ADVANCES IN
NMR AND MS BASED METABOLOMICS

Venue: Complesso San Micheletto, Lucca, Italy
Via San Micheletto, 3, 55100 Lucca

TUTORIALS

Day 1: Wednesday, November 20th, 2019

09:15 – 10:15

MS-based metabolomics: sample preparation, data acquisition, data pre-processing

Pietro Franceschi, Unit of Computational Biology, Research and Innovation Centre,
Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy

Mass spectrometry, almost always coupled with chromatographic separation, is one of the techniques of election able to perform a comprehensive investigation of the "metabolome". MS is almost "universal" and characteristics like sensitivity and dynamic range make it an ideal tool for this task. For exactly the same reasons, however, the results of a MS-based metabolomic investigation are sensitive to any issue occurring during sample preparation, sample analysis and data preprocessing. Errors occurring in these phases will affect all the downstream statistical analysis following the well known "garbage in, garbage out" principle.
The aim of this tutorial is to highlight the most critical aspect which should be taken into consideration when designing a (successful!) MS-based metabolomics assay. The discussion will touch lab practice, quality assessment and data preprocessing. The objective is to describe the strategies which can be used to control the major sources of variability occurring in the early stages of an investigation, with the ultimate objective of providing a reliable data matrix to the subsequent statistical analysis.

10:15 – 11:15

NMR-based metabolomics: sample preparation, data acquisition, data pre-processing

Emanuela Locci, Department of Medical Sciences and Public Health,
Section of Legal Medicine, University of Cagliari, Cagliari, Italy

NMR spectroscopy is widely employed in metabolomic investigations. NMR shows some advantages in the analysis of biological samples, since it is non destructive and non selective. It allows to obtain a global profile of the low molecular weight metabolites present in the sample in one experiment without extensive sample manipulation. Moreover, it is extremely versatile, robust, and highly reproducible. However, compared to MS spectrometry, it has an intrinsically lower sensitivity, which requires high concentrations and precludes the analysis of small volumes. This tutorial addresses the principal steps and methodologies used in NMR-based metabolomics. In particular, sample preparation, data acquisition, and processing of spectra will be discussed.

11:15 – 12:15

Design of Experiments and Data Processing

Matteo Stocchero, Department of Women's and Children's Health,
University of Padova, Padua, Italy

The analytical platforms used for metabolomic investigations produce large data sets where variables are strongly correlated and redundancy is present in the data. Discovering the hidden information is usually a challenge, and suitable approaches for data analysis must be employed. Multivariate data analysis has been successfully applied to metabolomics. On the other hand, design of experiments is foundamental to obtain robust results in metabolomics. Confounding factors are often present in the design and it is not obvious how to take them into account in data analysis to avoid false discovery.

In this tutorial some relevant issues about design of experiments and data analysis are addressed. Principal Component Analysis and Projection to Latent Structures regression (PLS2) are introduced. Post-transformation of PLS2 is used to discover structured noise and to focus the model on the data variation useful to explain the response. Moreover, model validation is discussed. Constrained PCA and constrained PLS2 are introduced to explicitly include experimental design in model building.

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Conference Program

Day 1: Wednesday, November 20th, 2019

12:00 - 14:30 ***Participants Registration***

13:00 – 14:00 **Light Lunch**

14:30 – 15:00 **Welcome and Introduction to the Meeting**
Lucca city Major
IMass and GIDRM Presidents

Chairman: Pietro Franceschi, Marco Geppi

15:00 – 16:00 David Wishart, University of Alberta, Canada
(*Plenary Lecture 1)* Making Quantitative Metabolomics Faster and Cheaper

16:00 – 16:25 **Cristina Piras**, University of Cagliari, Italy
(Oral W1) Metabolomics analysis and modeling in fibromyalgia

16:25 – 16:50 **Alessia Vignoli**, CERM, University of Florence, Italy
(Oral W2) Serum NMR-based metabolomics as prognostic tool for acute myocardial infarction

16:50 – 17:15 **Claudia Napoli,** Bruker Italia S.r.l.
(Oral W3) New NMR tools for clinical research and integrated use of NMR and MS

17:15 – 18:15 Augustin Scalbert, IARC, France
(*Plenary Lecture 2)* The food exposome in cancer epidemiology: discovery, validation and application of dietary biomarkers

19:00 Wine Tasting

Day 2: Thursday, November 21st, 2019

Metabolomics in Clinical Medicine
Chairman: Luigi Atzori, Giuseppe Giordano

09:00 – 10:00 **Marta Cascante, University of Barcelona, Spain**
(*Plenary Lecture 3*) Unveiling the metabolic phenotypes and vulnerabilities underlying metastasis and drug resistance

10:00 – 10:25 **Daniel O. Cicero,** Università di Roma Tor Vergata, Italy
(*Oral T1*) Can a cardiac ischemic episode be anticipated by metabolic profiling? An untargeted NMR study

10:25 – 10:50 **Veronica Ghini,** CERM Università di Firenze, Italy
(Oral T2) NMR-fingerprint of blood: from methods to application in precision medicine

10:50 – 11:15 **Marco Roverso,** Università di Padova, Italy
(Oral T3) Metallome alterations in gestational diabetes: an investigation on maternal whole blood, placenta and cord whole blood samples

11:15 - 11:45 ***Coffee Break***

Chairman: Giuseppe Pieraccini

11:45 – 12:10 **David Heywood,** Waters Corp.
(Oral T4) Development of high throughput, multi-omic methods applied to a breast cancer study

12:10 – 12:35 **Giovanna Musco,** Istituto Scientifico San Raffaele, Milano, Italy
(Oral T5) NMR metabolic studies of the renal cortices changes in a mouse model of Renal Cell Carcinoma (RCC)

12:35 – 13:00 **Elena Cannas**, Università di Bologna & Istituto Italiano di Tecnologia, Italy
(Oral T6) LC-MS Lipidomics to characterize the altered lipid metabolism as a stress reaction to acid tumor microenvironment in osteosarcoma

13:00 – 13-25 **Greta Petrella**, Università di Roma, Tor Vergata, Italy
(Oral T7) How NMR data could assist MS hit classification in an untargeted metabolomics analysis? Our case study: bladder cancer

13:25 - 14:25 ***Lunch and Poster Session I***

Food / Nutrition

Chairman: Augustin Scalbert

14:25 – 14:50 **Cinzia Ingallina**, Università di Roma, Italy
(Oral T8) Torpedino and San Marzano tomato fruit metabolite profiling through NMR and MS methodologies

14:50 – 15:15 **Elisabetta Schievano,** Università di Padova, Italy
(Oral T9) Innovative qNMR methodology for carbohydrates quantification in complex mixtures

15:15 – 15:40 **Anatoly P. Sobolev**, CNR Roma, Italy
(Oral T10) A multi-methodological protocol to characterize the metabolite profile of "Bianco di Sperlonga" PGI white celery ecotype

15:40 – 16:05 **Nicola Cimino**, Agilent Technologies
(Oral T11) A New LC/Q-TOF Platform for Metabolomics Analysis: 6546 LC-QTOF workflows

16:05 – 16:35 ***Coffee Break***

Chairman: Andrea Armirotti

16:35 – 16:50 **Alberto Asteggiano**, Università di Torino, Italy
(Oral T12) HPLC-MS/MS untargeted metabolomic approach for disease-related molecular markers detection in quick decline syndrome

16:50– 17:15 **Sara Tortorella**, Molecular Horizon srl, Perugia, Italy
(Oral T13) LipostarMSI for mass spectrometry imaging: a case study in untargeted plant metabolomics

17:15 – 18:15 Fabien Jourdan, French National Institute for Agricultural Research, France (*Plenary Lecture 4)* Metabolic networks to interpret and predict metabolism

20:00 ***Social Dinner***
Sala Refettorio del Campus San Francesco

Day 3: Friday, November 22nd, 2019

Metabolomics Data Analysis and Applications
Chairman: Matteo Stocchero, Andrea Raffaelli

9:00 – 10:00 **Jasper Engel, Biometris, Wageningen University & Research, The Netherlands. (*Plenary Lecture 5)* One-class modeling in untargeted metabolomics: case studies in diagnosis and risk assessment**

10:00 – 10:25 **Vito Gallo**, Politecnico di Bari, Italy
(Oral F1) Harmonization of Data Processing procedures for non-targeted NMR analysis in metabolomics studies

10:25 – 10:50 **Antonio Pompeiano**, St. Anne's University Hospital, Brno, Czech Republic
(Oral F2) Multivariate data analysis of metabolomic data: data integration, feature selection and visualisation

10:50 – 11:20 ***Coffee Break***

Chairman: Paola Turano

11:20 – 11:45 **Marialuce Maldini,** SCIEX
(Oral F3) SWATH™: QUAL & QUAN metabolomics in the same run

11:45 – 12:10 **Alberto Chighine**, University of Cagliari, Italy
(*Oral F4*) Metabolomic profile of aqueous humour in a 24-hours period after death: an animal model for post-mortem interval estimation

12:10 – 12:35 **Valeria Righi**, Università di Bologna, Italy
(Oral F5) From Actinic Keratosis to Squamous Cell Carcinoma: NMR Analysis with clinical and histological aspects

12:35 – 13:00 **Gabriele Poloniato**, Università di Padova, Italy
(Oral F6) An integrated software platform to improve the identification of metabolites, on the untargeted LC-MS metabolomics

13:00 - 14:30 ***Lunch and Poster Session II***

14:30 - 15:30 **Group discussion: the new Italian Metabolomics Network**

15:30 - 16:00 ***Closing Remarks***

POSTER LIST

P1. Gaia Meoni
From beans to brew: NMR based metabolomic approach to assess traceability of coffee producers within a restricted geographical area of Colombia

P2. Riccardo Frizzo
NMR-based metabolite profiles in mytilus galloprovincialis: experimental set-up and preliminary data

P3. Cristina Licari
The importance of bucketing procedure for NMR-based metabolomic fingerprinting

P4. Camilla Marasca
Blood microsampling for untargeted lipidomics

P5. Alana Pereira
MS metabolomic overview of chemical interactions from leaf cutting ants symbionts

P6. Erica Pitti
Development of a green method to extract lipids from human plasma

P7. Valeria Righi
Non canonical Cyclic Nucleotides Monophosphates in Aphanizomenon flos-aquae: nuclear magnetic resonance and mass spectrometry

P8. Silvia Sabatini
Lipidomic Data Analysis for Non-Alcoholic Fatty Liver Disease

P9. Mattia Spano
Metabolic profile of hemp flowers from Lazio: an NMR study

P10. Vito Gallo
Effects of sample preparation procedures on non-targeted NMR analysis of tomatoes

P11. Michela Buonocore
An NMR-MS metabolomic study of brain tissue from D-aspartate oxidase knock-in mouse model

P12. Emanuela Di Gregorio
Real time metabolomics analysis of breath volatile organic compounds (VOCs) by selected ion flow tube mass spectrometry (SIFT-MS) in cancer patients

P13. Anna Di Porzio
Metabolomic investigation of the effects of nutraceuticals and potential drugs in murine inflammatory models

P14. Nunzia Iaccarino
NMR and MS-based metabolomic study on the effects of structurally different mixed linkage β-glucan in hypercholesterolaemic rats

P15. Hocelayne Paulino Fernandes
Identification of Citrus metabolites associated with the defense against *Phyllosticta citricarpa* using NMR and GC-MS profiling techniques

P16. Eleonora Quartieri
Metabolomic profiling of human saliva

P17. Laura Righetti

Mass spectrometry imaging as a tool to visualize the plant metabolome changes in response to mycotoxin accumulation

P18. Fabio Spreafico

'Functional Microbiomics' – Assessing Nutrition-Microbiome-Host Interaction in Blood and Feces

P19. Elena Michelucci

A targeted LC-MS/MS analysis of circulatory lipid profile to highlight biomarkers for patient stratification according to
coronary artery disease severity

P20. Andrea Armirotti

Quantification of twelve neurotransmitters in mouse cerebrospinal fluid