Tackling Complex Mixture by NMR

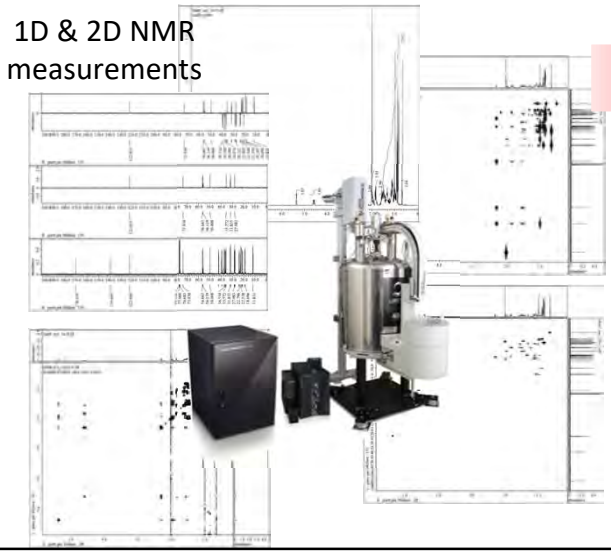
25th Jan., 2022

JEOL RESONANCE, Inc.

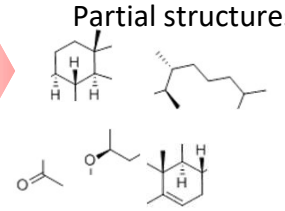
Takanori Komatsu, Ph.D.

NMR in laboratories (pure world): Structural Elucidation

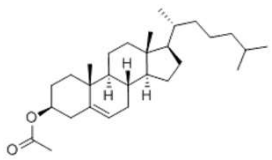
NMR is essential tool for *de-novo* structural elucidation of organic molecules in the laboratories. ☺



Partial structures



Structural elucidation by NMR



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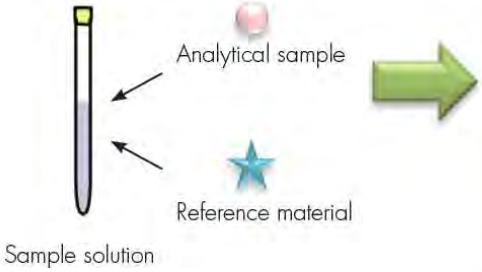
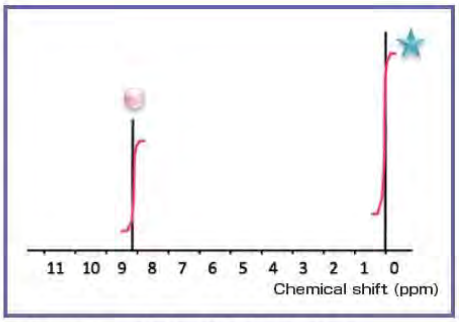
NMR in laboratories (pure world): Quantitative NMR

Quantitative nature of NMR is getting much attention for assay for pure chemicals. ☺





NMR spectrum of internal standard method

Formula (1) $$P_{sample} = \frac{I_{sample}}{I_{std}} \times \frac{H_{std}}{H_{sample}} \times \frac{m_{std}}{m_{sample}} \times \frac{M_{sample}}{M_{std}} \times P_{std}$$

I = signal intensity (integral value), H = number of protons (number of hydrogen atoms in the functional group), m = mass (weight), M = molecular weight, P = purity (%)

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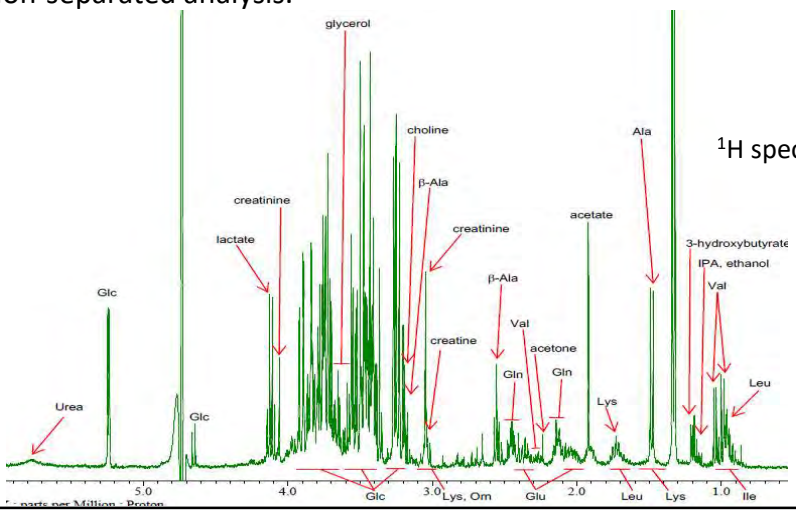
Toward laboratory (pure) to real world (mixture)

Real world: Very complex mixture -> Severe spectral overlapping ☹ Umm…

NMR measurements are highly reproducible with highly quantitative nature, but it is typically non-separated analysis.



^{1}H spectrum of human serum

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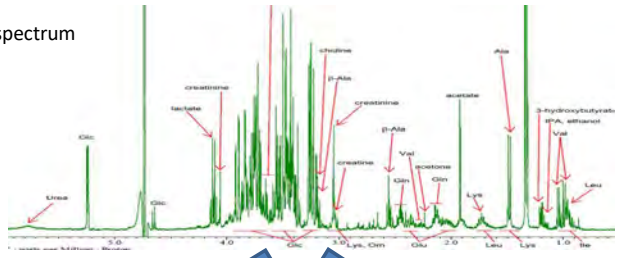
Strategies for Complex Mixture Analysis by solution NMR

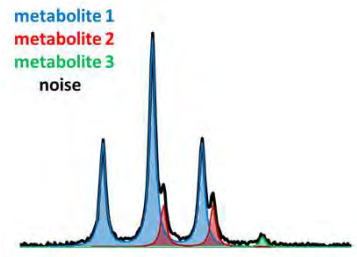
ex. Serum ^{1}H-NMR spectrum



Targeted Analysis

-"Reductionism"

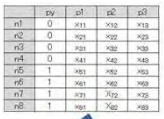
-Spectral library is required.

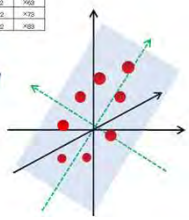


Non-Targeted Analysis

-"Holistic approach"

-Statistical analyses are typically used.





Today!

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Outline

Introduction (done)

Workflow of NMR Non-targeted analysis

Chemometrics tool in the Delta NMR software

Unsupervised Multivariate Analyses in the Chemometrics tool

- Classical and Robust Principle Component Analysis
- Hierarchal Cluster Analysis

Application Example

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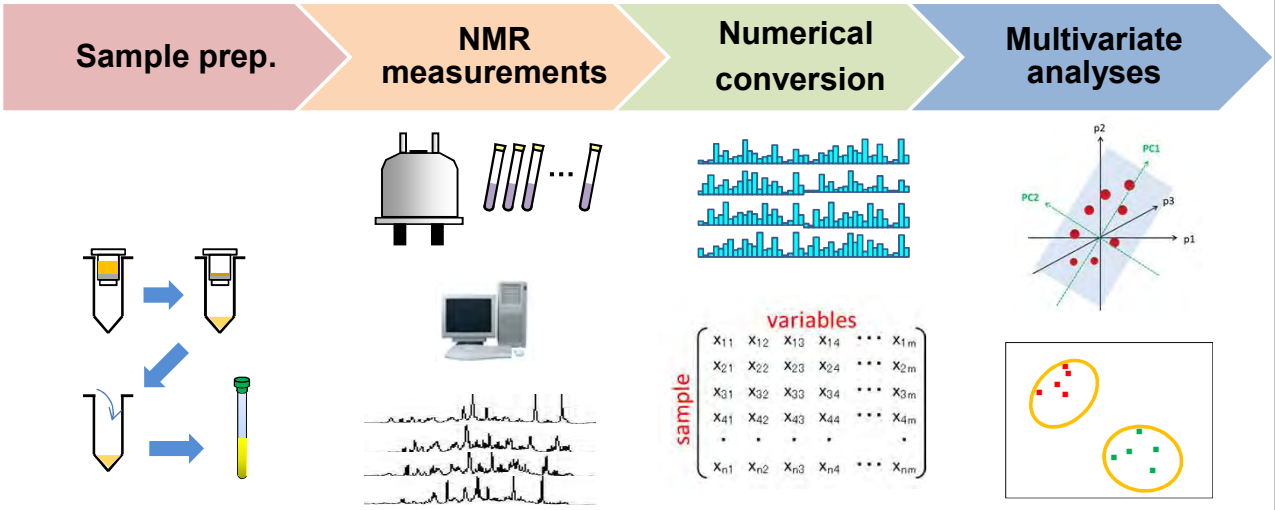
Non-Targeted Analysis –A Typical Workflow–



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Sample Preparation

Unfortunately there are no "golden recipe" for NMR sample preparation with all materials.

Generally simple sample preparation procedures are okay for NMR measurements (ex. add few deuterated solvent for 2H lock or even though without deuterated solvent), but that doesn't mean good spectral quality.

Liquid–liquid extraction (ex. variants of Bligh-Dyer method) is sometimes employed to remove non-polar (or polar) and neutral compounds.

Ultrafilterization is sometimes employed to remove maclomolecules (ex. biomacromolecules in serum).

Previous studies using similar material are helpful for choosing procedures of sample preparation.

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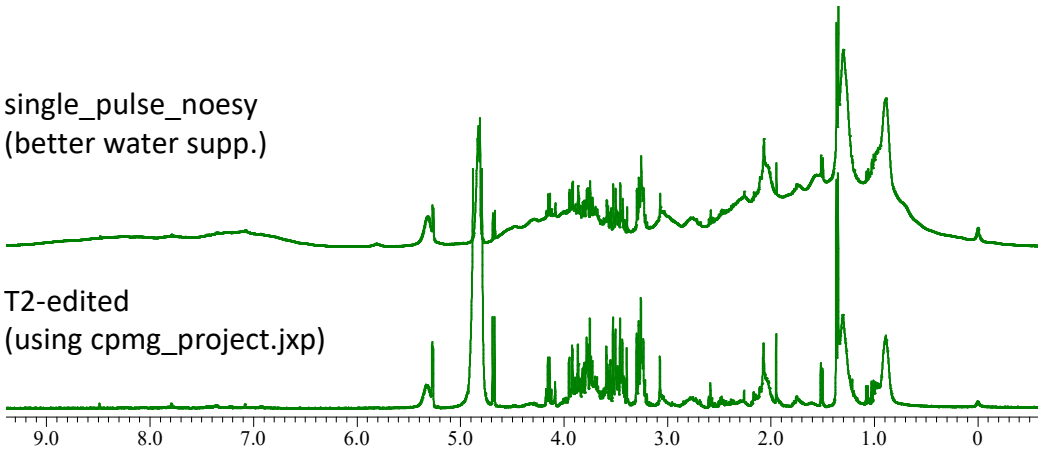
"Edited" or "As it is"?



Any 1D experiment is applicable.
Solvent suppression is often employed when solvent peaks hamper further analyses.
Spectral-editing is sometimes used for emphasizing what you want to see.

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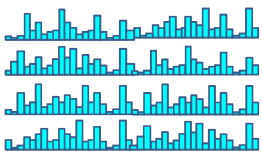
Numerical conversion (Spectra to Matrix)

Bucket Integration
(Chemometrics tool supports this)

Spectra

Spectra are divided into even width windows (ex. 0.04 ppm)



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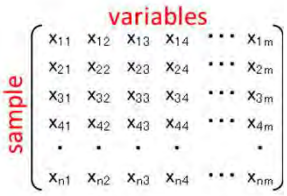
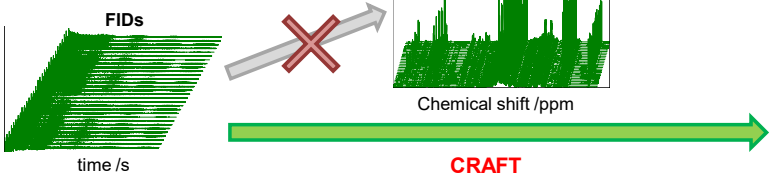
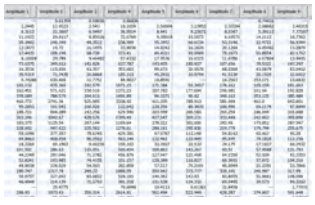
matrix



CRAFT
(Please be patient, it takes long time for converting…)





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Multivariate Analysis (Extract "something interesting" from "illegible matrix")

Unsupervised Analysis

Uses only explanatory variables (here NMR bins) and doesn't use objective variables.

PCA; Principal Component Analysis #The chemometric tool supports PCA.

HCA; Hierarchal Cluster Analysis #The chemometric tool supports HCA.

Supervised Analysis

Uses both explanatory variables and objective variables.

PLS-DA; Partial Least Squared-Discrimination Analysis

PLS-R; Partial Least Squared-Regression #The chemometric tool doesn't supports these supervised analyses.

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- Hierarchal Cluster Analysis

Application Example

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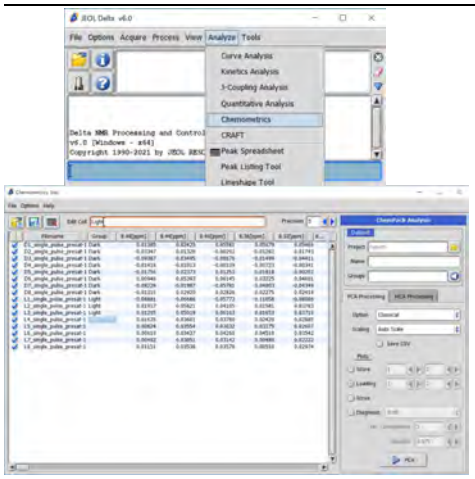
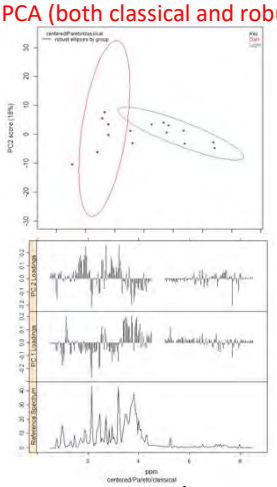
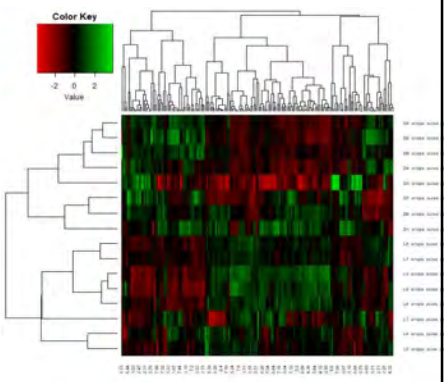
Chemometrics tool



PCA (both classical and robust)



HCA
(heatmap is optional)



- Delta NMR software has “Chemometrics tool” for non-target explanatory analyses.
- The “Chemometrics tool” supports bucket integration (convert multiple spectra into matrix), PCA*, HCA*.
- Robust version PCA is available as well as classical PCA*.

  * R (statistical language environment) and the ChemoSpec package (Develped by Dr. Bryan Hanson) are required.

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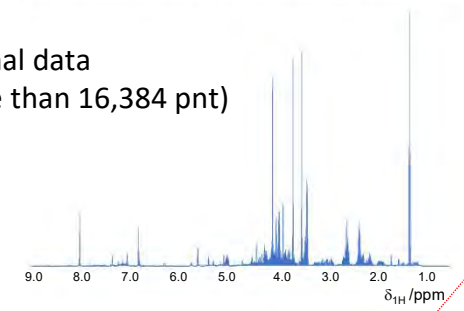
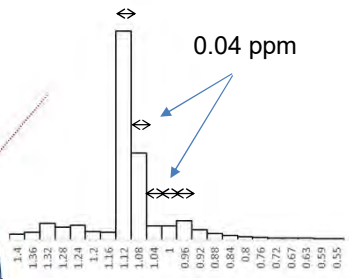
Bucket Integration in the Chemometrics tool (1/2)

Original data
(more than 16,384 pnt)



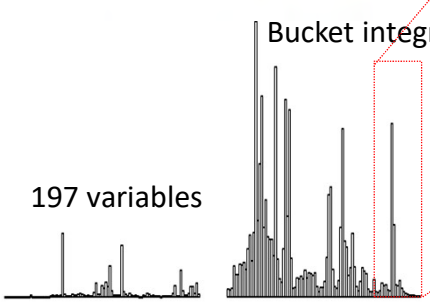
0.04 ppm

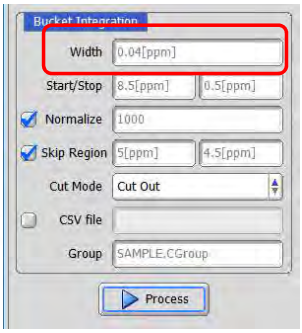
0.04 ppm



Bucket integration

197 variables





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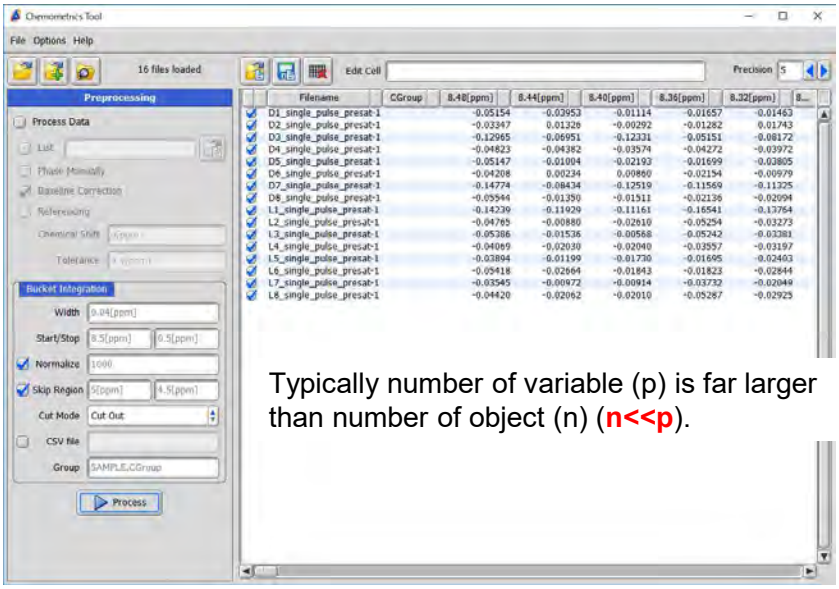
Bucket Integration in the Chemometrics tool (2/2)



Typically number of variable (p) is far larger than number of object (n) (**n<<p**).

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Application Example

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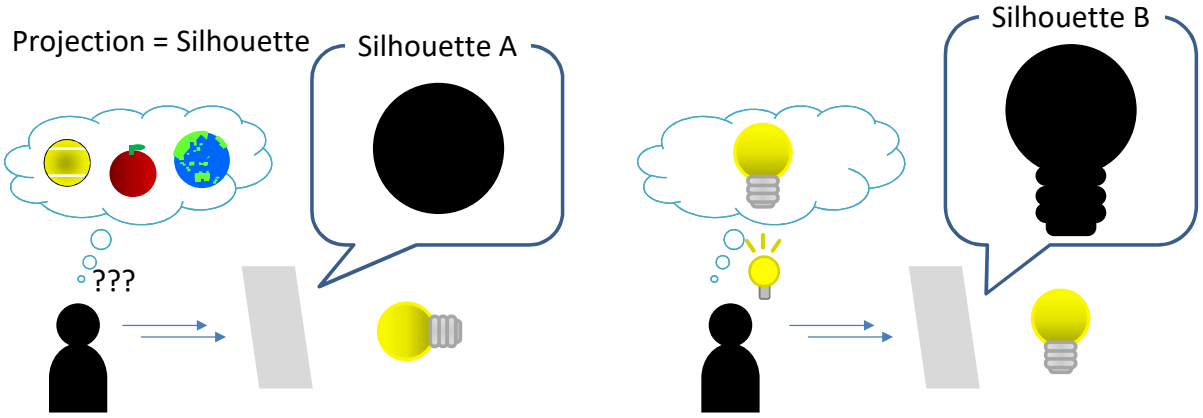
What is PCA?

PCA projects multivariate data set into the new orthogonal coordinate system.



Here silhouettes are 2D projections of (3D) shape of a light bulb.
Analogous to this, PCA projects high dimensional data into lower dimensional space.
How does PCA select "projecting angle"? → Maximizing variance of a projection.

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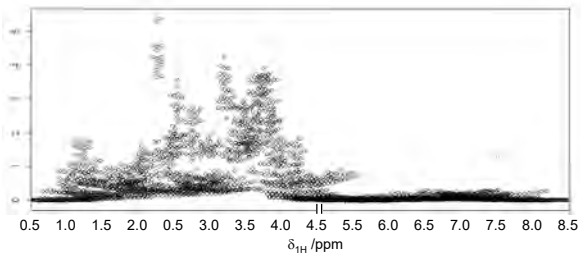
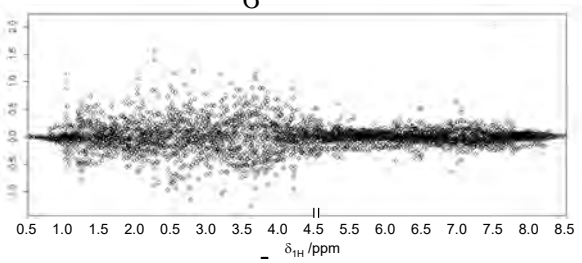
Data preprocessing (Scaling each variable)

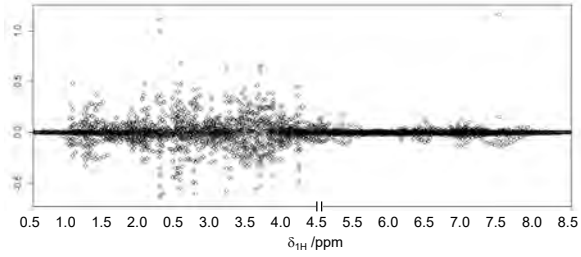
Original data (x_i)



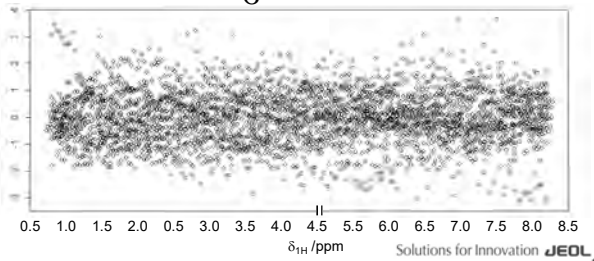
Pareto scale $\frac{x_i-\bar{x}}{\sigma^{1/2}}$



No scale (mean centering, $x_i-\bar{x}$)



Auto scale $\frac{x_i-\bar{x}}{\sigma}$



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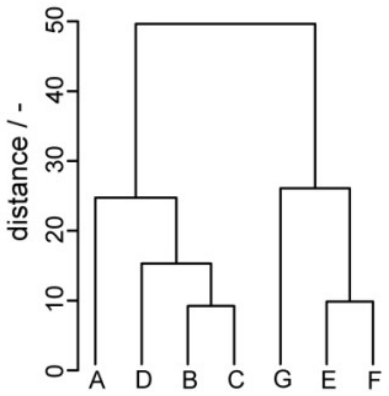
What is HCA (Hierarchal Cluster Analysis) ?

HCA, a clustering method, builds a hierarchal cluster which shows similarity based on particular distance between samples (or variables).

HCA connects the closet distance pair (observation or cluster) until whole data become one dendrogram.



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Application Example

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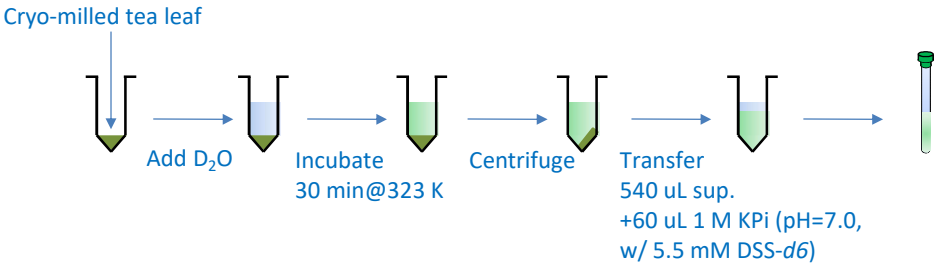
Exploratory Analysis of Japanese Green Tea

- 10 commercially available Japanese green tea leaves were purchased in a tea store.
- 5 teas are *gyokuro* tea, specialty tea (Tea trees are covered for several weeks before harvest in the production).
- Other 5 teas are *sencha* tea, normal tea.



- Three replicates were prepared for each tea leaf; therefore totally 30 NMR samples were prepared.
- ^{1}H-NMR spectra (with presaturation) were recorded with JNM-ECZ500R system equipped with ROYALPROBE^{TM} and ASC30.

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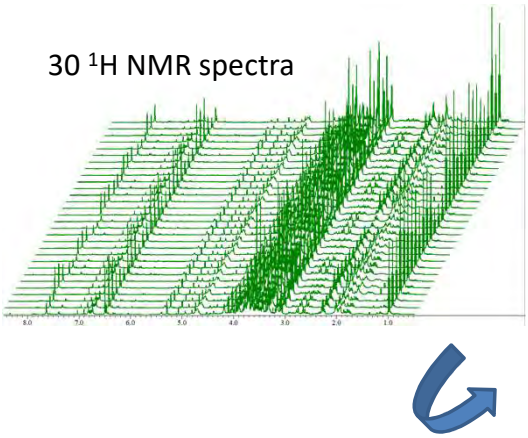
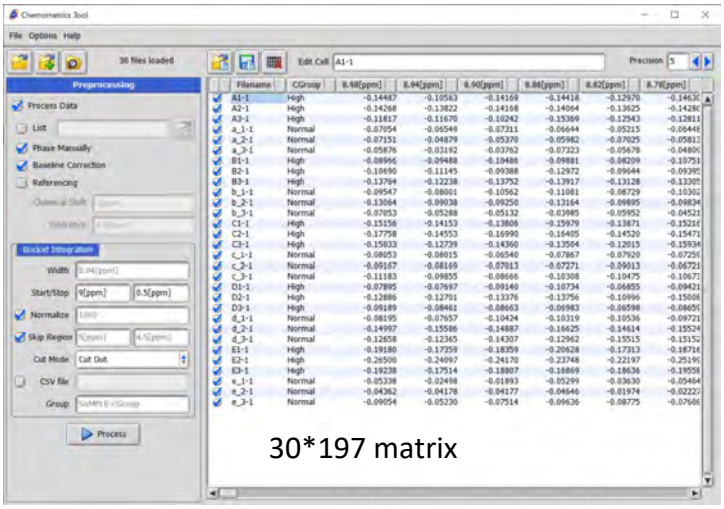
Convert ^{1}H NMR spectra into matrix by bucket integration





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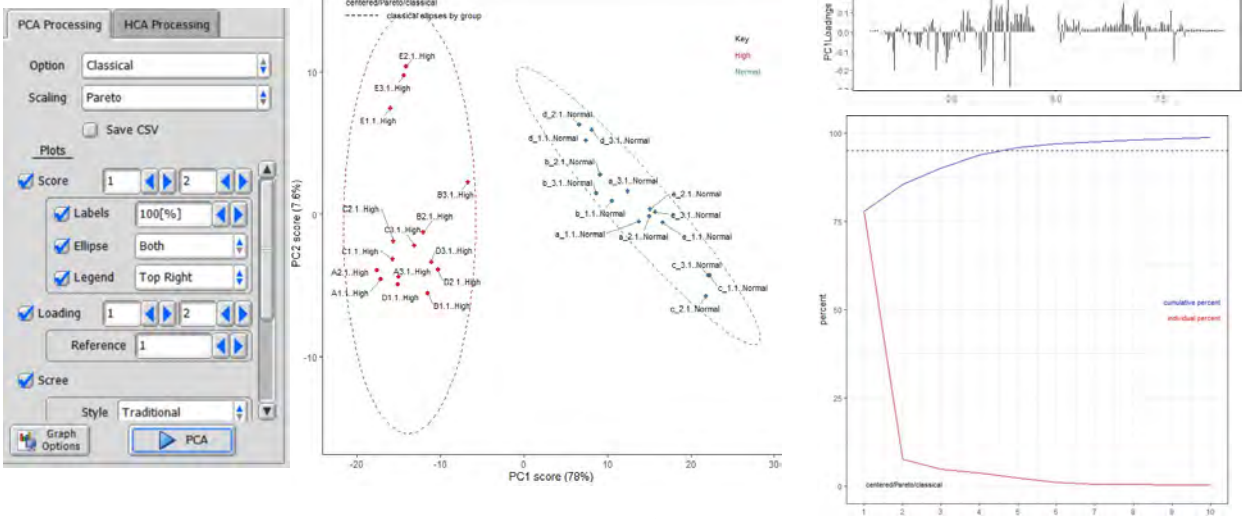
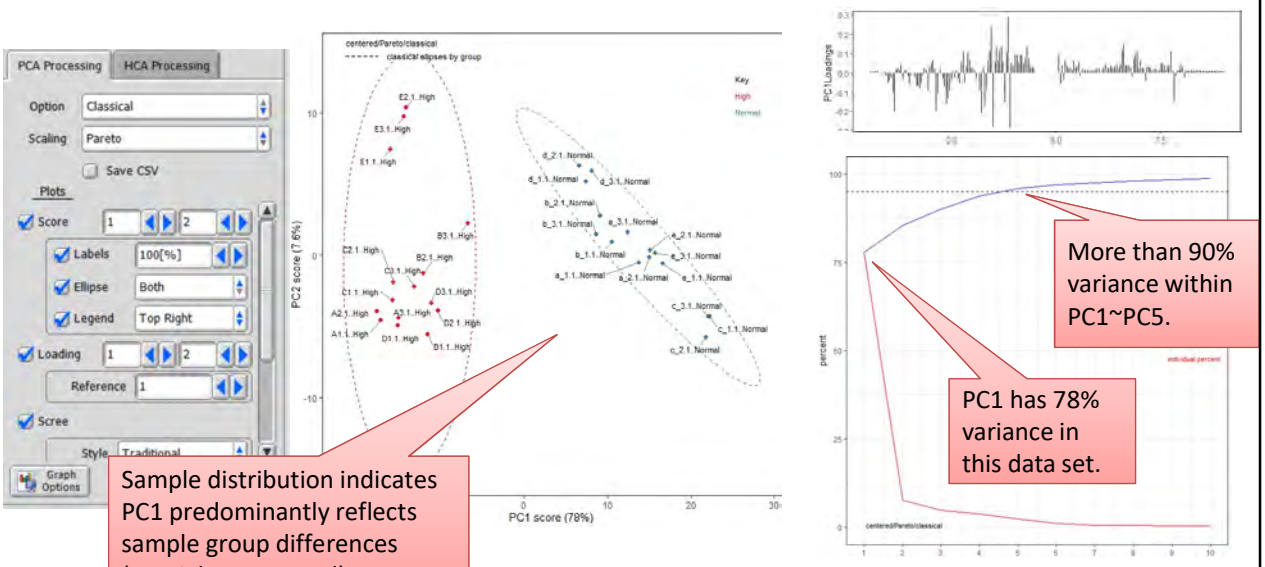
[Result] PCA

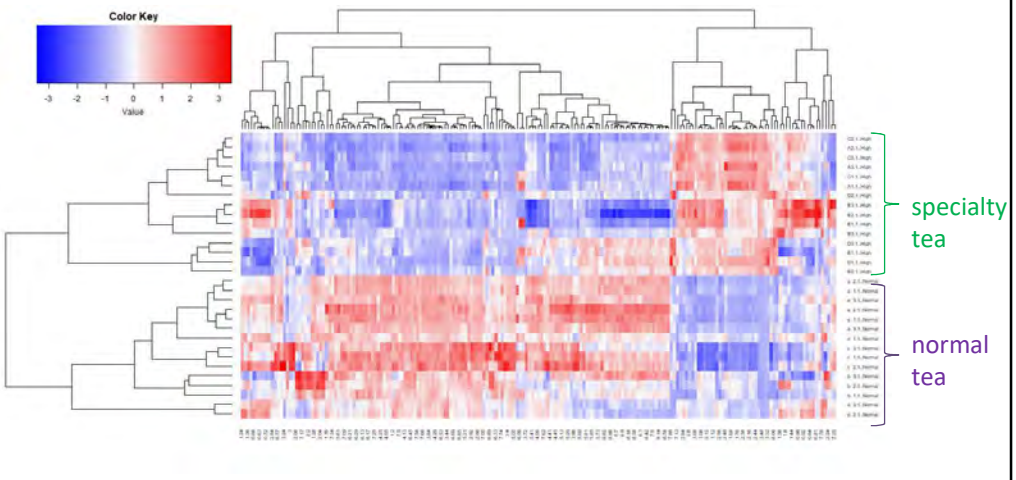


Classical PCA was performed with “Pareto scaling”. (Normalized with total sum)

[Result] PCA



[Result] HCA



HCA was performed using “Person dissimilarity” and “Complete clustering method”.
(Variables were scaled by variance (auto scale))

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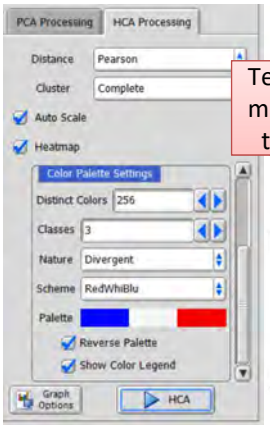
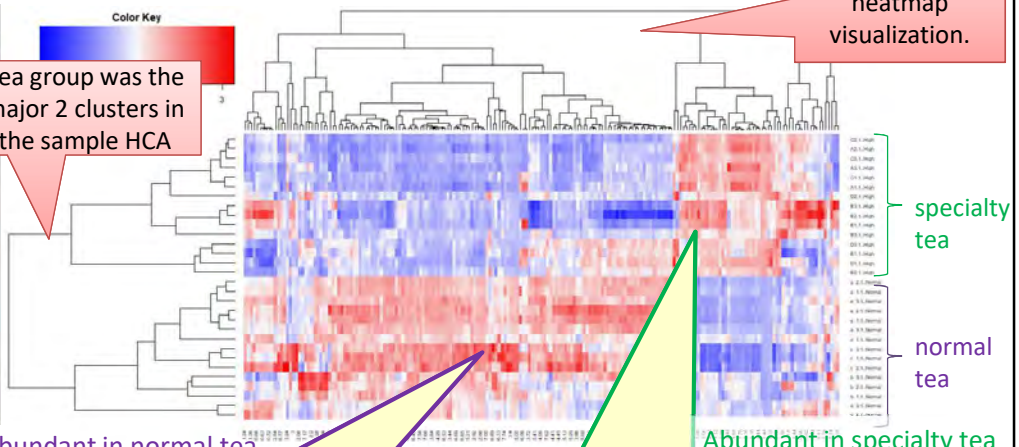
[Result] HCA

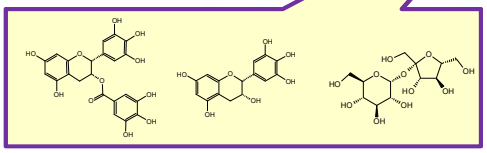
Variable HCA provides good heatmap visualization.

Tea group was the major 2 clusters in the sample HCA

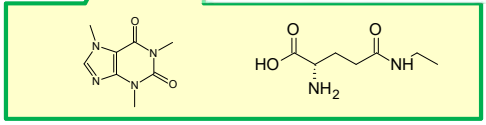




Abundant in normal tea



Abundant in specialty tea



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Summary

NMR is robust analytical platform for complex mixture analysis.

Multivariate analyses are typical employed for non-targeted analysis strategies with NMR data. The Chemometrics tool in Delta NMR software Ver. 6.0 (or later) supports PCA and HCA, unsupervised analyses.

Multivariate analyses are based on "simple" mathematics. Not mysterious.

The chemometrics tool is "Gateway to Chemometrics". Not only PCA and HCA but various data analysis are available with other both commercial and free software.

Acknowledgement

Dr. Bryan Hanson

-Prof. Emeritus of Chemistry & Biochemistry at DePauw University

Developing and maintaining the "ChemoSpec" package

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