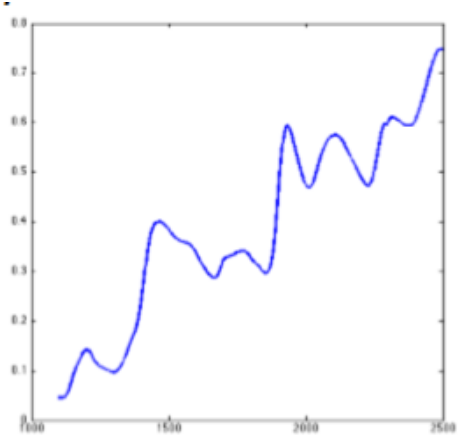
Source: ChemFlow-FUN (France Université Numerique)

To remove noise

$A_f(\lambda)$

Detrend

Spectrum of Wheat. Source: IRSTEA Montpellier



Delete the global tendency from the spectrum, modeled by a polynomium

Order 0
Average

Orden 1
Line

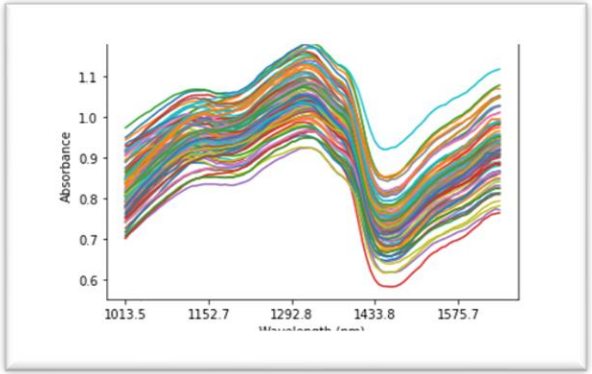
Orden 2
Parabola

The residuals that remain after removing the line correspond to the absorbance peaks related to the chemical components of the sample

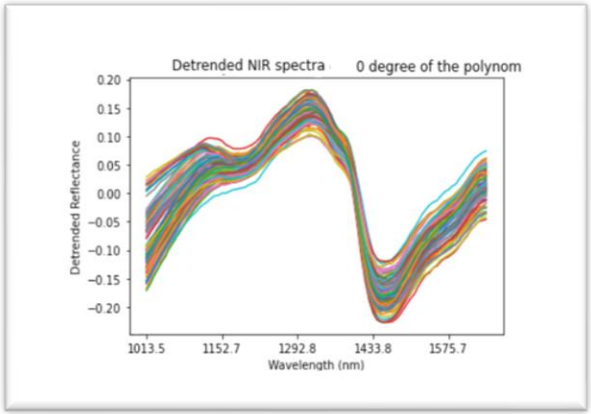
Source: ChemFlow-FUN (France Université Numerique)

Detrend

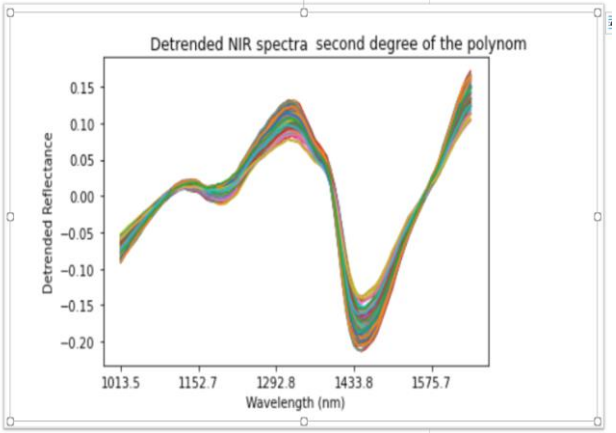
Raw spectra



Detrend order 0



Detrend order 2



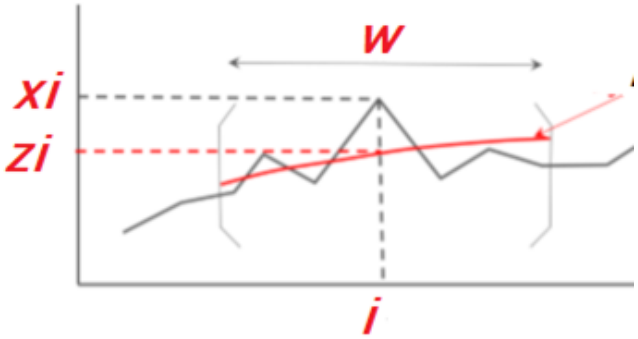
Source: ChemFlow-FUN (France Université Numerique)

To remove noise

$A_b(\lambda)$

polynomial of degree **d**

Savitsky Golay



w(odd)>d

At each point i in the spectrum, the raw value **xi** is replaced by **zi** from a polynomial fitted over a window around point **i.**

Source: ChemFlow-FUN (France Université Numerique)

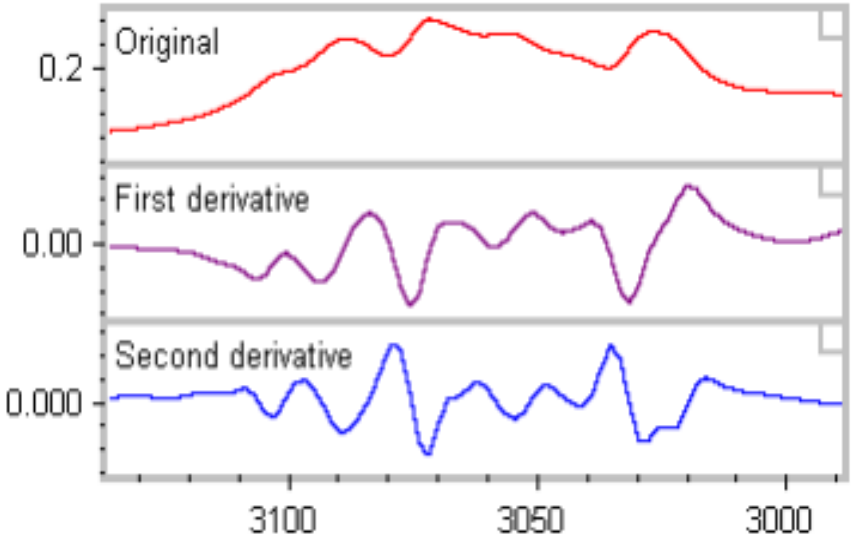
Derivatives

- They are also used to decrease the baseline. If the baselines are polynomials of degree **K**, then the derivatives of order **k+1** will make them disappear

$$\mathcal{A}_f(\lambda) = a\lambda + b$$

$$\mathcal{A}(\lambda) = k\varepsilon(\lambda)LC + a\lambda + b$$

$$\frac{\partial^2 \mathcal{A}(\lambda)}{\partial \lambda^2} = k\frac{\partial^2 \varepsilon(\lambda)}{\partial \lambda^2}LC$$

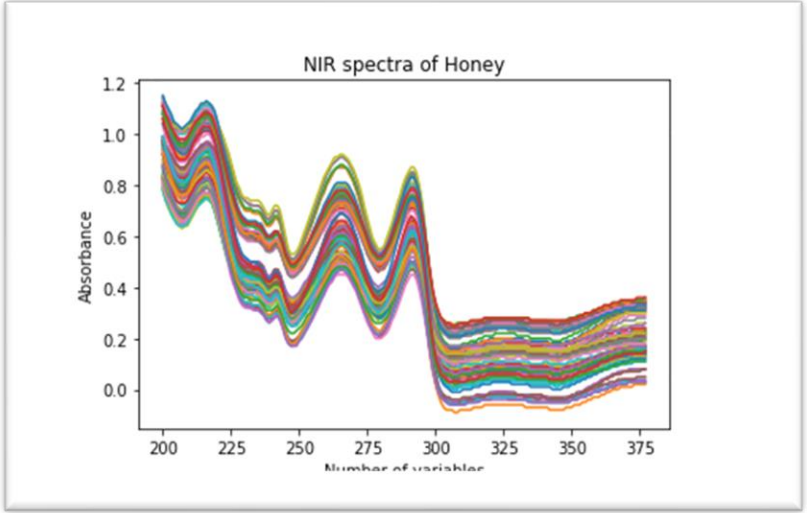


Standard Normal Variate (SNV)

The SNV pre-processing of a spectrum X consists of removing the mean of X at each of its points, then dividing them by the standard deviation of X.

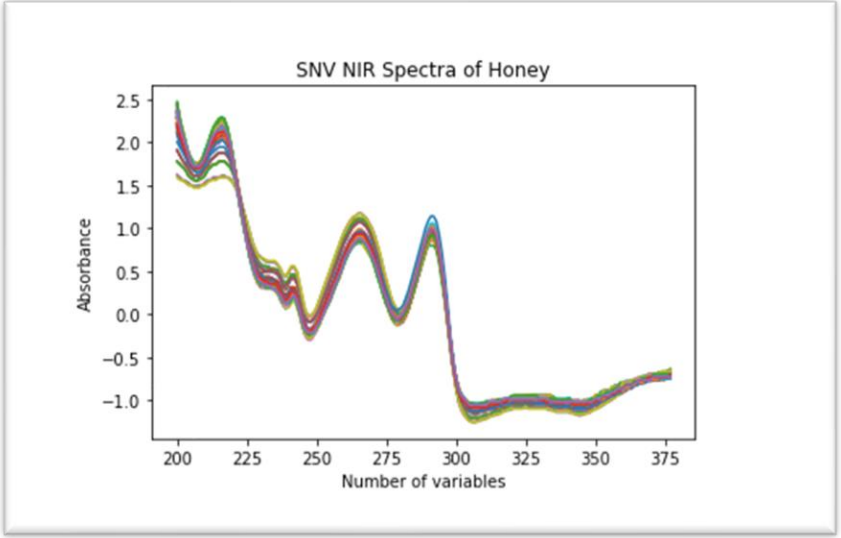
$$Xc = X - mean$$
$$XSNV = Xc / SD(Xc)$$

NIR spectra of honey, with both additive and multiplicative effects. Source: SENASA (Argentina)



SNV NIR spectra of honey. Additive and multiplicative effects are gone.

Source: SENASA (Argentina)



Multiplicative Scatter Correction (MSC)

- *"Mathematically, if we call Xm the mean spectrum, the multiplicative scatter correction is done in two steps.*

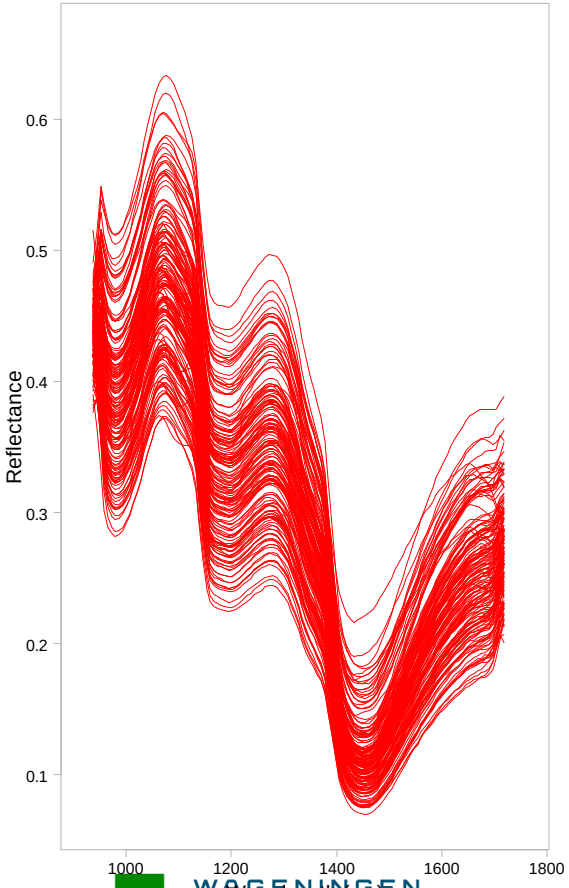
1. *We first regress each spectrum Xi against the mean spectrum*

   This is done by ordinary least squares: Xi≈ai+biXm .

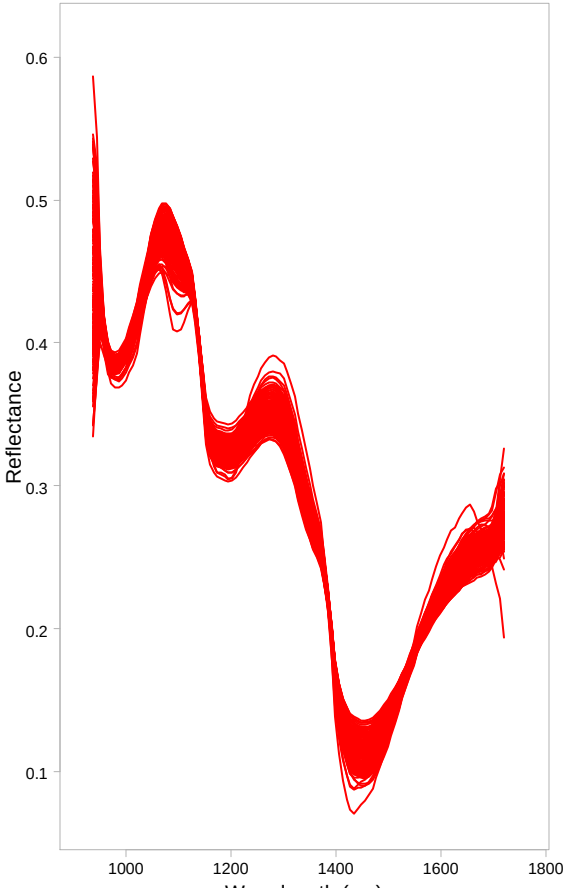
1. *We calculate the corrected spectrum msc=()/Ximsc=(Xi−ai)/bi"*

Source:https://nirpyresearch.com/two-scatter-correction-techniques-nir-spectroscopy-python/

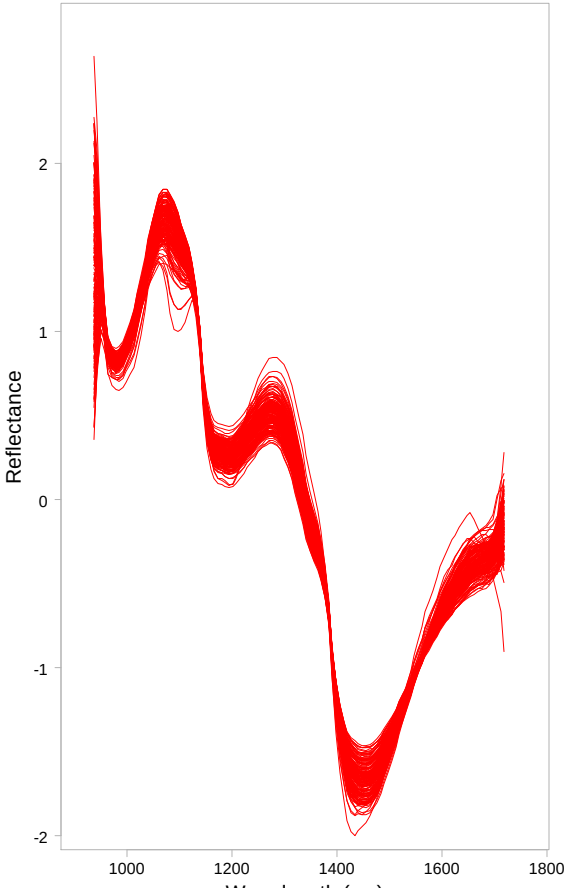
Before MSC



After MSC

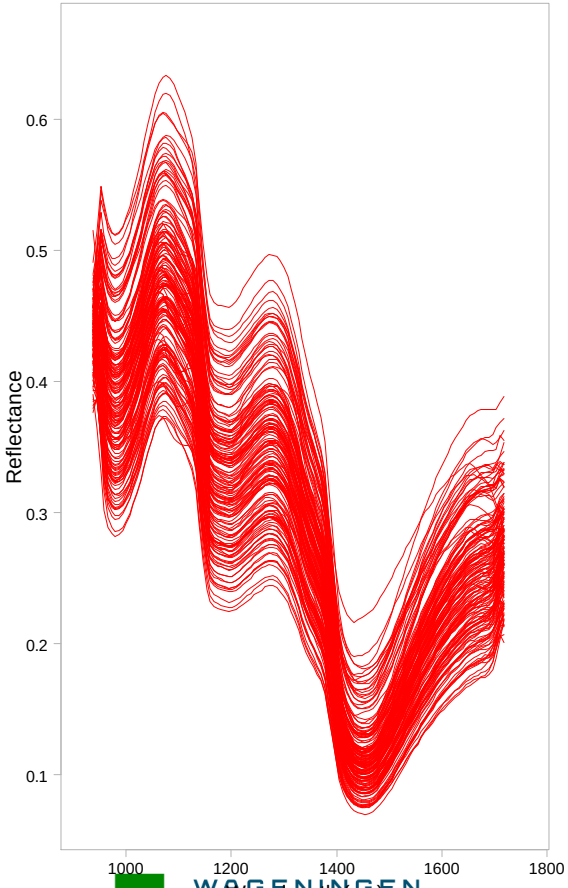


After SNV

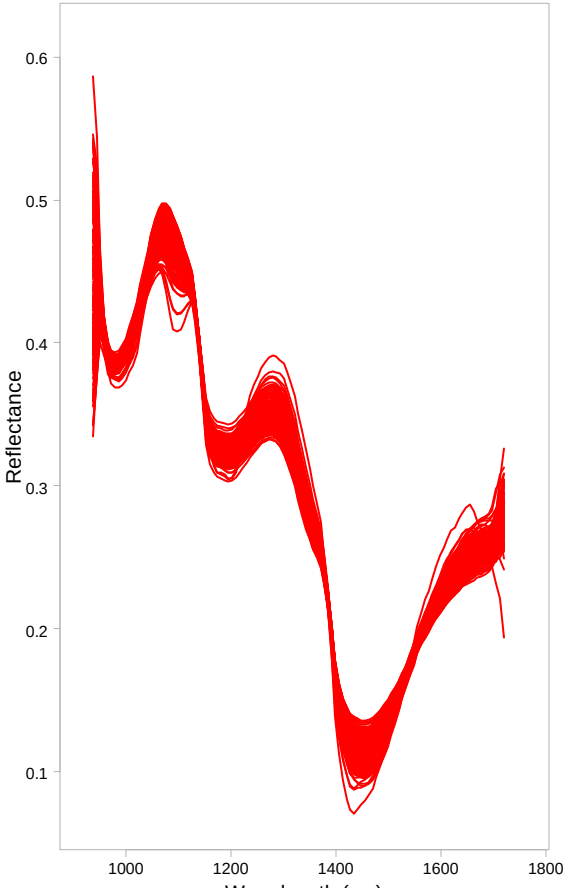


WAGENINGEN
UNIVERSITY & RESEARCH

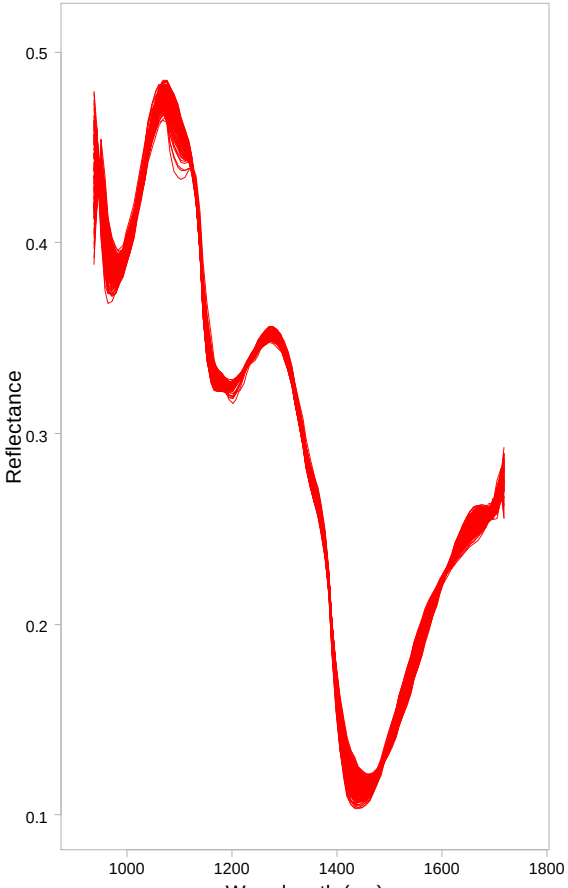
Before MSC



After MSC

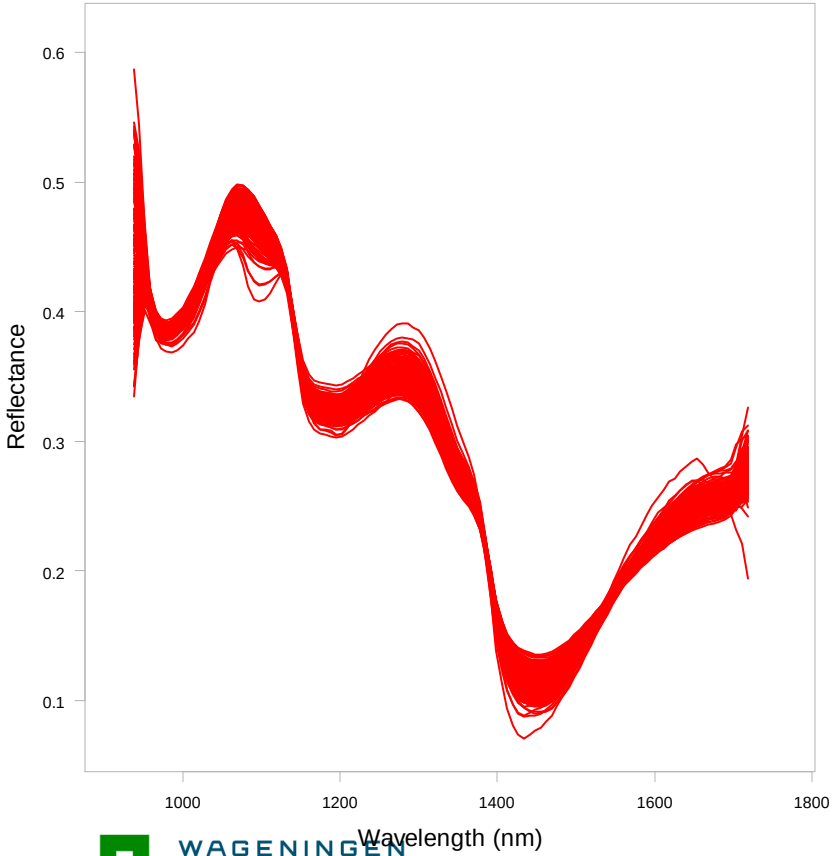


After EMSC

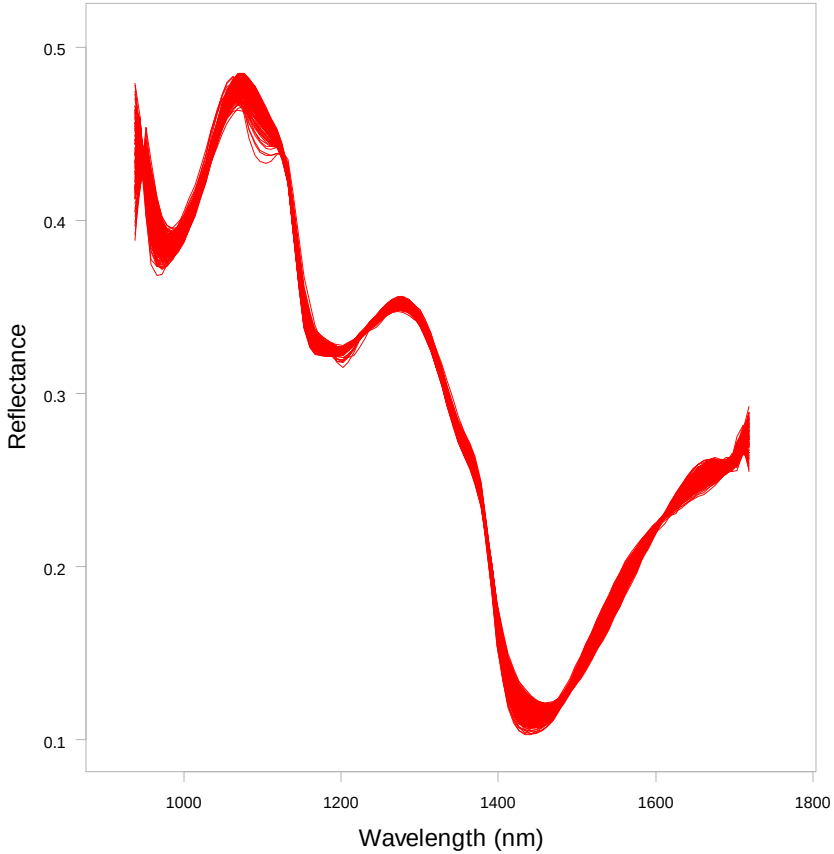


WAGENINGEN
UNIVERSITY & RESEARCH

After MSC

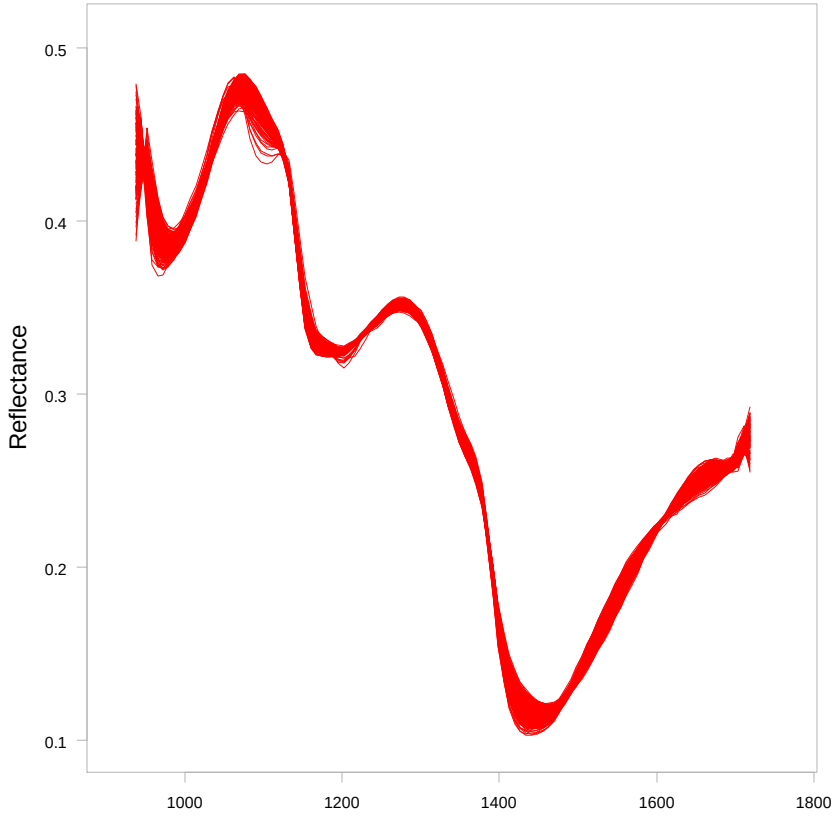


After EMSC, degree=6

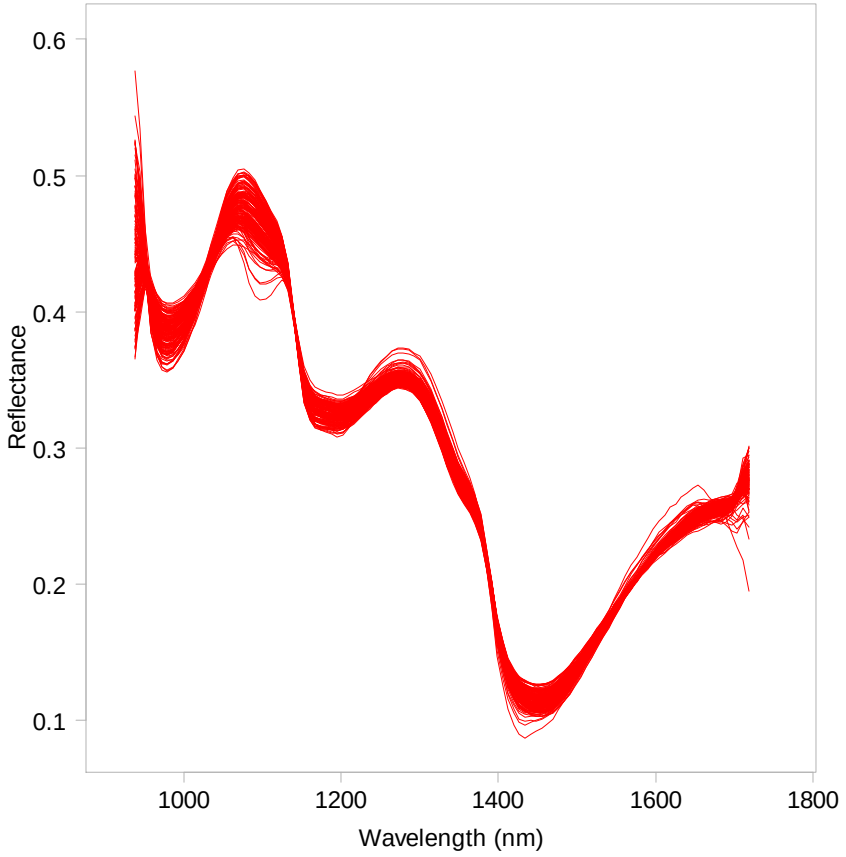


WAGENINGEN
UNIVERSITY & RESEARCH

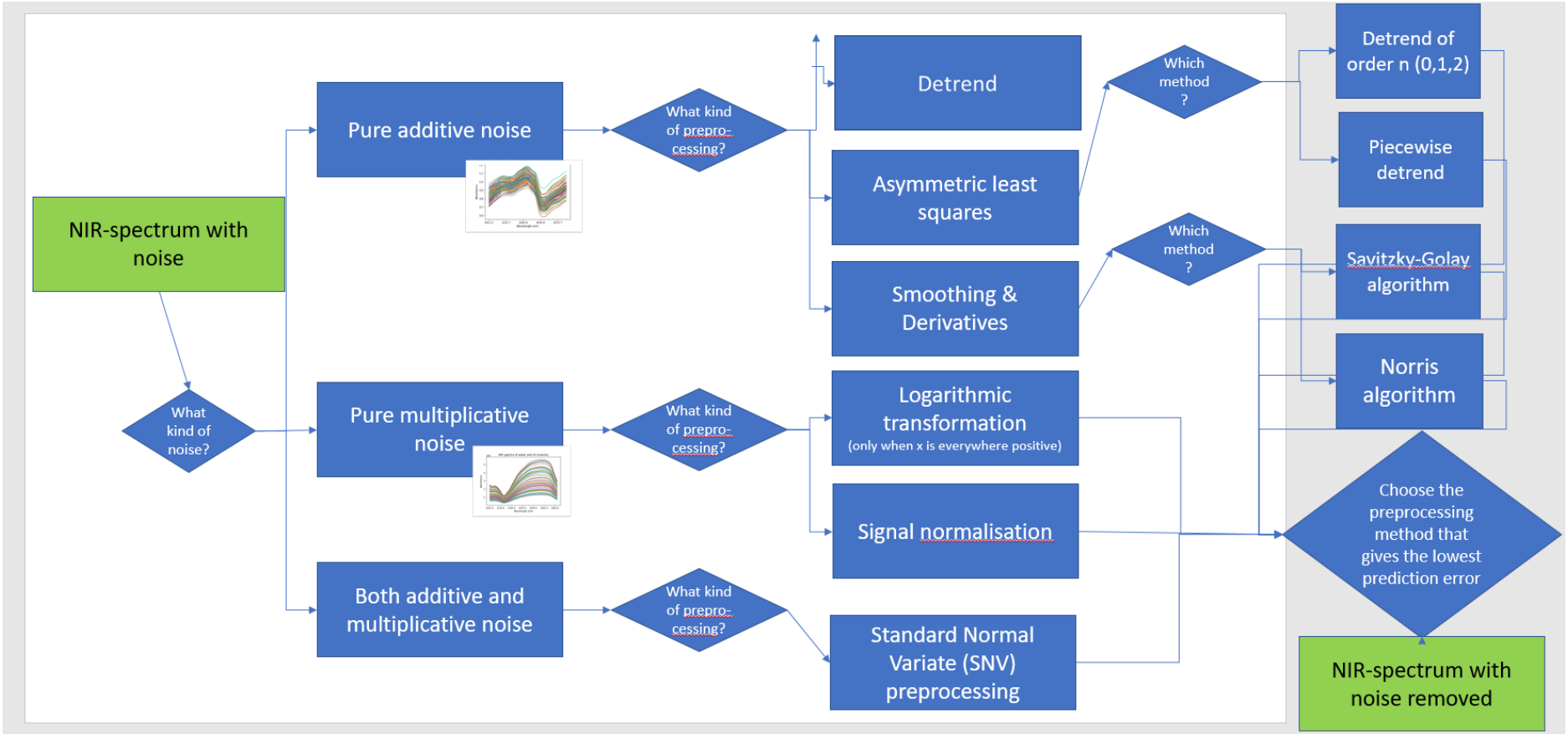
After EMSC, degree=6



After EMSC, degree=2



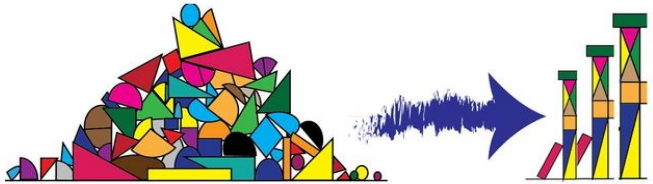
WAGENINGEN UNIVERSITY & RESEARCH


NIR-spectrum with noise
What kind of noise?
Pure additive noise
Pure multiplicative noise
Both additive and multiplicative noise
What kind of prepro-cessing?
What kind of prepro-cessing?
What kind of prepro-cessing?
Detrend
Asymmetric least squares
Smoothing & Derivatives
Logarithmic transformation
(only when x is everywhere positive)
Signal normalisation
Standard Normal Variate (SNV) preprocessing
Which method ?
Which method ?
Detrend of order n (0,1,2)
Piecewise detrend
Savitzky-Golay algorithm
Norris algorithm
Choose the preprocessing method that gives the lowest prediction error
NIR-spectrum with noise removed

Part 4: Feature selection methods

"Aristotle: Nature operates in the shortest way possible"

Feature selection



Increasing the number of variables:

Introduces unnecessary NOISE for discrimination, especially if they are strongly correlated

Carries a risk of OVERFITTING the models

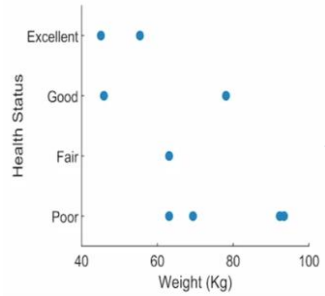
Using a simple model with few variables has a better chance of being generalized to a new sample than a model with hundreds of variables, which may fit the training set perfectly well but has limited generalization power

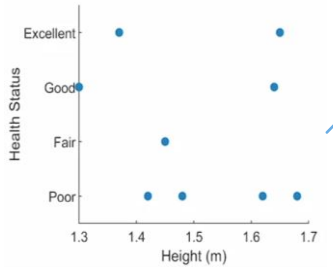
3 Approaches:
1. Variable Transformation and/or Selection
2. Discretization
3. Group Summary

Variable transformation

This involves applying an equation to existing variables to create a new feature

Example: Is it possible to accurately classify the health status of each individual from the original variables of age, height, weight and location?

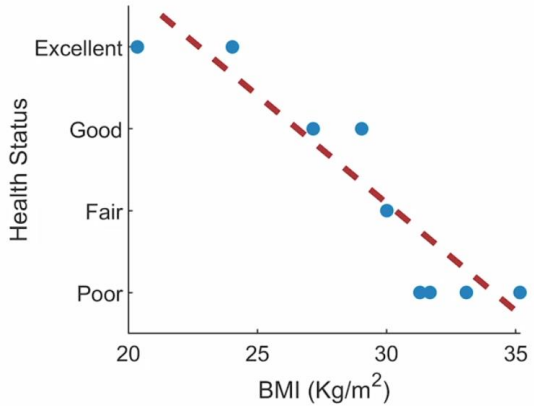




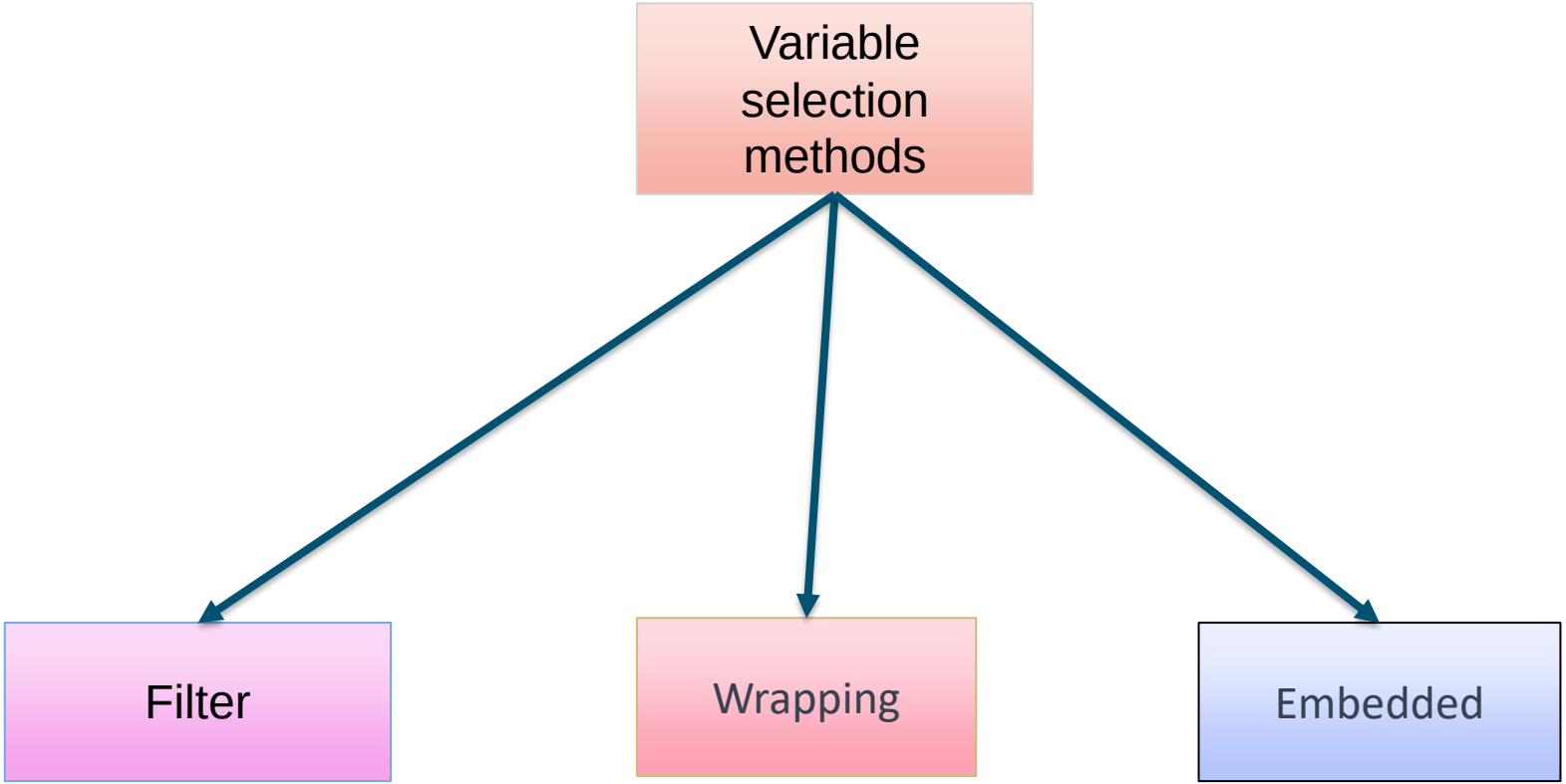
No clear correlation

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}$$

Correlation

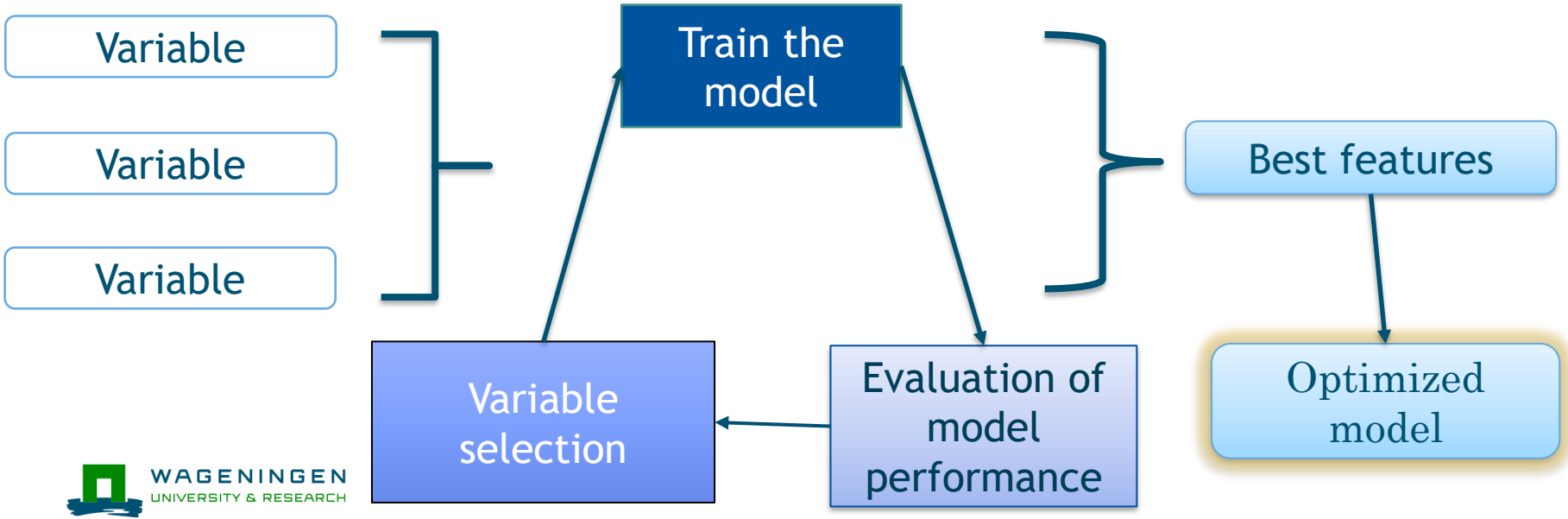


WAGENINGEN
UNIVERSITY & RESEARCH


Variable selection methods
Filter
Wrapping
Embedded

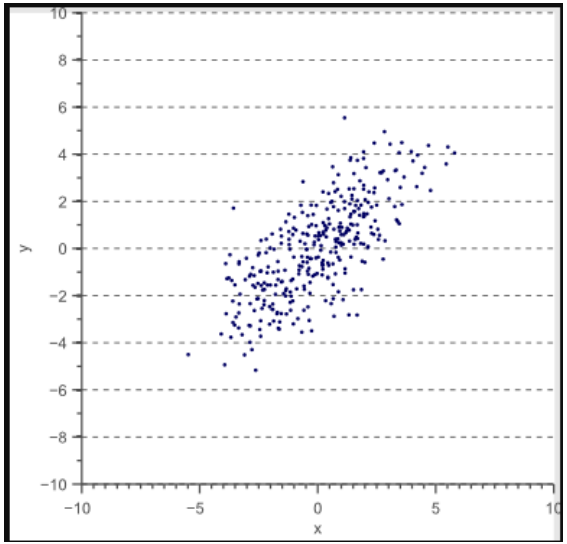
Wrapping methods

These variable selection methods depend on model performance. It is an iterative process of back and forth, where the chosen features are evaluated in relation to the final model performance in sequential stages.



Covariance

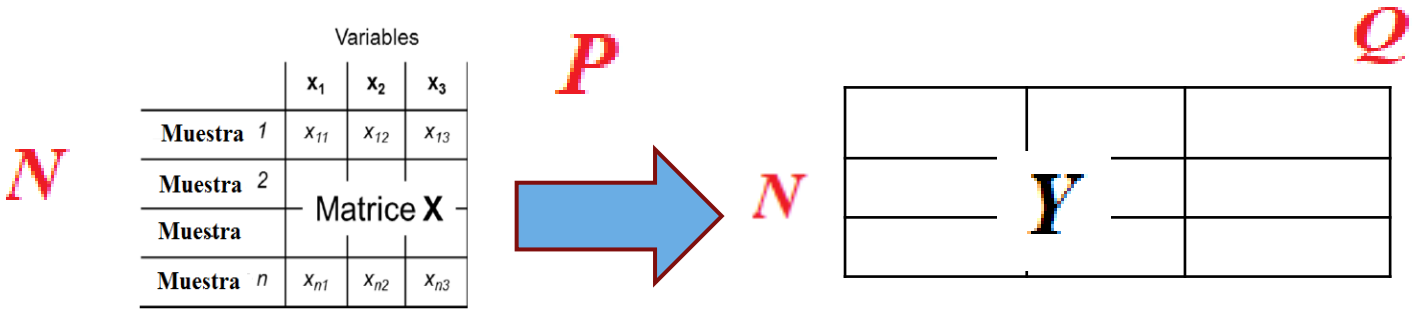
- Indicates the degree of joint variation between two random variables with respect to their means
- It can be used to understand the direction of the relationship between two variables
- The correlation coefficient is equal to the covariance divided by the product of the standard deviations of the variables



$$Cov(X,Y) = \frac{\sum_1^n (x_i - \bar{x})(y_i - \bar{y})}{n}$$

Wrapping method: Covariance selection (CovSel)

In each iteration, one variable **X** is selected on a criterion of the maximization of the covariances with **Y**



Once the variable with the highest covariance is isolated and selected, all other predictive factors and responses are orthogonalized with respect to it, and the process is repeated until the fixed number of variables has been selected

Covariance selection (CovSel)

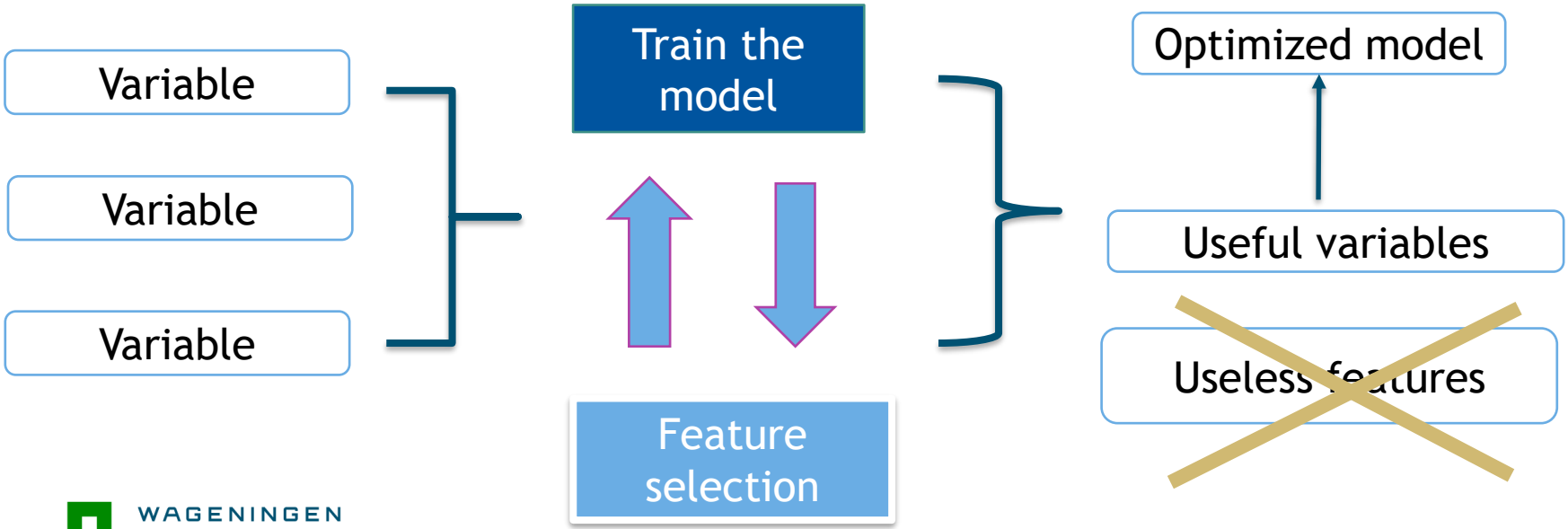
The selected variables from X should have:

- **Good predictive power for Y**
- **The highest possible variability**

CovSel can be applied to the problem of discrimination considering indicator variables as responses

Embedded methods

They automatically perform feature selection as part of the model training
The result is a trained model that highlights the useful features and disregards the rest

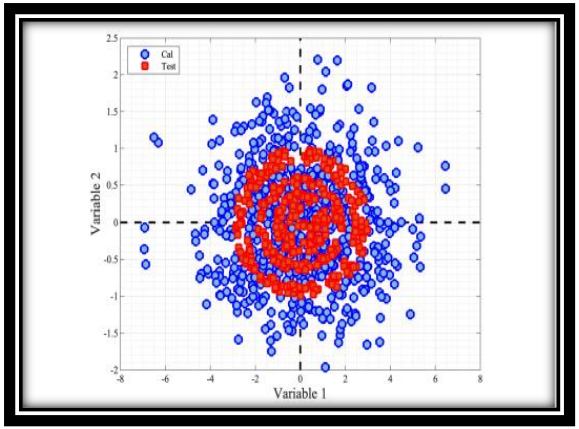


Part 5: Cross validation and Data Split

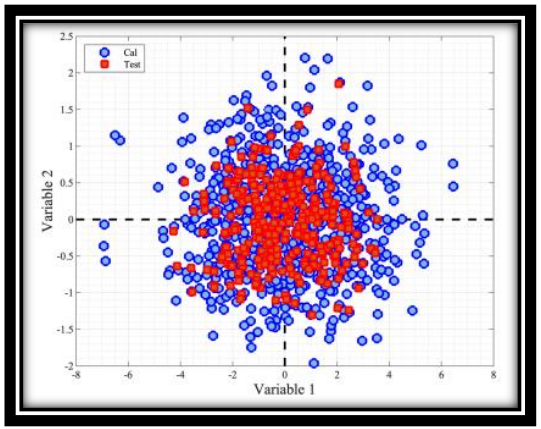
Some methods for the selection of Representative Learning and Test Sets

- Randomly
- Kernnand Stone
- Onion
- Duplex
- Reducennsamples

Onion



Kennard-Stone

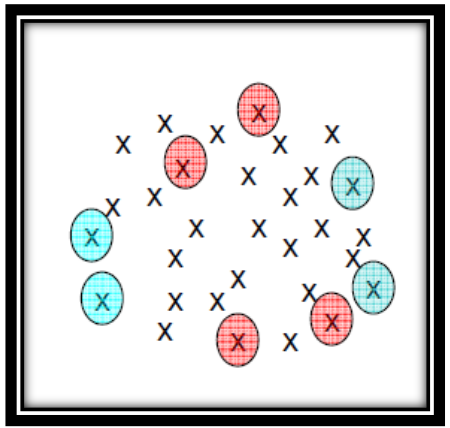


Source:https://eigenvector.com/wpcontent/uploads/2022/10/Onion_SampleSelection.pdf

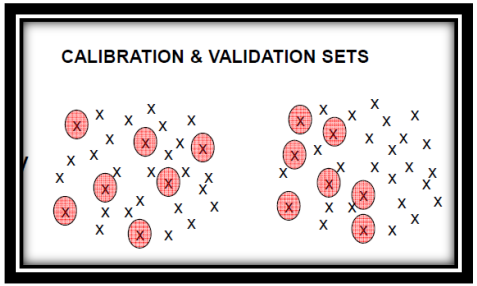
Some methods for the selection of Representative Learning and Test Sets

- Randomly
- Kernnand Stone
- Onion
- Duplex
- Reducennsamples

Duplex



Randomly

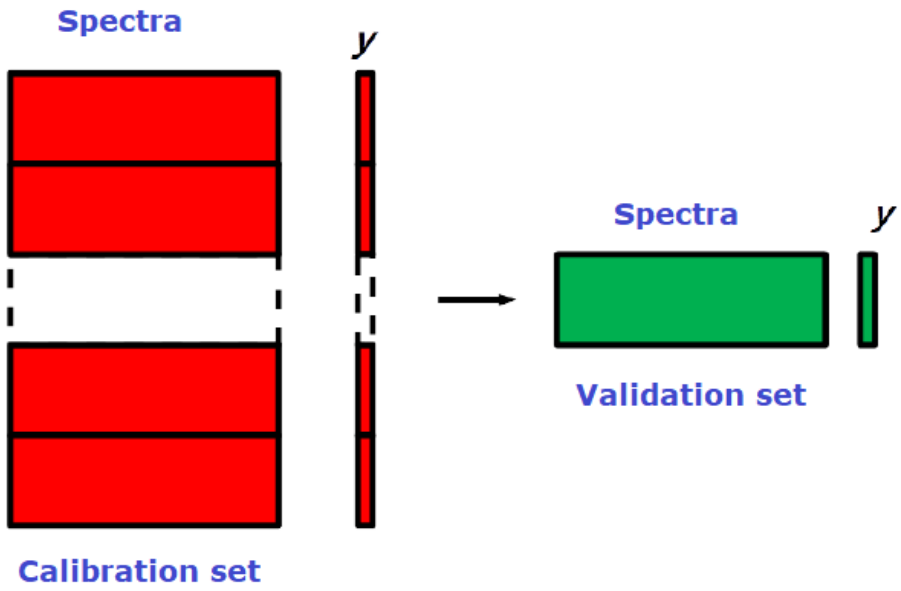


Source:https://eigenvector.com/wpcontent/uploads/2022/10/Onion_SampleSelection.pdf

Cross validation

Leave One Out: Over-estimates the predictive capacity of the model

Use only when data set has few samples



Part 6: Discrimination (PLSDA)

Principles and Objectives of Discrimination

- We have a data matrix **(nxp)** where **n** samples

  were measured for **p** quantitative variables, and a vector **Y**

  of size **n** measured on the same samples

- This vector represents the membership of each sample to each class **K**

- Each class contains at least one sample, and each sample belongs to a single class

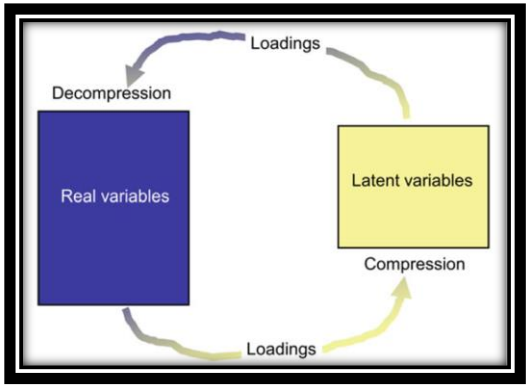
Partial Least Squares Discriminant Analysis

- Objective: To achieve a linear transformation that maps the data into a lower-dimensional space with the least possible error
- Supervised version of PCA
- In **PCA**, the transformation preserves (in its first principal component) the maximum possible variation in the original data
- In **PLS-DA**, the transformation preserves (in its first principal component) the maximum possible **covariance** between the original data and their labeling

Both can be described as iterative processes in which the error term is used to define the next principal component

Partial Least Squares Discriminant Analysis

- It consists of a classical PLS regression where the response variable is a category expressing the membership of samples in classes
- The relevant sources of data variability are modeled by the Latent Variables (LVs) which are linear combinations of the original variables



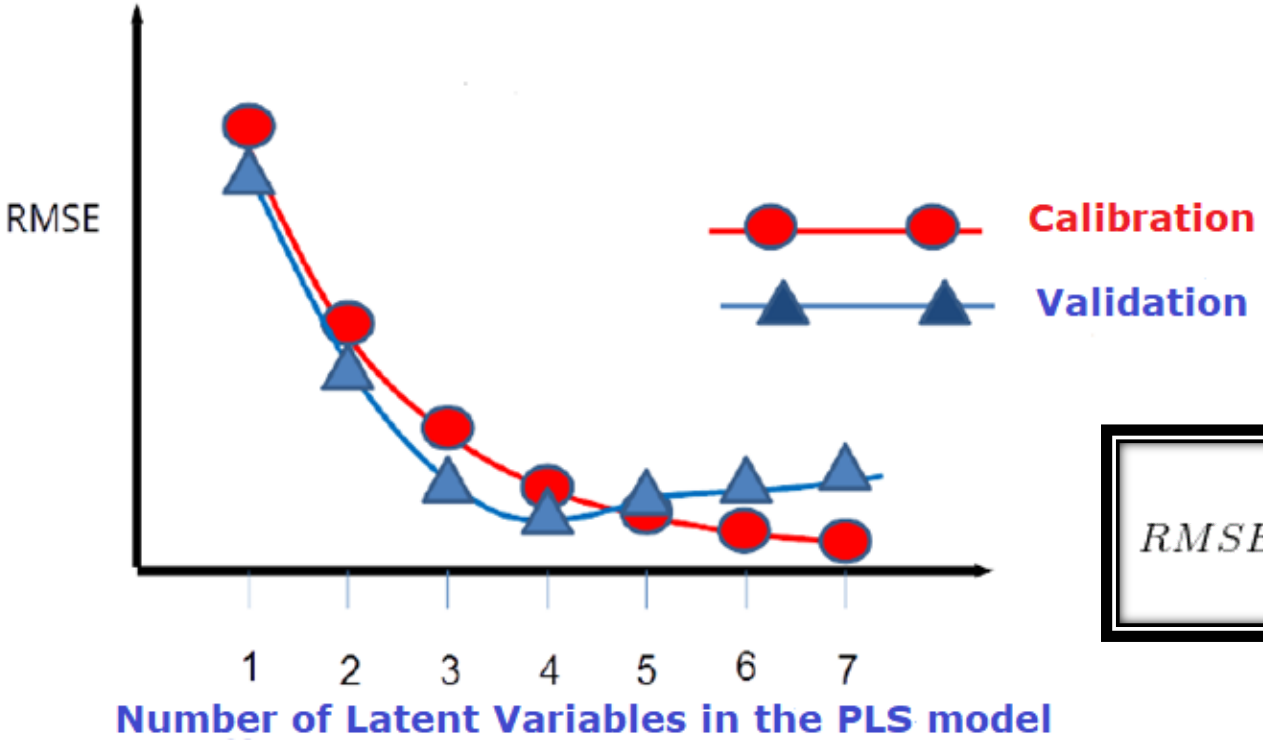
A fictitious matrix (Y) that records membership with 1s and 0s is combined with a spectral set (X), and PLS is implemented in the normal manner

$$y = \begin{pmatrix} 1 \\ 1 \\ 2 \\ 1 \\ 3 \\ 1 \\ 2 \\ 2 \\ 3 \end{pmatrix} \rightarrow Y = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

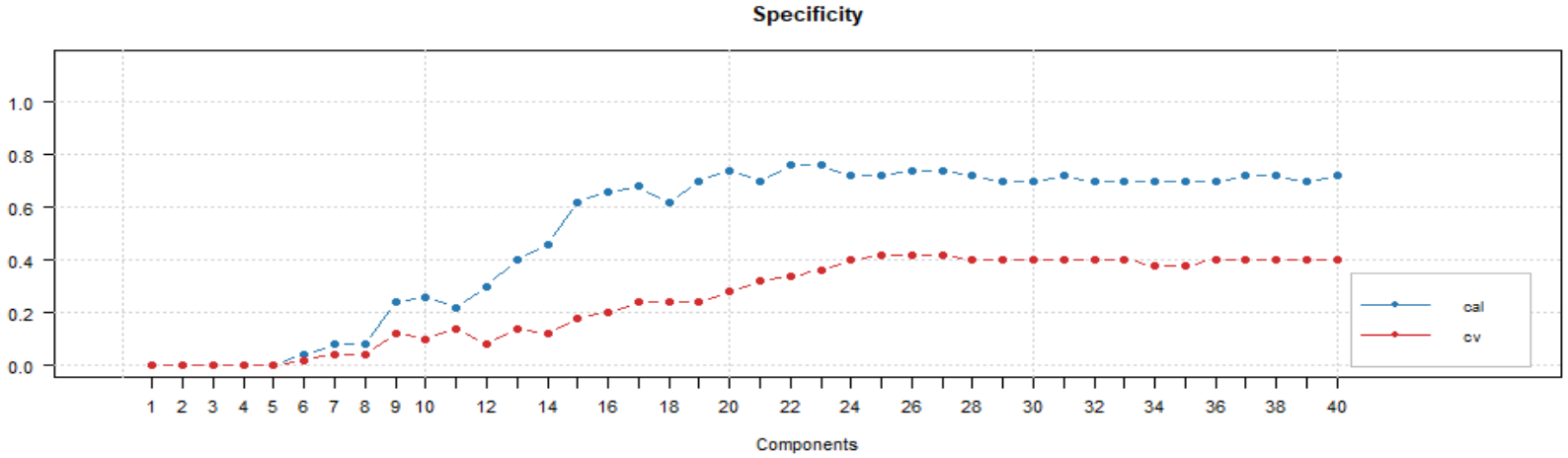
Partial Least Squares Discriminant Analysis

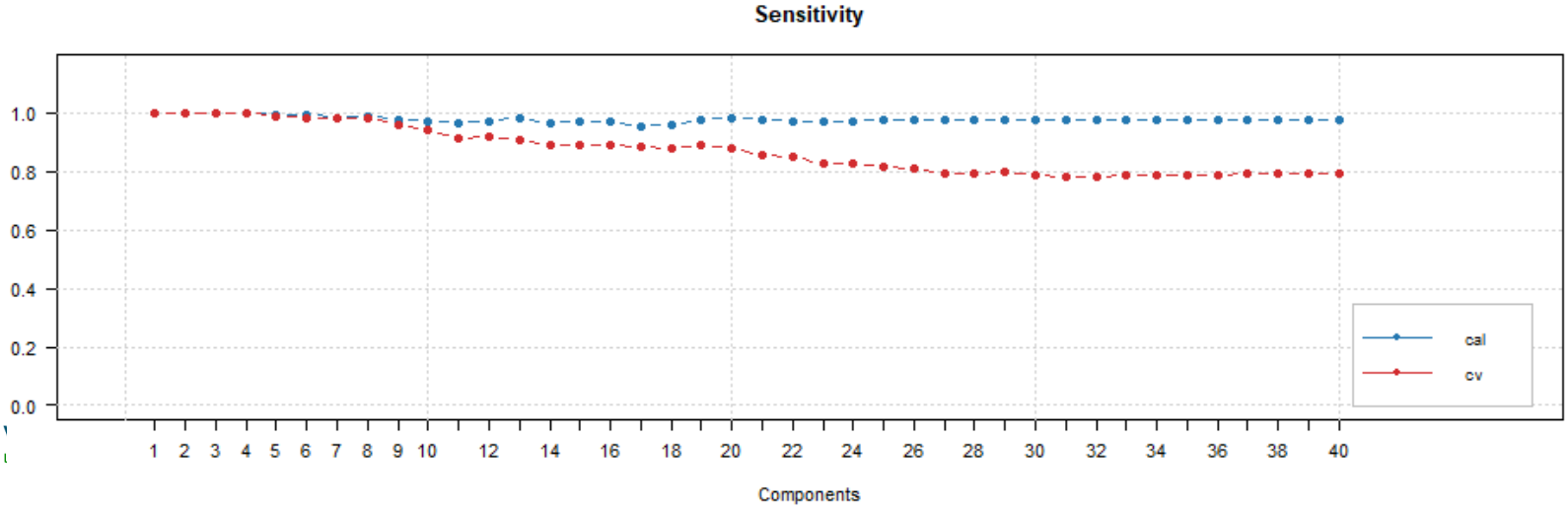
- PLS-DA provides estimated values for each sample and for each class.
- These values will not be exactly 1 or 0; however, if the calculated **y** is closer to 0, then the sample likely does not belong to that class, while a value closer to 1 would indicate the opposite
- To make a class assignment, a **threshold** can be defined for each class
- Thresholds can be calculated on the basis of the Bayes theorem

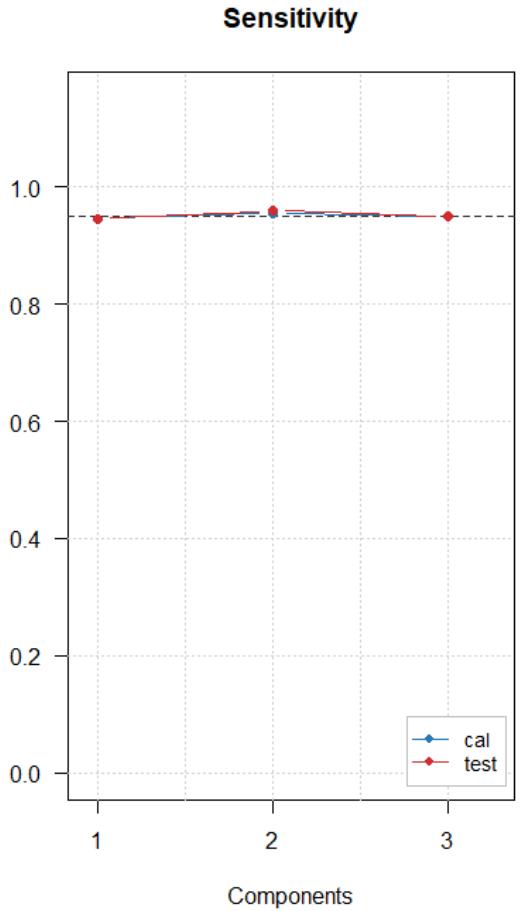
Study of Error as a Function of Dimensionality

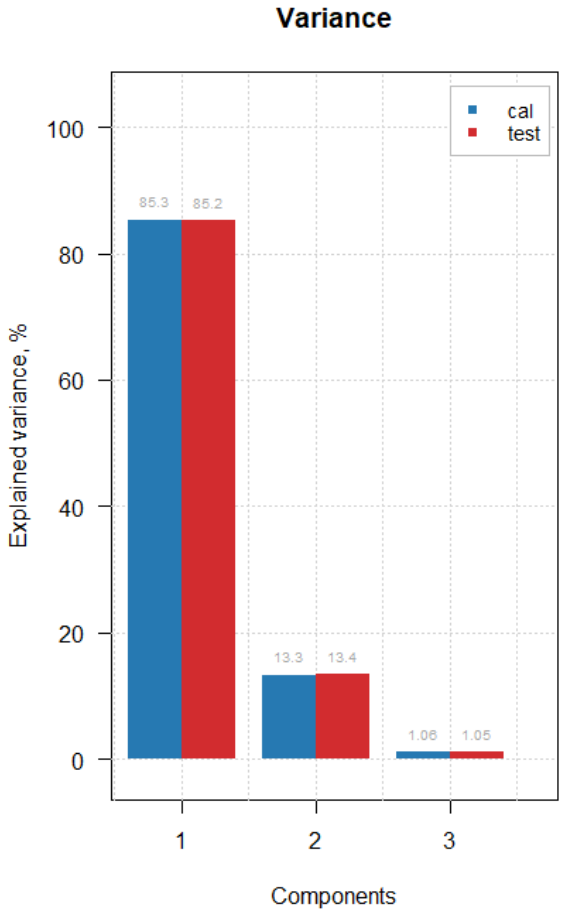


$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$


Specificity
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Components
cal
cv


Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Components
cal
cv


Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
cal
test
1
2
3
Components


Variance
cal
test
100
80
60
40
20
0
Explained variance, %
85.3
85.2
13.3
13.4
1.06
1.05
1
2
3
Components

Part 7: Hands on data

Hands on data: R code

https://github.com/MMBDD/Serbia

Name	Functionality
OutlierDetection.R: NEEDED	Removes outliers Averages Spectra from the same Sepal into only One Spectrum per Sepal
GlobalModel.R: NEEDED	Creates PLSDA Classification models with different number of important variables as input for PLSDA
PLSDA.R: OPTIONAL	Creates PLSDA Classification models with optimized parameters using Rchemo
PLSDAmdatools.R: OPTIONAL	Creates PLSDA and SIMCA Classification models using mdatools
MSC.R: OPTIONAL	Pretreats raw data and plots the results

Description of initial datasets

Cultivar name/ number of	Pixels per sepal	Sepals per tomato	Tomatoes per image	Images	Spectra in the initial dataset	Spectra in the averaged dataset	Variables
Provine	Between 119 and 90	5 or 6	16	2	16156	159	112
Brioso	Between 45 and 53	5 or 6	32	1	6497	164	112
Cappricia	Between 81 and 124	5 or 6	16	2	12816	165	112

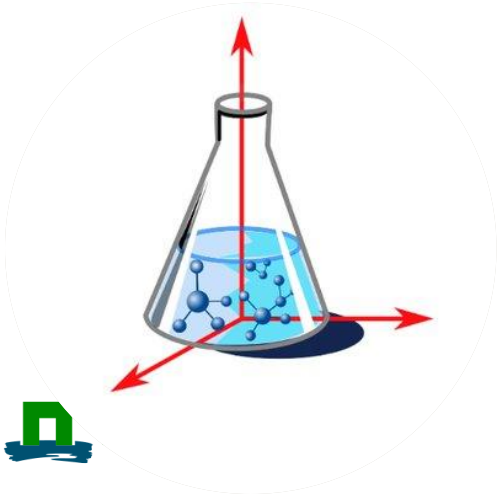
Number of spectra in each class (Healthy: Class 1; Diseased: Class 2) when dataset was split according to different labelling scenarios (Label 1: 0/123; Label 2: 01/23 and Label 3: 0.5/123).

Cultivar	n	Label 1		Label 2		Label 3	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Cappricia	163	139	24	85	78	117	46
Brioso	153	145	8	78	75	126	27
Provine	152	137	15	72	80	129	23

References

- https://www.chemproject.org/ChemHouse
- https://www.fun-mooc.fr/en/courses/?limit=21&offset=0
- Personal Material from the International School of Chemometrics 2023
- https://eigenvector.com/wp-content/uploads/2022/10/Onion_SampleSelection.pdf
- https://nirpyresearch.com/two-scatter-correction-techniques-nir-spectroscopy-python/
- J.M. Roger, B. P., D. Bertrand, E. Fernandez-Ahumada (2011). "CovSel: Variable selection for highly multivariate and multi-response calibration. Application to IR spectroscopy." Chemometrics and Intelligent Laboratory Systems 106(2).
- J.M. Roger, B. P., D. Bertrand, E. Fernandez-Ahumada. (2011). CovSel: Variable selection for highly multivariate and multi-response calibration. Application to IR spectroscopy. Chemometrics and Intelligent Laboratory Systems, 106(2). doi:ff10.1016/j.chemolab.2010.10.003f

Thank you for your attention

Please, open your R studio!

Any questions or comments?