NON-DESTRUCTIVE SPECTROSCOPY COMBINED WITH CHEMOMETRICS AS A TOOL FOR GREEN CHEMICAL ANALYSIS OF LIGNOCELLULOSE

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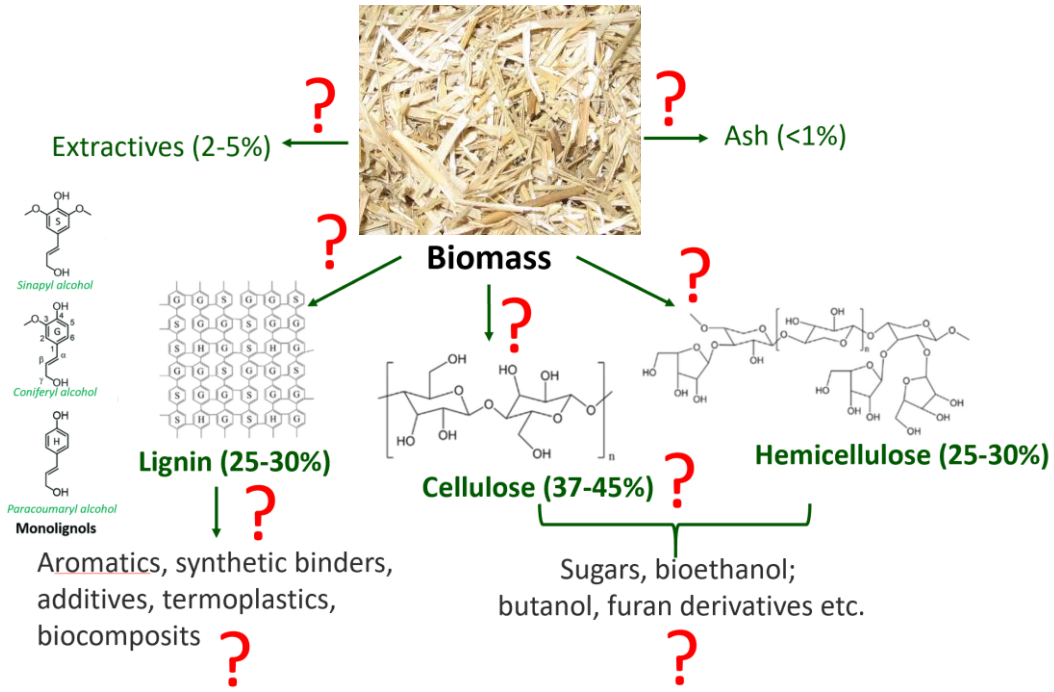
10th International Conference on Sustainable Solid Waste Management Chania, Greece, 21 - 24 JUNE 2023

THE WHY: BIOMASS ANALYSIS

- Wood species type and composition determine their suitability for pulping and other biorefinery strategies and processing technologies
- Mostly „wet" chemistry methods are currently used to yield accurate results
    - Composition – Van Soest, Klason, CASA (ASTM D1106-96, NREL /TP-510-42618, TAPPI)
    - Cellulose/hemicellulose – hydrolysis + HPLC, CE
    - Extractives – Soxhlet/gravimetry + GC/MS
    - Monolignols – thioacidolysis + GC/MS
    - Molecular weight distribution - SEC
    - Structural characteristics - FTIR and NMR + multivariate analysis
    - Mineral/element content – AAS, ICP-MS, CHNOS analysis
    - ……



Questions addressed to **analytical chemistry**

TAL TECH

DATA ANALYSIS - CHEMOMETRICS

- 6 logging areas
- 3 different wood species

93 objects (wood samples)

Area	Species	C %	H %	N %	O %	Cl %	P %	Zn, mg/kg	Na, mg/kg	K, mg/kg	Cu, µg/kg	Pb, µg/kg	Cd, µg/kg	Ni, µg/kg	EA, %	ASL %	KL %
Vana-Saaluse	Birch	48,9	5,5	0,11	47,5	< 0,01	< 0,01	13,6	6,7	265,1	1128,5	1052,0	50,3	71,6	2,54	3,15	25,34
Vana-Saaluse	Birch	49,1	5,8	0,11	47,6	< 0,01	< 0,01	19,8	< 3,0	200,4	919,1	1190,1	61,4	66,0	1,97	3,00	26,08
Vana-Saaluse	Birch	49,2	6,0	0,10	47,7	0,01	0,01	22,3	3,1	315,8	867,4	733,0	118,4	< 40,0	2,83	3,21	25,71
Vana-Saaluse	Birch	48,6	6,2	0,10	47,8	< 0,01	0,01	17,0	< 3,0	255,7	837,7	582,4	52,8	< 40,0	8,87	1,56	23,79
Vana-Saaluse	Birch	48,6	6,3	0,09	48,3	0,01	0,01	29,6	3,1	419,4	910,5	688,6	84,3	81,9	4,98	3,01	21,10
Vana-Saaluse	Spruce	50,0	6,3	0,06	46,2	0,01	< 0,01	8,5	< 3,0	285,6	794,5	283,8	68,5	208,0	3,20	0,39	31,07
Vana-Saaluse	Spr	50,3	5,8	0,07	46,7	0,01	< 0,01	12,3	< 3,0	200,7	565,3	182,4	33,2	< 40,0	1,74	0,36	30,50
Vana-Saaluse	Sp		5,8	0,07	46,9	0,01	< 0,01	11,6	< 3,0	271,5	655,3	358,0	95,7	< 40,0	2,08	0,41	26,40
Vana-Saaluse	Sp			0,05	46,4	< 0,01	< 0,01	21,3	< 3,0	330,8	1056,0	241,1	39,5	77,1	1,44	0,39	31,90
Vana-Saaluse	S			04	46,6	0,01	< 0,01	8,3	< 3,0	201,3	616,5	583,3	36,2	< 40,0	2,86	0,37	29,75
Vana-Saaluse	Pine				44,2	0,01	< 0,01	8,1	< 3,0	263,6	2110,2	66,2	43,7	224,5	3,01	0,57	33,79
Vana-Saaluse	Pine	52,3				0,01	< 0,01	8,3	< 3,0	182,0	654,9	56,2	76,9	44,8	4,97	0,63	35,20
Vana-Saaluse	Pine	52,0	6,2			< 0,01		6,1	< 3,0	218,4	877,1	56,4	71,0	< 40,0	2,87	0,67	35,03
Vana-Saaluse	Pine	51,5	6,1	0,0				6,9	< 3,0	254,3	641,5	116,4	84,4	< 40,0	3,29	0,57	34,34
Vana-Saaluse	Pine	50,5	6,0	0,05	45,				< 3,0	262,9	835,0	63,7	62,1	50,0	4,32	0,44	29,08
Pikakannu	Birch	49,0	6,0	0,14	47,1	0,			5,3	703,9	1386,4	2204,1	195,3	54,4	2,22	2,44	24,62
Pikakannu	Birch	48,6	6,5	0,13	46,7	0,01	0,			609,5	1256,5	1704,8	183,4	76,9	1,50	2,50	28,13
Pikakannu	Birch	48,6	6,6	0,09	47,3	0,01	0,01			783,3	756,8	45,1	106,0	1,23	2,40	26,64
Pikakannu	Birch	48,3	6,5	0,11	47,0	< 0,01	0,01	30,3			26,5	950,4	170,1	46,4	1,28	2,93	24,19
Pikakannu	Birch	48,2	6,6	0,09	47,3	< 0,01	0,01	35,1	10,			689,0	63,1	75,9	2,46	2,90	23,22
Pikakannu	Birch	49,1	6,6	0,14	47,1	< 0,01	0,01	26,1	< 3,0				62,0	53,1	3,18	2,70	25,18
Pikakannu	Spruce	49,5	6,5	0,06	46,4	< 0,01	< 0,01	5,6	< 3,0	448,9				< 40,0	5,10	0,35	33,00
Pikakannu	Spruce	49,6	5,6	0,06	46,6	0,01	< 0,01	7,2	< 3,0	172,8	1019,			09,2	3,16	0,36	32,47
Pikakannu	Spruce	49,7	6,7	0,06	47,2	0,01	< 0,01	5,0	< 3,0	221,9	829,8			4,19	0,36	30,03
Pikakannu	Spruce	49,6	6,6	0,07	46,7	0,01	< 0,01	8,6	< 3,0	281,5	1027,2	< 50,0				0,37	31,00
Pikakannu	Spruce	50,1	6,5	0,07	45,6	0,01	< 0,01	9,1	< 3,0	553,8	1731,7	286,6	151,1				35,07
Pikakannu	Pine	49,7	6,7	0,07	46,5	< 0,01	< 0,01	< 3,0	< 3,0	338,0	1096,2	58,8	19,9	42,			30,15
Pikakannu	Pine	50,1	6,7	0,07	46,0	0,01	< 0,01	4,6	< 3,0	481,5	1185,4	56,1	19,2	< 40,0	2,		32,18
Pikakannu	Pine	49,6	6,6	0,08	46,9	0,01	< 0,01	8,0	< 3,0	487,2	974,7	75,6	24,0	153,3	2,14	0,	30,32
Pikakannu	Pine	49,2	6,6	0,09	46,5	< 0,01	0,01	5,6	< 3,0	466,8	1043,2	65,8	34,4	98,4	0,89	0,40	30,66
Pikakannu	Pine	50,0	6,7	0,08	46,3	0,01	< 0,01	8,0	< 3,0	503,8	1191,8	< 50,0	7,2	60,8	2,12	0,42	29,96
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
Madala	Birch	48,2	6,3	0,08	47,9	0,01	< 0,01	35,2	3,8	192,4	442,0	555,3	63,0	< 40,0	3,36	1,66	22,49
Madala	Birch	49,2	6,3	0,13	46,2	< 0,01	< 0,01	43,3	3,5	471,4	953,4	800,0	129,3	< 40,0	7,56	2,38	28,76
Madala	Birch	48,6	6,4	0,08	47,5	0,01	< 0,01	21,1	8,0	273,8	514,8	486,3	58,1	40,0	2,79	2,19	24,04

Data ≠ Information ≠ Knowledge/Interpretation

16 variables

EA – extractives
ASL – acid soluble lignin
KL – Klason (total) lignin

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SIMPLE QUESTIONS

- How do the different wood species compare?
- What is the relation between the variables?
- Do we need all 16 descriptors?
- **Does the chemical composition of the wood depend on growing region?**
- **Are all woods similar?**

While these are simple questions to ask, they are quite difficult to answer by looking at the table

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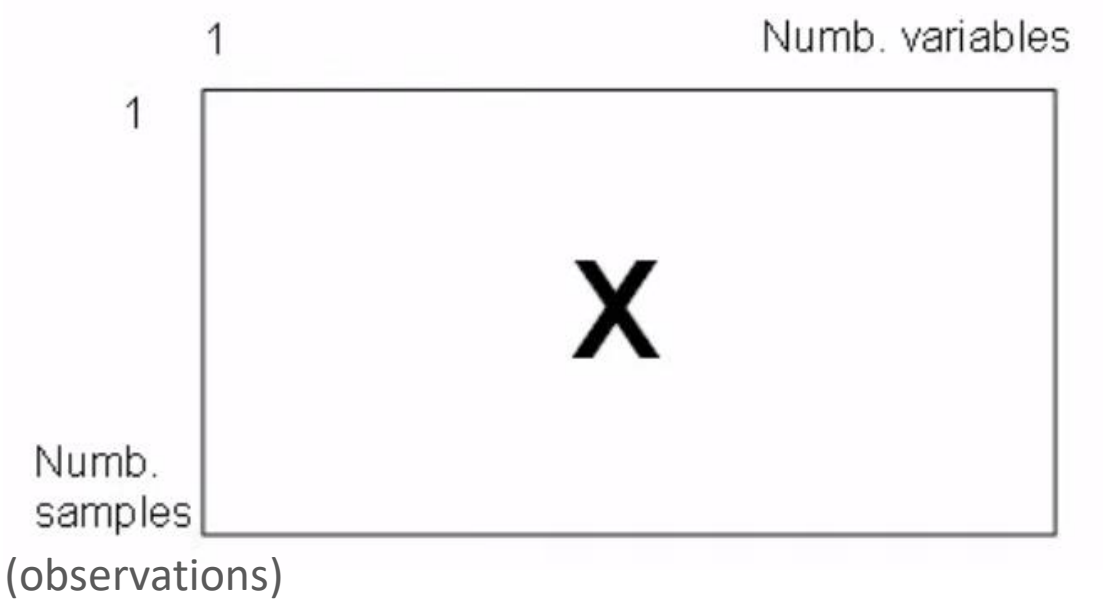
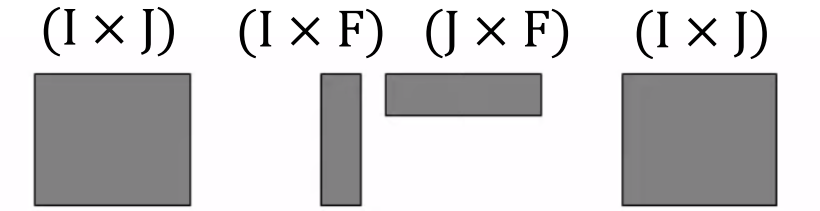
PRINCIPAL COMPONENT ANALYSIS

PCA reduces the number of variables of a data set, while preserving as much information as possible

PCA is used for analysing *one* matrix/table



Graphically it looks like this:



$$\mathbf{X} = \mathbf{TP}' + \mathbf{E}$$

The data X are modelled as a product TP' plus residuals E.

T - the score matrix,
P - the loading matrix
} *they are determined in a least square sence*
F - the number of components

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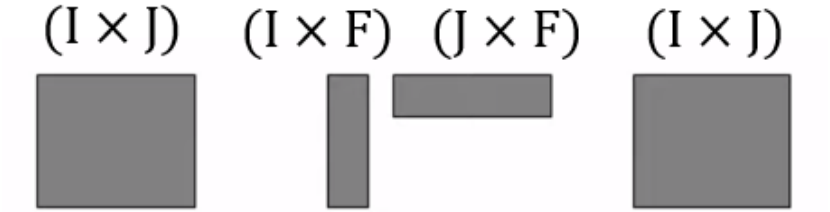
PRINCIPAL COMPONENT ANALYSIS

- Each principal component consists of one score and one loading vector
- Component one has highest possible variance
- Component two is orthogonal (in scores and loadings) and has the next highest possible variance, etc.

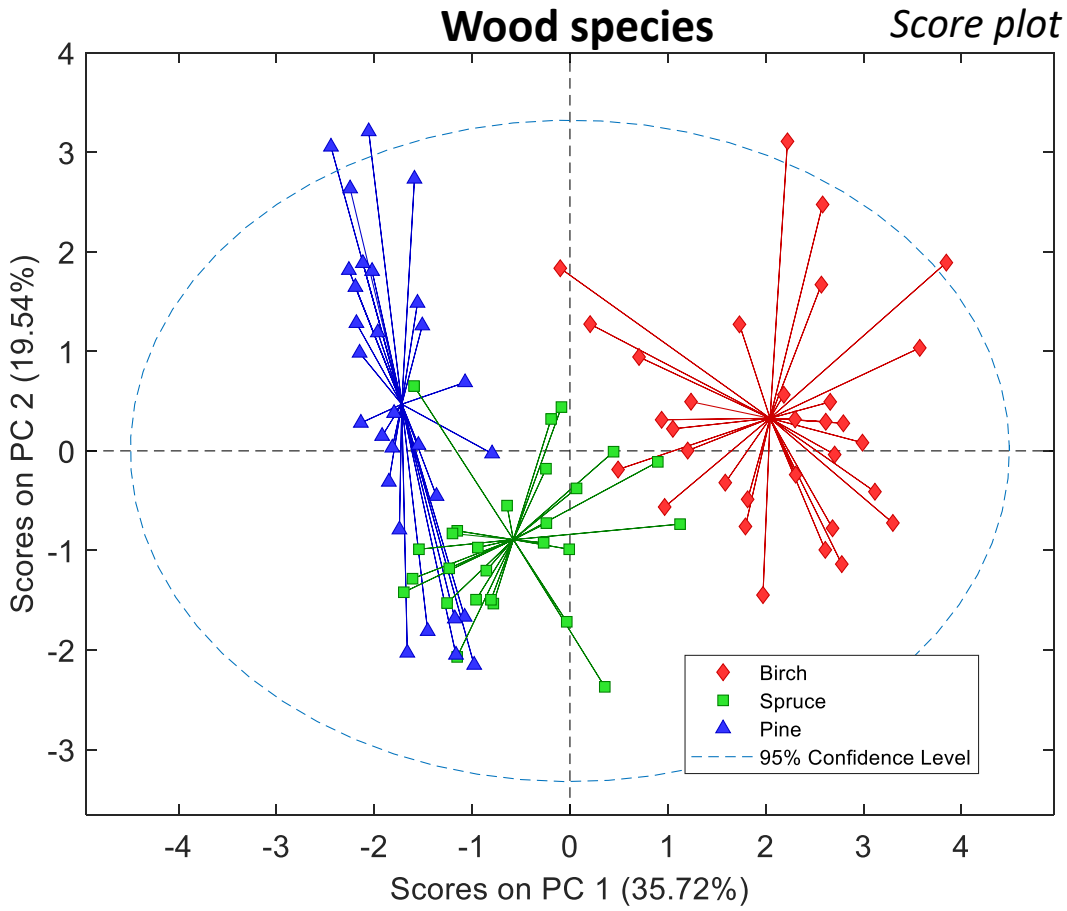


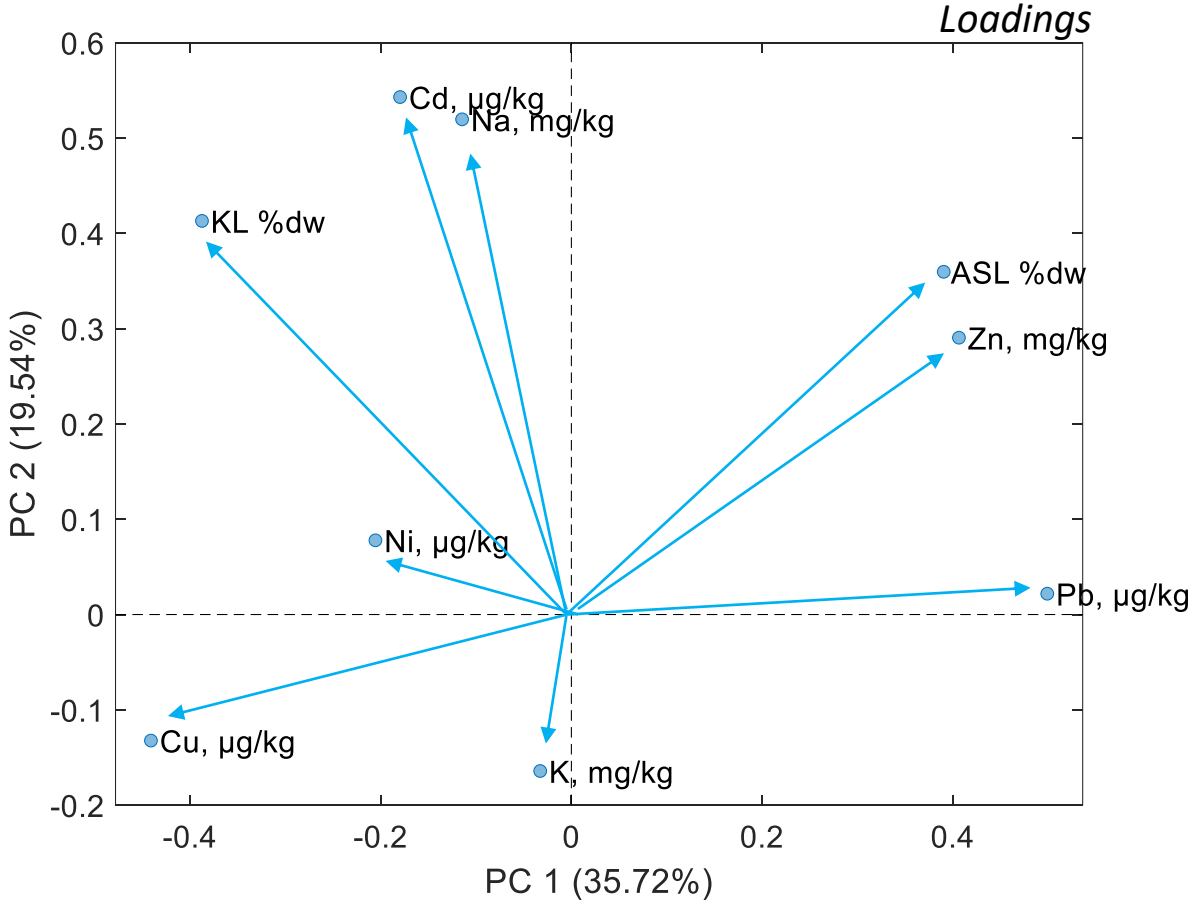
$$\mathbf{X} = \mathbf{TP}' + \mathbf{E}$$

TAL TECH

PCA ON THE WOOD-SPECIES DATA
(METALS+LIGNIN)

Is it possible to classify woods based on measured data?





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After two components, 55% of the variations are captured. Rather than looking at 9 original variables, two different kinds of weighted averages provide most information in the data.

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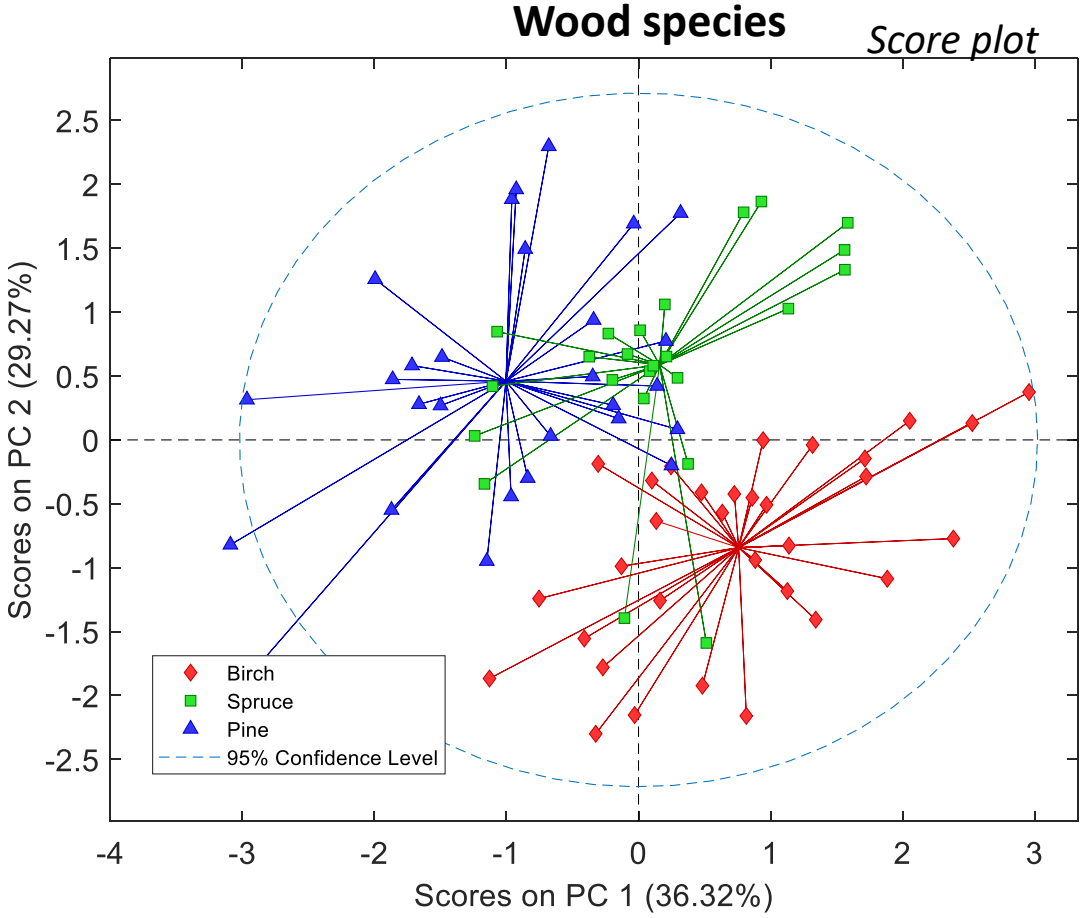
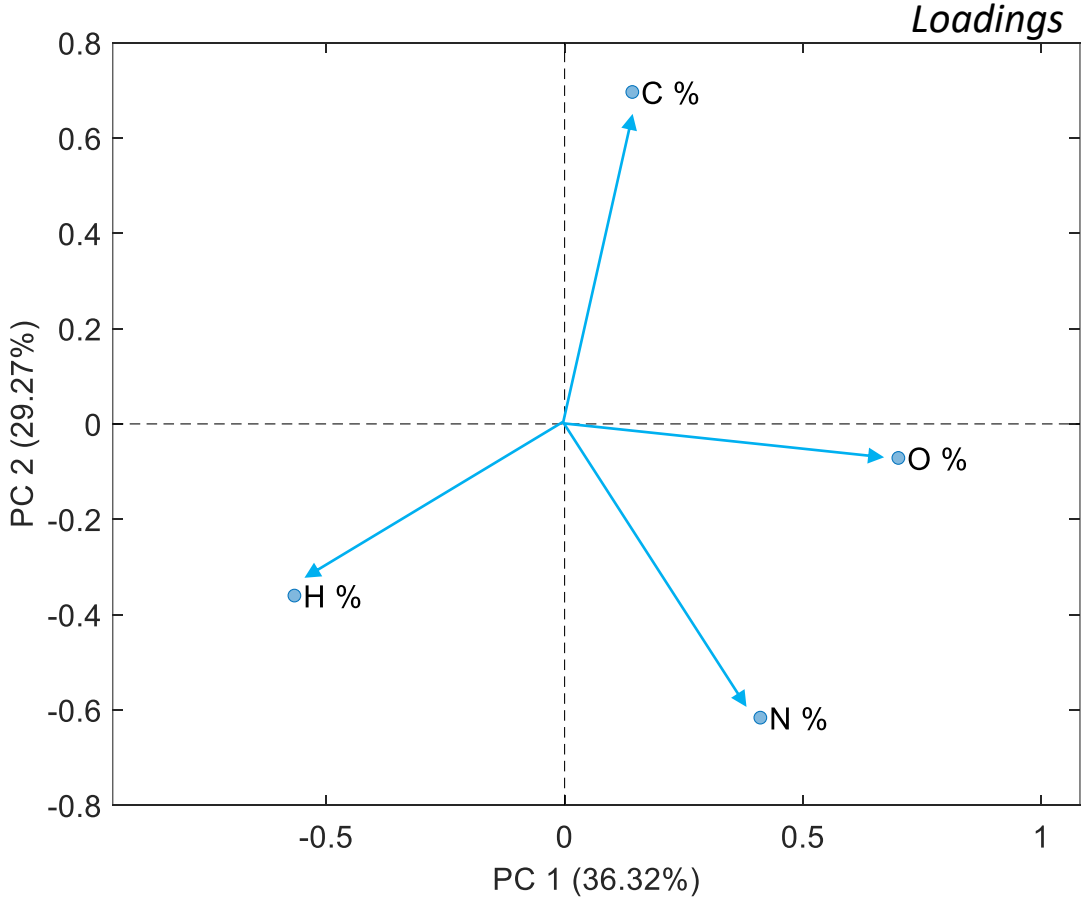
PCA ON THE WOOD-SPECIES DATA
(ELEMENTS C, H, N, O)

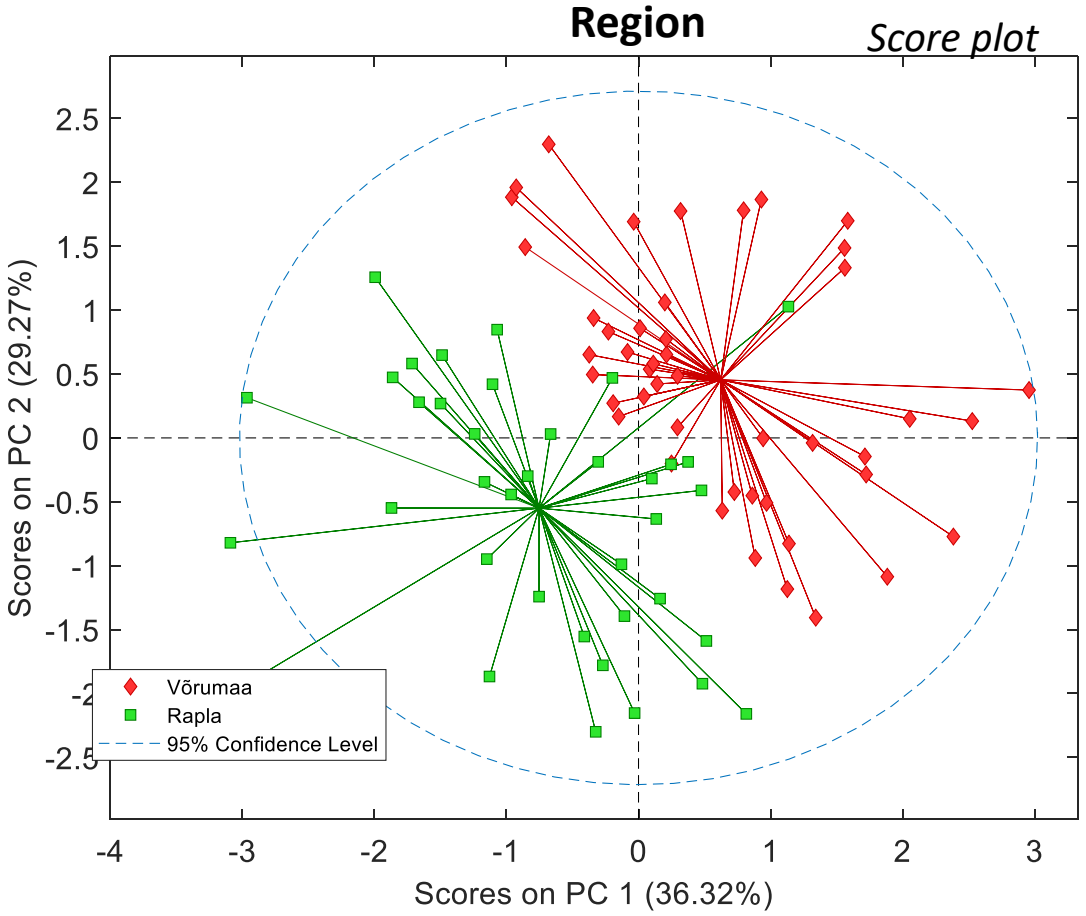


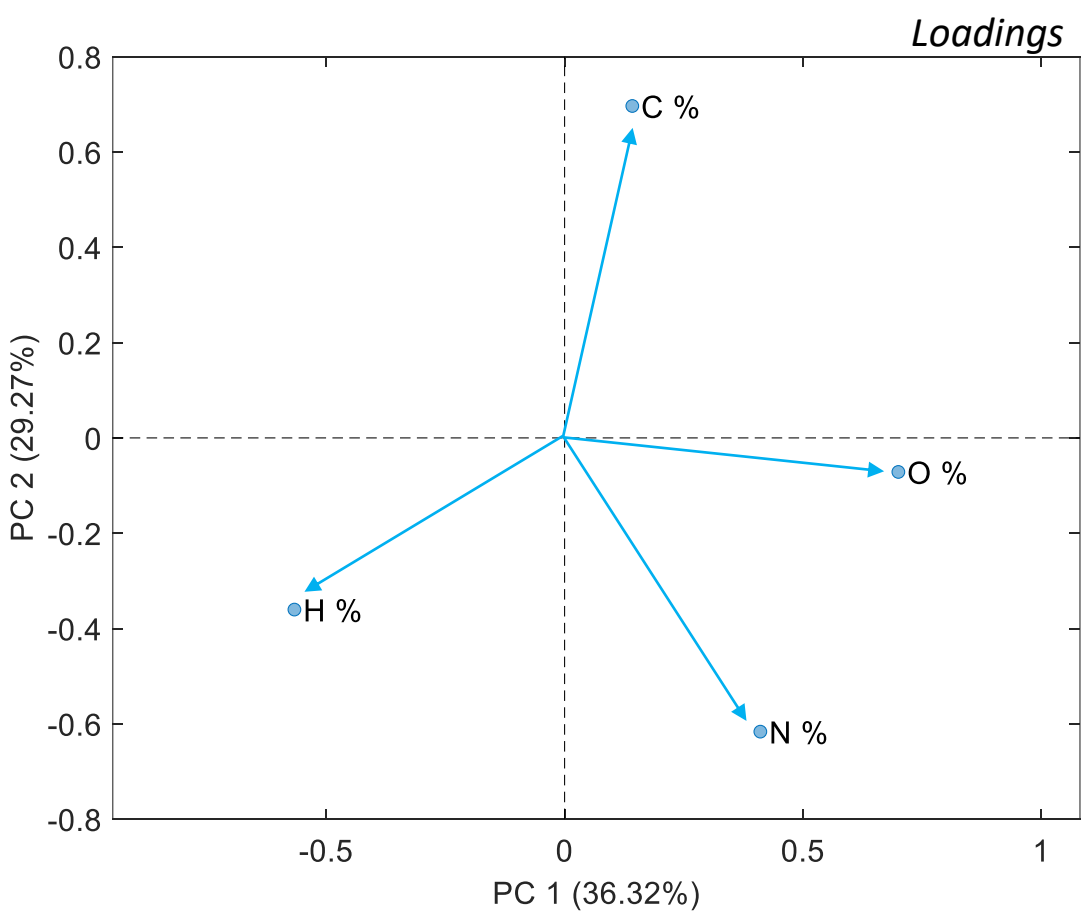


TAL TECH

EIGENVECTOR RESEARCH INCORPORATED

PCA ON THE WOOD-SPECIES DATA
(ELEMENTS C, H, N, O)





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Does growing region/area correlate with chemical composition of the wood?

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USING PCA ANALYSIS

- You can handle many variables rationally
- You do get more information by doing so
- You can overview, diagnose, monitor, classify even in complex situations
- You can learn about a problem from the data. You *do not* need to know everything beforehand. You don’t even need a hypothesis.

TAL TECH

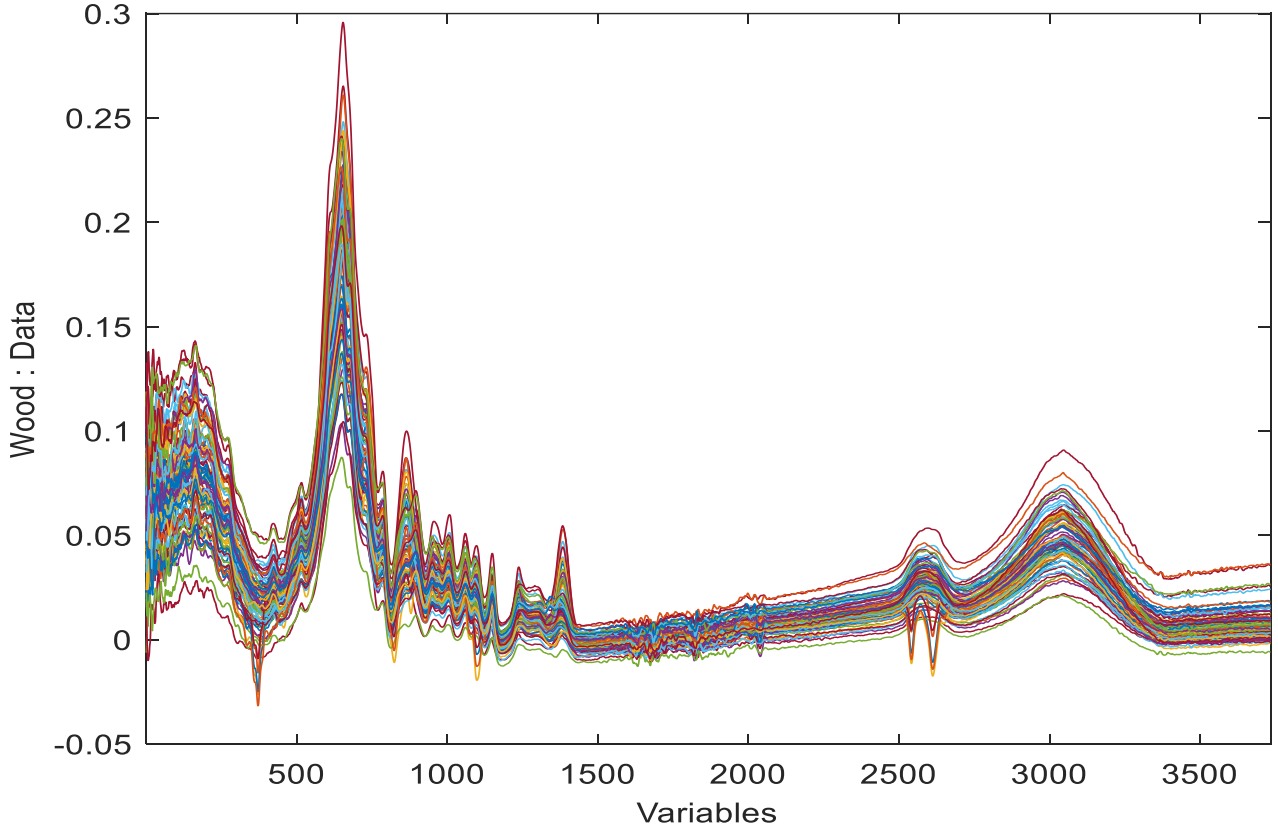
FTIR COMBINED WITH PCA AS GREEN ALTERNATIVE TO "WET" CHEMISTRY

Used dataset obtained by chemical/instrumental methods of analysis:

- Elemental analysis (CHNO)
- Metal AAS analysis
- Gravimetric analysis (KL, ASL, EA)

These methods are laborious, expensive, time-consuming, require large sample sizes and toxic chemicals

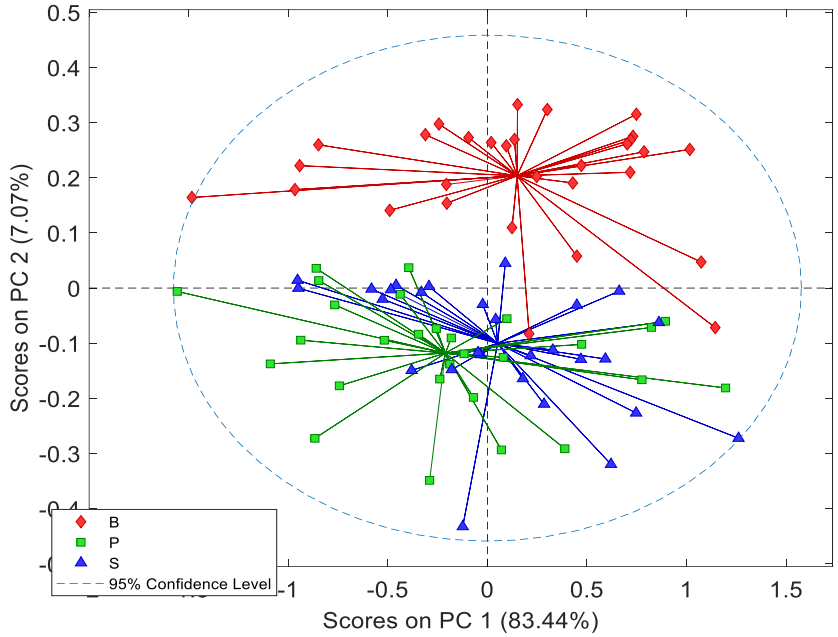
FTIR spectra of 93 wood samples (birch, spruce, pine)



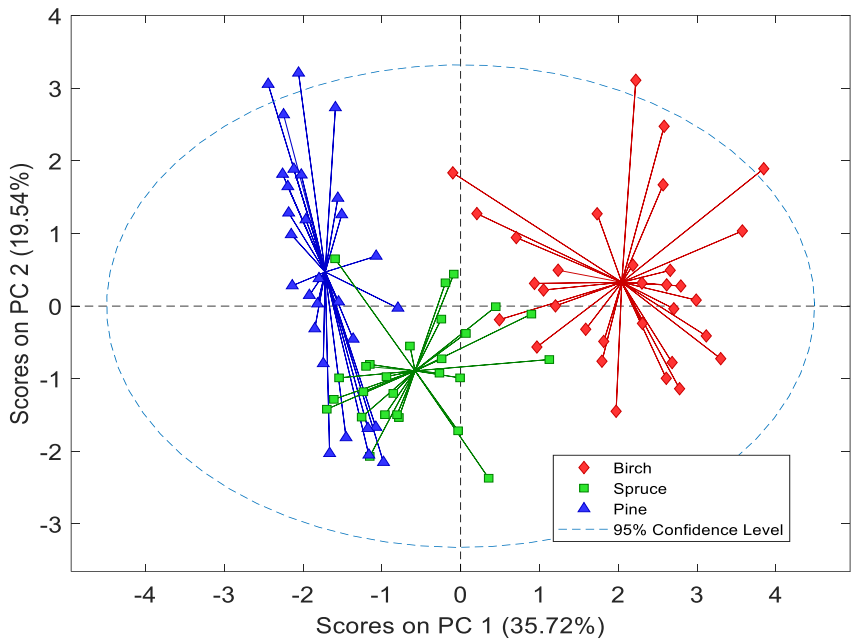
FTIR spectroscopy is low cost, rapid, nondestructive and reliable

FTIR COMBINED WITH PCA AS GREEN ALTERNATIVE TO "WET" CHEMISTRY

Spectral data



Chemical data



Note the similarity with the plot obtained from the spectral data. The traditional chemical data though, are much more expensive and time- and labor-intensive to obtain.

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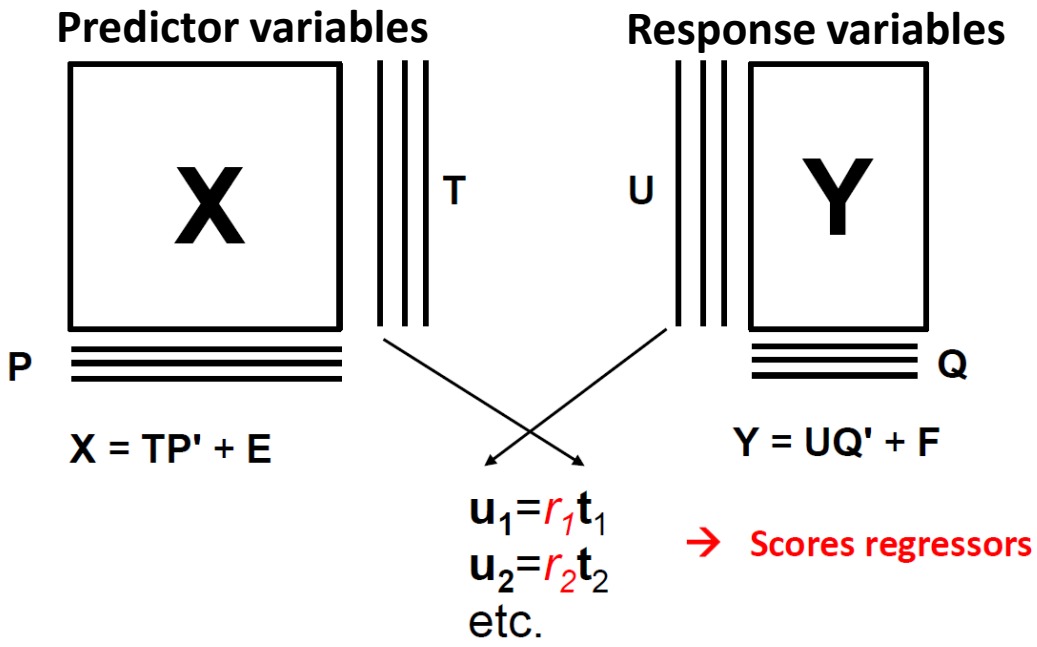
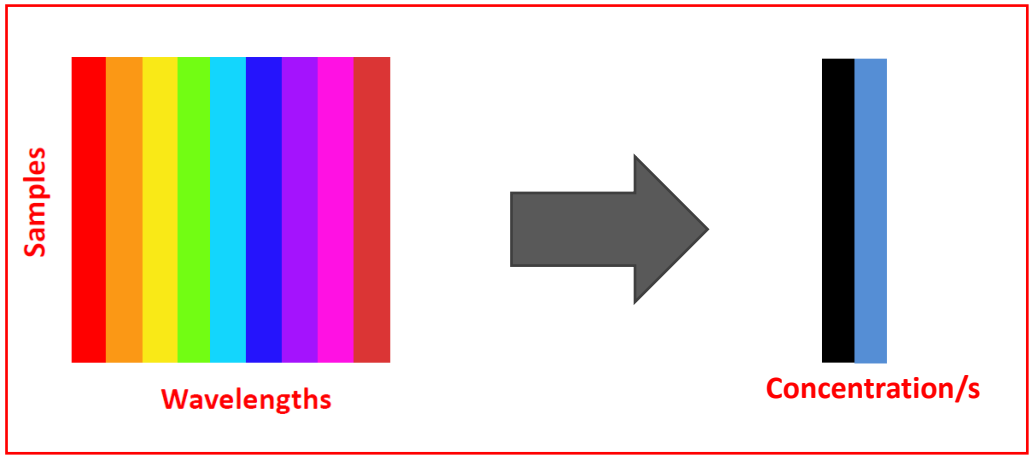
PARTIAL LEAST SQUARE REGRESSION (PLS-R)

$$\left.\begin{aligned} X &= TP' + E \\ Y &= UQ' + F \end{aligned}\right\} U = RT$$

This is the main core of PLS!!!



- PLS looks for calculating the correlation between X and Y by maximizing the COVARIANCE between t and u
- It is used to develop predictive models



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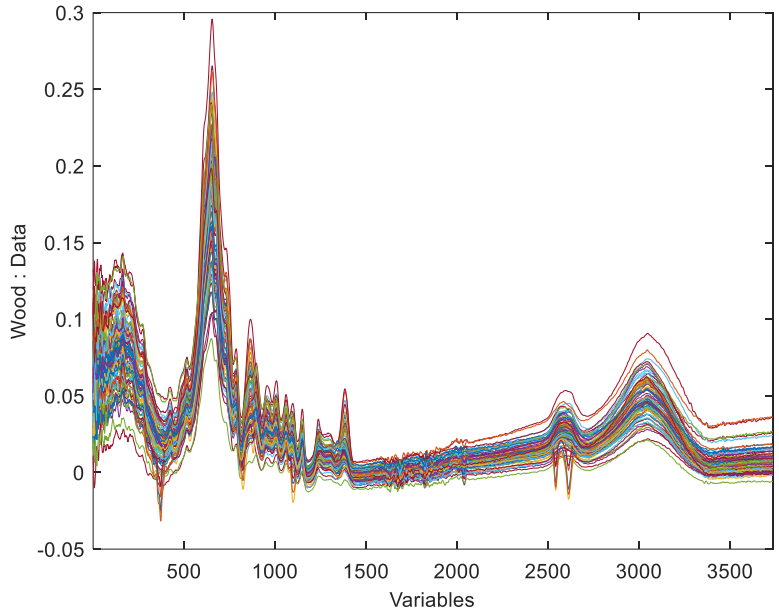
PARTIAL LEAST SQUARE REGRESSION (PLS-R)

93 FTIR spectra of wood samples



Predictor variables

NIPALS algorithm

Quantitative data

Species	EA, %	ASL %	KL %
Birch	2,54	3,15	25,34
Birch	1,97	3	26,08
Birch	2,83	3,21	25,71
Birch	8,87	1,56	23,79
Birch	4,98	3,01	21,1
Spruce	3,2	0,39	31,07
Spruce	1,74	0,36	30,5
Spruce	2,08	0,41	26,4
Spruce	1,44	0,39	31,9
Spruce	2,86	0,37	29,75
Pine	3,01	0,57	33,79
Pine	4,97	0,63	35,2
Pine	2,87	0,67	35,03
...	...	...	...
Pine	3,29	0,57	34,34
Pine	4,32	0,44	29,08

Response variables

PLS-R model

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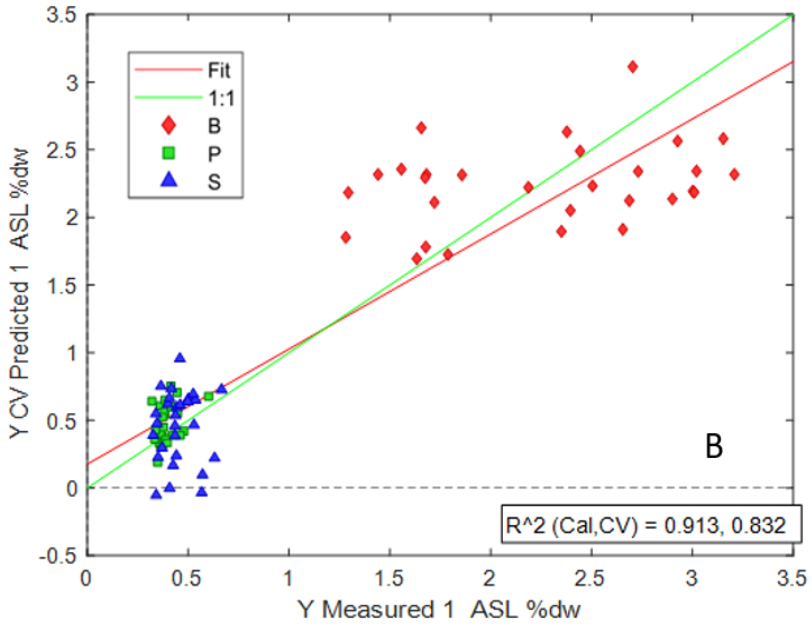
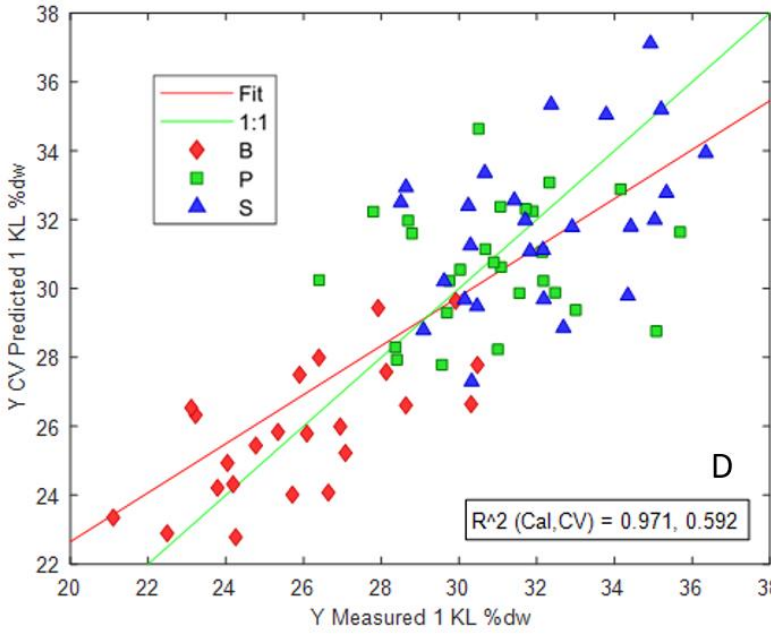
Nonlinear iterative partial least squares (NIPALS) algorithm reduces the number of predictors to extract a set of components that describes maximum correlation between the predictors and response variables

PLS-R MODELS FOR *ASL*, *KL* AND *EA* QUANTIFICATION IN WOOD

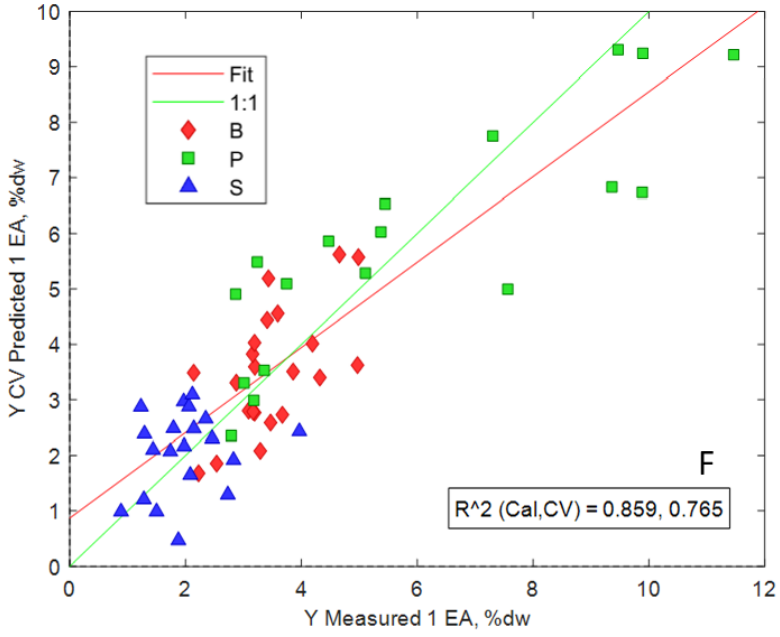
Acid soluble lignin (ASL), %



Klason lignin (KL), %



Extractives (EA), %



Partial least squares regression models using five PLS factors were able to predict ASL, KL and EA contents in wood with a correlation coefficient of ~0.9 and ~0.8 in training and full cross-validation, respectively.

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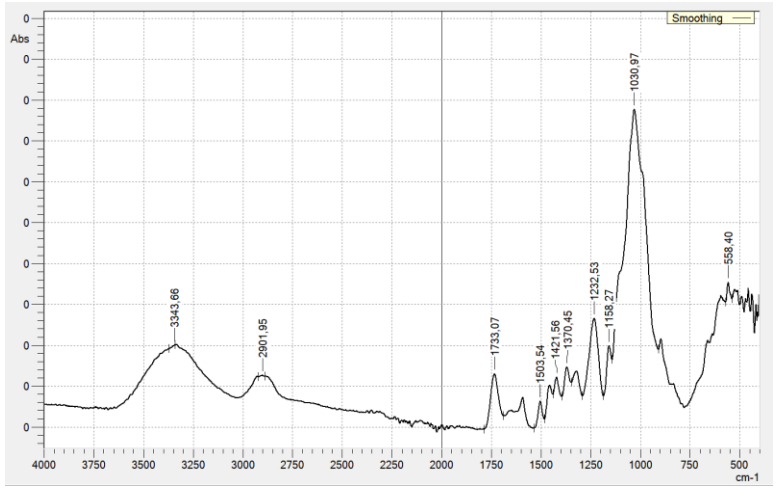
ASL, KL AND *EA* QUANTIFICATION IN WOOD



+ PLS-R model

Characteristics	Predicted value	Chemically measured value
ASL,%	2,9	3,1
KL,%	25	24
EA,%	2,2	2,4

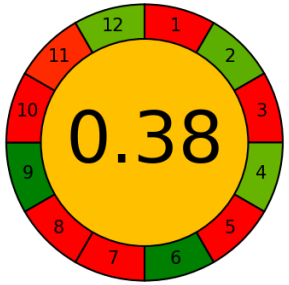
Measuring FTIR spectrum - 2 min

Using PLS-R model - 1 min

Quantitative results in 3 min

Green analytical chemistry metrics

Klason method



1. Sample treatment
2. Sample amount
3. Device positioning
4. Sample prep. stages
5. Automation, miniaturization
6. Derivatization
7. Waste
8. Analysis throughput
9. Energy consumption
10. Source of reagents
11. Toxicity
12. Operator's safety

ATR-FTIR + PLSR prediction

Much more *greener* approach if compared to classical "wet" chemical analysis!

AGREE—Analytical GREEnness Metric Approach and Software**,** F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, *Analytical Chemistry* **2020** *92* (14), 10076-10082

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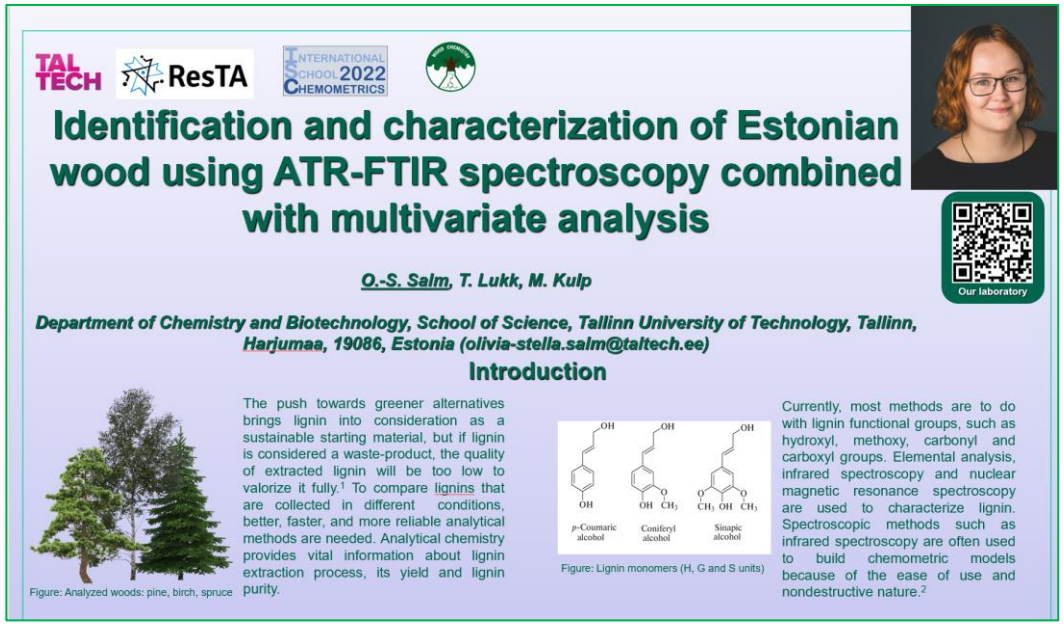
ANALYTICAL CHEMISTRY LAB

CHANIA 2023 CONFERENCE



TAL TECH ResTA International School 2022 Chemometrics

Identification and characterization of Estonian wood using ATR-FTIR spectroscopy combined with multivariate analysis

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Our laboratory

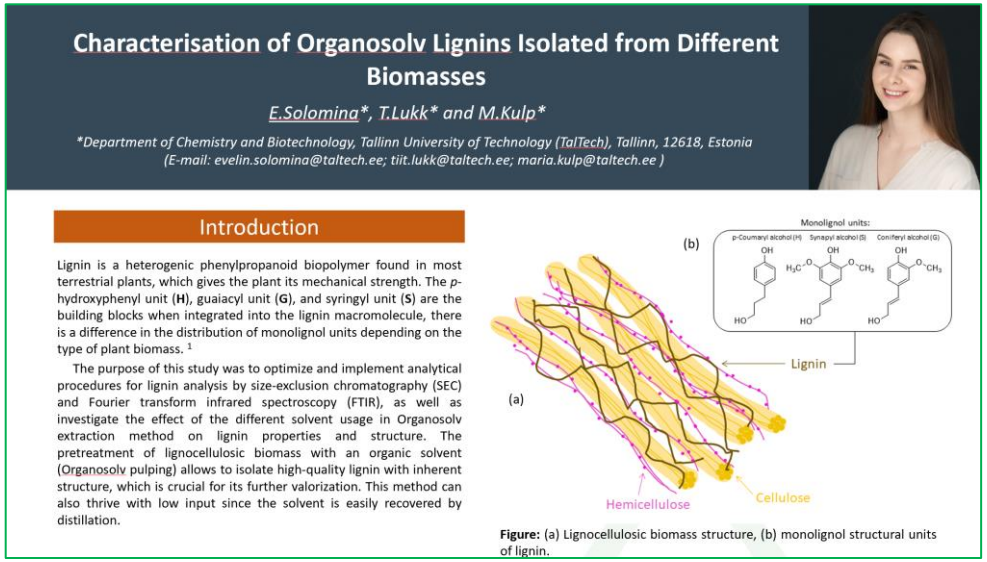
Introduction

Figure: Analyzed woods: pine, birch, spruce

The push towards greener alternatives brings lignin into consideration as a sustainable starting material, but if lignin is considered a waste-product, the quality of extracted lignin will be too low to valorize it fully.[1] To compare lignins that are collected in different conditions, better, faster, and more reliable analytical methods are needed. Analytical chemistry provides vital information about lignin extraction process, its yield and lignin purity.

Figure: Lignin monomers (H, G and S units)

Currently, most methods are to do with lignin functional groups, such as hydroxyl, methoxy, carbonyl and carboxyl groups. Elemental analysis, infrared spectroscopy and nuclear magnetic resonance spectroscopy are used to characterize lignin. Spectroscopic methods such as infrared spectroscopy are often used to build chemometric models because of the ease of use and nondestructive nature.[2]



Characterisation of Organosolv Lignins Isolated from Different Biomasses

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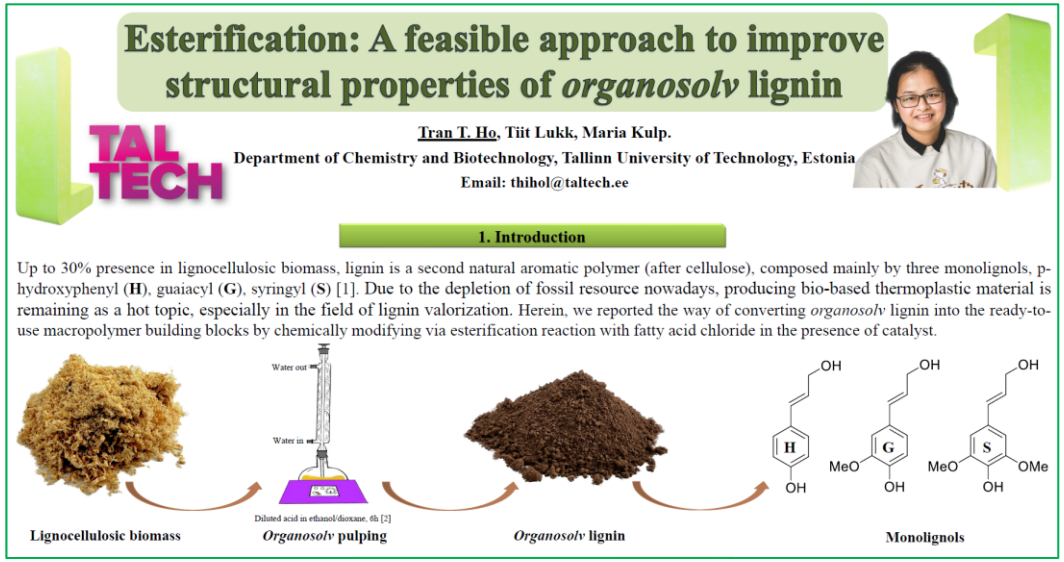
Introduction

Lignin is a heterogenic phenylpropanoid biopolymer found in most terrestrial plants, which gives the plant its mechanical strength. The *p*-hydroxyphenyl unit (**H**), guaiacyl unit (**G**), and syringyl unit (**S**) are the building blocks when integrated into the lignin macromolecule, there is a difference in the distribution of monolignol units depending on the type of plant biomass. [1]

The purpose of this study was to optimize and implement analytical procedures for lignin analysis by size-exclusion chromatography (SEC) and Fourier transform infrared spectroscopy (FTIR), as well as investigate the effect of the different solvent usage in Organosolv extraction method on lignin properties and structure. The pretreatment of lignocellulosic biomass with an organic solvent (Organosolv pulping) allows to isolate high-quality lignin with inherent structure, which is crucial for its further valorization. This method can also thrive with low input since the solvent is easily recovered by distillation.

Figure: (a) Lignocellulosic biomass structure, (b) monolignol structural units of lignin.



Esterification: A feasible approach to improve structural properties of *organosolv* lignin

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1. Introduction

Up to 30% presence in lignocellulosic biomass, lignin is a second natural aromatic polymer (after cellulose), composed mainly by three monolignols, p-hydroxyphenyl (**H**), guaiacyl (**G**), syringyl (**S**) [1]. Due to the depletion of fossil resource nowadays, producing bio-based thermoplastic material is remaining as a hot topic, especially in the field of lignin valorization. Herein, we reported the way of converting *organosolv* lignin into the ready-to-use macropolymer building blocks by chemically modifying via esterification reaction with fatty acid chloride in the presence of catalyst.

Lignocellulosic biomass — ***Organosolv* pulping** — ***Organosolv* lignin** — **Monolignols**

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Analytical chemistry laboratory at TalTech

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THANK YOU!

Support for R&D activities of resource valorization

CONCLUSIONS

- PCA is one of the most important and powerful methods in chemometrics as well as in a wealth of other areas
- PCA model provides an overview of your data
  - Scores
  - Loadings
- PCA model is an exploratory model, generating hypotheses
- Data reduction
- Useful for classification and multivariate calibration

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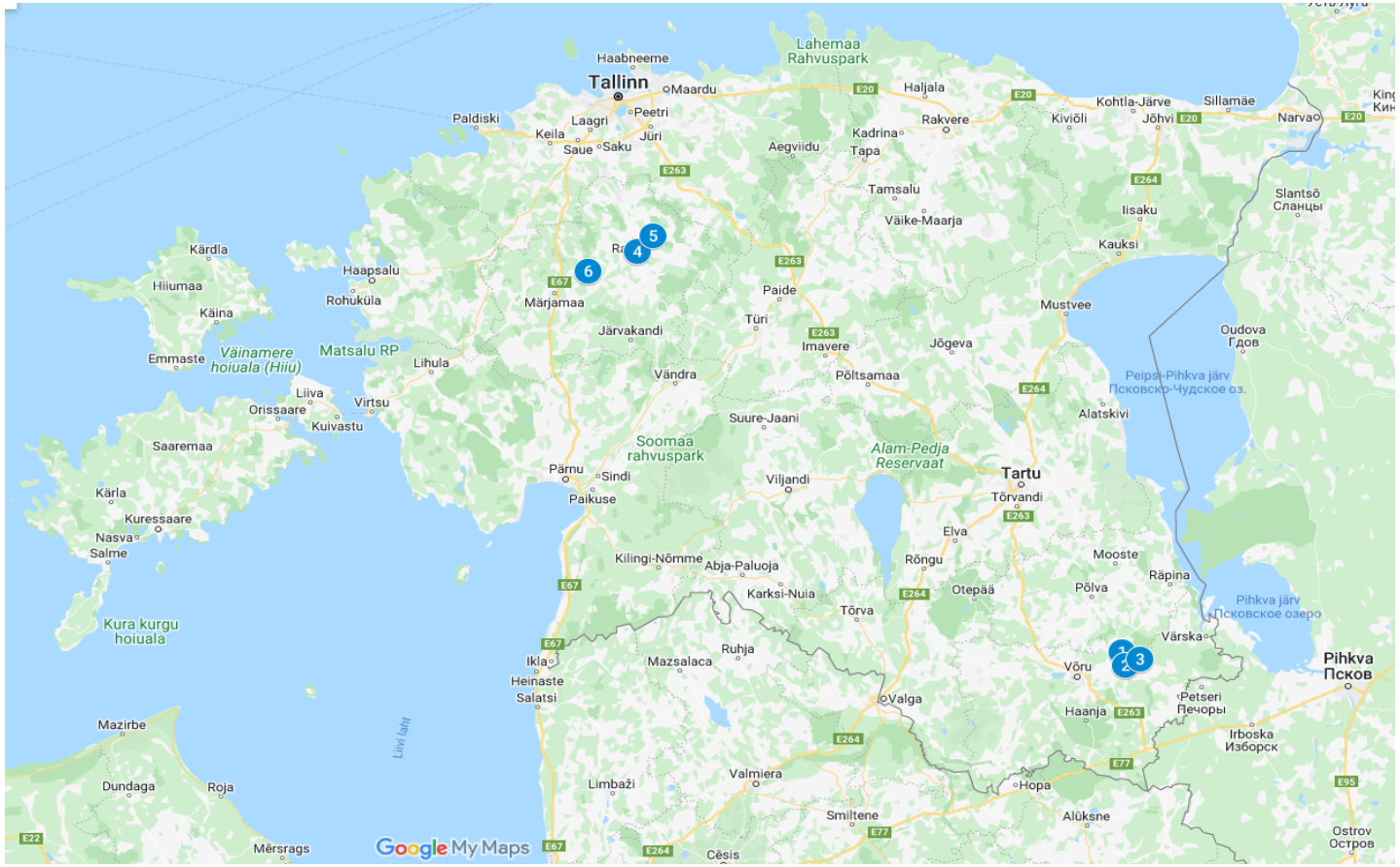

1. Vana-Saaluse
2. Pikakannu
3. Madala
4. Ridaküla
5. Maidla
6. Jalase

Principle of PCA

In the final plot in the movie, a plane is shown. That is the PCA model plane spanning the space of the two components chosen. If three principal components were chosen, we would have a three-dimensional space etc.

In the plane, the observations as well as the variable axes, can be shown by projecting these orthogonally onto the plane. The interpretation of such a *bi-plot* is in many cases, the main purpose of the initial analysis.

Two observation points that are close to each other imply that these observations are similar (as in a traditional scatter). This holds to the extent that the samples are close to the plane. If the variance explained by the model is high, then the samples are close to the plane.

The original variable axes can be projected to the new coordinate system and from these projections it can be evaluated how important these variables are. If they are positioned close to origo, then they are not influencing the model significantly and if they are located close to each other, then these variables carry essentially the same information.

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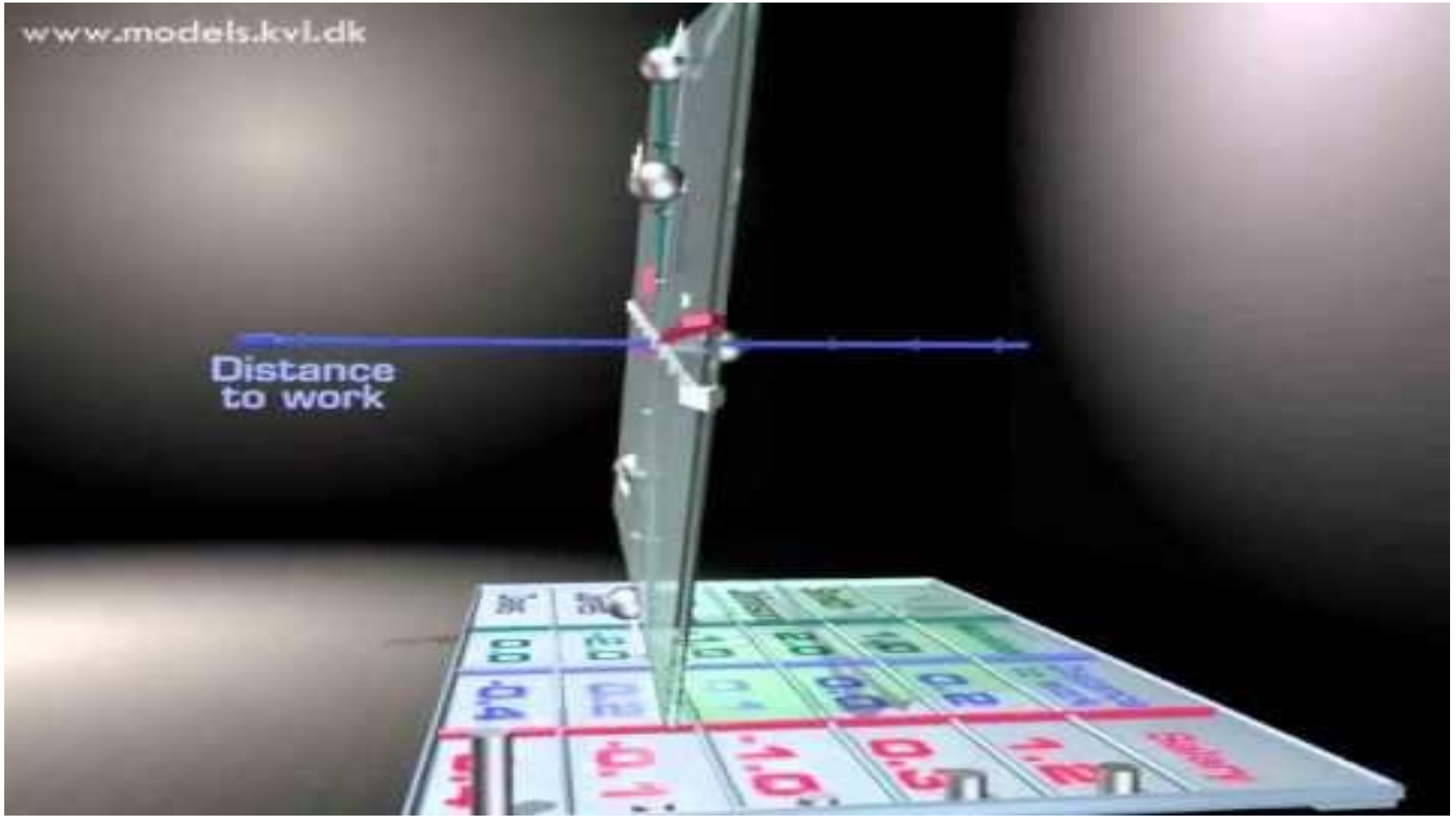
PRINCIPAL COMPONENT ANALYSIS: SIMPLIFIED



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