Μεταβονομικη

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-OMICS

GENOMICS

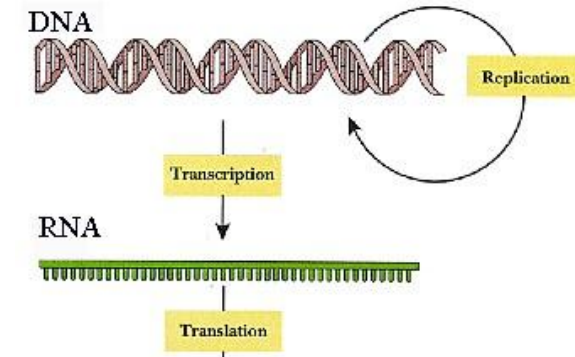
TRANSCRIPTOMICS

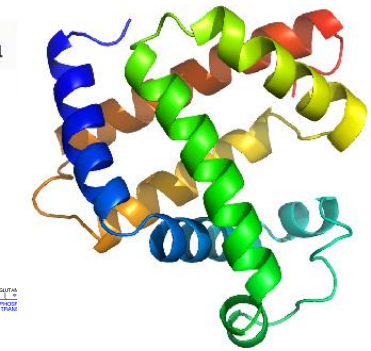
PROTEOMICS

METABONOMICS

PHENOTYPE

ENVIRONMENT



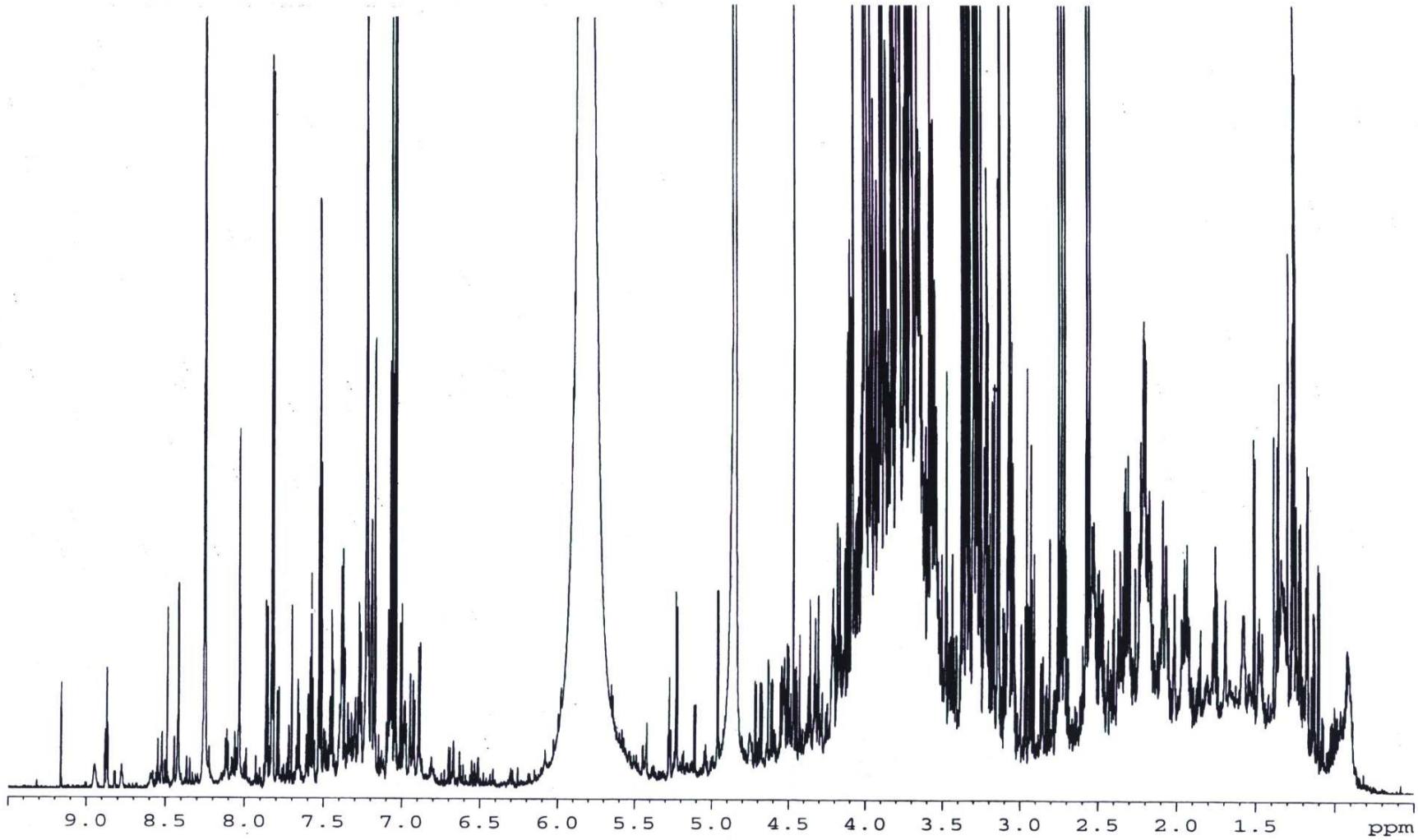


Metabonomics

Quantitative measurement of multivariate metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification

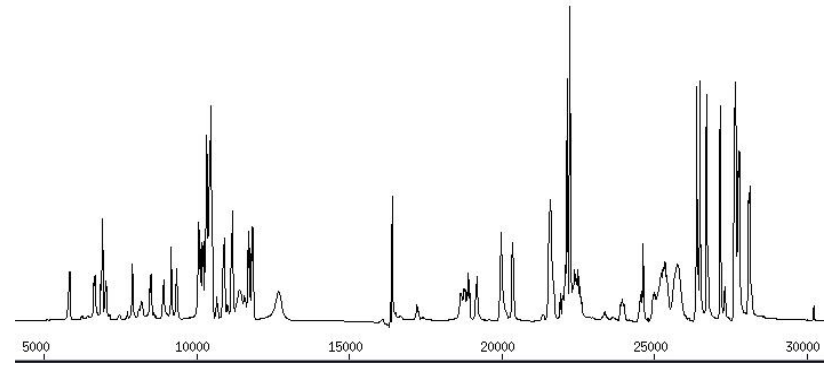
J.K. Nicholson 1999

NMR and complex mixtures

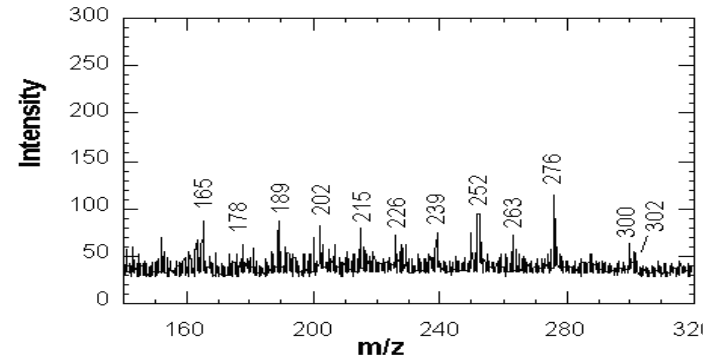


^{1}H NMR Spectrum of Untreated Human Urine

Analytical Approaches



NMR



MS

CHEMOMETRIC ANALYSIS

(pattern recognition for classification, diagnostics & biomarker analysis)

Metabolites

- Any organic molecule detectable in the body with a MW < 1000 Da
- Includes peptides, oligonucleotides, sugars, nucelosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids and drugs (xenobiotics)
- Includes mammalian & microbial products
- Concentration > 1μM

What's the Difference Between Metabonomics and Traditional Clinical Chemistry?

Throughput

(more metabolites, greater accuracy, higher speed)

Advantages

- Measure multiple (10's to 100's) of metabolites at once – no separation!!
- Allows metabolic profiles or "fingerprints" to be generated
- Mostly automated, relatively little sample preparation or derivatization
- Can be quantitative (esp. NMR)
- Analysis & results in ~15 min

NMR versus MS

NMR

- Quantitative, fast
- Requires no work up or separation
- Allows ID of 300+ cmpds at once
- Intact tissues
- Robustness
- Not sensitive
- Needs MS or 2D NMR for positive ID

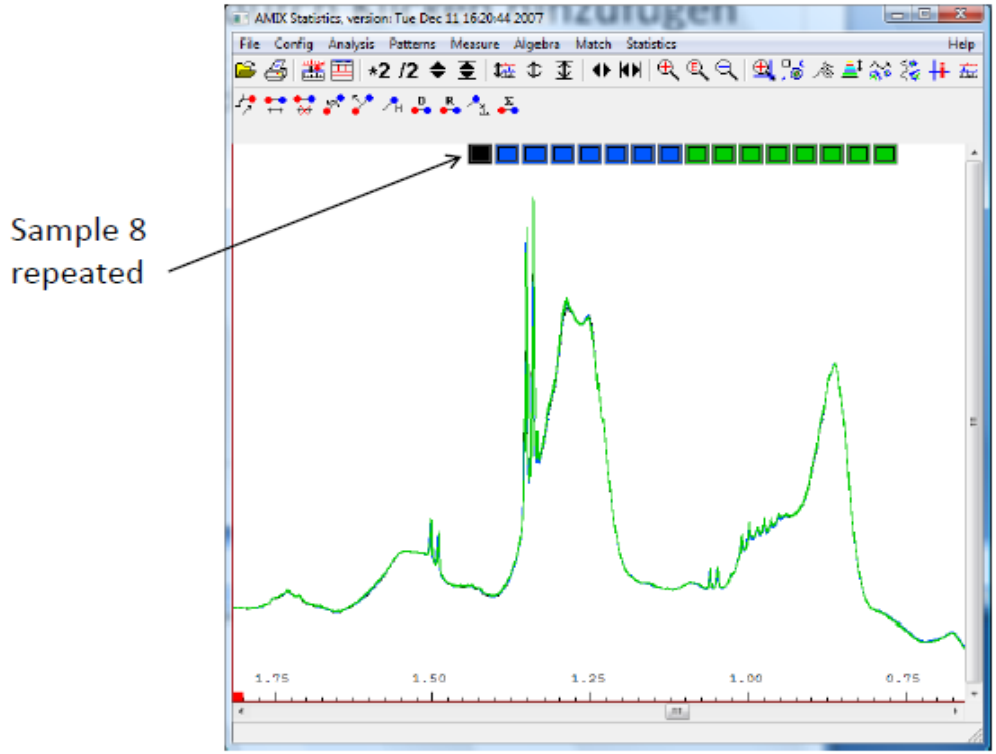
MS

- Very fast
- Very sensitive
- Allows analysis or ID of 3000+ cmpds at once
- Not quantitative
- Ion suppression
- Requires work-up
- Needs NMR for ID
- Peak alignment of LC-MS

NMR reproducibility

Plasma NMR

2 sample sets split into 2 aliquots each
1 measured in Germany, 1 in Holland



Sample 8
repeated

Bruker
LUMC

Leiden
University
Medical
Center

NMR reproducibility

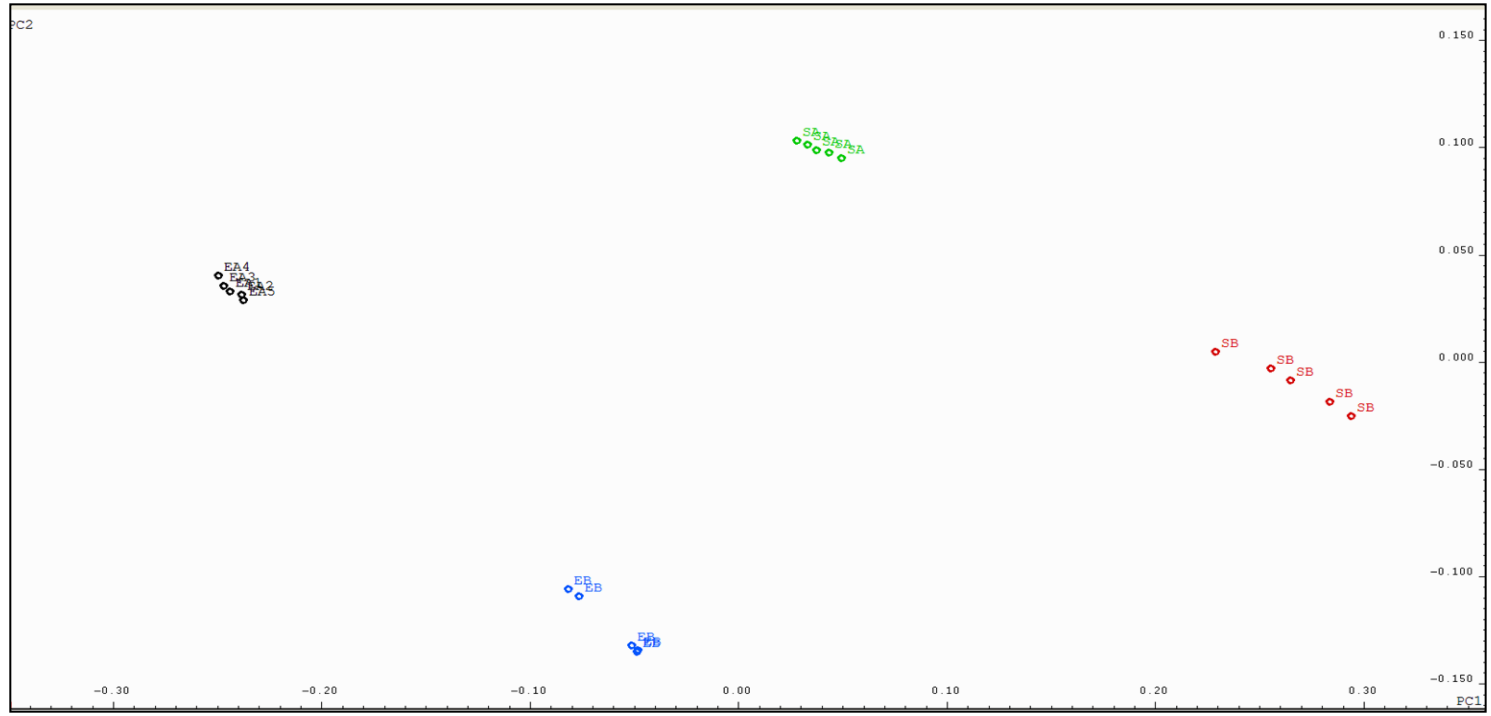
Urine samples Athens

- Buffer A (PBS pH=7.4): original from Bruker with NaN_3
- Buffer B (PBS pH=7.4): local preparation

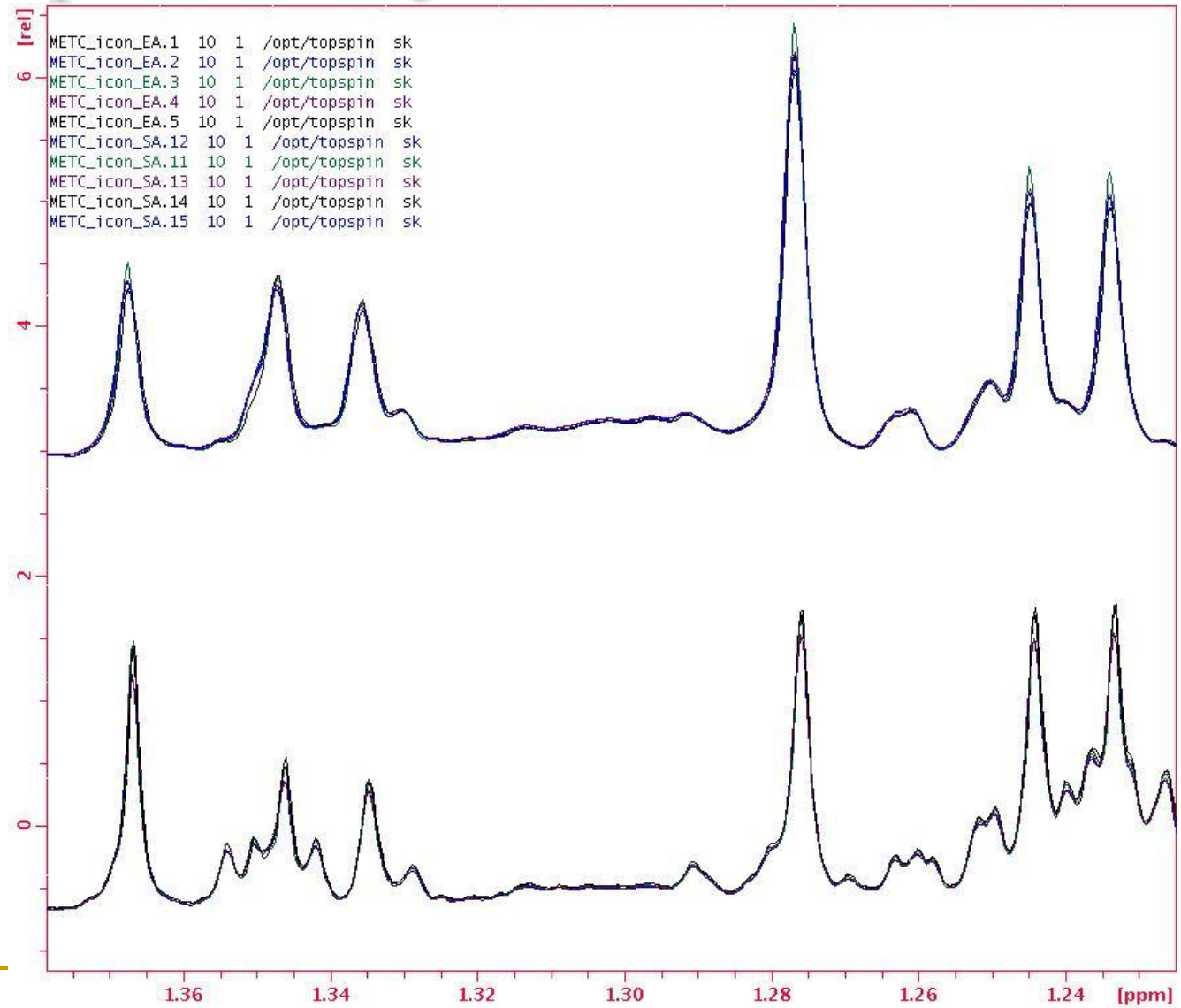
- Urine:
  - Sample 1-5 person E buffer A 1EA – 5EA
  - Sample 1-5 person E buffer B 1EB – 5EB
  - Sample 1-5 person S buffer A 1SA – 5SA
  - Sample 1-5 person S buffer B 1SB – 5SB

NMR reproducibility

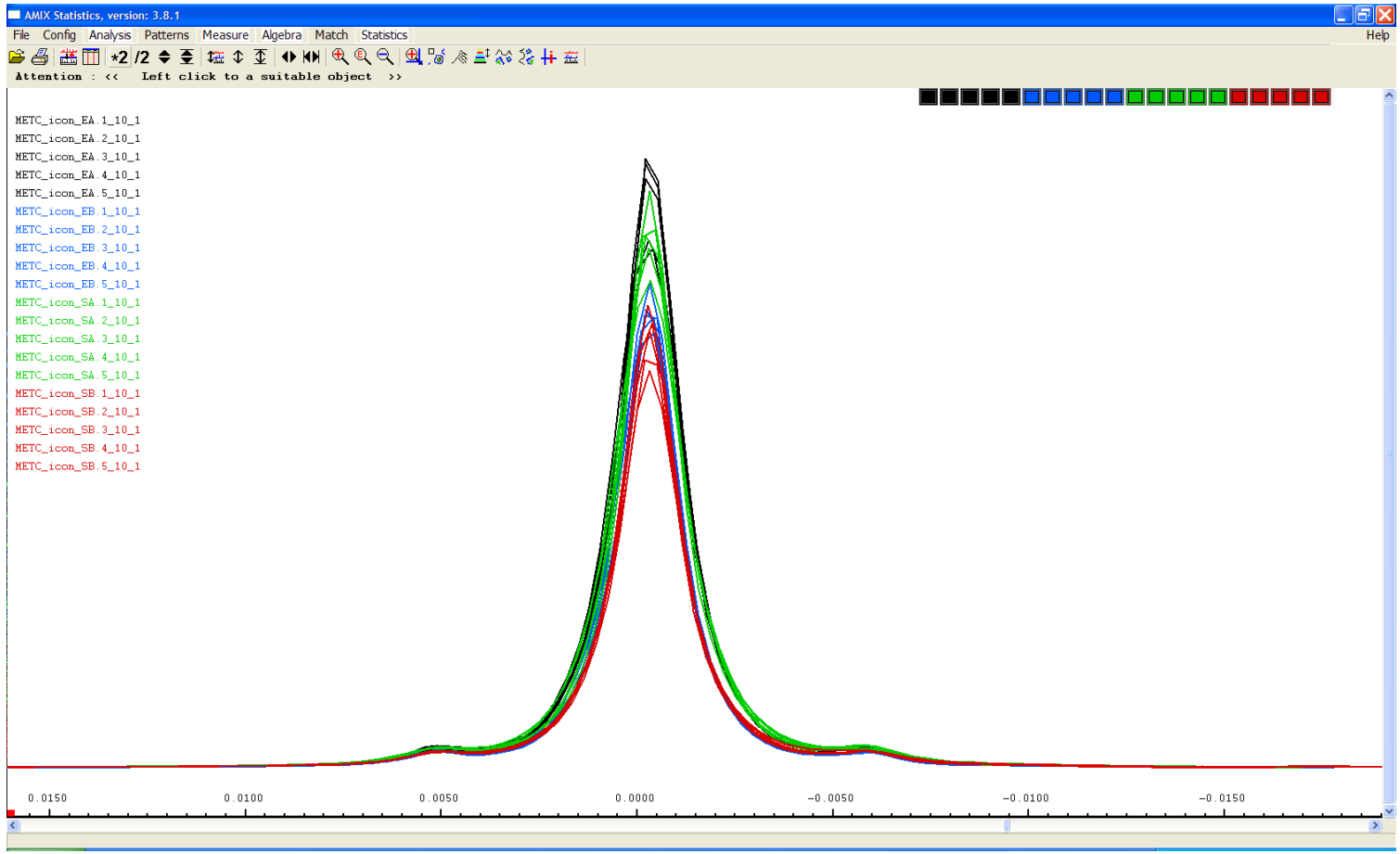
Spectral data scaled on TSP
in-group variance mainly caused by variation urine/buffer mixing procedure



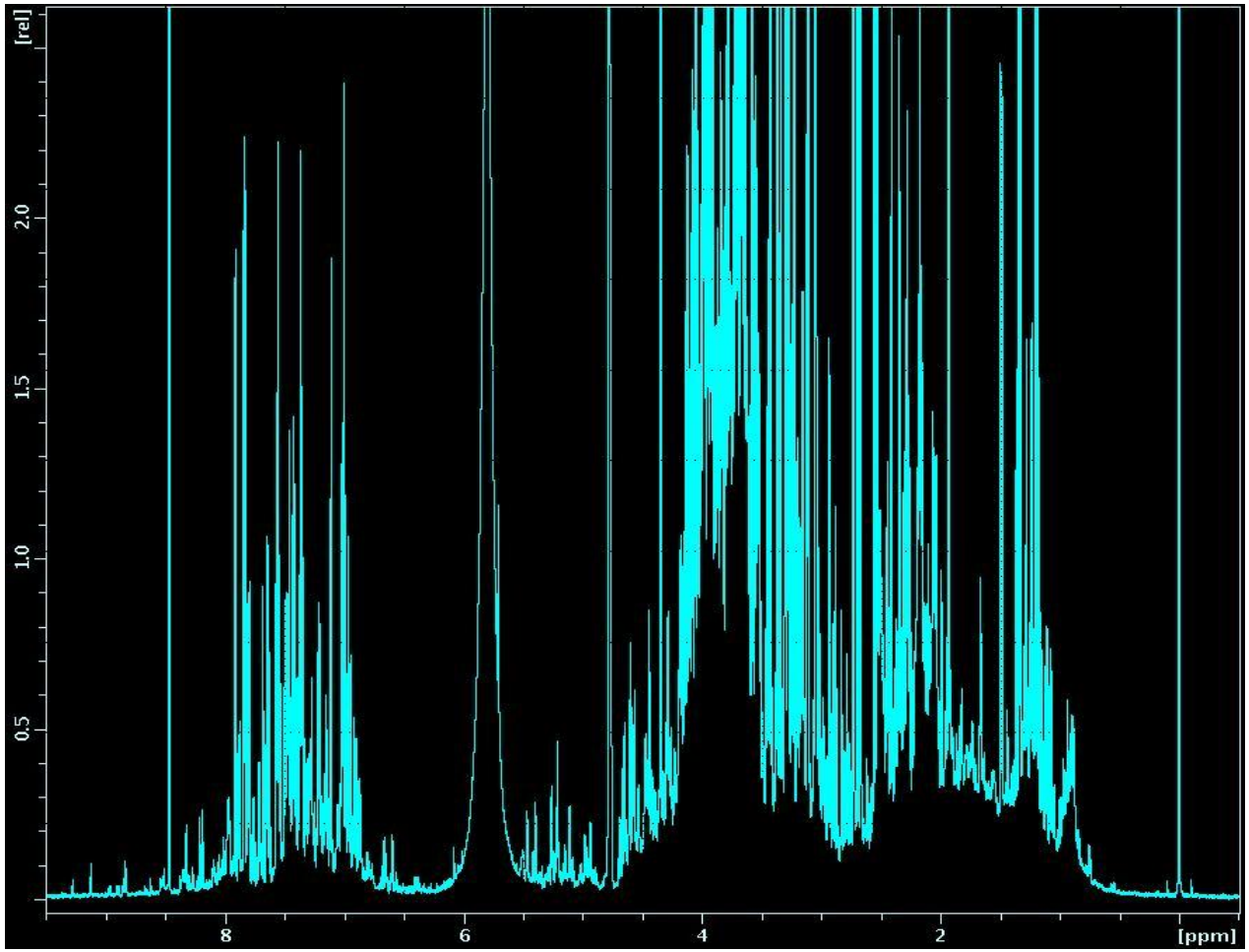
NMR *reproducibility*



NMR *reproducibility*



Resulting 1D NOESY spectra of urine



600 MHz 1D NOESY spectrum of urine

Assignment process

Literature and
web databases (HMDB)

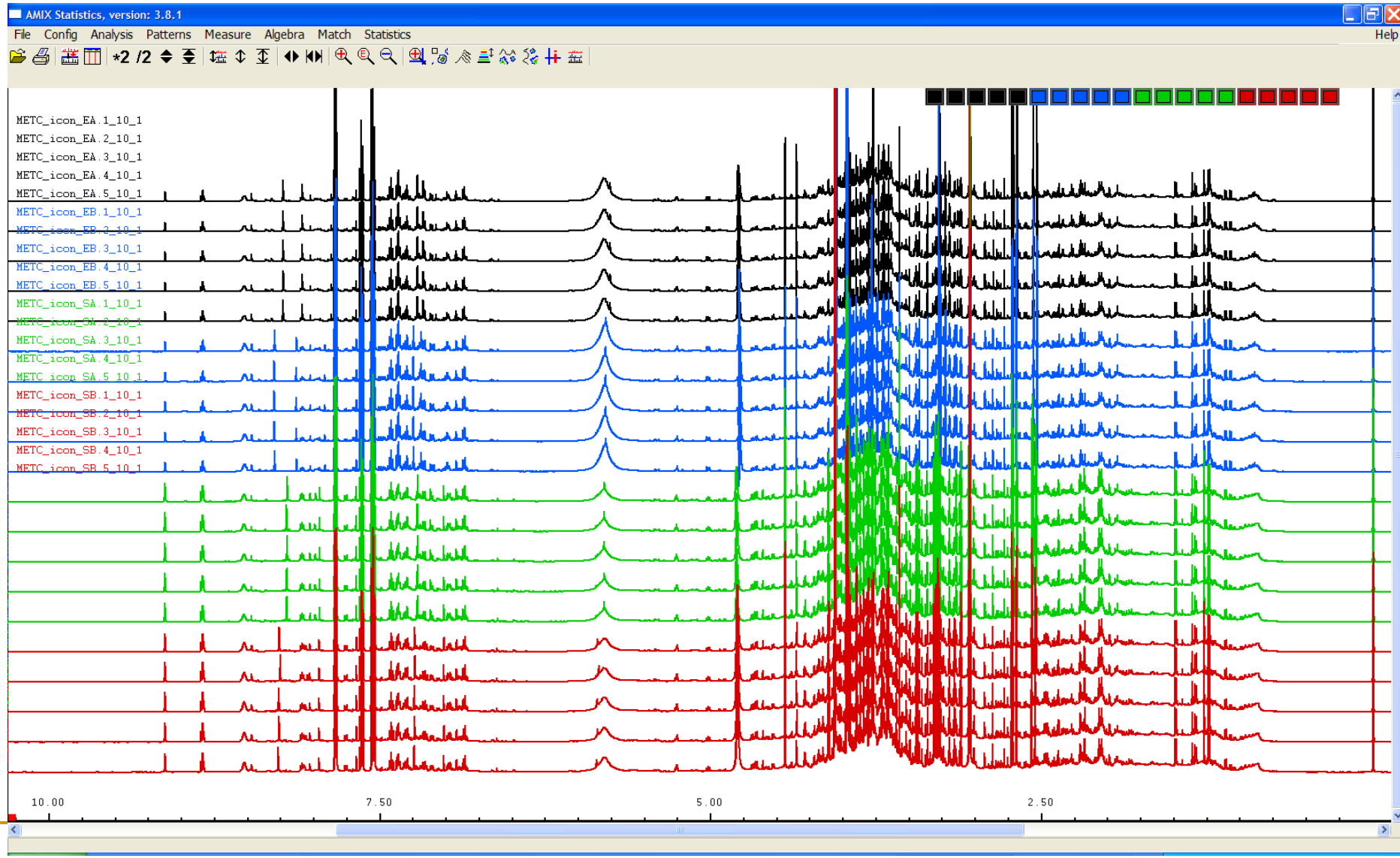
Urine spectra:

Highly complex

Contains tens or hundreds of metabolites

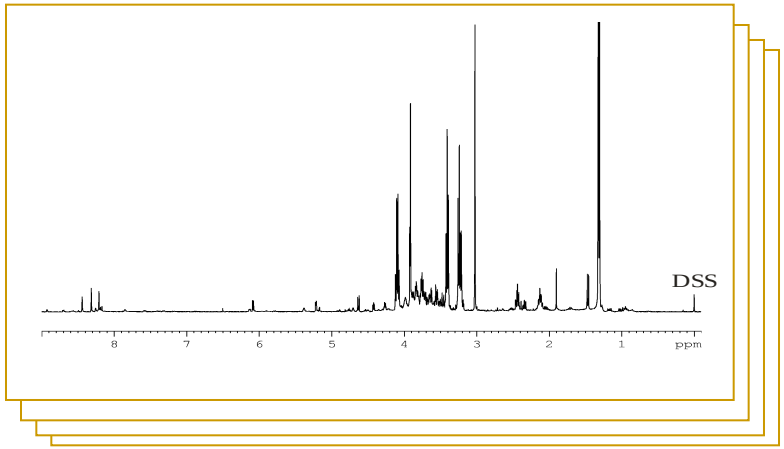
Assignment cannot be unambiguous without additional tools

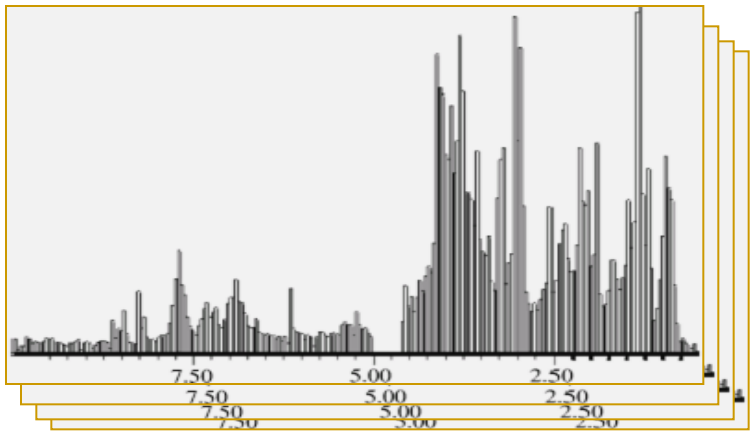
Need for chemometrics



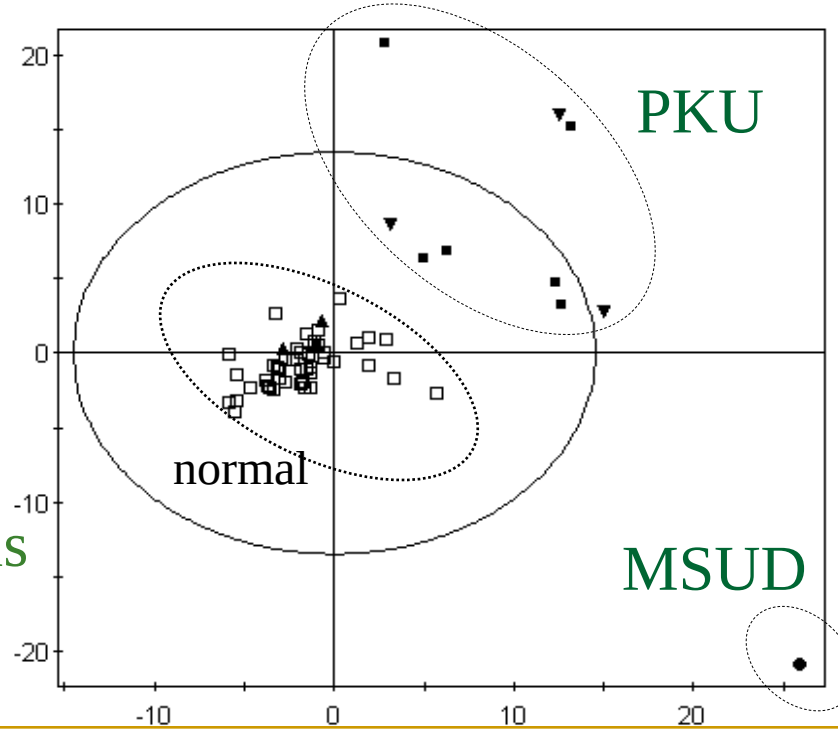
Standard Procedure

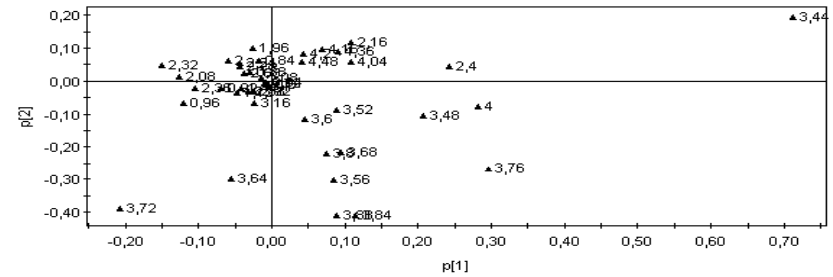
Measure the metabolite profile





statistical analysis
classification





gain mechanistic insight

Application of NMR spectroscopy combined with principal component analysis in detecting inborn errors of metabolism using blood spots. A metabonomic approach

M.A. Constantinou, E. Papakonstantinou, M. Spraul, K. Shulpis, M.A. Koupparis, E. Mikros *Analytica Chimica Acta*, 511, 303-312, 2004

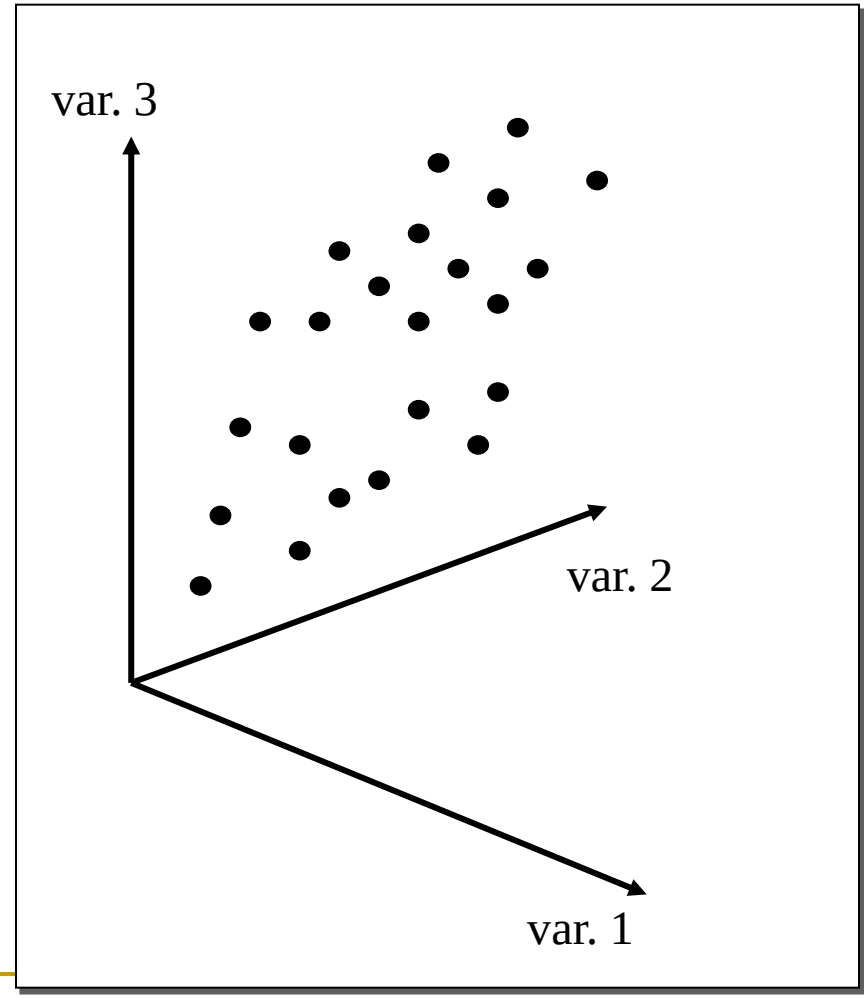
Principal Component Analysis
PCA

A multivariate statistical approach that facilitates the identification of differences or similarities between groups

Data preparation

Data table → variable space

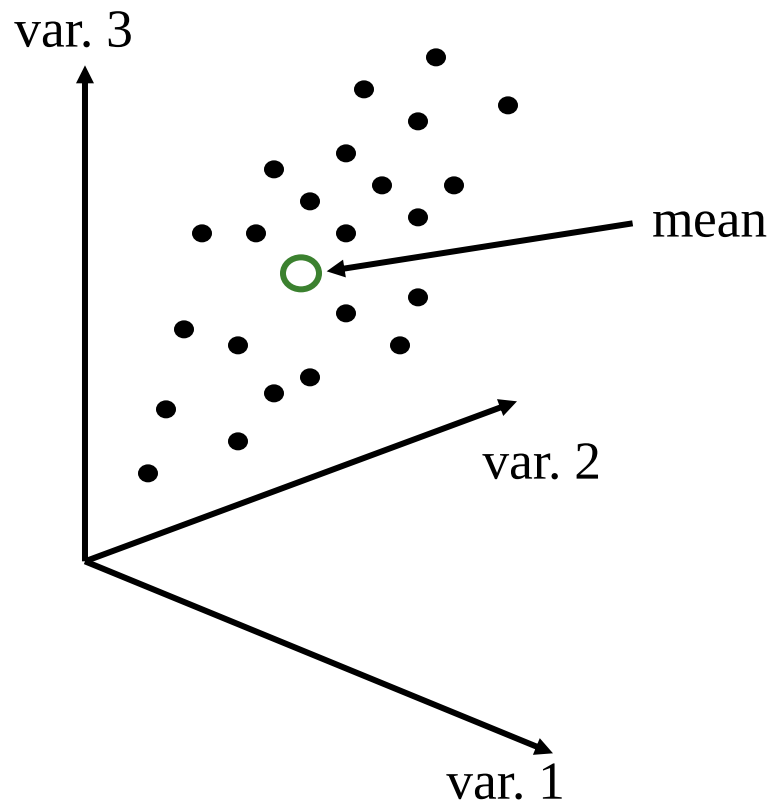
	var. 1	var. 2	var. 3
1			
2			
3			
4			
5			
6			
N			



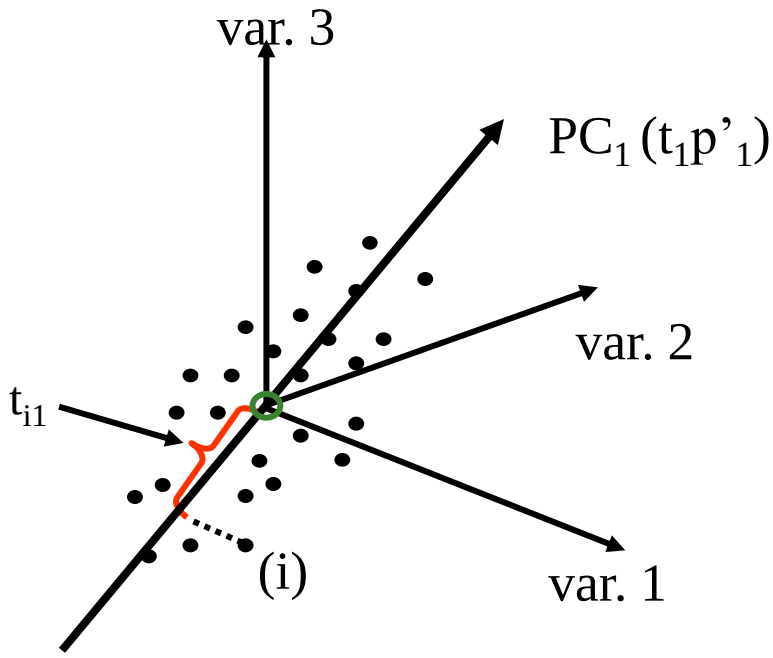
The whole table yields a swarm of points in variable space

Pre-processing

•Centering – move centre of point swarm to the variable origin



PCA theory – step by step



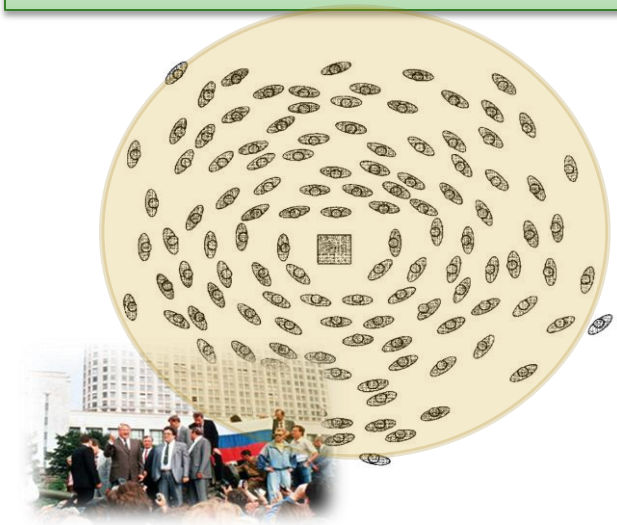
The first principal component (PC_1) is set to describe the largest variation in the data, which is the same as the direction in which the points spread most in the variable space

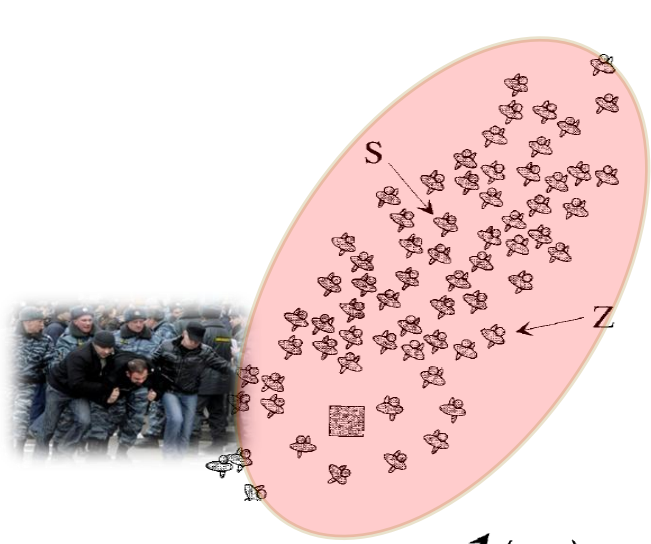
The Score value (t_{i1}) for the point i is the distance from the projection of the point on the 1:st component to the origin.

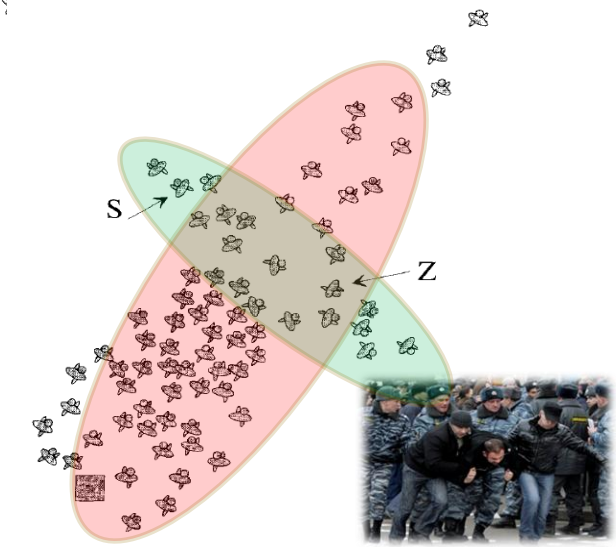
PC_1 hence is the first latent variable in a new coordinate system that describes the variation in the data.

Πολυ-μεταβλητή ανάλυση δεδομένων πολυ-γρήγορα – το παράδειγμα του Yeltsin

Introduction to multivariate methodology, an alternative way?
Olav H.J. Christie, Chemometrics and Intelligent Laboratory Systems 29 (1995) 1777188

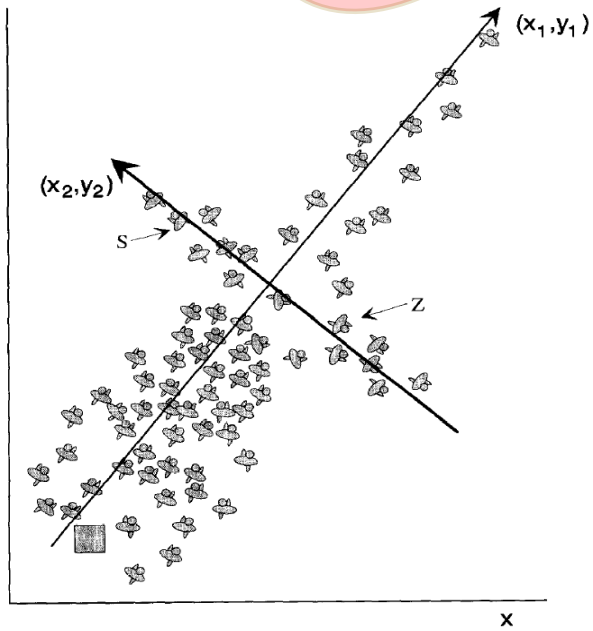






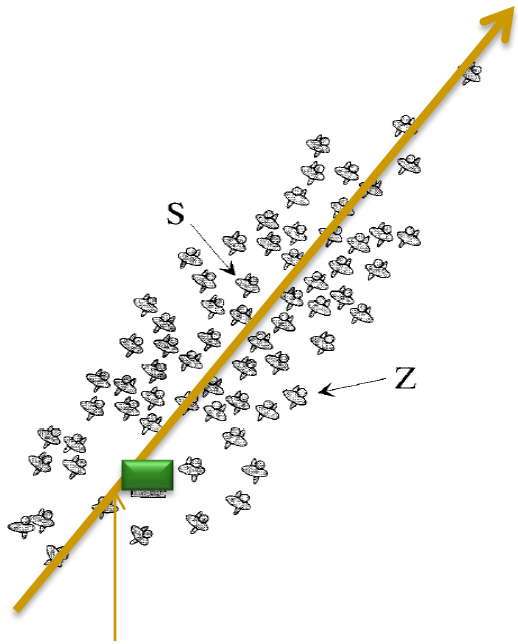
Εαν μια διαδικασία επηρρεάζει ένα σύστημα δημιουργεί μια δομή στα δεδομένα και... Αντίστροφα

Η επέμβαση της αστυνομίας δημιουργησε ροή στην κατεύθυνση των θεατών



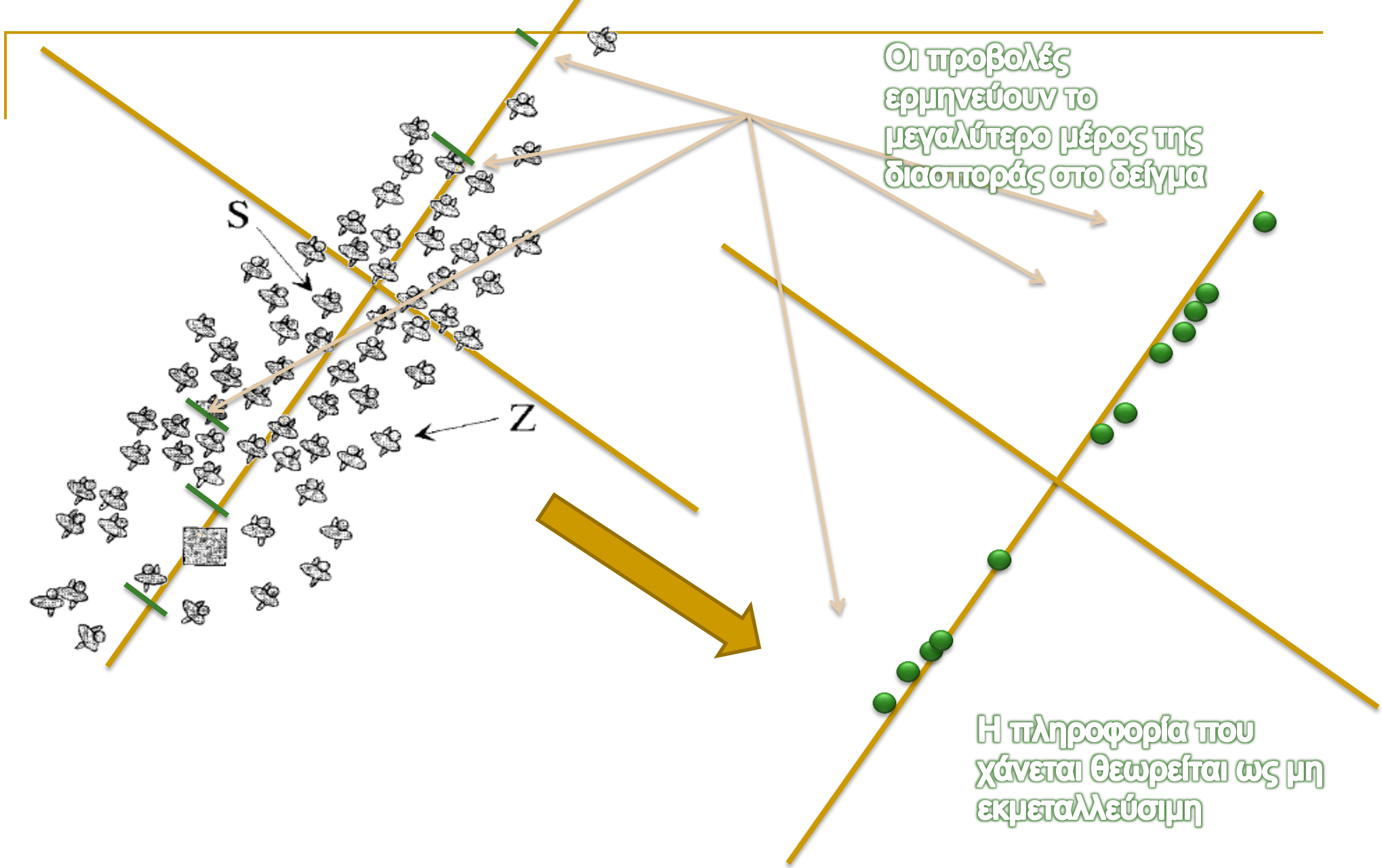
π.χ. αν υπάρχει μια ασθένεια τότε δημιουργεί μια δομή δεδομένων στους μεταβολίτες

Μας ενδιαφέρει η κύρια δομή στα δεδομένα



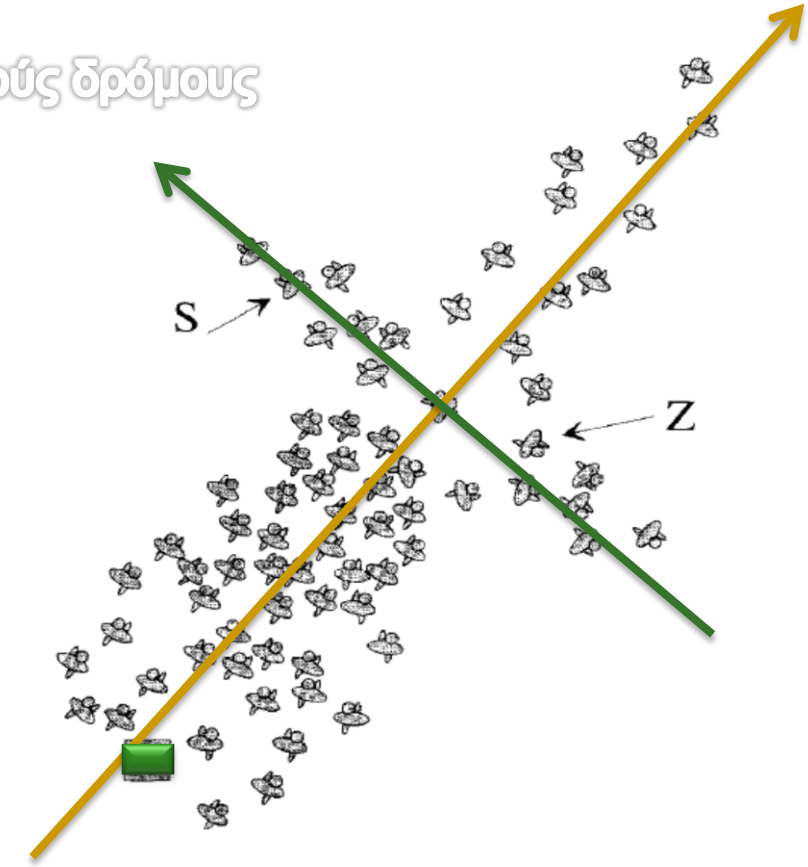
Οι διαδηλωτές απομακρύνονται από την εξέδρα

Η πληροφορία (variability) βρίσκεται προς αυτή την κατεύθυνση

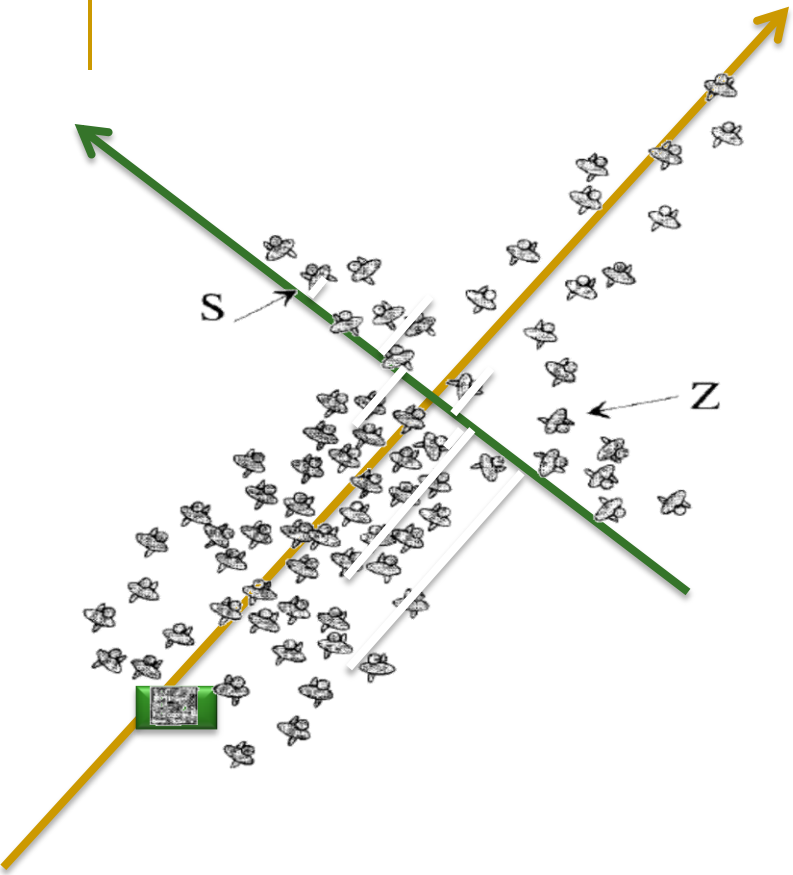


Αλλά τώρα είναι πολύ πιο εύκολη η ερμηνεία!!!

Διασπορά σε γεπονικούς δρόμους

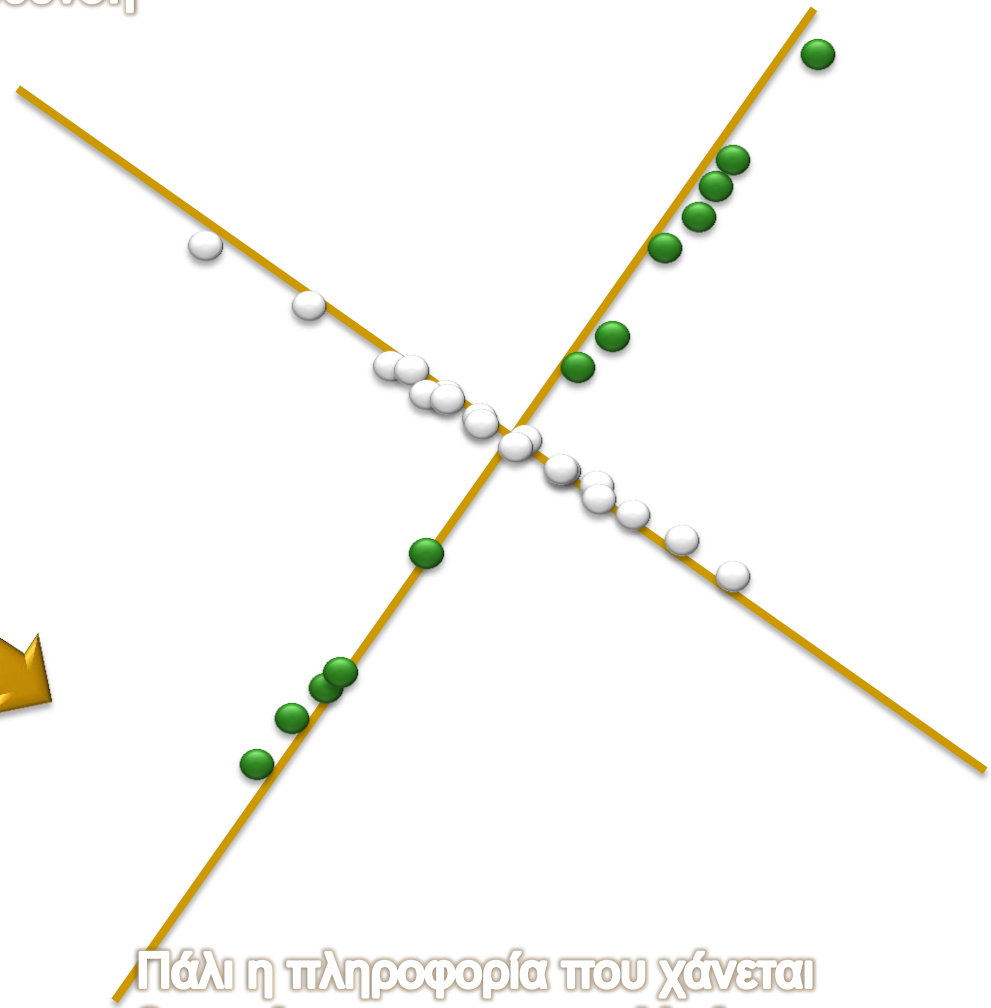


Η πληροφορία – διασπορά (variability) βρίσκεται τώρα και προς αυτή την **κατεύθυνση** που είναι ορθογώνια (ασυσχέτιστη) ως προς την πρώτη



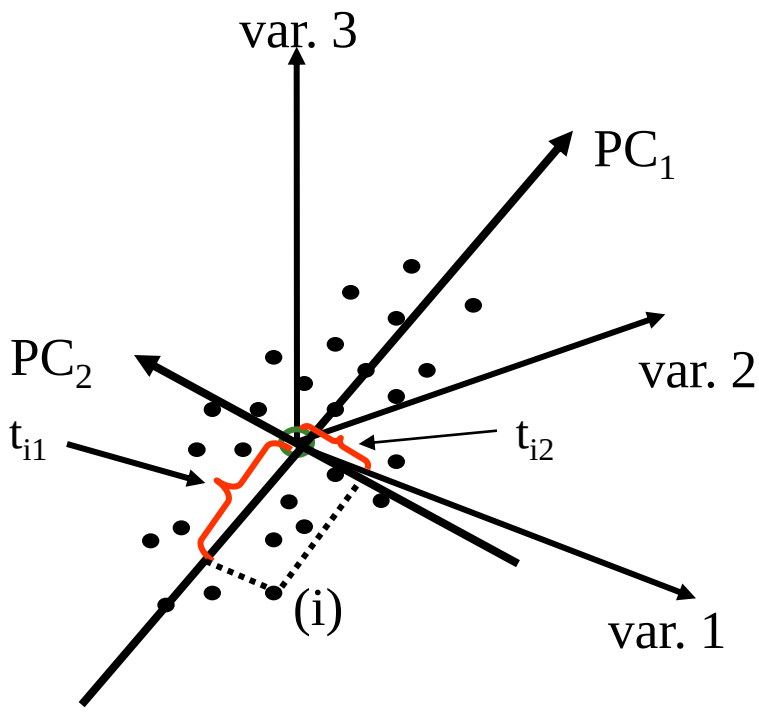
Principal Component Analysis
(ανάλυση κυρίων συνιστωσών)

Προβολή στον άξονα των Y: Ερμηνεύουν το δεύτερο μεγαλύτερο μέρος της διασποράς στο δείγμα που δεν συσχετίζεται με την πρώτη κατεύθυνση



Πάλι η πληροφορία που χάνεται θεωρείται ως μη εκμεταλλεύσιμη (redundant)

PCA theory – step by step



The second principal component (PC_2)
is set to describe the largest variation in the data,
Perpendicular (orthogonal) to the 1:st component

PCA theory – step by step

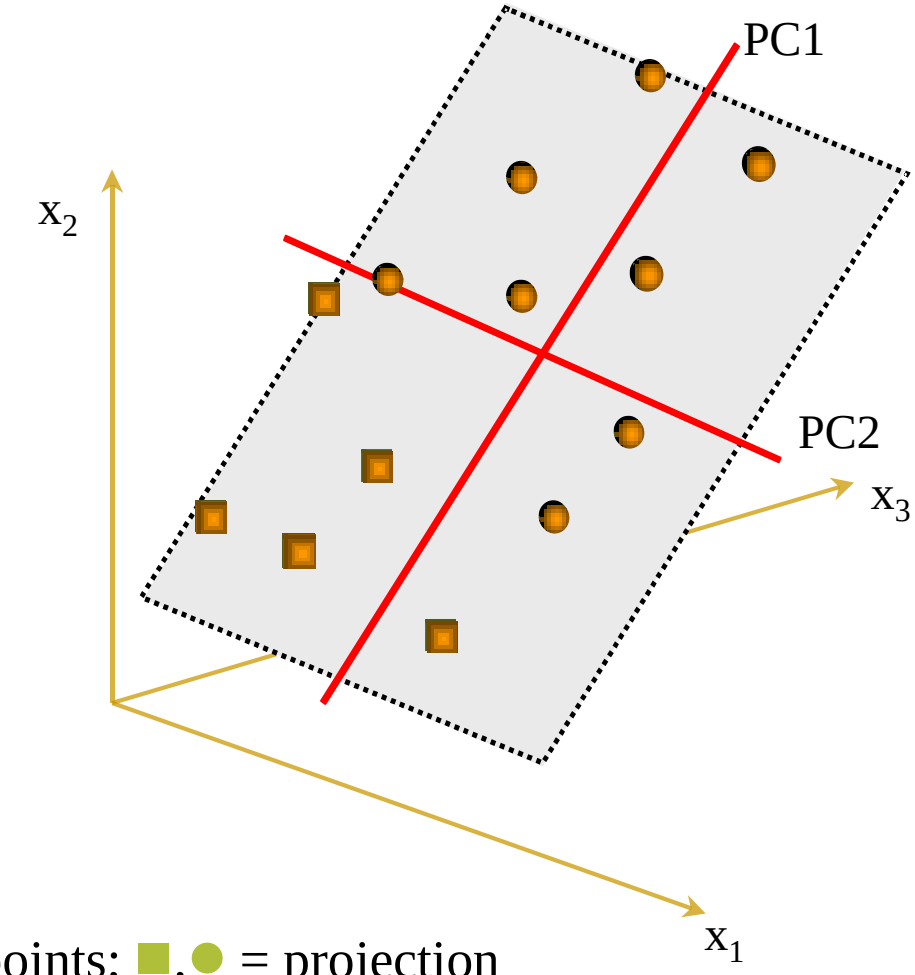
•Two PCs make a plane (window) in the K-dimensional variable space. The points are projected down onto the plane which is lifted out and viewed as a two dimensional plot.

•This is the scores plot

- •similarities or differences between samples can now be seen.

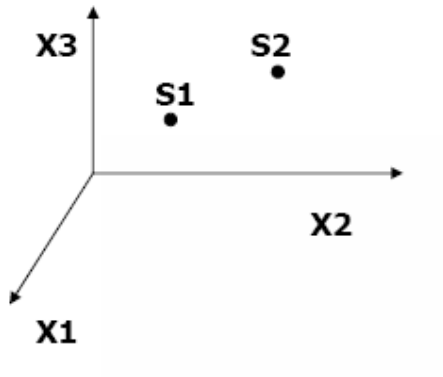
•A corresponding loading plot describes the variables relationships

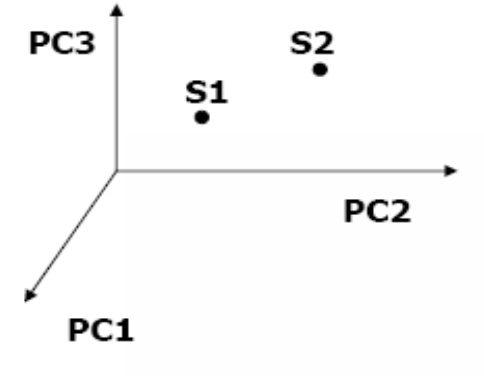
- •allows interpretation of the scores plot by showing which variables are responsible for similarities and differences between samples.



■,●=data points; ■,● = projection

PCA



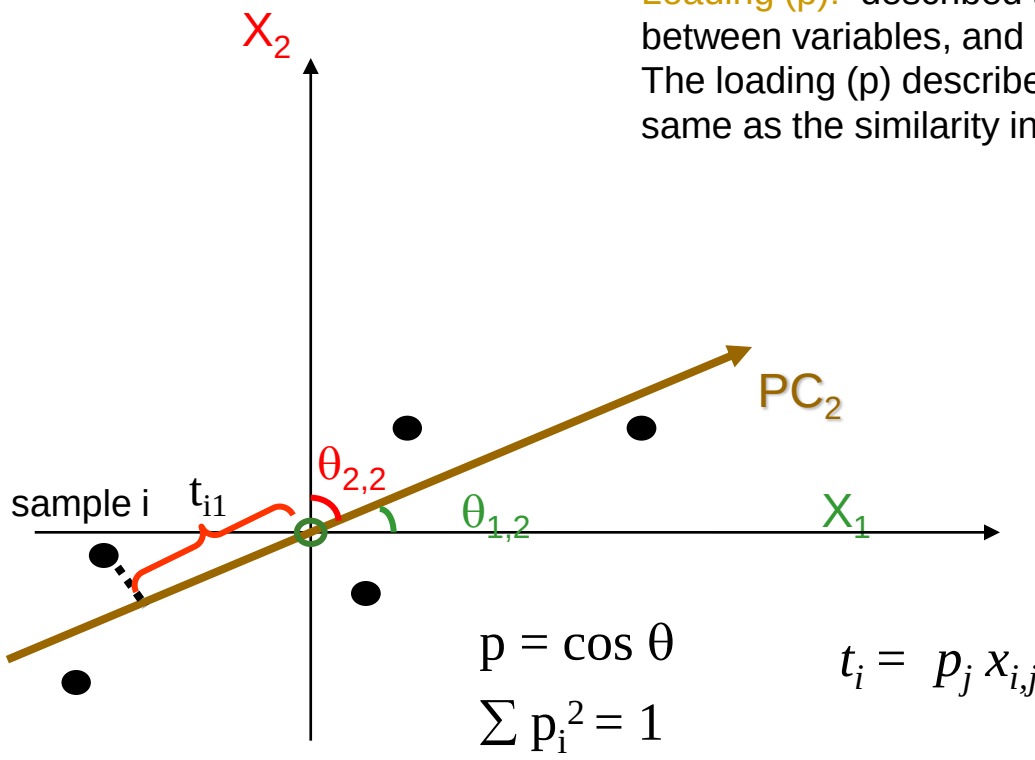


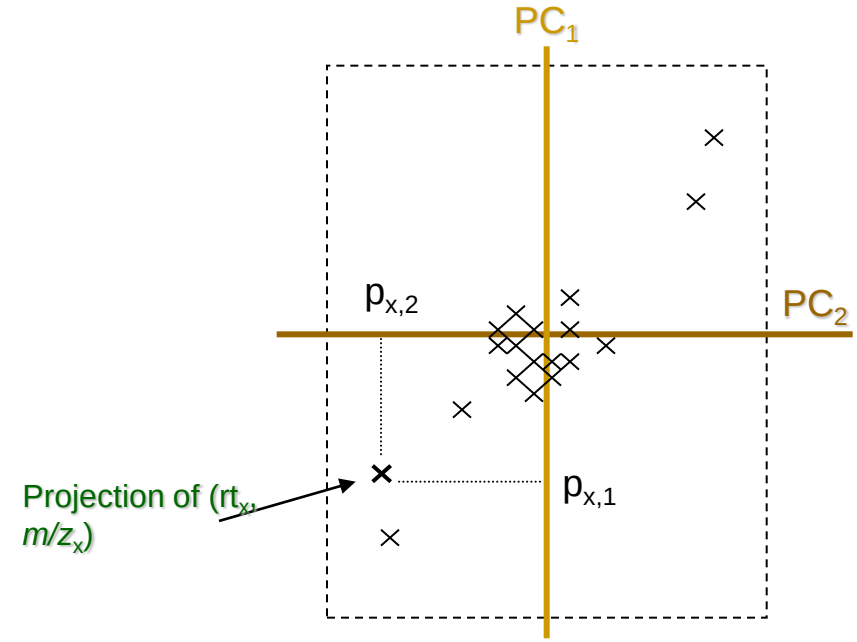
	X1	X2	X3
S1	a11	a12	a13
S2	a21	a22	a23

	PC1	PC2	PC3
S1	t11	t12	t13
S2	t21	t22	t23

The Loadings Plots

Loading (p): described the variation in the variable direction i.e. similarity/ dissimilarity between variables, and also explains the variation in scores.
The loading (p) describes the original variables importance for respective PC. This is the same as the similarity in direction between the original variable and the PC.

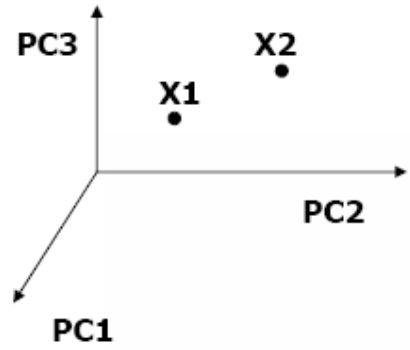




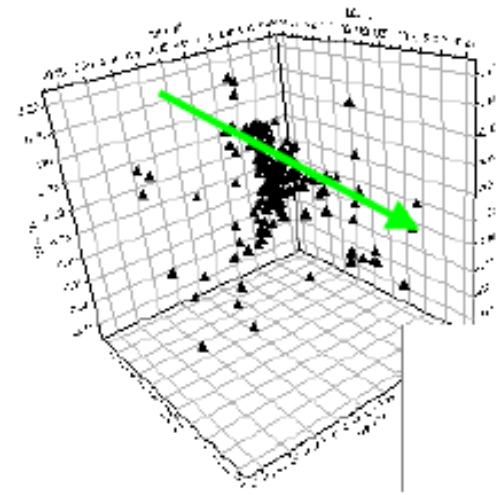
The loading (p) is described as the cosine of the angle between the original variable and the PC.

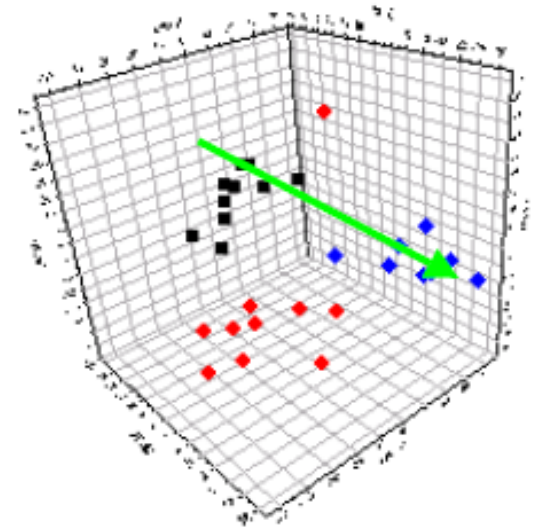
With $p_{x,1} = \cos(\theta_{x,1})$ and $p_{x,2} = \cos(\theta_{x,2})$
and $\theta_{x,1}$: angle between axe (rt_x, m/z_x) and PC1
and $\theta_{x,2}$: angle between axe (rt_x, m/z_x) and PC2

The Loadings Plots



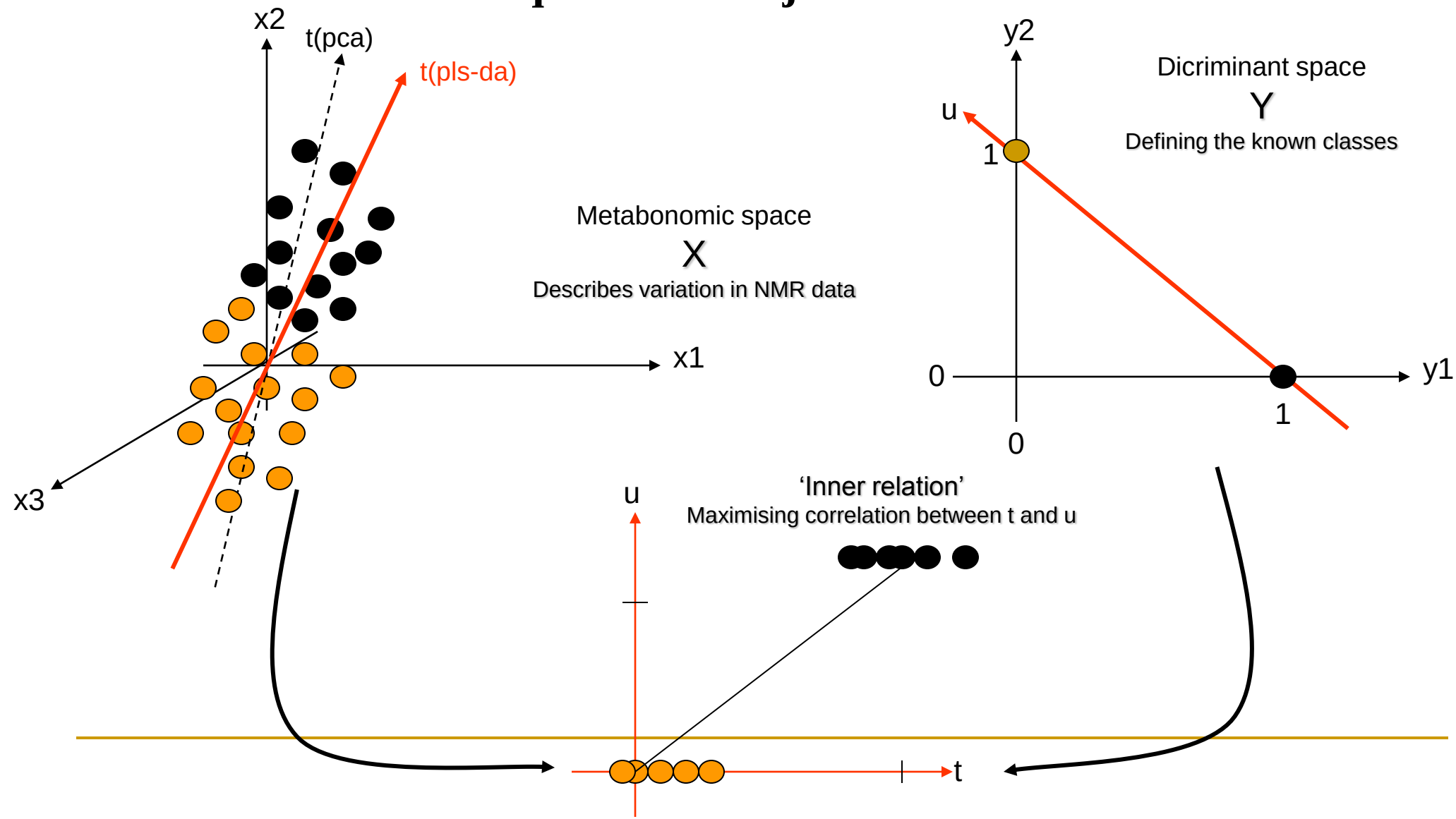
	PC1	PC2	PC3
X1	p11	p12	p13
X2	p21	p22	p23





PLS-DA

Partial Least Squares or Projection to latent structure.

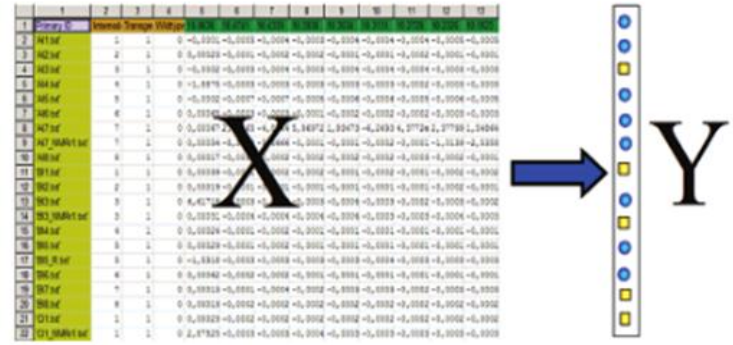


PLS

PLS Partial Least Squares ή Projections to Latent Structures

Παρόμοια αρχή με PCA
Χρησιμοποιούνται δύο πίνακες εισαγωγής δεδομένων: ένας X (όπως στο PCA) και ένας Y που περιέχει ποιοτικές μεταβλητές όπως π.χ. βιολογική απόκριση
Ο αλγόριθμος μεγιστοποιεί την συμμεταβολή μεταξύ των X και Y.

Εποπτευόμενη μέθοδος → π.χ. ο χρήστης αποδίδει σε ομάδες τις παρατηρήσεις → κατασκευή μοντέλου → χρήση ως μοντέλου πρόβλεψης

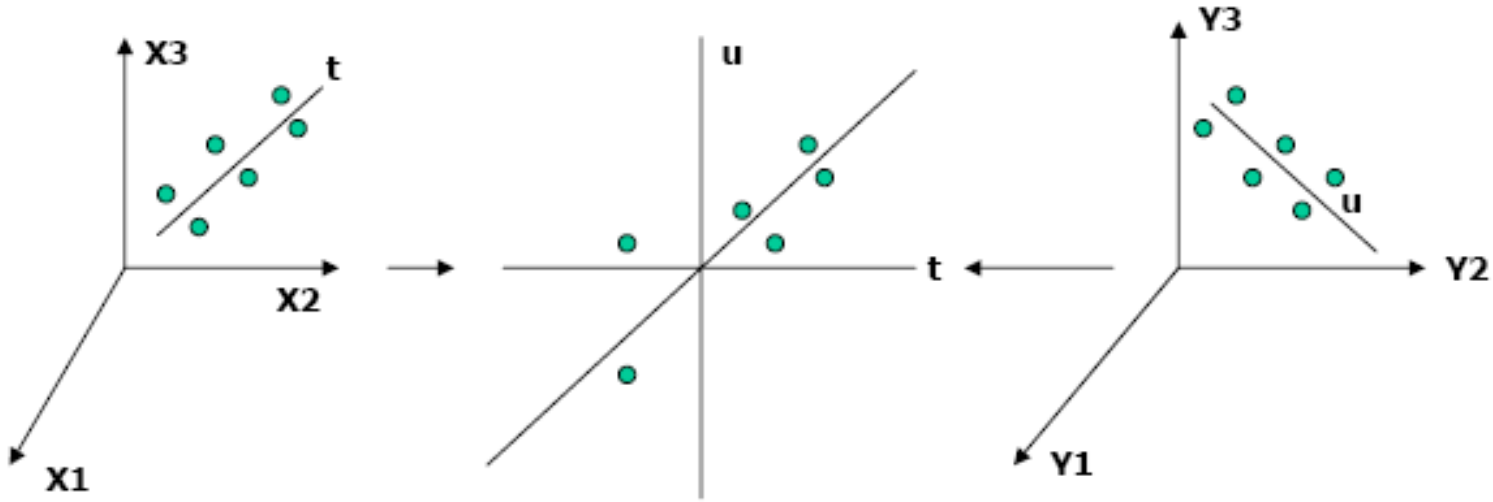


Class information can also be used to construct an additional matrix, hereinafter called the **Y** matrix, consisting of a discrete 'dummy' variable where [1]/[0] indicate the class belonging.

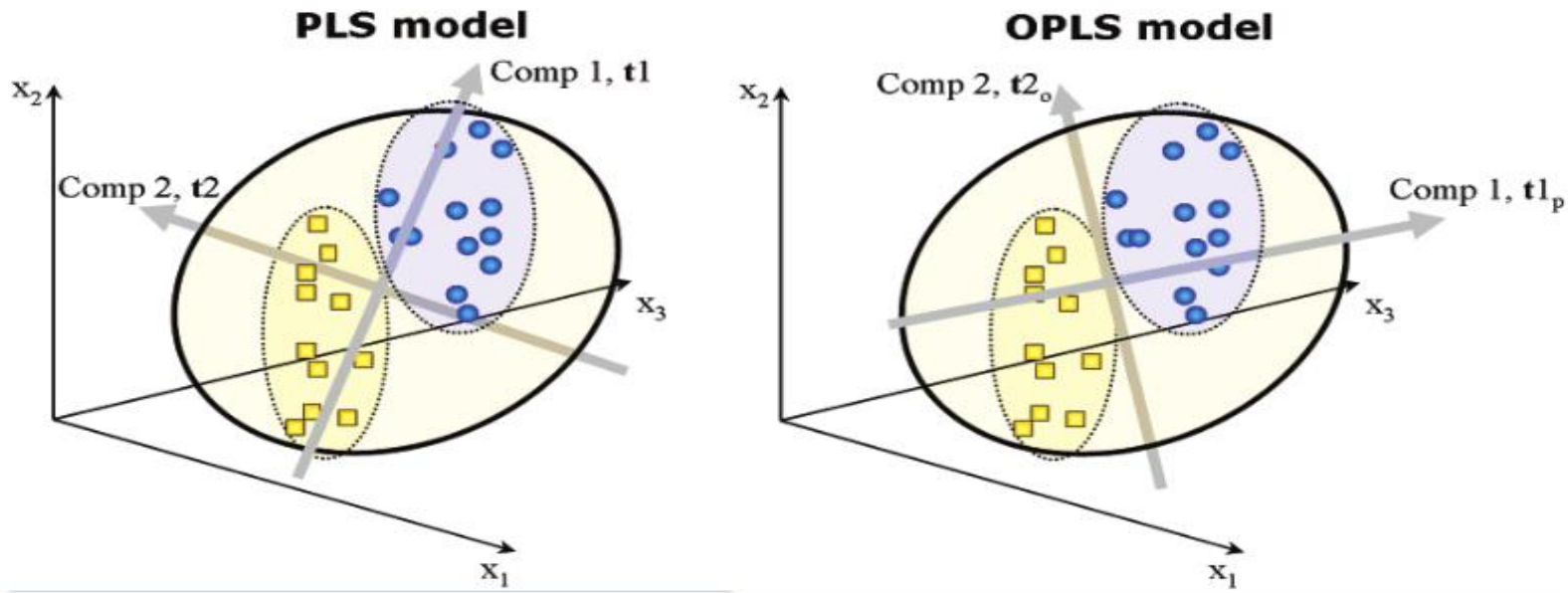
PLS-DA

Partial Least Squares or Projection to latent structure.

Partial least squares (PLS) is a method for constructing predictive models when the factors are many and highly collinear.



Models both the X & Y matrices simultaneously to find the latent variables in x that will predict the latent variables in Y the best. These PLS-Components are similar to principal components and will also be referred to as PCs.



A geometrical illustration of the difference between the PLS-DA and OPLS-DA models. In the left panel, the PLS components cannot separate the between-class variation from the within-class variation, and the resulting PLS component loadings mixes both types of variations. In the right panel, the OPLS components are able to separate these two different variations. Component 1 ($\mathbf{t1}_p$) is the predictive component and displays the between-class ([blue circles], [yellow squares]) variation of the samples. The corresponding loading profile can be used for identifying variables important for the class separation. Component 2 ($\mathbf{t2}_o$) is the **Y**-orthogonal component and models the within group (within-class) variation.

Metabonomics Applications

- Diagnosis
- Drug toxicity
- Phenotype variations

Inborn Errors of Metabolism (IEM)

APS EDITION

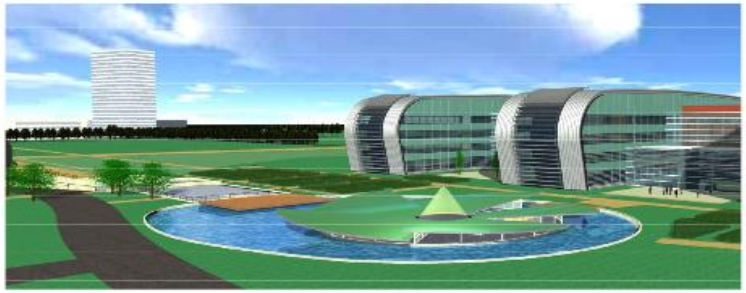
Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen

in der Deutschen Gesellschaft für Kinderheilkunde und Jugendmedizin

Handbook of ^{1}H-NMR spectroscopy in inborn errors of metabolism:

body fluid NMR spectroscopy and in vivo MR spectroscopy

U.F.H. Engelke, S.H. Moolenaar, S.M.G.C. Hoenderop, E. Morava, M. van der Graaf, A. Heerschap and R.A. Wevers



UMC St Radboud

SPS PUBLICATIONS

NMR-based newborn screening

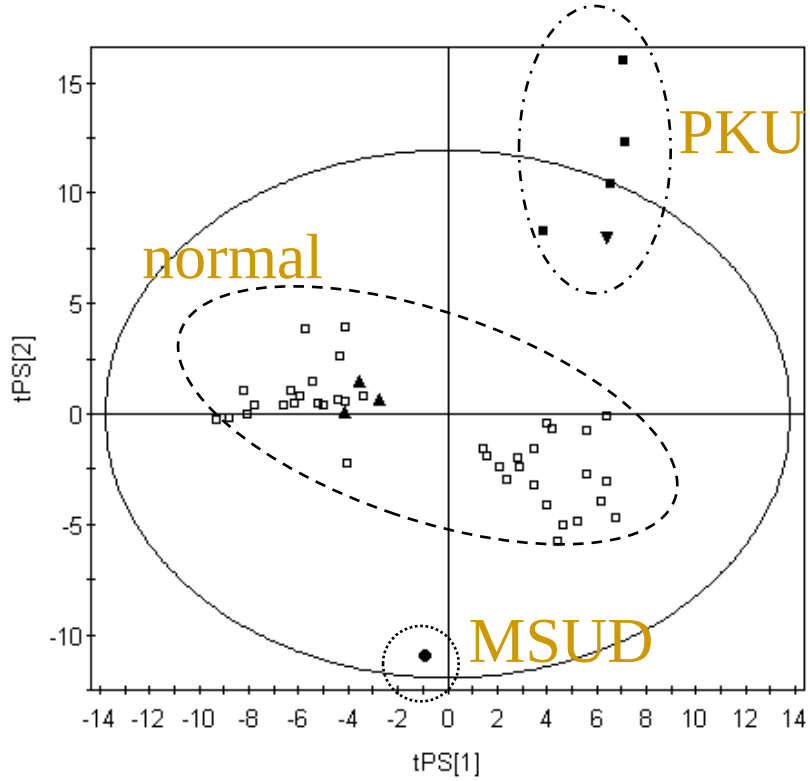
Targeted and non-targeted screening method

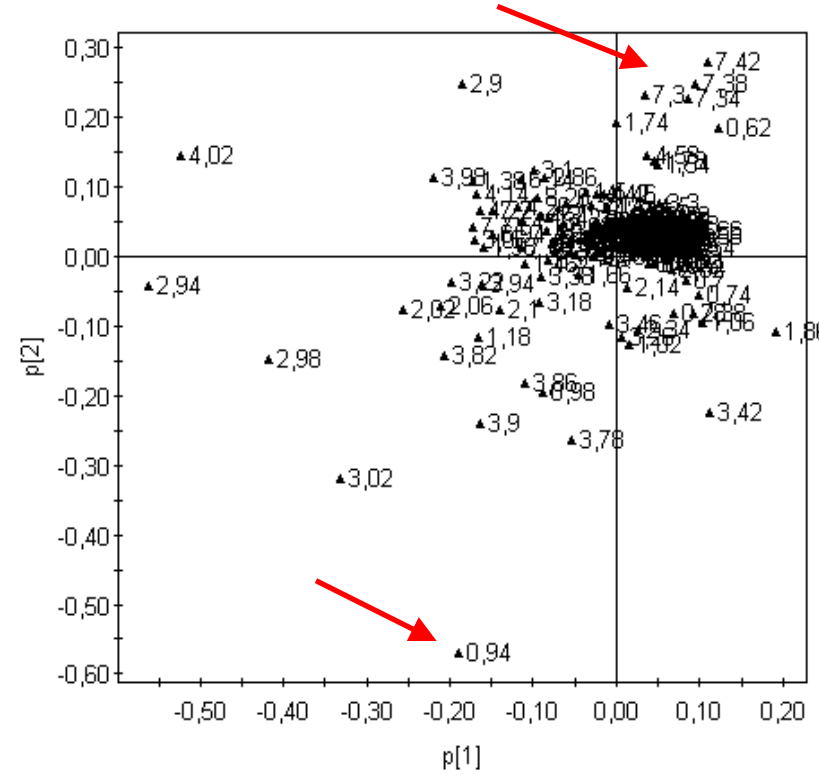
R.A. Wevers (2007)

Includes the ^{1}H NMR spectra of urine (mainly) from 82 IEM

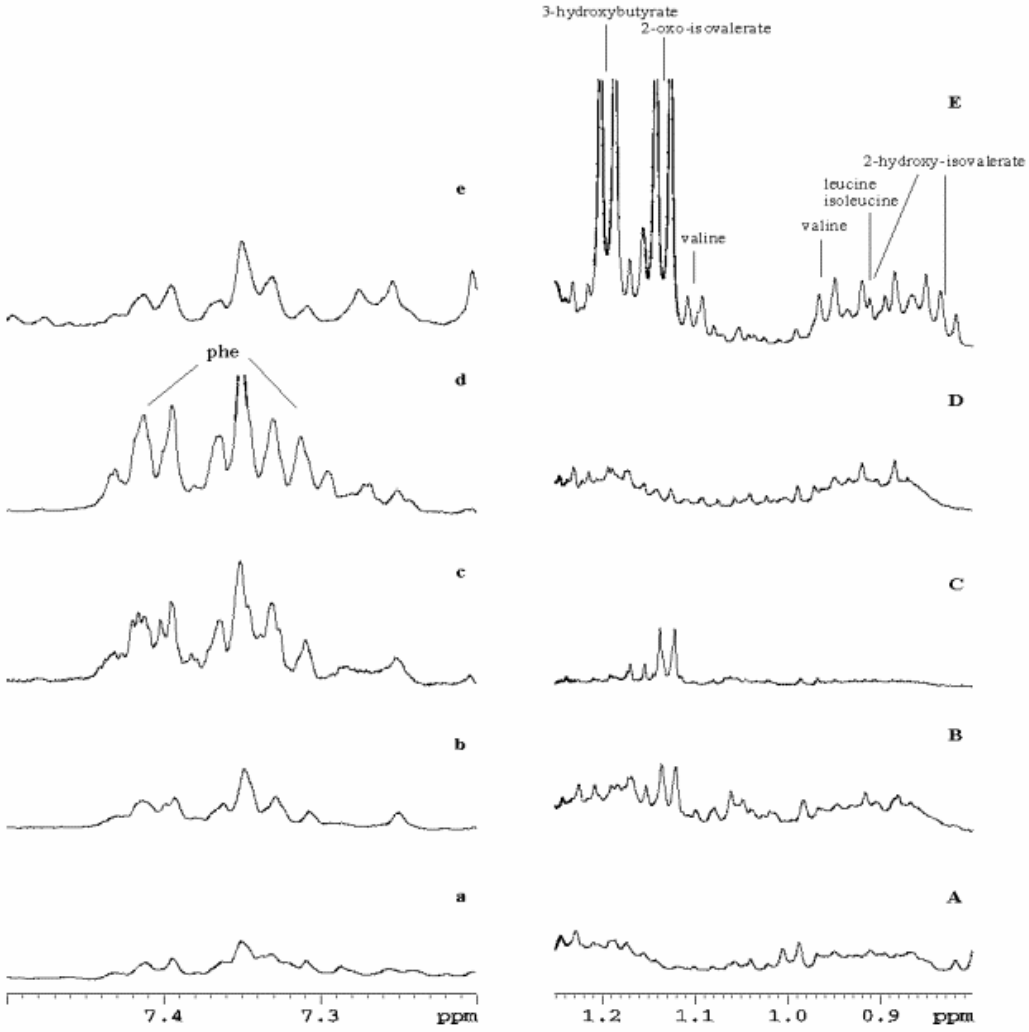
Inborn errors of metabolism (IEM)

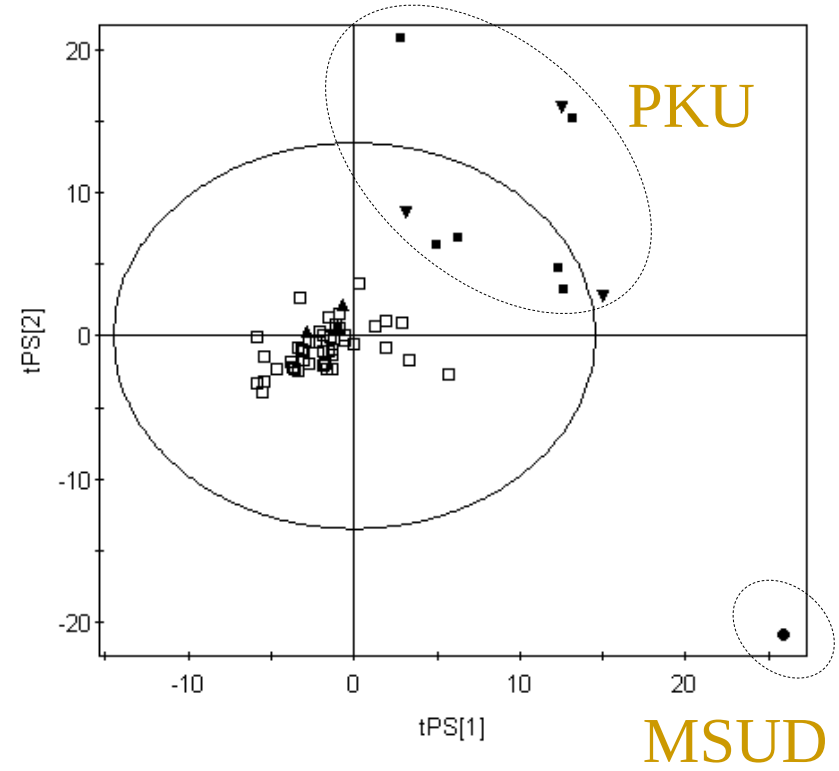
Blood spot extraction





Urine





PLS-DA

Constantinou MA, Papakonstantinou E, Spraul, M, Sevastiadou S, Costalos C, Koupparis MA, Shulpis K, Tsantili-Kakoulidou A, Mikros E* *Anal. Chim. Acta* 542 169-177, 2005

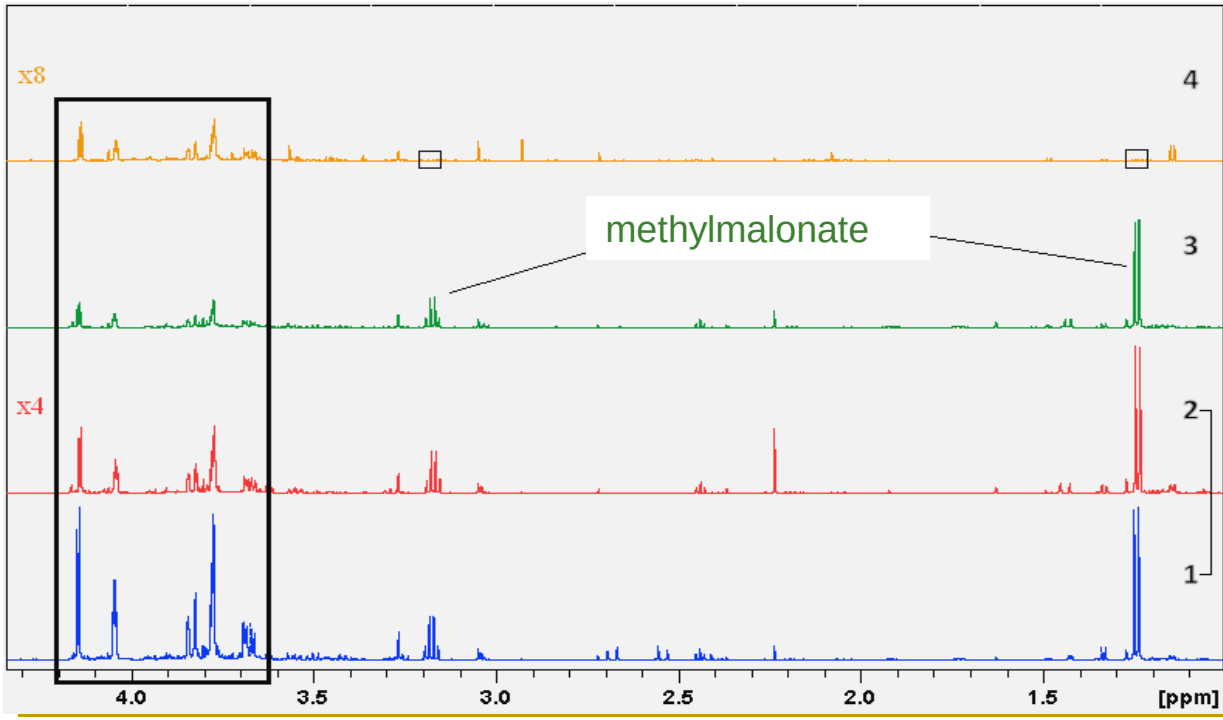
Newborns Metabolic Profile

Example 1

Methyl Malonic Acidurea

Characterized by high levels of methylmalonate

Conventional newborn screening: high levels of methylmalonate



NMR (urine):

- increased levels of methylmalonate (expected)
- increased levels of urocanate (biomarker for Urocanic Acidurea)
- urocanate was not detected by conventional screening and remains unknown whether Urocanic Acidurea is related to Methylamalonic Acidurea or the newborn had both IEM.

Newborns Metabolic Profile

Example 1

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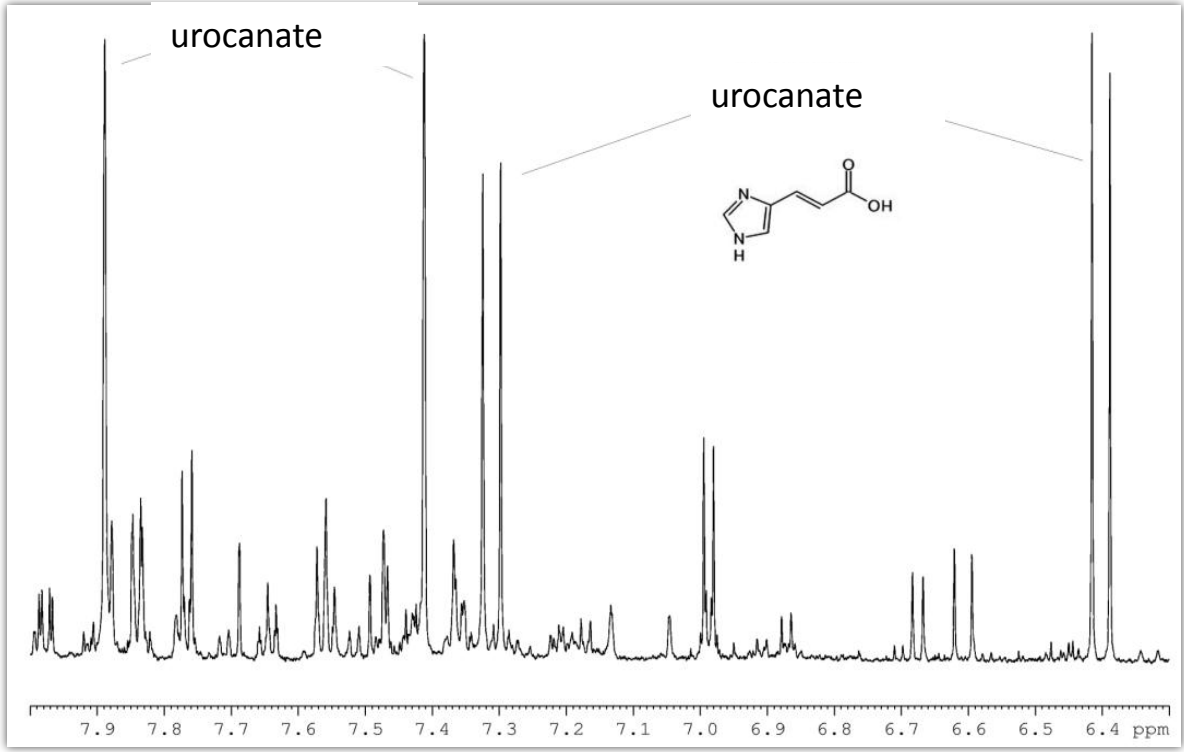

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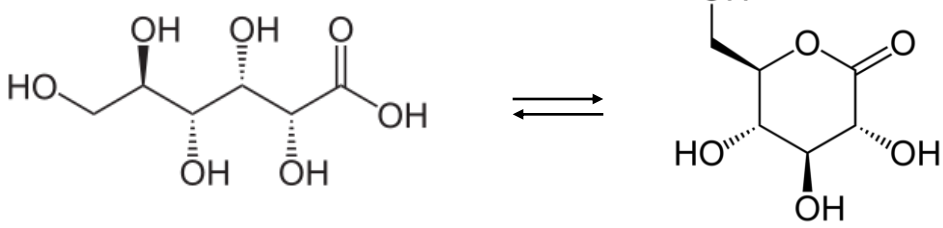
- increased levels of urocanate (biomarker for Urocanic Acidurea)
- urocanate was not detected by NEOLAB and remains unknown whether Urocanic Acidurea is related to Methylamalonic Acidurea or the newborn had both IEM.

Newborns Metabolic Profile

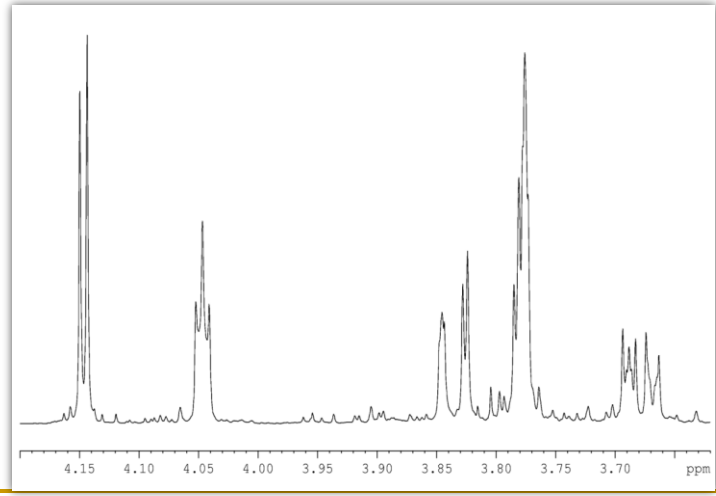
Example 2

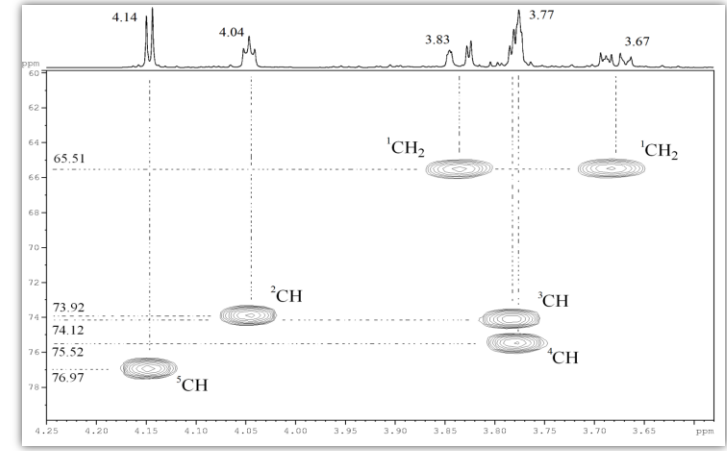
Unknown metabolite with characteristic NMR peak pattern

Assigned in 4 newborns urine samples

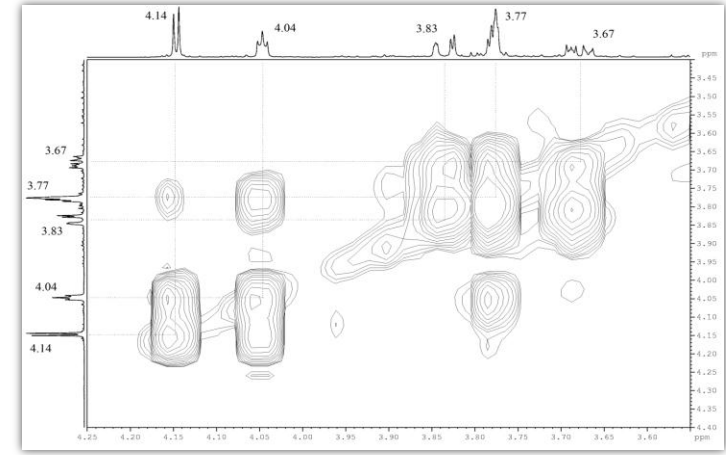


Gluconic acid Gluconolactone



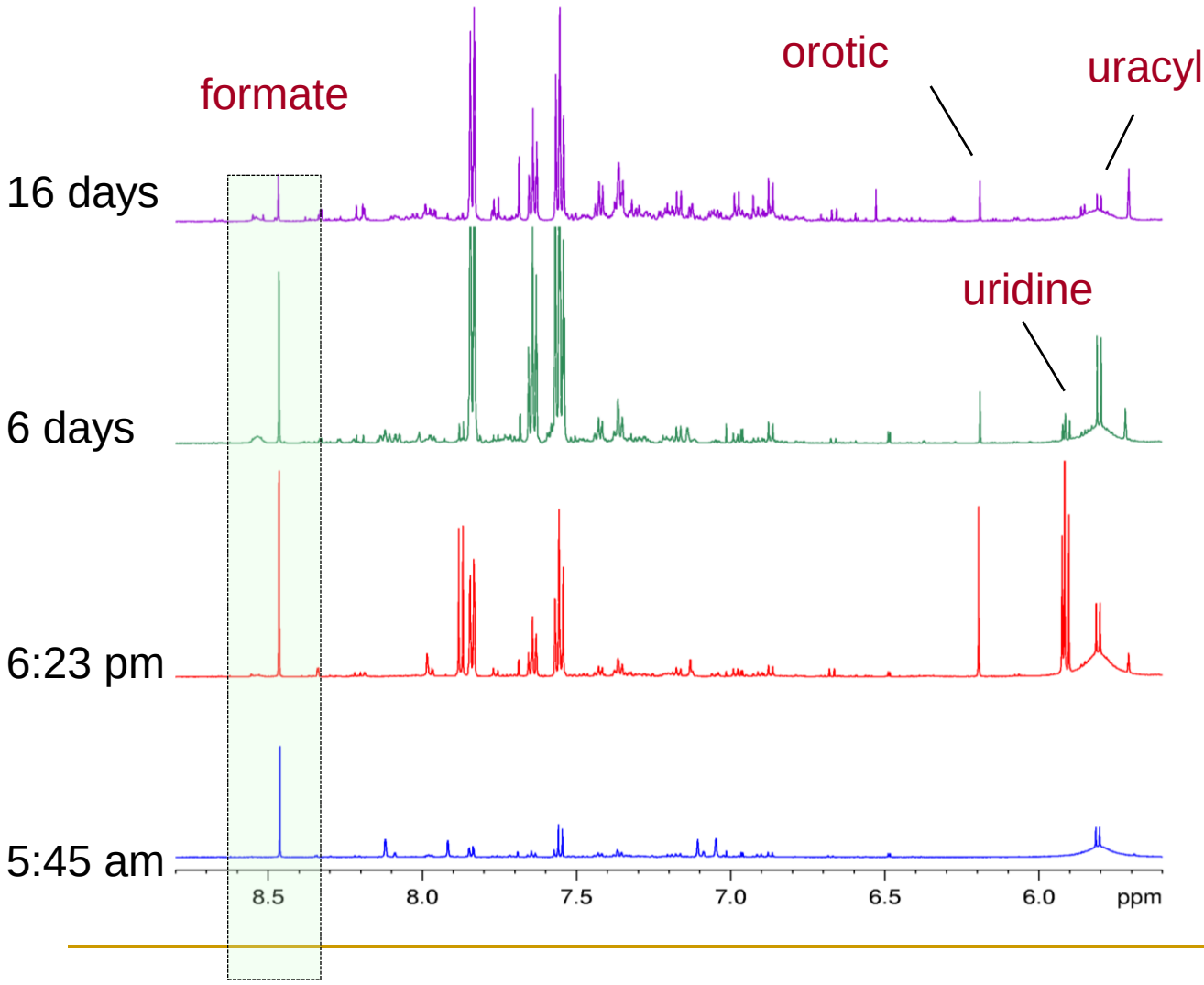


^{1}H-^{13}C HSQC-DEPT135



^{1}H-^{1}H TOCSY

Newborn urines. Appearance of formic acid just before (blue) and during crisis of OTC disease



Newborn 10 months old, diagnosed with OTC and follow medical treatment

NMR (urine):

- increased levels of uridine, uracyl and orotic acid during crisis (expected)
- Appearance of formic acid just before (blue) and during crisis of OTC disease

nature news

Published online 14 December 2009 | Nature | doi:10.1038/news.2009.1128

News

Surgeons get real-time tissue profiling

Nuclear magnetic resonance technology could reduce time spent under the knife.

Ananyo Bhattacharya

Could tomorrow's surgeons be guided by nuclear magnetic resonance?
R. McVay/Getty

“This is huge for NMR.”

Metabonomics Toxicity

- **Kidney cortical toxins**
  - mercury II chloride
  - *p*-aminophenol
  - uranyl nitrate
  - the anticancer drug ifosfamide
  - cephaloridine
  - the kidney medullary and papillary toxin, propylene imine
  - renal papillary toxin
  - 2-bromoethanamine hydrochloride

- **Liver toxins**
  - hydrazine
  - allyl alcohol
  - thioacetamide
  - 1-naphthylisothiocyanate
  - Allyl formate
  - galactosamine
  - bromobenzene
  - acetaminophen
  - carbon tetrachloride

Metabonomics Applications

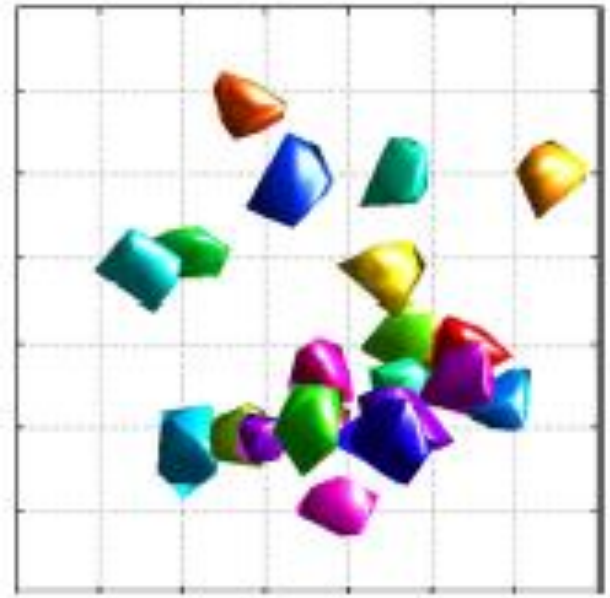
- Phenotype variations
- Diagnosis
- Drug toxicity

Metabonomics - Phenotyping

Evidence of different metabolic phenotypes in humans

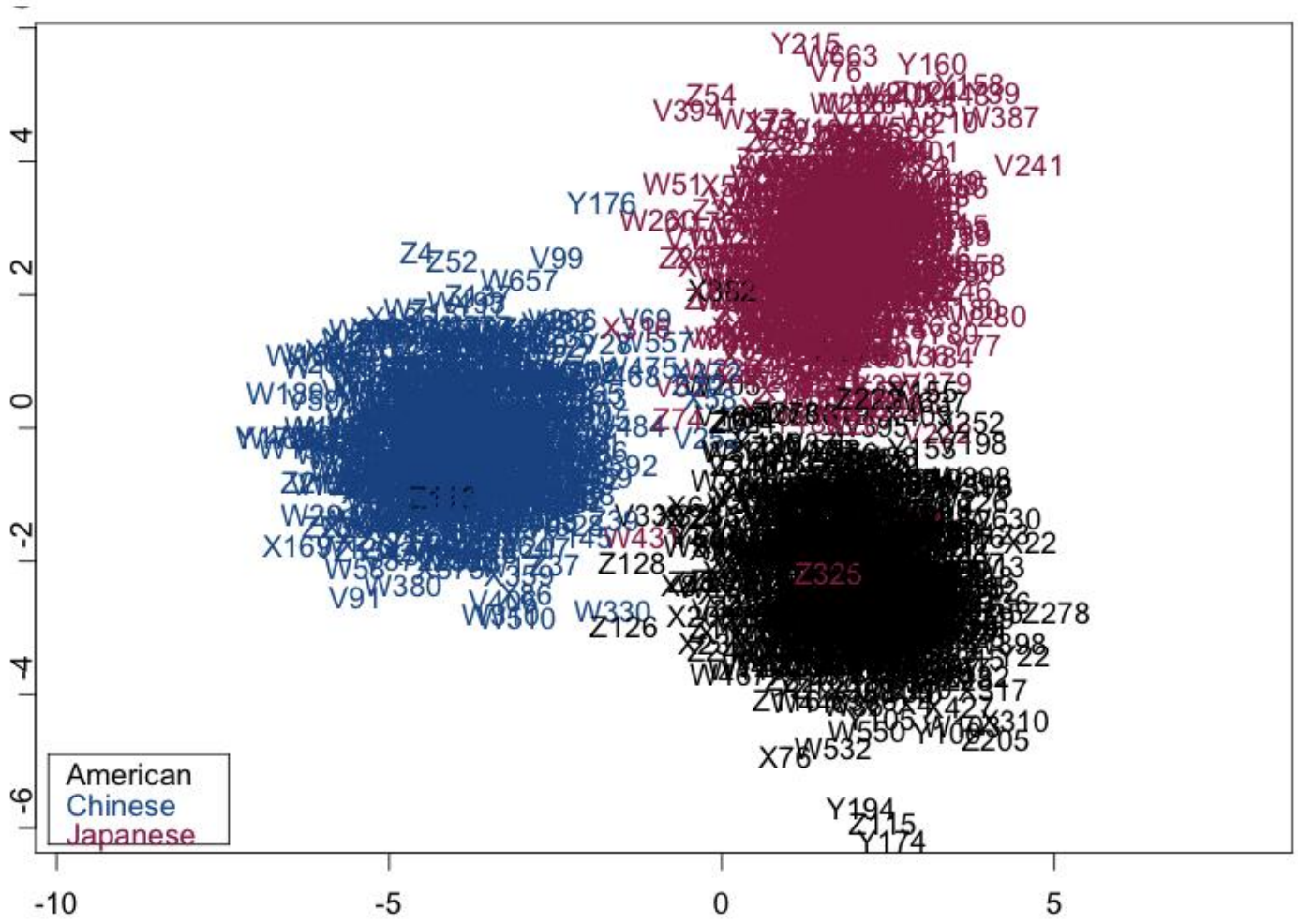
Michael Assfalg*†, Ivano Bertini‡§¶, Donato Colangiuli‖**, Claudio Luchinat‡††, Hartmut Schäfer‡‡, Birk Schütz‡‡, and Manfred Spraul‡‡

1420–1424 | PNAS | February 5, 2008 | vol. 105 | no. 5



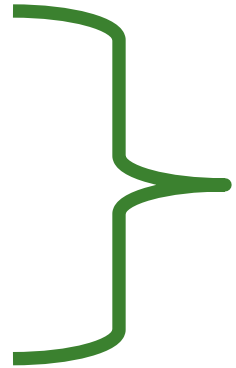
Metabotype Variability

- INTERMAP
- Nicholson and coworkers



Metabonomics Applications

- Diagnosis
- Drug toxicity
- Phenotype variations



Personalized therapy

Pharmaco-metabonomics

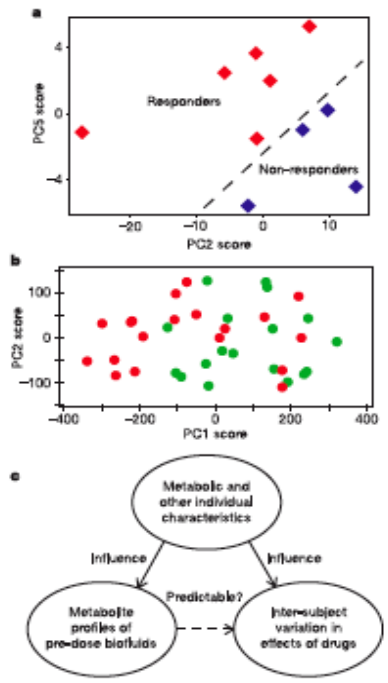
nature Clayton et al 440 (20) 1073-1077, 2006) LETTERS

Pharmaco-metabonomic phenotyping and personalized drug treatment

T. Andrew Clayton[1], John C. Lindon[1], Olivier Cloarec[1], Henrik Antti[2], Claude Charuel[3], Gilles Hanton[3], Jean-Pierre Provost[3], Jean-Loïc Le Net[3], David Baker[4], Rosalind J. Walley[5], Jeremy R. Everett[5] & Jeremy K. Nicholson[1]

There is a clear case for drug treatments to be selected according to the characteristics of an individual patient, in order to improve efficacy and reduce the number and severity of adverse drug reactions[1,2]. However, such personalization of drug treatments requires the ability to predict how different individuals will respond to a particular drug/dose combination. After initial optimism, there is increasing recognition of the limitations of the pharmacogenomic approach, which does not take account of important environmental influences on drug absorption, distribution, metabolism and excretion[3–5]. For instance, a major factor underlying inter-individual variation in drug effects is variation in metabolic phenotype, which is influenced not only by genotype but also by environmental factors such as nutritional status, the gut microbiota, age, disease and the co- or pre-administration of other drugs[6,7]. Thus, although genetic variation is clearly important, it seems unlikely that personalized drug therapy will be enabled for a wide range of major diseases using genomic knowledge alone. Here we describe an alternative and conceptually new 'pharmaco-metabonomic' approach to personalizing drug treatment, which uses a combination of metabolite profiling before drug administration and chemometrics to model and predict the responses of individual subjects. We provide proof-of-principle for this new approach, which is sensitive to both genetic and environmental influences, with a study of paracetamol (acetaminophen) administered to rats. We show pre-dose prediction of an aspect of the urinary drug metabolite profile and an association between pre-dose urinary composition and the extent of liver damage sustained after paracetamol administration.

^{1}H nuclear magnetic resonance (NMR) spectroscopy has been widely applied as a metabolite profiling tool for metabonomic studies, as it enables many endogenous metabolites to be quantified rapidly and reproducibly without derivatization or separation[8–11]. In one of many potential applications, NMR-based metabonomic analysis of post-dose rodent biofluids has been developed as a



A new paradigm for personalized predictive drug metabolism and toxicology.

Pharmaco-genomics

Individual predisposition to drug therapy due to genetic factors

exogenous factors ?

diet, foreign chemicals, environment, other drug therapies, gut microflora, age, hormonal status

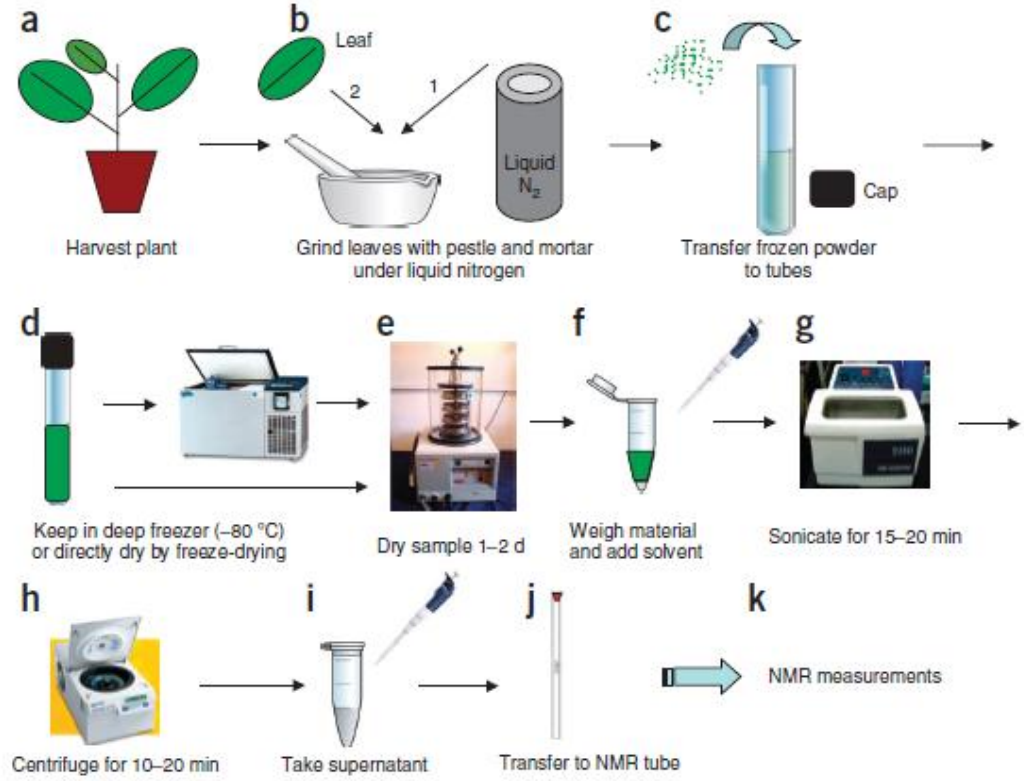
HERBAL METABONOMICS

PROTOCOL

NMR-based metabolomic analysis of plants

Hye Kyong Kim, Young Hae Choi & Robert Verpoorte

536 | VOL.5 NO.3 | 2010 | NATURE PROTOCOLS



Fruit Juices quality control

Nutrients **2009**, *1*, 148-155; doi:10.3390/nu1020148

OPEN ACCESS

nutrients

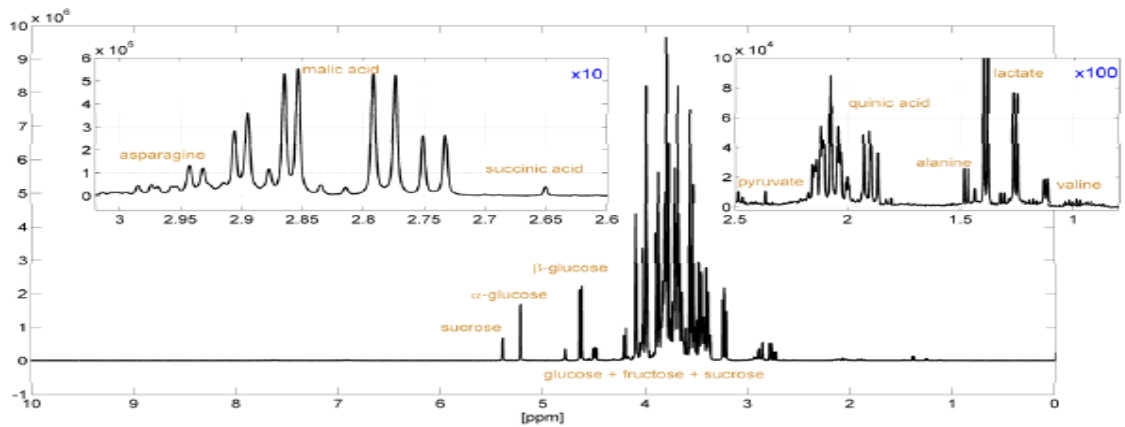
ISSN 2072-6643

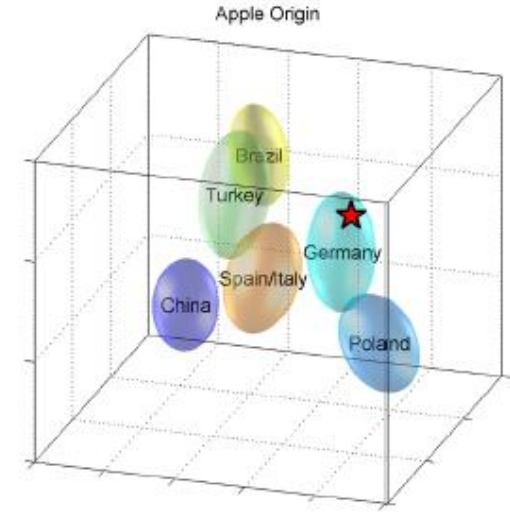
www.mdpi.com/journal/nutrients

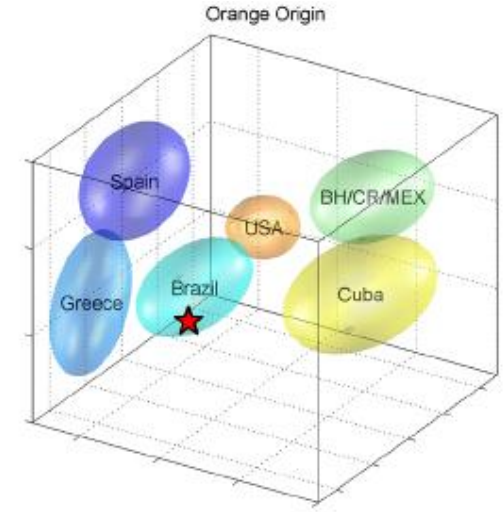
Communication

NMR-Based Multi Parametric Quality Control of Fruit Juices: SGF Profiling

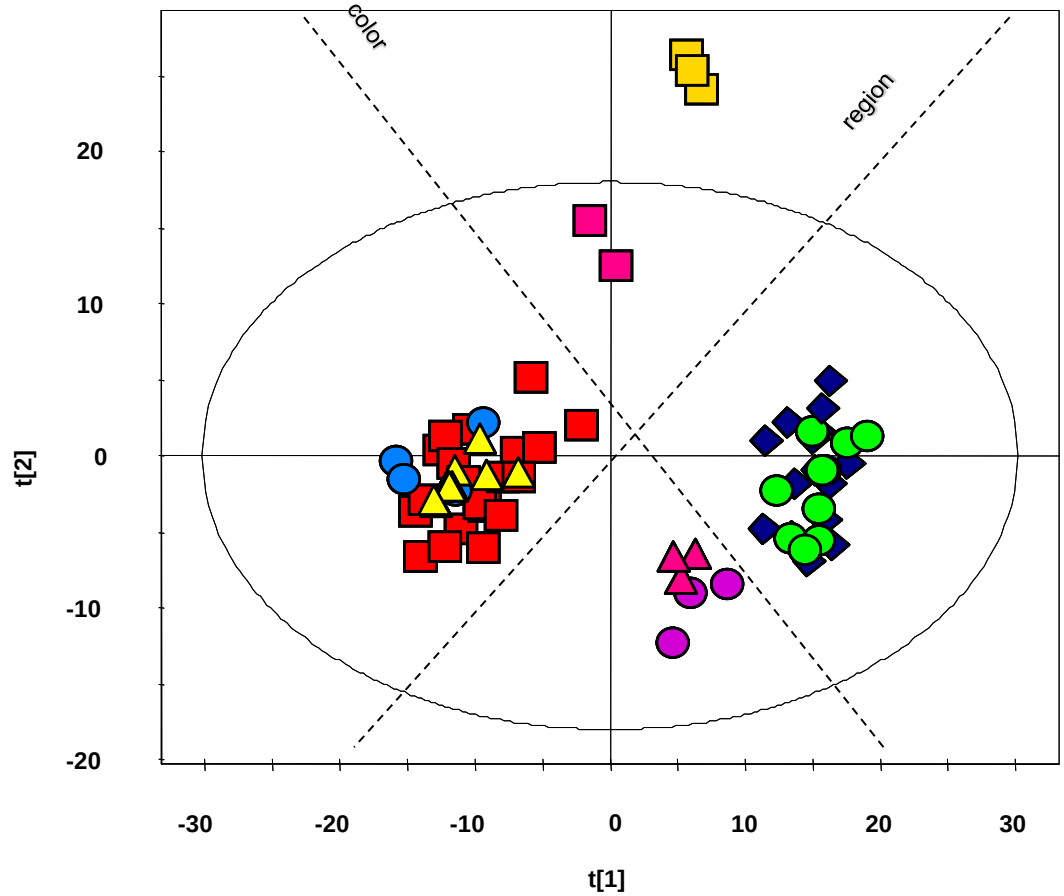
Manfred Spraul [1,*], Birk Schütz [1], Peter Rinke [2], Susanne Koswig [2], Eberhard Humpfer [1], Hartmut Schäfer [1], Monika Mörtter [1], Fang Fang [1], Ute C. Marx [1] and Anna Minoja [3]







Greek Wines classification



1H NMR-Based Metabonomics for the Classification of Greek Wines According to Variety, Region and Vintage – Comparison with HPLC Data.
Anastasiadi, M; Zira, A; Magiatis, P; Haroutounian, S; Skaltsounis, AL; Mikros E;
J. Agr. Food. Chem. (2009); 57; 11067-11074