# OurCon 2012

Ourense Conference on Imaging Mass Spectrometry

## Book of Abstracts

3rd - 5th September 2012

Co-organized in cooperation with COST action BM 1104

Co-organized in cooperation with ProteoRed

## Laias Caldaria

## Ourense, Spain

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# Book of Abstracts of the Ourense Conference on Imaging Mass Spectrometry– OurCon 2012

Ourense - Vigo, Spain

3rd – 5th SEPTEMBER 2012

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## Welcome

In the name off the Bioscope group, I am honoured to welcome you to Orense to participate in the Ist OurCon conference. The organization of this event was decided in 2011 during the celebration of the II-ICAP that was also held in Ourense, in the exactly the same location where the OurCon is going to take place this year. The challenge to organize this conference was made between Dr. L. A. Mcdonnell, Dr. G. Corthals, Dr. C. Lodeiro and Dr. J. L. Capelo based on the fact that imaging mass spectrometry was turning into a mature technique. In the words of Dr. Corthals, "we are creating a community" and indeed this is what it has been created between all of us. To this end, the I OurCon is going to offer a number of interesting possibilities to help create such a community. Experienced researchers are going to be strategically seated during meals, so they, students and young researches can interact together. Also a meeting room will be permanently available with food and drinks, so researchers can meet together at any time to talk about science and future projects. In this way, we hope to make of the I Ourcon a nest of new and great ideas to further develop imaging mass spectrometry. The bar is going to be open till 2 A. M. "to help" those who prefer to work by night in the making of science. Also, the "jogging morning" has been established so every morning those who like jogging can meet with "master" Garry and run/jog together. Not to mention the excellent SPA facilities free available on the venue. Further to these events, the Gala dinner is going to be open to all participants, and it will have some entertainments (uncountable noun) such as regional food and music. But this is not all. Two Gold prizes of 500€ are going to be offered to the best posters by the Sunchrom company and the Congress venue, whilst two silver prizes of 250 € are going to be offered by the organizing committee. Indeed, the organizing committee hopes to make of the Ist OurCon the beginning of a community united by the passion for imaging mass spectrometry. We have done our best to me you feel as if you were in your own home.

On behalf of the organizing committee,

J. L. Capelo
C. Lodeiro

# *Preface*

***Dear Colleagues,***

*The potential of imaging MS has long been recognized. The first elemental imaging MS experiments were performed in the 1960's using secondary ion mass spectrometry (SIMS) [1, 2], and the first molecular imaging experiments in the 1970s using SIMS and laser microprobe mass analysis (LAMMA) [3, 4]. These techniques are highly adept at elemental and small molecule analysis and have subsequently become widely established for semi-conductor research.*

*The advent of electrospray ionization [5] and matrix assisted laser desorption/ionization (MALDI) [6] revolutionized mass spectrometry by enabling the analysis of biomolecular macromolecules. At the 1994 ASMS Conference on Mass Spectrometry Spengler et al. reported a LAMMA analysis of a dried-droplet MALDI preparation [7]. High spatial resolution images were presented of the peptide substance P, the matrix 2,5-dihydroxy benzoic acid, and potassium ions. However it was the pioneering work of Richard Caprioli, demonstrating how MALDI imaging MS could be applied to study the distributions of biomolecules in tissue sections [8-10], which initiated the current wide interest in biomolecular imaging MS. The essential difference is the sample preparation that enables rich biomolecular mass spectrometry profiles to be obtained from the small number of cells in each pixel. Indeed sample preparation is still the most important factor that determines the success of an experiment. Imaging MS is often used as shorthand for imaging sample preparation and spatially correlated mass spectrometry analysis.*

*Biomolecular imaging MS has now grown into a sizeable sub discipline within biomolecular mass spectrometry. For example the 2012 ASMS Conference on Mass Spectrometry (Vancouver, Canada) included 139 poster presentations and 3 dedicated oral sessions. Biomolecular imaging MS is now a focus topic of the European Proteomics Association and of a dedicated European intergovernmental network that currently includes 17 participating countries (COST Action BM1104).*

*The field continues to rapidly develop new methodologies. New ionization methods, such as DESI, enable imaging MS analysis in ambient conditions and without the need for matrix application. The implementation of atmospheric pressure sources, DESI and MALDI, has not only increased the practicality of the experiments but also led to a reassessment of the fundamentals of macromolecular ionization. The culmination of these developments means it is now possible to analyze a diverse array of molecular classes, with high sensitivity, high spatial resolution, high mass resolution, and high mass accuracy at high speed.*

*In part the developments in biomolecular imaging MS reflect the demands of the application areas, as well as the active involvement of scientists from different disciplines. Pharmaceutical analysis is establishing itself as one of the principal applications of imaging MS. It is used to gather an early assessment of the accumulation of drugs and their metabolites in tissues (which can lead to strong toxicological effects). Imaging MS of pharmaceuticals necessitates greater quantitation than had been reported for protein imaging. The targeted analysis of pharmaceuticals is much more amenable to quantitation than protein/peptide/lipid imaging MS experiments, but the contrast between the different application areas has lead to renewed interest in improving the quantitative abilities of imaging MS. Similarly the active involvement of pathologists in the clinical application of imaging MS has led to a much greater appreciation of the importance of histologically classifying the cell types in the tissue during a biomarker discovery project. Indeed it is becoming increasingly accepted that it is the combination of histology and imaging MS, enabling MS profiles specific to distinct histopathological features to be obtained, that is the unique strength of imaging MS based biomarker discovery.*

*The application of imaging MS is highly multidisciplinary and depending on the application area may necessitate expertise in tissue preparation, pharmacology, histology, disease biology, and statistical analysis in addition to mass spectrometry. One of the goals when putting together the programme of the OurCon conference was to provide a setting where experts in the different elements and applications of imaging MS could come gather and discuss the challenges facing*

*imaging MS, to help it make the transition from an up-and-coming technique with high potential to a robust (semi) quantitative bioanalytical tool for biomedical research. Hence the conference focuses on the fundamental aspects of imaging MS as well as the principal application areas. The conference begins with dedicated sessions on sample preparation, instrumentation, quantitation and data analysis – the basics that underpin its application. We have plenary lectures from leading figures in the clinical and pharmaceutical application of imaging MS, Prof. Axel Walch and Dr. Markus Stoeckli respectively, to introduce the principal application areas. A series of dedicated sessions will then discuss the latest developments in biomarker discovery, molecular histology, and pharmaceutical analysis.*

*The final sessions are focused on new experimental approaches and the future of the imaging MS field. This will span multiple different aspects, including the implementation of imaging MS within a routine clinical setting, integration within the wider biomedical research field, and standardization. To date most imaging MS experiments have been individual experiments, utilizing individual infrastructure and based on the individual expertise of the group and their immediate collaborators. For imaging MS to develop into a trusted technique in its own right, rather than a discovery tool that is then validated by more established methods such as immunohistochemistry or LC-MS/MS, it will be necessary to develop standardized methods with standardized reporting protocols.*

*In many regards the OurCon conference maybe considered the successor of the highly successful, and highly enjoyable, ASMS Sanibel Conference on Imaging Mass Spectrometry, which took place on 19-22 January 2007. In fact the origins of COST Action BM1104, a large European network of imaging MS research groups and co-organizer of the OurCon conference, can be traced to informal discussions that took place during the Sanibel conference. Likewise it is hoped that new science, new capabilities and new interactions result from OurCon.*

*We sincerely welcome you to OurCon and wish you a very pleasant stay and conference. In fact, the biggest goal of the meeting is to foster a friendly collegiate atmosphere where good science can be discussed and better friendships forged.*

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***OurCon: Ourense Conference on Imaging Mass Spectrometry***
*3-5 September 2012, Ourense, Spain*

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# *Plenary Lectures*

# Approaching MALDI molecular imaging for preclinical and clinical research: current state and fields of application.

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## Abstract

In the last years, Matrix-Assisted Laser Desorption/Ionization (MALDI) imaging mass spectrometry ("MALDI imaging") has emerged as promising technique for combined morphological and molecular tissue analyses. It enables a spatially resolved, unlabeled and multiplexed analysis of different molecule classes, ranging from small molecules (drugs) to proteins, lipids and other analytes, directly in tissue sections. Molecules can be imaged in the histological context of tissues, and therefore, molecular profiles can be allocated to specific cell types.

MALDI imaging has proven to provide novel and clinical relevant information in a variety of different biomedical questions, with focus in oncology and inflammatory diseases. While several studies so far have worked on a proteomic level, of increasing interest is also the ability of MALDI imaging to visualize the spatial distribution of drugs and their metabolites in tissues, which are valuable for drug development and efficacy studies in animal models and even in individual patients.

This presentation will give an update on the application of MALDI imaging in preclinical and clinical research. We discuss the use of MALDI imaging in clinical proteomics and put it in context with classical proteomics techniques for tissue analysis. In the research area of gastrointestinal disorders MALDI imaging has already been used to address several questions of upper- and lower gastrointestinal diseases, which will be briefly presented. We also highlight a number of upcoming challenges for personalized medicine, development of targeted therapies and diagnostic molecular pathology where MALDI imaging could help.

Because of its practical simplicity and ability to gain reliable information even from smallest tissue amounts, which may also originate from endoscopic biopsy sections, we believe that MALDI imaging might have the potential to complement histopathological evaluation for assisting in diagnostics, risk assessment, or response prediction to therapy.

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### Acknowledgements

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# We Can See - Mass Makes the Difference

Markus Stoeckli

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## Abstract

Molecular imaging is broadly applied in biomedical research to depict the distribution of analyte molecules of interest. MALDI mass spectrometric imaging (MSI) in a newer member in the family of available technologies, which has the distinct advantage of allowing completely label free imaging of multiple analytes simultaneously. This makes it an ideal tool for research, where either labels are not available, or one searches for unknown molecules with a distinct spatial distribution. The MALDI MSI acquisition starts with a flat sample, typically a biological tissue section, which is mounted on a surface and dehydrated. The sample is coated with some organic matrix and introduced in a mass spectrometer, where a laser is used to raster over the sample, ablating and ionizing molecules by assistance of the deposited matrix. These ions are measured using an analyzer appropriate for the molecular class to be detected, e.g. quadrupole instruments for small molecules or TOF instruments for biopolymers. From the acquired data set, on can obtain intensity images for each species of interest. Simplified, one can describe a MSI analyzer as a digital camera, which has instead of 3 color channels a large number of mass channels.

This technology has been successfully applied to a wide range of molecules and analytical problems in academic and industry. In this presentation an overview is given on the technology applied to pharmaceutical research, including examples where MALDI MSI was applied to drug discovery projects adding unique value. Capabilities in spatial resolution, molecular classes, sensitivity and quantification are shown and discussed.

### References

http://maldi.ms

### Acknowledgements

I like to thank Dieter Staab, Nicole Ehrhard, Brendan Prideaux and Gregory Morandi for their contribution to the data shown in this presentation.

# *Shot-Gun Presentations*

# S1.Compatibility between histological staining and cluster-TOF-SIMS for in situ lipidomic studies

Claudia Bich, Vincent Guérineau, David Touboul, Alain Brunelle.

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## Abstract

Cluster-TOF-SIMS is successfully utilized since several years to acquire images of a large variety of organic compounds, such as lipids (up to *m/z* 1500), at the surface of biological tissue sections, and with a spatial resolution of 1 micron or less [1]. This technique can be associated with histology for medical diagnosis in order to correlate structural features with ion images. The possibility to use the same tissue section for both histology and mass spectrometry imaging would be a major advantage in terms of sample preparation and precision on the histological structure localization, provided that none of the two methods disturbs the performances of the other [2].

Rat brains sections (16µm) cut at -20°C were deposited on glass slides and dried under vacuum during 10 minutes. Staining and TOF-SIMS analyses were performed on the same sections. Standard Hematoxylin-Eosin (HE) and Nile red protocols were used, as well as washing with various amounts of ethanol. For the plates analyzed by TOF-SIMS after staining, dehydration baths were not done.

TOF-SIMS imaging was performed on a TOF-SIMS IV mass spectrometer (ION-TOF GmbH, Münster, Germany), equipped with a bismuth liquid metal ion gun ($Bi_3^+$ were selected). 500 x 500 $\mu m^2$ (256 x 256 pixels) positive ion images were recorded with a fluence of $3 \times 10^{11}$ ions/$cm^2$.
On the one hand, rat brain sections were stained with HE after a TOF-SIMS surface analysis, and on the other hand, lipid mapping with TOF-SIMS was performed after the HE staining procedure. In the first case we evidenced that the high vacuum conditions applied in TOF-SIMS imaging did not disturb the staining neither the recognition of the brain structures. Optical pictures obtained when HE staining was applied after the TOF-SIMS analysis were compared with a reference slide (without TOF-SIMS analysis). These two images are similar, with nuclei and the aspect and thickness of the *corpus callosum* easily identified. In the second case, a cholesterol fragment ion (*m/z* 369), chosen for imaging, was still detected in the brain structure after HE staining. However, a difference appears between the picture of the tissue reference and the picture of the tissue after HE staining: the *corpus callosum* looks wider than in the reference suggesting a distention of the tissue. This modification of the tissue can be explained by the absence of fixation in the protocol of HE staining prior to the surface analysis by TOF-SIMS. In spite of this distention of the tissue, the cholesterol remains detected in the same region, with no significant delocalization encountered.

Thus, it is possible to work on the same tissue section with both analysis methods, preferably by acquiring the TOF-SIMS images prior to the HE staining. Other staining procedures more specific to lipids, such as Nile red, will be also investigated, always by testing their compatibility with TOF-SIMS. Washing with various amounts of ethanol are also tested to investigate the extraction of lipids for TOF-SIMS imaging.

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**Acknowledgements**

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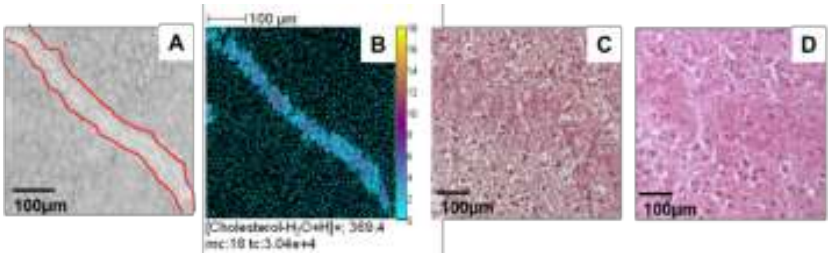
**Figure**




ToF-SIMS analysis prior Hematoxylin-Eosin (HE) stain. (A) Optical image of the extremity of the corpus callosum recorded with the video camera of the ToF-SIMS spectrometer. (B) Ionic image of the same area, *m/z* 369.4 [M-$H_2O$+H]+, (C) Optical image of the rat brain section after the ToF-SIMS analysis and HE staining. (D) Optical image of the rat brain section after having been stained with HE.

# S2. MALDI-imaging as a powerful tool for comparing various human skin states

Bernd Enthaler[1,2], Claudius Rapp[1], Markus Fischer[2], Julia K. Pruns[1].

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## Abstract

Since the introduction of matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) by Caprioli et al. in 1997 [1] there has been an increasing number of publications in the last couple of years. However, only a few groups have dealt with *ex-vivo* human skin and subsequent MALDI-MSI measurements so far [2]. The localization of endogenous and exogenous compounds within intact *ex-vivo* tissue itself, including the investigation of biological processes within tissue, is a challenging task in the field of skin research.

In this manuscript, we report opportunities offered by MALDI-MSI for comparing various skin states, based on the analysis of tryptic peptides (tryptic peptides MSI). Unfortunately, the identification of proteins *via* their tryptic peptides by use of MS/MS measurements directly on tissue is not trivial due to high biological background, resulting in fragment spectra, which are often difficult to interpret. By use of the spatial proteomics approach, MALDI-MSI measurements are used to generate two-dimensional maps of proteins *via* their tryptic peptides and eluates of tryptic peptides obtained from adjacent tissue sections are used to enable accurate identification of the corresponding amino acid sequences by liquid chromatography mass spectrometry investigations (LC-MS/MS). Strikingly, results of both workflows are correlated.

The specific distribution of several proteins, identified *via* their tryptic peptides, is presented as follows: In Fig. 1 a and c *m/z* 1015 is localized specifically in the area of eccrine sweat glands for both skin states. Furthermore, *m/z* 1354 is distributed only in the lower dermis [Fig. 1 b] for skin state 1, whereas for skin state 2 it is localized in the entire dermis [Fig. 1 d]. The identified proteins and the data on the correlation with LC-MS/MS results will be provided.

To summarize, two-dimensional maps of several proteins, identified by use of LC-MS/MS, were generated. Our work reports for the first time the opportunities of spatial proteomics in generating protein maps directly in skin tissue sections. The spatial proteomics approach presented here shows new insights into localizing and identifying endogenous proteins in skin tissue sections simultaneously without the need of labeling. Thus, MALDI-imaging is a powerful tool for comparing various skin states and for biomarker discovery.

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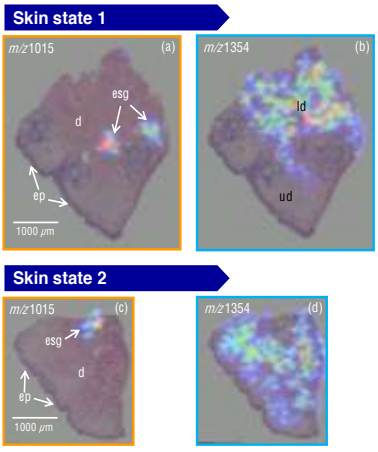


**Figure 1**

Fig. 1 Overlays of ion density maps and corresponding HE staining images.
Overlays of the ion density maps of *m/z* 1015 and *m/z* 1354 and the corresponding HE staining image for skin state 1 is shown in a and b, respectively. Overlays for skin state 2 are depicted in c and d.

Abbreviations:
d – dermis
ep – epidermis
esg – eccrine sweat glands
HE – hematoxylin and eosin
ld – lower dermis
ud – upper dermis

Intensity scale:

# S3. New Advances for In Situ Protein Identification by MALDI In-Source Decay FTMS Imaging

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## Abstract

MALDI imaging mass spectrometry has proven to be effective for the discovery and the monitoring of disease-related proteins [1, 2]. With this technique a molecular analysis could be performed directly from tissue sections in the region of the diseased area. The use of in-source decay (ISD), allowing fast and reliable sequences assignments of proteins termini, has proven to be a crucial tool for proteins identification in solution [3] and tissue slices [4]. However, it is necessary to develop additional tools that allow unambiguous assignment of proteins sequences in complex tissue slices. The development of bioinformatic tools and the use of ultra-*high mass resolution* and high mass *accuracy* of Fourier transform ion-cyclotron (FTICR) mass spectrometry are ideal for this purpose. In this study, we show that FTICR mass spectrometry data filtered with a software that subtracts matrix peaks aids protein identification.

All measurements were carried out on a SolariX FTMS (9.4 Tesla) equipped with a Dual Source with a smartbeam™II laser that includes a robust solid state 1 kHz laser with advanced optics for molecular imaging (Bruker Daltonics). Mouse brain tissue slices of 14 µm thickness were rinsed sequentially with 70% ethanol (30 s), 100% ethanol (30 s), Carnoy's fluid (2 min), 100% ethanol (30 s), H2O (30 s), 100% ethanol (30 s) to obtain optimal sensitivity and high-quality ions. 1,5-Diaminionaphtalene (DAN), at a concentration of 5 mg/mL in ACN/0.2% trifluoroacetic acid (TFA) 70:30 vol/vol, was sprayed using an ImagePrep (Bruker Daltonics, Bremen, Germany). Results were interpreted using BioTools™ 3.2 in combination with Mascot™ (Matrix Science) for ISD spectra and FlexImaging™ 3.0 for MALDI-ISD imaging experiments. Matrix peaks were subtracted using an in-house written Java code that sequentially scans all peak lists from acquired spectra against the DAN mass list. Then, another Java code allows to create 2D ion images at selected *m/z* ratios.

The studies were carried out by MALDI-ISD imaging to create interest on FTICR mass spectrometer for proteins identification in the field of biomarkers characterization. It is demonstrated that protein ISD leads to the same pattern of fragmentation observed during MALDI-TOF analyzes. Fragmentation generates $c_n$- and $z_n$-series ions of myelin in presence of DAN. The internal calibration of all the data provides a mass accuracy neighboring 2.5 ppm over the *m/z* range of interest (300-2500 Da) and a mass resolution of 70000 at *m/z* 400 Da. The use of our software "cleans" MS imaging data by reducing/eliminating MALDI matrix peaks that are isobaric to an analyte peak.

The mass accuracy and high mass resolution coupled with software allow unequivocal assignment of ISD fragments of proteins, in the low mass range (*m/z* between 300 and 900), whether from pure solutions or from tissue slices.

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## Acknowledgements

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# S4. Study of breast cancer adaptation to anti-angiogenic therapies by molecular imaging on tissue slides

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## Abstract

Breast carcinoma is the most common and second leading cause of cancer mortality in women1. The recognition of the "angiogenic switch" as a rate-limiting secondary step in tumorigenesis led to extensive pre-clinical researches on angiogenesis and finally the approval of VEGF-neutralizing antibodies (bevacizumab) and VEGF receptor tyrosine kinase inhibitors (RTKs:sunitinib). The Sunitinib has been used clinically in patients with breast cancer refractory to other therapeutic agents2. Unfortunately, like the cytotoxic therapies, these drugs do not produce lasting effects and resistance to treatment appeared clinically3. Recently, independent laboratories have reported experimental data demonstrating that anti-angiogenic treatments inhibit tumor growth, but also stimulate the formation of lung metastases after treatment discontinuation4. The field of imaging mass spectrometry provides new tools to visualize and study the profiles of proteins and small molecules associated with biomedical problems5.

To this aim, we conducted a series of experiments to setup a reproductible model of resistance to sunitinib. The cells MDA-MB-231 triple negative, from human breast cancer and expressing luciferase are injected subcutaneously into mice RAG1-/-. The mice were divided into four experimental groups including, on the one hand, control mice treated with placebo (Carboxymethyl cellulose, CMC) sacrificed on day 30 (group 1) or when the tumor reached a volume of 300 mm3 (group 2). On the other hand, Sunitinib-treated mice (LC Laboratories, 40mg/kg/day), sacrificed at day 30 (group 3), or when the tumor reached a volume of 300 mm3 (group 4). MALDI mass spectrometry imaging was performed on tissue sections of tumors and organs subsequently colonized by metastases. Matrix sublimation was used to coat tumor sections (14 µm-tick) with 1.5 Diaminonaphthalene (1.5 DAN) for lipids analysis and Sinapinic acid (SA) for entire proteins analysis. Ion cartographies were recorded with a Solarix9.4T FTMS instrument for lipids and with an Ultraflex II TOF-TOF instrument for entire proteins (BrukerDaltonics, Bremen, Germany) with a spatial resolution of 100 µm.

The analysis of differential protein/lipid profiles with high mass accuracy and broadband resolution allows detection of intense signals from lipid families such as Phosphatidylcholine (PC), Triglyceride (TAG), Sphingomyelin (SM) and precise lipid droplets or tumor cells differentiated location in the Sunitinib resistant tumor cells compared to control cells.The protein profiles of the 4 groups of mice show differences in intensity and location, enabling a correlation to inflammatory (highlighted by histological staining) and angiogenic phenomenon.

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Acknowledgements

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# S5. MALDI-MSI and Conventional Proteomic Techniques to Compare Protein Induction in Combretastatin Resistant and Susceptible Tumours

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## Abstract

Variability of protein induction in tumours following vascular-targeted therapy can help to determine both dose response relationships and drug resistance mechanisms. Mass spectrometry (MS), imaging, profiling, tandem MS and conventional proteomics have been used here. Proteins induced in two mouse fibrosarcoma models (expressing VEGF120/ VEGF188 isoforms only) following treatment with the tubulin-binding tumour vascular disrupting agent, combretastatin A-4-phosphate (CA-4-P) have been studied. The nature of the fibrosarcoma models are such that those expressing VEGF 120 are more sensitive to vascular disruption by CA-4-P compared to the more resistant VEGF 188 type. Employment of ion mobility separation with MALDI provided further separation of isobaric ions to assist analysis of numerous species resulting from *in- situ* tryptic digests. *In situ* tissue digestion was performed with trypsin (20µg/ml) solution containing Octyl-α/ β-glucoside. Tissue was incubated overnight 37°C/ 5% $C0_2$. CHCA was deposited using the Sun Collect spraying system. Peptide mass fingerprints, MS/MS analyses, MALDI Images for PCA and PLSDA were performed using an Applied Biosystems Q-Star Pulsar *i* Quadrupole ToF Mass Spectrometer and HDMS SYNAPT ™ G2 system (Waters corporation, Manchester, UK). LC-MS/MS analysis was performed using a *Bruker maXis*™ and Q-Star XL. Regional heterogeneity in tumour tissue has been apparent from initial 60µm spatial resolution MALDI imaging leading to more detailed images at 40µm and 30µm. HDI software (Waters Corporation, UK) provides a novel interactive tool for detailed observation of MALDI-IMS imaging in the comparison of both susceptible and resistant tumour models. PCA displayed clustering of tumour time points supported by PLS-DA regression vector analysis. Statistical analysis of spectra from fibrosarcoma120 tumour tissue confirmed the increase in haemoglobin due to CA-4-P (m/z 1274) this indicated that gross pharmacological responses by CA-4-P can be depicted by MALDI-MS/MSI.[1] However further investigations of other peptides induced by CA-4-P appeared to be hampered by haemoglobin dominance within spectra and PCA/ PLSDA plots. Experimental work with the resistant fibrosarcoma 188 tissue appears to enable further inspection of low abundant tryptic peptides. Conventional proteomic work has revealed numerous proteins and work is underway to relate these findings to MALDI imaging and correlate their relevance to tumour angiogenesis and progression. Immunohistochemical work aims to support this proteomic study.

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**Figures** (a) 40µm MALDI-MSI localisation of Rho GTPase activating protein 2 at m/z 844.5 within fibrosarcoma 188 tumour tissue (b) SYNAPT MALDI imaging plate indicating tissue image regions. (c) 40µm MALDI-MSI inverse localisation of Histone 2AX.

# S6. Analysis of Latent Fingermarks by Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI)

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## Abstract

Fingerprint pattern recognition remains an integral method of identification in forensic investigations. Many techniques are currently available which allow the enhancement and recovery of fingermarks left at a crime scene. The choice of enhancement method is based on the surface a fingermark was deposited on and the chemical composition of the mark, requiring different enhancement protocols for optimum recovery of the mark.[1] One limitation of these techniques is that only one image of the fingermark can be obtained, which could potentially not be of a sufficient quality to ascertain an individual's identity. In this circumstance, more effective methods of visualisation, particularly analytical, are still deemed necessary.
We have previously demonstrated the use of matrix assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) to map the distribution of a variety of endogenous lipids present in latent fingermarks (as well as some exogenous species), thus reconstructing the image of the fingermark ridge pattern at a molecular level[2,3]. Here we present subsequent work involving the development of a novel matrix deposition method named the dry-wet method, which is forensically applicable and enables the optical image of the fingermark to be obtained prior to analysis by MALDI-MSI.[4]

The novel protocol enables the distribution maps of a wide range of endogenous compounds from amino acids to triglycerides to be visualised within ungroomed fingermarks in a single analysis, as well as exogenous contaminants, and can potentially provide chemical information about a donor even if the fingermark is unsuitable for comparison and match in the Police Database. The dry-wet method can also be applied to fingermarks lifted from a variety of non-porous and semi-porous deposition surfaces including glass, metal, plastic, leather and cardboard, demonstrating the potential of the dry-wet method in conjunction with MALDI MSI to be applied as a credible tool in the forensic analysis of fingermarks in the future.

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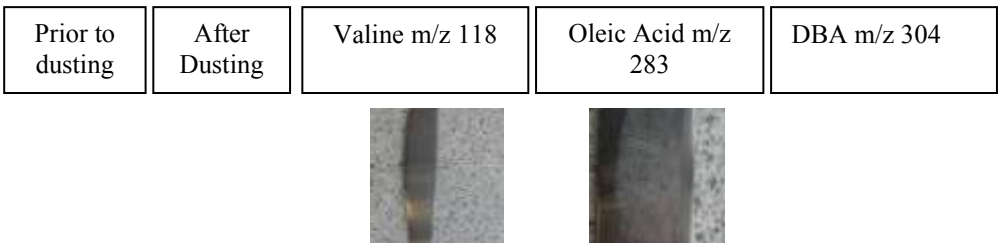


**Figure 1**. MALDI MSI of ungroomed fingermarks recovered from a metal surface, showing the distribution maps of two endogenous compounds ( putative valine at a m/z of 118 and oleic acid at a m/z of 283), and an exogenous compound dimethylbenzylammonium ion (DBA) at a m/z of 304.

# S7. MALDI imaging and top down by MALDI ISD, new approaches for glioma analysis

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## Abstract

The molecular characterization of tissues necessitates the identification of thousands of biomolecules each participating in physiopathological processes. Glioblastoma multiforme (GBM) are the most commonly intracranial tumors encountered in adults. They are assigned a very poor prognosis with a survival median of patients approximately 14 months. The identification of prognostic and predictive factors of response to treatment is a major issue to better understand the biology of these tumors and to optimize their therapy. MALDI molecular imaging enables location of thousands of molecules in tissues at the same time without any kind of labeling. However, molecule identification *in situ* is not an easy task; MALDI in-source decay (ISD) fragmentation has already proven to be effective for protein characterization.

We provide MALDI molecular images at 80 and 30 µm resolution of mouse brain horizontal sections showing protein distributions and reporting the heterogeneity of different anatomical localization pattern, brain structure and glioblastoma localization. We show also a direct molecular printout by top down identification of tubulin isotypes and of tumor progression biomarker in mouse brain tissue section using MALDI ISD.

This is a milestone that should allow progress at the clinical stage for tumor prognosis but also for improvement of targeted chemotherapy of various solid tumors.

# S8. MALDI MSI analysis of fingermarks following forensic fingermark enhancement techniques (FFET's)

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## Abstract

The Home Office currently employs a range of forensic fingermark enhancement techniques (FFET's) which are used to obtain ridge detail from fingermarks deposited at crime scenes. Interestingly, Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI MSI) has been proven to be applicable to latent fingermarks providing fingermark ridge detail and chemical information simultaneously, potentially leading to more investigative leads[1,2,3]. In this work, the compatibility of MALDI MSI with many of the current FFET's and the feasibility of its integration into the current fingermark examination workflow is evaluated. The range of FFET's employed included; A) Powders/ powder suspensions B) Stains C) Chemical developers D) Superglue fuming and E) Vacuum Metal Deposition.

Ungroomed fingermarks were prepared as previously described[1] and deposited onto a range of surfaces including porous and non-porous materials. FFET's were used to enhance the fingermarks before matrix application and MALDI MSI analysis. Matrix application was performed using the dry-wet method[3] (with α-CHCA) or auto-spraying[1] with 5 mg/mL α-CHCA in 70:30 ACN:0.1% TFA, according to the deposition surface. Mass spectrometric analyses were conducted using a modified Applied Biosystems API Q-Star Pulsar i hybrid QTOF instrument (Concord, Ontario, Canada) incorporating a SPOT 10 kHz Nd:YVO4 solid-state laser (Elforlight Ltd.).

Preliminary data suggests that the integration of MALDI MSI with FFET's currently employed in the Home Office fingermark examination workflow can provide additional chemical information that could be potentially advantageous in forensic investigations. In particular, it was possible to obtain fingermark ridge detail simultaneously with chemical information of endogenous and exogenous compounds contained within latent fingermark residue. Fresh fingermarks provided ridge detail that was comparable to those physically enhanced using FFET's, though the quality of the fingermark and the chemical information available decreased through ageing.

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## Figures

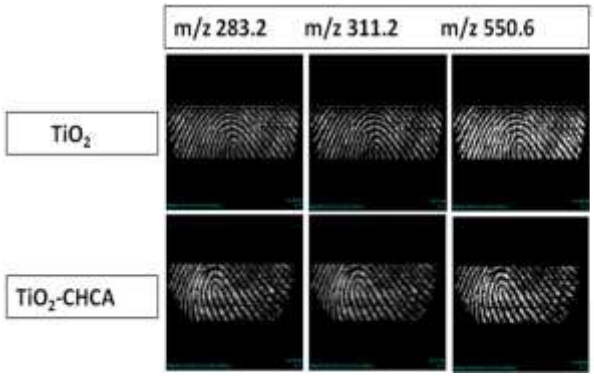


**Figure 1**. Partial MALDI MSI image of ungroomed fingermarks enhanced using a) $TiO_2$ and b) $TiO_2$-CHCA. Molecular images were generated for endogenous species at m/z 283.2 (oleic acid) and m/z 311.2. Also a molecular image of an exogenous species, dimethyldioctadecylammonium, was obtained at m/z 550.6.

# S9. Repeat MALDI analysis of tissue sections provides additional information.

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## Abstract

MALDI-MS is an extremely powerful, label free technique for detection and imaging of the distribution of small molecules in tissue. One advantage of MALDI is the ability to detect a wide range of species without specific labelling. However, it has been shown that different matrices promote the ionization of different biomolecules which argueably limits its power as a truly label free technique. To help address this issue binary matrix systems have been suggested and has recently shown some promise.[1] It has previously been shown that a single tissue section may be analysed multiple times and still provide useful biochemical data.[2] In this study we investigate the information that can be obtained from a single tissue section undergoing MALDI analysis using sequential matrix coatings and whether sensitivity can be improved by aggregation of data from the repeat analysis of a single tissue section.

Initial studies were carried out on sagittal rat brain sections airspray coated with matrix. Repeat analysis data sets acquired using MALDI-QqTOF (QSTAR XL, Applied Biosystems) with matrix having been reapplied between each raster image collection. This study was conducted first using α-CHCA (15 mgmL$^{-1}$ in 80 % $CH_3OH$ / 0.1 % TFA) as the matrix for positive ion mode analysis.
Repeat analysis of a single tissue section after re-coating with the same matrix showed that high quality data could be collected on each occasion. The opportunity for improved sensitivity through the co-registration and aggregation of spectra from the same tissue position after repeat analysis is demonstrated for selected lipids.

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## Acknowledgements

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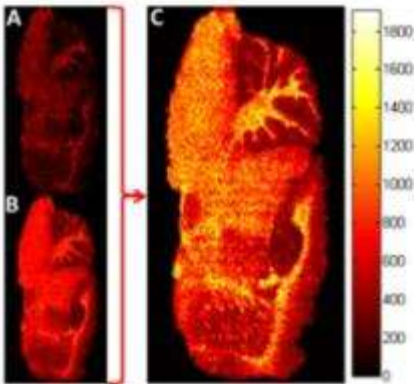


**Figure 1.** Ion images of *m/z* = 826.6 from reanalysis of single tissue section with reapplication of CHCA prior to second analysis. Potential for increased sensitivity is demonstrated by combining ion data from both acquisitions. Acquisition one – A; acquisition two – B; sum of acquisitions

# S10. Analysis of the biocompatibility of different intraocular lens (IOL) material using Mass Spectrometry Tissue Imaging

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## Abstract

The cataract corresponds to the total or partial opacification of the lens of the eye preventing the passage of the light. At present, the surgery is the only effective treatment to overcome the cataract. The surgical intervention consists in removing the cloudy lens and to replace it by an artificial intraocular lens (IOL). The *in vivo* implantation of these synthetic lenses involves the evaluation of several factors as their physico-chemical properties, their capacities to interact with lens epithelial cells and proteins, as well as their biocompatibility. During a previous study, we demonstrated major differences concerning the tackiness (atomic force microscopy), the cellular adhesion and the protein adsorption of various polymer disks intended for the manufacturing of intraocular lenses. The aim of this work was to correlate a histological analysis to a mass spectrometry imaging analysis performed on the same sample. To estimate the biocompatibility of the biomaterials, an animal testing was realized in rabbits. The various polymers were implanted subcutaneously. After one month, the 2 cm x 3 cm pieces of rabbit skin and underlying muscle with a 2 cm thickness were removed, fixed with formaldehyde 10% during six days, treated for the paraffin inclusion and stored at room temperature until use. Slices of 5 µm thickness were performed using a microtome. Paraffin was removed and tissue sections were washed in graded ethanol baths. The slices were then stained with the hematoxylin and eosin dyes. The analysis of stained sections showed different histo-morphological features according to the implanted polymer. For MALDI MSI purposes, on tissue protein digestion was performed using trypsin (1) and the MALDI matrix (α-cyanohydroxycinnamic acid, 5 mg/mL in ACN/0.2% TFA 70:30) was deposited using an ImagePrep automated sprayer (Bruker Daltonics, Bremen, Germany). Experiments were carried out using an UltraFlex II TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). MALDI imaging can show the detection of different proteomic profiles according to the tested biomaterials, which may be considered as biocompatibility markers. The MALDI images of these markers are then correlated with the histo-morphological profiles. Consequently, mass spectrometry imaging can become a powerful tool in the evaluation of the biocompatibility of artificial implants in biomedical application.

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# S11. MALDI/MS$^n$ Imaging of Lipid Oxidation Products in Nervous Tissue

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## Abstract

There is increasing evidence that phospholipid oxidation products play an important role in the pathogenesis of various diseases, including diabetes.[1,2] Following oxidative stress, unsaturated phospholipids undergo oxidative modifications resulting in alterations to the fluidity, permeability, and integrity of cellular membranes.[3] Isolation and characterization of these products is challenging, as there can be multiple oxidative modifications to each phospholipid. Nevertheless, tandem mass spectrometry (MS$^n$), coupled with soft-ionization techniques, provides a method to elucidate the structural alterations to phospholipids following oxidative stress.[3] This investigation characterizes phospholipid oxidation products by matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS$^n$) using a linear ion trap (LIT) mass analyzer. The MS$^n$ capabilities of the LIT were exploited to provide enhanced selectivity, resulting in more reliable identification of oxidized phospholipids. Once the fragmentation patterns of these oxidation products were characterized, the spatial distributions of these products in nervous tissues from diabetic rats were determined by MALDI-MS$^n$ imaging.

The MS$^2$, MS$^3$, and MS$^4$ fragmentation patterns of commercially available phosphatidylcholine (PC) short-chain oxidation products were characterized. These standards are analogous to the short-chain products formed by oxidative cleavage along the *sn*-2 fatty acid tails of PCs. MS$^3$ imaging was utilized to differentiate the spatial distribution of characteristic oxidation product ions from other isobaric species abundant in tissue (e.g., ceramides and heme). One of the oxidation products studied was 1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (POVPC, MW = 593 Da). Figure 1 illustrates the MS$^2$, MS$^3$, and MS$^4$ spectra of the [M+Na]$^+$ ion of POVPC from diabetic rat spinal cord tissue. Figure 2 illustrates the ability of MS$^3$ imaging to differentiate the distribution of POVPC (a) from heme (b).

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## Acknowledgements

The authors would like to thank Dr. Peter Stacpoole and Dr. Albert Shroads for helpful discussions of oxidative damage to nervous tissues and the Yost group members for their insightful discussions and review of this work.

## Figures

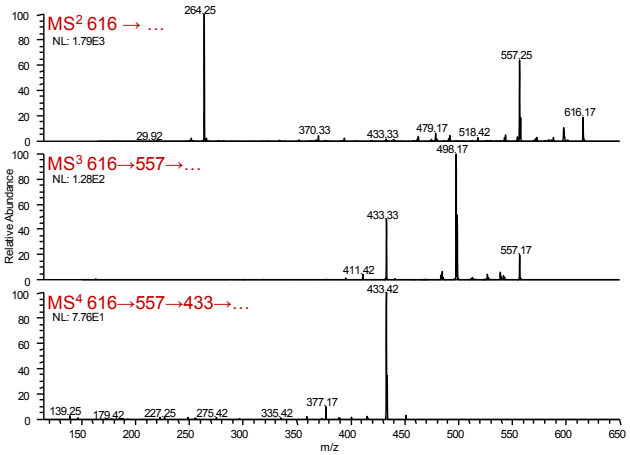


Figure 1. $MS^2$, $MS^3$, and $MS^4$ spectra of the $[M+Na]^+$ ion of POVPC from diabetic rat spinal cord tissue . The fragent ion at m/z 264 (in $MS^2$) is indicative of an isobaric ceramide at *m/z* 616. The isboaric heme species is evident by the fragement ion at *m/z* 498 in the $MS^3$ spectrum. Although the signal and fragmentation efficiency is low in $MS^4$ of POVPC in tissue, the fragmentaion pattern of *m/z* 433 is similar to that observed using the standard.

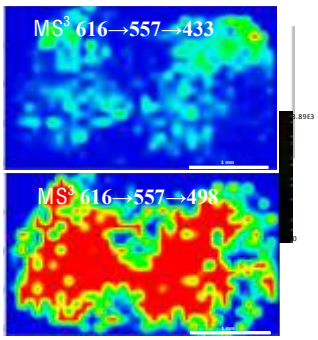


Figure 2. $MS^3$ images of POVPC (a) and heme (b) in diabetic rat spinal cord. $MS^3$ is necessary to differentiate these species since the parent ions are isobaric (*m/z* 616) and CID fragmentation of both species yields a predominant daughter ion at *m/z* 557 (NL of 59). $MS^3$ 616→557→498 results from another NL of 59, indicative of heme (a), and $MS^3$ 616→557→433 results from a NL of 124, indicative of POVPC (b). The $MS^3$ image of POVPC shows localization in the gray matter and dorsal horn region.

# *S12. MS Imaging of small metabolites in Fruits*

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## Abstract

MS Imaging of large molecules, e.g. proteins and lipids, have been reported with MALDI. On the other hand, few works have been done on mapping the small molecules by MALDI imaging. This is mainly due to the high chemical noise background interference in the low mass region cause by chemical matrices. DESI Imaging, however, could be complementary to MALDI in that sample can be analyzed directly without matrices.

A large group of small metabolites are of considerable physiological and morphological importance in plants, e.g. flavonols, involve in plant defense against environmental stresses and organic acids, are one of important factors for fruit quality, but knowledge of their precise functions is limited due to insufficient characterization of their spatial responses.

In this communication we will discuss methodological details about MS imaging of small metabolites in apple and grape in terms of sample preparation, imaging methods, and other experimental concerns by using MALDI [1] and DESI source coupled with a high resolution/accurate LTQ-Orbitrap mass spectrometer. Finally, we will describe the spatial distributions of flavonols and organic acids in apple and grape, respectively.

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Figures

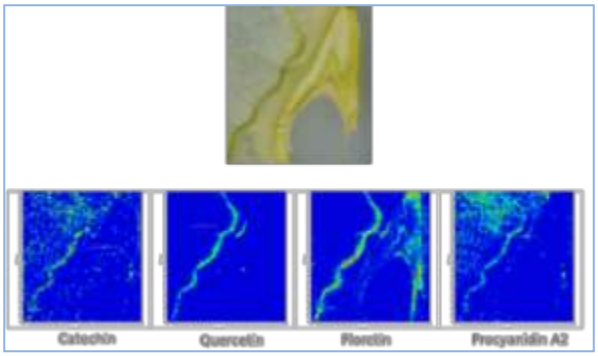


Figure 1. MALDI Imaging of flavonols in apple

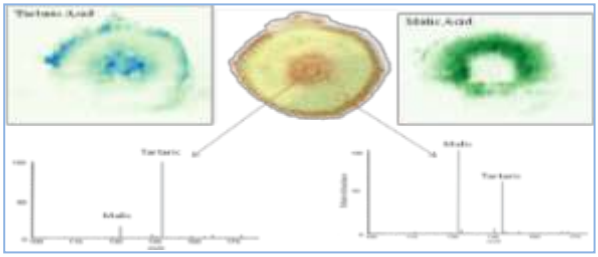

Figure 2. DESI Imaging of organic acids in grape

# *Oral Presentations*

# *O1. New Approaches for Mass Spectrometry Imaging*

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## Abstract

Mass spectrometry imaging is a versatile and powerful analytical technique. Our work is focused on further increasing the the biologically relevant information that can be obtained by mass spectrometry imaging. Here we present a number of improvements in instrumentation, sample preparation, measurement parameters and data processing. The discussion will be based on phospholipids in mammalian samples, but the method was also successfully used for other applications including insect and plant specimen. Even though experiments are partially based on home-built instrumentation, a large part of the presented concepts and approaches is generally applicable.

MS imaging experiments were performed with a high resolution atmospheric-pressure imaging source [1] attached to 'LTQ Orbitrap', 'Exactive Orbitrap' or 'Q Exactive' mass spectrometers (Thermo Scientific GmbH, Bremen). Pixel size was between 3 and 10 µm. Mass accuracy was better than 2 ppm (root mean square) under imaging conditions. Tentative identification based on accurate mass was confirmed by on-tissue MS/MS experiments. MS images were generated with a bin size of $\Delta m/z = 0.01$, which largely eliminates interferences from neighboring peaks in complex samples.

A dedicated sample preparation protocol was established for the analysis of cell cultures. Phospholipids and smaller metabolites such as nucleic acids and cholesterol were imaged in single cells. A full metabolic profile of was obtained from a single 7 µm pixel.

Phospholipids were investigated in detail in mouse brain and human tumor samples. 'All ion fragmentation' experiments were used to image intact phospholipids simultaneously with their acyl chain and headgroup fragments. This allowed the (tentative) differentiation of isomeric lipid structures throughout the whole section within one experiment. A complete set of positive and negative ion images was obtained simultaneously by periodically switching the polarity of the ion optics throughout the imaging experiment. This significantly increased the number of lipids that could be identified in one experiment and thus improves the differentiation of tissue types. MS image acquisition was increased to up to 10 pixels per second while maintaining high mass accuracy.

Statistical analysis tools (including PCA and LDA) were adopted and applied for semiautomic assignment of tissue types in mouse and human tissue sections. Additional data analysis tools were made accessible by conversion to the common data format for MS imaging - imzML (www.imzml.org). This enables a flexible choice of the software best suited for a given application. Measurements from different instruments (data formats) were converted to imzML and displayed in one software tool with identical settings in order to allow easy comparison.

### References

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# *O2. Combining lipidomics and proteomics by MALDI-MSI*

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## Abstract

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is a method that allows the investigation of the molecular content of tissues within its morphological context.[1] Recently, the use of multiple matrices by applying inkjet-printing onto a single tissue surface has been presented.[2]

The aim of this contribution is to show its useful application by analyzing two different compound classes, lipids and proteins, in a variety of different tissue organs. This developed methodology is inparticular interesting when precious tissue samples are employed.

Organs, such as liver, brain, and kidney were rapidly extracted from a C57BL/6J mouse and snap frozen in liquid nitrogen and stored at -80 °C. For the MALDI-MSI analysis 12 µm thick sections were cut in a microcryotome (Leica CM 1850, Microsystems GmbH, Wetzlar, Germany) and thaw mounted on ITO glass slides (Bruker Daltonics, Bremen, Germany). Prior to MALDI-MSI analysis the sections were dried in a vacuum desiccator for 10 minutes. For the analysis of lipids α-cyano-4-hydroxy cinnamic acid (10 mg/mL α-CHCA and 1 mg/mL LiTFA in acetonitrile/water/TFA 70:30:0.1, v/v/v) and for proteins sinapinic acid (10 mg/mL in acetonitrile/water/TFA 50:50:0.1, v/v/v) were used. The matrices were applied with a spatial resolution of 500 x 500 µm across the tissue section with an offset of 250 µm x 250 µm using an inkjet-printer (microdrop Technologies GmbH, Norderstedt, Germany). A volume of 3 drops per spot during each pass and 12 passes of matrix application were applied. Droplets for both matrices were generated using a voltage of 144 to 148 V, a pulse width of 30 to 40 ms, and a fixed frequency of 200 Hz. Mass spectra were acquired on an Ultraflex III MALDI-TOF/TOF instrument (Bruker Daltonics) running in the positive mode. A m/z range of 500-1,600 for lipids and of 2,000-23,000 for proteins were analyzed, respectively. A total sum of 500 shots per spot was acquired in steps of 50 shots in a random pattern. MS acquisition and visualization was performed using FlexImaging and FlexControl software (Bruker Daltonics).

The methodology was first tested on mouse liver tissue, since this is the most homogeneously available tissue. As expected the distribution of lipids as well as proteins was more or less homogeneously as determined by a series of MALDI-MSI images, as shown in Figure 1 (a) and (b). The next investigated tissue type was mouse brain. Since the limit of resolution is currently 500 µm for the application of two matrices on a single tissue slice, some anatomical features of the mouse brain are presented by only a few pixels in the recorded MALDI-MSI images, however a lower resolution caused the generation of a homogeneous film instead of discrete spots. Further improvements are still ongoing. Finally, mouse kidney was analyzed and the distributions of lipid and protein signals were compared. The developed methodology shows the simple combination of two compound classes or areas (lipidomics and proteomics) in the emerging technique MALDI-MSI.

## References

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## Acknowledgements

A. C. Crecelius acknowledges the financial support of the Friedrich-Schiller University for the ProChance 2010 project and U. S. Schubert thanks the Dutch Polymer Institute (DPI, technology area HTE) for financial support.

## Figures

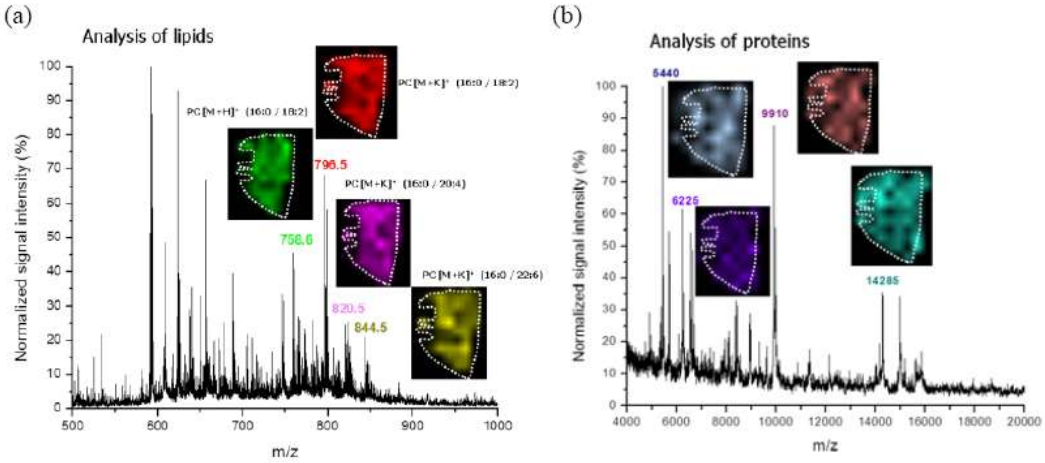


Figure 1. MALDI-MSI mass spectra and corresponding images of (a) lipids, and (b) proteins obtained from the analysis of a single mouse liver tissue.

# O3. Approaching MALDI molecular imaging for preclinical and clinical research: current state and fields of application.

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## Abstract

In the last years, Matrix-Assisted Laser Desorption/Ionization (MALDI) imaging mass spectrometry ("MALDI imaging") has emerged as promising technique for combined morphological and molecular tissue analyses. It enables a spatially resolved, unlabeled and multiplexed analysis of different molecule classes, ranging from small molecules (drugs) to proteins, lipids and other analytes, directly in tissue sections. Molecules can be imaged in the histological context of tissues, and therefore, molecular profiles can be allocated to specific cell types.

MALDI imaging has proven to provide novel and clinical relevant information in a variety of different biomedical questions, with focus in oncology and inflammatory diseases. While several studies so far have worked on a proteomic level, of increasing interest is also the ability of MALDI imaging to visualize the spatial distribution of drugs and their metabolites in tissues, which are valuable for drug development and efficacy studies in animal models and even in individual patients.

This presentation will give an update on the application of MALDI imaging in preclinical and clinical research. We discuss the use of MALDI imaging in clinical proteomics and put it in context with classical proteomics techniques for tissue analysis. In the research area of gastrointestinal disorders MALDI imaging has already been used to address several questions of upper- and lower gastrointestinal diseases, which will be briefly presented. We also highlight a number of upcoming challenges for personalized medicine, development of targeted therapies and diagnostic molecular pathology where MALDI imaging could help.

Because of its practical simplicity and ability to gain reliable information even from smallest tissue amounts, which may also originate from endoscopic biopsy sections, we believe that MALDI imaging might have the potential to complement histopathological evaluation for assisting in diagnostics, risk assessment, or response prediction to therapy.

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## Acknowledgements

**Grant support:** The author gratefully acknowledges the financial support of the BMBF (grants no. 01EZ0803, no. 0315508A and no. 01IB10004E) and the Deutsche Forschungsgemeinschaft (SFB 824 TP B1, SFB 824 TP Z2, and WA 1656/3-1).

# O4. Distribution and identification and of molecular interactions between tomato roots and bacterial biofilms

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## Abstract

Some non pathogenic microorganisms evolving in the root micro-environment can trigger a positive effect on plant, increasing host defense against disease or/and directly inhibiting growth of pathogen in soil (1). To initiate both phenomena leading to biocontrol activity, microorganisms use plant exudates to grow on roots and to produce *in-situ* active compounds. In Bacilli, cyclic lipopeptides of the surfactin, iturin and fengycin families represent important antibiotics involved in biocontrol (2). Recent studies in microbiology allowed a better understanding of plant microorganism interactions but few has been done at the molecular level. In this study, MALDI MS imaging has been used to study the nature of the secreted lipopeptide molecules, their relative quantity and their distribution in the root's environment.

Disinfected tomato seeds were first germinated at 28°C in sterile conditions for germination. Seedlings were then placed in Petri dish on ITO glass slide recovered with a thin layer of plant nutritive solution (Hoagland) containing 1,75% of agar and treated with freshly-grown cells of *Bacillus amyloliquefaciens* S499. Petri dishes were finally incubated vertically in phytotron at 28°C with a 16h photoperiod. Different root age / time of incubation were studied: 13 / 3; 13 / 7; 21 / 14 and 39 / 32. Control tomato root (without bacterial treatment) of the same ages were also analyzed (13 / 0; 21 / 0 and 42 / 0. For MALDI imaging experiments, the ITO slide was removed from the agar and dried in a dessiccator under vacuum. The matrix solution (α-cyano-hydroxycinnamic acid, 5mg/mL in ACN/0.2% TFA 70/30) was applied with an ImagePrep automated sprayer (Bruker Daltonics). An UltraFlex II TOF/TOF and a Solarix FT-ICR mass spectrometers were used to record molecular cartographies.

The average mass spectra recorded around the tomato root (2-3 mm on both sides of the root) showed that lipopeptides were major compounds detected on the agar. The relative intensity of lipopeptides families varied with respect to the age of the root/biofilm system. In the 13/3 system, 3 homologues of surfactins were essentially detected (C13, C14 and C15), with very few iturins and fengycins. Their localizations were identical, whatever the considered homologue. Then the production of iturin and fengycin families increases in older systems (13/7 and 21/14) and a novel homologue of surfactin is detected (C12). Some variations in localizations within families may be observed (around the root or at the close vicinity of it in function of the considered homologue or alkali adduct). Then for the oldest system we studied, iturins and fengycins are not detected anymore and the localization of surfactins is less precise. In the 39/32 system, we also detected unknown compounds at 986.6, 1000.6, 1014.7 and 1028.7 m/z. The mass range of these compounds allied to the mass difference between two consecutive ion peaks let us think that these unknown compounds could be a new lipopeptide family. Investigations are in progress to identify these new secondary metabolites of *Bacillus amyloliquefaciens*.

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## Acknowledgements

The FRS-FNRS (Fonds National de la Recherche Scientifique, Belgium) is acknowledged for a postdoctoral fellowship to D.D. and E.J. M.O. is senior researcher associate at the FNRS.

# O5. SCiLS Lab: software for analysis and interpretation of large MALDI-IMS datasets

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## Abstract

3D imaging has a significant impact on many challenges of life sciences. 3D matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) is an emerging label-free biochemical analytical technique visualizing the distribution of hundreds of molecular compounds in 3D by providing a MALDI mass spectrum for each spatial point of a 3D sample. Currently, 3D MALDI-IMS cannot tap its full potential due to the lack of efficient computational methods for building, processing, and visualizing large and complex 3D IMS data. In this talk we present a collection of new efficient computational methods which enable creation, analysis, and interpretation of a 3D MALDI-IMS dataset.

We present our software SCiLS Lab for visualization and analysis of 3D MALDI-imaging data. It takes several 2D MALDI imaging datasets as an input, each dataset corresponding to a consecutive slice. Then, it creates a 3D cloud of measurement points in the 3D Cartesian space with coordinates (x,y,z), with a spectrum assigned to each point. For mining large 3D MALDI-IMS data, we propose using the spatial segmentation approach which is well-known in 2D MALDI-IMS [1]. In order to reduce significant spectrum-to-spectrum variation, we propose to use edge-preserving 3D image denoising prior to segmentation [2]. For segmentation, we developed a new efficient clustering method, called as bisecting k-means, which is suitable for hierarchical clustering of a large 3D MALDI-IMS dataset.

For demonstration of the software we present a simulated 3D MALDI-TOF-IMS dataset based on the anatomical model of mouse brain provided by Allen Institute for Brain Science (http://mouse.brain-map.org). After importing this atlas into our internal format, we simulated a MALDI-TOF spectrum for each voxel using our statistical simulator based on [3]. Moreover, we demonstrate the software with a measured 3D dataset of the central part of a mouse kidney built up of 115 slices. Individual serial sections were measured using 2D MALDI-IMS following the standard protocols with the high spatial resolution of 50 µm. Altogether, more than two million mass spectra were acquired that corresponds to approximately 200 GB of data.

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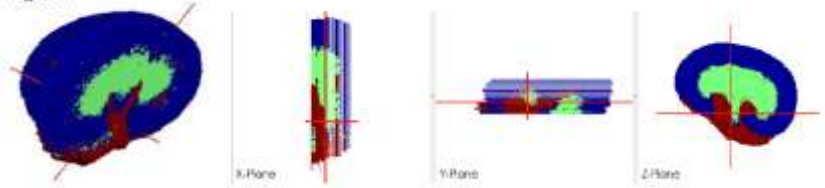
Figures



# O6. Imaging Mass Spectrometry Based Molecular Histology: Defining Tissues by MS Profiles Provides New Diagnostic Capabilities

Emrys A. Jones[1], René J.M. van Zeijl[1], Pancras C.W. Hogendoorn[2], Judith V.M.G. Bovée[2], André M. Deelder[1], Liam A. McDonnell[1]

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## Abstract

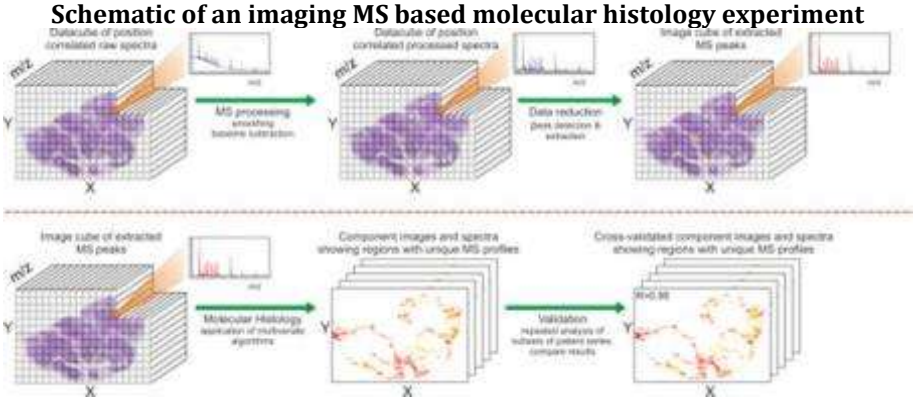
There is growing awareness that imaging MS can be used to annotate tissues based solely on the detected MS profiles and thereby identify regions that are not distinct using established histological methods but which are characterized by distinct MS profiles. For example it was recently demonstrated how imaging MS could provide a novel structural definition of the claustrum, an ill-defined forebrain structure owing to its vague borders. It is in the field of pathology in which imaging MS based molecular histology could have its greatest impact, as it is able to highlight biomolecular changes that occur prior to, or without, an analogous morphological change. Imaging MS based molecular histology has revealed neuropeptide and metabolite changes in rodent models of neurological disorders; differentiated between tumors with overlapping/ identical histologies; highlighted biomolecular intratumor heterogeneity; and demonstrated that tumor associated protein profiles may extend far beyond histological borders into the surrounding healthy tissues. Here we will compare and contrast the different data analysis algorithms that have been reported, review the applications in which it has complemented established histological or histochemical practice, and demonstrate how imaging MS based molecular histology can be simultaneously applied to 3D imaging MS datasets and patient series of tissues.

## Acknowledgements

This work is financially supported by NWO Horizon project 93511027 and the ICT consortium COMMIT project 'e-biobanking with Imaging' and the Cyttron II project 'Imaging Mass Spectrometry.'

## Figures

**Schematic of an imaging MS based molecular histology experiment**



# O7. Combining SIMS imaging mass spectrometry and CARS spectro microscopy to reveal patterns in developmental biology

Florian Marty[1,2] Gianluca Rago,[2], Gert Eijkel[2],Don F. Smith [2], Luke MacAleese[2,3], Andrei Kharchenko[2], Mischa Bonn[2], Konrad Basler[1], Erich Brunner[1], Ron M.A.Heeren[2]

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## Abstract

A tightly controlled and time-resolved genetic program guarantees that an organism is properly assembled and reliably consists of the necessary and precisely positioned structures. A paradigm for studying organ development is the wing imaginal disc of *Drosophila melanogaster*. It is known for decades that spatially restricted gene expression in the wing primordium leads to the formation of sharp boundaries between distinct cell populations, called compartments. The compartment boundaries not only serve as borders, preventing the cells from mixing with each other, but also act as signaling centers, which organize patterning and growth of the developing tissue. Importantly, our current understanding is primarily based on the genome and the proteins encoded therein. However, there is increasing evidence that also small molecules may play essential roles during patterning and growth of the disc. The ability to gather information on the chemical composition and the microscopic imaging capabilities in one experiment makes secondary ion mass spectrometry imaging (SIMS-IMS) a powerful tool to screen for small molecules relevant for developmental processes.

Using SIMS-IMS single wing imaginal discs were imaged in positive and negative ion mode on a SIMS TRIFT-II TOF. Principle component analyses of these images revealed patterns reminiscent of the known compartments that drive the patterning and growth of the wing disc[1] (Figure 1). Currently, the interpretation of SIM-IMS spectra underlying the identified Principle Components (PCs) is challenging and a direct identification of the molecules that correspond to these PCs fails due to the lack of MS/MS possibilities. To narrow down the space of candidate molecules we measured wing discs using Coherent anti-Stokes Raman Scattering spectro-microscopy (CARS). Applying canonical cross correlation analysis between the SIMS-IMS and CARS datasets we identified some of the patterns observed by SIMS-IMS with a high significant correlation score. Inspection of the CCA components revealed a differential occurrence of lipids and proteins in distinct regions of the disc. From this we conclude that lipids may - at least in part – underlie the PCs observed by both, CARS and SIMS. We are currently generating a detailed lipid map of the wing disc in order to identify and functionally characterize lipids that mediate wing disc development.

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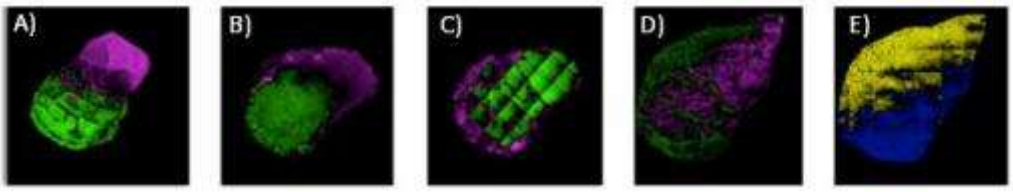


Figures

**Figure 1: Patterns identified by SIMS IMS: A)** Pattern identified on the wing imaginal disc ressembling the differentiation between hinge and notum region. **B)** Division of pouch versus non pouch tissue. **C)** Visualization of the ventral versus the dorsal compartment as seen in SIMS IMS. **D)** Pattern reminiscent of the antagonistic activity of brinker/dpp. **E)** Anterior versus posterior compartment know to be established by cell lineage restriction.

# *O8. Quantitation in MALDI-MSI: What can we learn from MALDI-SRM/MS?*

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## Abstract

In matrix-assisted laser desorption/ionization (MALDI) the mass spectrometric response depends on many factors which are specific for the analyte, the matrix, the sample preparation and the mass spectrometric system. For quantitative MSI the influence of the various parameters should carefully evaluate so that one can determine the precision and accuracy windows of the measurements. However it remains challenging to find a model system to evaluate the influence of the various parameters. Also as for any technique the use of an adequate internal standard (structural or labeled analogue) allows to compensate for sample preparation and ionization variability but his analyte dependent**.** MALDI with a high repetition laser mounted on a triple quadrupole linear ion trap has been successfully applied for the direct quantification of low molecular weight compounds and peptides in complex matrices such as plasma or cell extracts[1,2]. MS acquisition was performed in the selected reaction monitoring mode. Precision and accuracy better the 15 % could be obtained in rastering mode over almost three orders of magnitude and using labeled internal standards or structural analogs. Laser frequency, MS dwell time and rastering speed, intra and inter spot averaging were found to affect overall quantitative performance. The described approach will be used as a model system to discuss the response variability for metabolites and peptides signal form a MSI perspective at a pixel level with and without internal standards.

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# O9. Maldi Imaging of small histological tissue applied to Ophthalmology: Qualitative and Quantitative approach in Drug discovery

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## Abstract

Mass Spectrometry Imaging (MSI) has become a common technique to detect the localization of molecules directly on the surface of biological tissues without labeling. Recently, numerous studies have dealt with the growing interest in combining quantitative and distribution analyses using MSI. This application may play a significant role in early phases of pharmaceutical discovery to evaluate small molecule concentration, notably drugs. Moreover, unlike classical imaging and quantification techniques, MSI can provide precise and selective quantitative information at micrometer level to differentiate fine histological structures. Our approach, first described on model tissues, will then be applied in an ophthalmic study to assess the distribution and quantification of several compounds in specific areas of the eye after instillation.

Our experimental section is divided into two parts; first, olanzapine-dosed rat kidney tissue (at 10 mg/kg, 2 hrs post injection) and patented "spiked tissues" models. Secondly, rabbit eyes were treated with Benzalkonium chloride (BAK) and others compounds, respectively. Cryosections of each tissue were carried out with a Microm cryostat HM560 (Thermo Scientific), specific thicknesses were used depending on the sample. All sections were mounted on conductive ITO glass slides, then, dried and coated with the adapted matrix solution. MALDI-MSI analysis was performed on an Autoflex Speed MALDI-TOFMS (Bruker Daltonics) and a SolariX 7.0T FTMS (Bruker Daltonics) both equipped with a Smartbeam-II (λ=355 nm, 1000 Hz).

Our methodology of quantification by MSI consists in three steps:

Take into account tissue suppression effect by estimating loss of signal intensity related to specific type of tissue and molecule. TEC (Tissue Extinction Coefficient) is calculated by comparing average intensity of a drug standard applied to a control tissue with the same value directly on the slide.

Calibration curve of targeted molecule is calculated using imaging data. From standard dilution series and for each concentration spot, molecular image is constructed, mean intensity values are extracted and correlated to amount of drug per surface unit.

Dosed tissue analysis performed following same analytical parameters. Data are then integrated into software to obtain an amount of compound in sample in gram per gram of tissue.

For the reference sample, we developed a model tissue that can be evaluated by MSI by homogenizing a control rat kidney spiked with a fixed amount of drug. The tissue is reconstituted via flash freezing, then, undergoes traditional MSI sample preparation. Homogenate distribution of the target drug is evaluated by MSI through transversal and longitudinal section of the spiked tissue, which in turn, allows us to validate our methodology.

Afterward, the distribution and quantification of Benzalkonium chloride, BAK C12 *m/z* 302.3 and BAK C14 *m/z* 332.3, were investigated in small specific histological regions of the eye (such as iridocorneal angle or sclera, choroid, retina regions) in order to estimate efficiency of action or adverse effects of the treatment. Local drug concentration differences were observed according to histological area and position on the eye section (anterior, posterior, temporal or nasal side). Molecular distribution was also correlated to tissue histology using H&E staining.

# O10. "Mass Spec Imaging Develeopments for Targeted Drug Treatments"

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## Abstract

Pulmonary Diseases such as Lung Cancer and Chronic Obstructive Pulmonary Disease (COPD) are currently the major leading cause of death and its prevelance is increasing. The leading cause of COPD is smoking and an estimated 600 million patients suffer from the disease. We collected blood plasma from gefitinib-treated NSCLC patients in Japan in a pharmacoepidemiologic cohort study. Today, we are able to optimise lung retention and correlate drug Pharmaco Kinetics in lung with effect and toxicity when only total concentration of drug in lung tissue homogenate can be measured (1). We performed a study for direct measurement of drug uptake in terms of a time kinetic and concentrations attained at the local sites has not been readily available as a clinical index for most drugs. A proof-of-principle study was conducted to test the utility of applying matrix-assisted laser desorption ionization-mass spectrometry imaging (MALDI-MSI) to demonstrate the qualitative distribution pattern of a locally administered drug within tissue sites of targeted action. We have also measured the occurrence of an inhaled the muscarinic receptor antagonist ipratropium, within human bronchial biopsies obtained by fiber optic bronchoscopy shortly after dosing exposure (2-3). Cryo-preserved biopsy samples from five subjects being evaluated for airway obstruction or potential tumor development were prepared as thin frozen sections. Samples were analyzed by a MALDI LTQ Orbitrap XL mass spectrometer at high (30 μm) spatial resolution, of drug localization within organ compartments using normal clinical dosing schemes. Our results demonstrate the ipratropium is rapidly absorbed into the airway wall and localized in activated smooth muscle cells.

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# *O11. The Cell by cell chemical characterizations of the brain via mass spectrometry: from profiling to imaging*

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## Abstract

In the postgenomic era, one expects the chemical players in a brain region to be known and their functions uncovered. However, many cell-to-cell signaling molecules remain poorly characterized and for those that are known, their localization and dynamics are oftentimes unknown. A suite of mass-spectrometry-based approaches are described that allow the investigation of individual neurons and small brain regions for their metabolites and peptides. Direct single cell MALDI-MS and MALDI-MSI provide unmatched information on the contents of the cells.[1] Single cell MALDI-MS has been used for the discovery of hundreds of novel peptides and in the characterization of the heterogeneity of cellular populations.[2] While this approach typically requires manual sample preparation and therefore is low throughput, it provides unmatched performance. MALDI-MSI has evolved into a sensitive approach capable of probing tissues at cellular-level resolutions. We have adapted MALDI MSI to dissociated cell populations and hyphenate it to other information rich measurement capabilities. MALDI-MSI can acquire thousands of mass spectra from cell-sized samples, and can even be used to directly acquire spectra only from cells and not the spaces between them.

The high sensitivity of MALDI-MS, requiring attomole to zeptomole amounts of analyte, allows a range of analytes to be characterized in individual cells.[2,3,4] One often overlooked advantage of single cell MALDI-MS and MALDI-MSI is its low analyte consumption. MALDI consumes just a small fraction of the material at a specific location; one can re-measure the same sample on the MALDI target spot and obtain the same results. We have confirmed this using absolute quantitation and even standard addition from a selected cell.

Several applications of single cell MSI are highlighted including metabolite profiling of selected cells. As examples, new serotonin-related compounds and literally hundreds of new neuropeptides have been characterized in well-defined neuronal networks, and in several cases, the functional roles of these molecules described. MSI and dynamic sampling of the extracellular environment are used for elucidating novel cell to cell signaling molecules in a range of neuronal model systems. Current efforts involve extending the depth of metabolome coverage and adapting these analytical approaches to higher throughput single cell assays. We highlght one example looking for selected neurons within the rat dorsal root ganglia that respond to pain; while most neurons do not respond, a small subopoplation of neurons s have changes in their chemical profiles that correlate to noniceptive stimuli. Our overarching goal is to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

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# O12. Optimising data acquisition and data handling methods for MALDI-MS and MALDI-MSI of small molecules in tissue

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## Abstract

MS imaging is a powerful method for mapping molecules directly in tissue with applications in a diverse range of disciplines. In this presentation we address optimization of methods for data acquisition and data handling and describe routes to maximizing information obtained in imaging studies.

Raster mode MALDI imaging permits significantly shorter analysis times in MALDI imaging. Faster stage speeds require access to high repetition rates in order to generate sufficient ions for detection from each pixel area. Optimization and assessment of laser delivery is vital for improved performance. We present results from a systematic study of the effects of high repetition rates in raster mode MALDI conducted on a QStar XL (AB SCIEX) using an Nd:$YVO_4$ laser (Elforlight). A new method of profiling beam characteristics via capture of laser-induced fluorescence was developed and used to assess laser beam profiles and fluence following delivery through a selection of fibres.

Optimized methods were applied to the study of human liver disease. Images of stained sections labeled by a histologist are projected onto a MALDI image and used to label all pixels in the dataset. Effective classification of disease type and tissue region from MS data was afforded by memory efficient PCA routines and higher order clustering of PCA results. The MALDI image data were used to inform spatial and spectral locations for further, high resolution, in-situ mass spectrometry profiling using liquid extraction surface analysis (LESA, Advion) coupled to nano-electrospray and high resolution MS (LTQ Orbitrap Velos, Thermo Scientific).

# O13. ProteoRed experience in the development of MIAPE-related tools

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## Abstract

From its foundation, ProteoRed, the Spanish networked proteomics platform, has worked in the incorporation of proteomics standards, participating in the HUPO-PSI [1] activities and developing standard-related bioinformatics tools. Significant efforts have also been made to develop a toolkit [2] that manages the MIAPE (Minimum Information About a Proteomics Experiment) [3] information.

The "MIAPE Generator tool", an online tool accessible at http://www.proteored.org/MIAPE-Generator, is based on a set of web forms dedicated to compile MIAPE information. In order to create a MIAPE-compliant report the user has to go through all requested fields typing in the data manually, or loading previously entered data, while it is stored in a database. This tool has been used by ProteoRed members in the ProteoRed Multicentric Experiments (PME) since all experiments were reported using this tool.

Further MIAPE-related developments have wanted to reach the automation of the MIAPE generation. The "MIAPE extractor tool", free accessible at http://www.proteored.org/MIAPE-Extractor, allows the automatically extraction the MIAPE information from proteomics standard files (mzML, mzIdentML or PRIDE). Once the MIAPE-compliant information is extracted, it is remotely stored in the ProteoRed MIAPE repository, where can be edited and consulted using the previously described "MIAPE Generator tool". Furthermore, that MIAPE information can be exported as a PRIDE XML file for its submission in the PRIDE repository, assuring its MIAPE-compliance.

Additionally, the tool provide a new functionality which allows performing reproducibility analysis of different experiments containing different replicates, presenting different plots representing different qualitative aspects.

This tool has resulted extremely useful for qualitative comparisons under the multi-centric experiments organized by ProteoRed, as well as for the comparison between ProteoRed participants of the last study (2011) of the Proteomics Standards Research Group (sPRG) of the Association of Biomolecular Resource Facilities (ABRF).
Very useful information has been extracted from these studies, and some of them will be presented here.

In conclusion, ProteoRed MIAPE related tools provide to the scientific community an appreciating help to manage MIAPE information from proteomics experiments, that is, to assure that our experiments are reported with the minimal information required by journals. Additionally, we also propose the MIAPE-Extractor tool as a tool for multiple qualitative experiment comparison.

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# O14. Imaging mass spectrometry provides fingerprints for distinguishing hepatocellular carcinoma from cirrhosis

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## Abstract

MALDI imaging mass spectrometry (MALDI IMS) is a powerful tool for comprehending the spectrum of peptides/proteins expressed in tissue sections. The aim of the present study was to investigate, using MALDI IMS, the proteome of hepatocellular carcinomas (HCC) and to compare it with peritumoral cirrhosis so as to characterize new biomarkers of HCC. Frozen liver tissues corresponding to HCC and background cirrhosis (n = 30) were selected and subjected to MALDI IMS. We found a set of proteins/peptides with a differential intensity level that most accurately delineated cancer from adjacent cirrhotic tissue. Using a support vector machine algorithm, we generated a classification model in the train set that enabled segmenting images from the independent validation set, and that in most cases matched histologic analysis. The most discriminating peak (m/z 8,565) more intense in HCC was characterized as the monomeric ubiquitin. An immunohistochemical study in a large series of HCC/cirrhosis sampled on tissue microarray supported that ubiquitin was overexpressed in HCC. We demonstrated also that this increase was not related to an upregulation of ubiquitin gene transcription in HCC, thus suggesting a post-transcriptional mechanism. This approach might provide a new tool for diagnosis of difficult HCC cases and an opportunity for identifying candidate biomarkers.

## Acknowledgements

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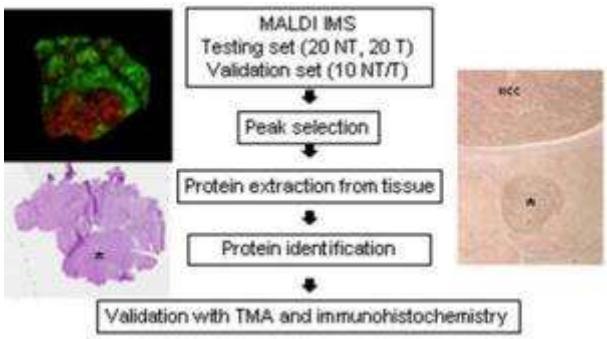
### Figures



# O15. Sample preparation: the key to success for MALDI-MSI

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## Abstract

MALDI Mass Spectrometry Imaging (MALDI-MSI) has shown important potential to study the spatial distribution of endogenous and exogenous compounds including, drugs [1], metabolites [2], lipids [3], peptides [4] and proteins [5] from both vegetal [6] and animal models. MALDI MSI was found to be a powerful technology for many fields of research such as pharmaceutical applications, biomarkers discovery and tracking for various pathologies e.g. brain disorders [7] or oncology[8].
Since several years, many groups have given many efforts to improve the detection of lipids, peptides and proteins. Tissue treatments, choice of matrices and its deposition were then greatly investigated. It was clearly shown that tissue sections submitted to bath of organic solvents have improved the detection of proteins [9]. In addition, the use of ionic matrices (IMs) were presented as a good alternative than common matrices for the detection of lipids, peptides and proteins [10]. Moreover, these IMs were found to be very suitable for the devices used for the matrix deposition [11]. All developments have allowed a better detection of peptides and proteins up to 30kDa with a high sensitivity. However, larger proteins are still difficult to unmask and to overcome this drawback some groups are investigated the use of low polar organic solvents including Trifluoroethanol (TFE) and 1,1,1,3,3,3 hexafluoroisopropanol (HFIP). Proteins up to 70kDa were then observed directly from tissue sections [12, 13].

MALDI-MSI data combined with the use of proper statistical analysis tools [14] can be used to classify cell groups according to their molecular content profiles and can be therefore used in clinical applications as a Molecular Histology Tool. Such a strategy also helps to determine the m/z of molecules showing regulation within the classified Regions of Interest (ROIs) [15]. It's then possible to highlight regulation of molecules with important functions related to a specific biological process by a simple MALDI-MSI acquisition. However, these molecules are generally unknown and require further characterization.

For this, few groups have developed strategies for on tissue identification of proteins [16]. On tissue Bottom-Up approaches through *in situ* enzymatic digestion have demonstrated to allow direct identification of proteins from both frozen [11] and Formalin Fixed Paraffin Embedded (FFPE) [17] tissues. More recently, on tissue Top-Down strategy was introduced by means of In Source Decay (ISD) [18] directly on a tissue section [19]. However, in the most of cases, with both strategies, only most abundant proteins are generally identified. To get deeper information, the extraction of compounds from tissue section is generally required leading to the identification of more proteins [20].

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# O16. Imaging Mass Spectrometry – New Possibilities in Pathology

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## Abstract

Imaging mass spectrometry (IMS) is a new technology to assess spatial molecular arrangements in tissue sections, going far beyond microscopy in providing hundreds of different molecular images from a single scan.[1] The possibility to correlate distribution maps of multiple analytes with histological and clinical features makes it an ideal tool to discover diagnostic and prognostic markers of diseases. IMS based studies of exemplary diseases that would benefit from new insights will be highlighted. One example is prostate cancer (PCa), the most common cancer in men in the United States. A major challenge in treatment decisions remains due to the use of unreliable markers (such as PSA) to distinguish between aggressive and non-aggressive PCa. Although highly curable at an early stage the overall death toll remains high due to recurrence of "cured" cases and progression to hormone-refractory and/ or metastatic disease. Elucidating changes at the protein level involved in PCa progression would provide invaluable information. Another example are follicular lymphomas (FL) that account for approximately 30% of all B-cell non-Hodgkin lymphomas. Recent findings suggest distinctive genetic differences between FL with and without translocation t(14;18). Elucidating differences between both entities at the protein level would provide valuable insights.

Formalin-fixed and paraffin-embedded (FFPE) samples were subjected to on-tissue tryptic digestion.[2] Briefly, sections were mounted onto conductive glass slides and underwent paraffin removal as well as antigen retrieval. On-tissue digestion was achieved by spotting trypsin onto the tissue in an array pattern using a Portrait 630 reagent multi-spotter. Following digestion, matrix was spotted directly onto the array of tryptic spots. Samples were analyzed utilizing an UltrafleXtreme MALDI TOF/TOF mass spectrometer. Additionally, MS/MS measurements of selected peptides were acquired. Data analysis was performed by using the ClinProTools 2.2 and FlexImaging 2.1 software.

On tissue tryptic digestion revealed many changes between different disease states, even if indistinguishable by histology. For example, from regions bearing hormone-refractory PCa, peptides detected at *m/z* 831.5, 1701.9 and 1743.9 were at significantly higher intensity compared to localized PCa. Classification of spectra from localized PCa and hormone-refractory PCa samples could be achieved by combining 19 peaks in a support vector machine based model resulting in an overall cross validation of 91.1% with a sensitivity of 84.8% and a specificity of 97.5%. The tryptic peptide at *m/z* 831.5 could be identified as a peptide from histone H3. Additionally, localized PCa could be distinguished from PCa metastases with an overall cross validation of 97.1%. Similarly, peptides at *m/z* 850.5, 1287.7 and 1791.1 were observed in FL samples without translocation with significantly higher abundances compared to FL samples with translocation. By combining 8 peaks in a support vector machine based model 100% of FL with translocations and 84.2% of FL without translocation could be classified correctly. The tryptic peptide at m/z 850.5 could be identified as a peptide from histone H2A.

Further identification of differentially expressed peptides and validation of these initial promising findings by immunohistochemistry are ongoing and could facilitate the discovery of proteins that could aid in the diagnosis of these diseases.

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# O17. European Cooperation in Science and Technology. Mass Spectrometry Imaging: New Tools for Healthcare Research

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## Abstract

Imaging mass spectrometry has enabled the levels and distributions of panels of biomolecules to be simultaneously measured in tissue sections. The goal has been to exploit the intrinsic capabilities of mass spectrometry for pathohistological and pharmaceutical analysis, specifically:-

- Analysis of multiple molecular classes.
- Parallel analysis of panels of biomolecules containing 100's of distinct species.
- Ability to distinguish between isoforms and metabolites by exploiting the difference in their mass.
- Improved relative quantitation.
- Label free analysis – thus prior knowledge of the biomolecular content is not required.

An array of methods has been developed to achieve these goals:-

- Sample preparation strategies for peptides, proteins, lipids, pharmaceuticals and metabolites.
- Mass analysis methods optimized for sensitivity, spatial resolution, mass resolution, high mass molecules, or combined with an additional ion-separation dimension.
- Combined imaging MS – histological/histochemical analysis of tissues for the identification of candidate biomarkers.
- Imaging MS based molecular histology for revealing molecular changes that occur prior-to/without morphological change.
- Correlation with patient outcome for the identification of prognostic biomarkers.
- Correlation with patient response-to-therapy, thereby potentially aiding personalized medicine.

The field is evolving rapidly and there is now ample evidence that imaging MS can complement established histological and histochemical methods and aid patient stratification. Despite the increasing use of imaging MS and its widespread commercial availability the majority of studies are independent research projects, utilizing individual infrastructure and based on the individual expertise of the research group and their collaborators. Cross-laboratory investigations, which demonstrate robust data acquisition and data analysis capabilities, are conspicuous by their absence. Standardized protocols, or at least established best practice guidelines, are necessary to ensure cross-laboratory robustness.

Only with sufficient training and co-operation can the full potential of imaging MS be utilized to test the capabilities of these highly multidisciplinary tools against an array of diseases of present day concern, both in terms of improved diagnosis and pharmacological development. Central to this purpose is the dissemination of the complementary techniques and expertise and their sustained interaction. Interaction between imaging MS researchers is crucial for devising best practice guidelines and web-based experimental resources; the involvement of healthcare researchers is essential in order to ensure the imaging MS efforts target real needs in healthcare research, e.g. differentiation patient subgroups, and pharmaceutical development, e.g. more cost-effective methods for differentiation of lead compounds. Here we will describe the activities of a recently awarded European network, COST Action BM1104, whose principal aim is the establishment of best practice guidelines, the provision of imaging MS training and the creation of resources to help standardize imaging MS and to increase its accessibility.

## Acknowledgements

This work is financially supported by COST Action BM1104.

# O18. Molecular subdivisions in the mouse brain as revealed by imaging mass spectrometry

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## Abstract

Regional differential molecular expression profiles play a key role in the anatomical subdivision of the healthy brain into distinct subregions with specific morphology, physiology and function. Pathological conditions can perturb the complex protein-protein interaction networks in cells and tissues, reflected in measurable differential protein levels. Direct tissue analysis of such molecular expression patterns can therefore provide invaluable information towards better insights into the biology of different types of nervous system disorders. In search for proteins and possible posttranslational modifications that are involved in normal structural and functional regionalization of the brain we initiated an extensive mass spectrometry imaging (MSI) analysis of the adult mouse brain with special emphasis on the posterior pole comprising the visual circuitry. Once the expression patterns in healthy subjects have been established the goal of our research is to apply this research strategy to detect the molecular signature of visual cortex plasticity which drives the structural and functional adaptations to sensory deprivation as a result of retinal deficits like in glaucoma or age-related macular degeneration[1,2]. The advantages of the MSI approach are that a multivariate statistical unbiased analysis of the MS dataset can reveal specific subdivisions not apparent from histology alone. A combination of principal component analysis and hierarchical clustering allows interactive exploration of the MSI dataset based on both histological and molecular information. We will show molecular partitions in the mouse brain that fit known histology, e.g. cortical areas and layers or brain nuclei, but also region-specific chemical profiles that do not correlate unambiguous with standard histological subdivisions. In conclusion, our approach provides new insights into the intricate spatial relationship of morphology and biochemistry in the healthy and diseased mouse brain.

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### Acknowledgements

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# O19. Semiquantitative imaging MS: application to ischemic disease and cancer models

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## Abstract

Imaging mass spectrometry (IMS) combined with $MS^2$ analyses allowed us to collect micrographs of many different metabolites in a single frozen section, and combination with capillary electrophoresis (CE)-MS data collected from the serial section provided semiquantitative information of individual signals. In an ischemic disease model such as mouse middle cerebral artery occlusion (MCAO), the technique revealed that ischemic penumbra, regions formed between ischemic core and non-ischemic contralateral hemisphere, exhibited paradoxical ATP elevation, despites that the regions were exposed to reduced blood flow[1]. Detailed analyses suggested that such compensatory back-up mechanisms in the hypoxic regions appear to involve roles of endogenous CO in neurons and cystathionine β-synthase, a CO receptor occurring in astrocytes[2]. Refinement of spatial resolution of microscopic IMS allowed us to show that human colon cancer xenografts metastasized in livers of super-immunodeficient NOD/scid/γnull (NOG) mice deprives L-alanine to support their metabolic demands for synthesizing glutathione and nucleotides[3]. In this model, hepatic metastasis triggered regenerative responses of the host liver concurrently with hypoglycemia and accumulation of glutathione and nucleotides in the tumor-bearing liver. $MS^2$ analyses under loading $^{13}C_3$-L-alanine provided evidence for earlier filling of glutathione with $^{13}C_2$-γ-glutamylcysteine structure in metastases than surrounding liver parenchyma. Our results collected by a novel semi-quantitative IMS suggest that human colon cancer metastases utilize gluconeogenic substrates of the host not only through pentose phosphate pathway but through glutaminolysis, supporting their metabolic demand of glutathione and its reducing equivalents NADPH (Figure), both of which are necessary to protect cancer cells against oxidative stress[4], causing hypoglycemia. Overall, IMS combined with CE-MS metabolome analyses serves as a potentially useful strategem to carry out semi-quantitative analyses of varied disease models.

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## Acknowledgements

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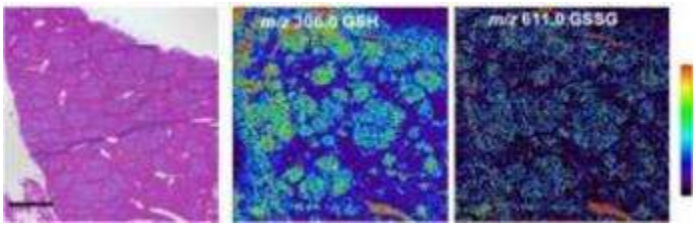
## Figures



# O20. The "After-Life" Experiment - Mass Spectrometry Imaging Used to Demonstrate the "Cycle of Life".

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**Abstract**

**Introduction**

In May 2011 the MS Imaging group at Sheffield Hallam University was approached by the BBC to take part in a project to demonstrate the science of decay. Set up in Edinburgh Zoo in summer 2011 a fully equipped kitchen and garden together with all the detritus from a family barbeque was sealed in a glass box. The programme a joint venture between the BBC and the Discovery Channel followed the events as maggots, moulds, bacteria, flies and mushrooms transformed the contents beyond all recognition. As part of the experiment the programme makers wished to demonstrate that decay and decomposition was only part of the story with atoms and molecules from dead plants and animals being incorporated into new life.

**Methods**

"Caliente" leaf mustard plants (84% Brassica juncea, 16% Sinapsis Alba) were grown hydroponically using a nutrient system containing 15N KNO3 (98% labelled) as the only source of nitrogen. Plants were cropped and left to ferment in water for two weeks to create a mustard "tea". The mustard "tea" was used a source of nitrogen for a second hydroponics experiment where radish (Raphanus sativus) and marigold (Tagetes petula) plants were grown in an experiment set up in a sealed container within the chimpanzee house of Edinburgh Zoo. After ten weeks of growth radish and marigold plants were harvested sent to Sheffield where they were cryosectioned and sections imaged by negative ion MALDI imaging and LDI imaging mass spectrometry.

**Preliminary Data**

The original intention of the experiment was to image the distribution of 15N labelled arginine to demonstrate the incorporation of nitrogen from the now dead mustard plants into protein synthesis for the new living plants, however this proved difficult owing to the complex overlapping isotope peaks in this region of the positive ion mass spectrum. Therefore a different approach was adopted. Using negative ion laser desorption mass spectrometry it was possible to detect 15N labelled NO3- at m/z 61.99 in the plant sections and profiles are presented. The distribution of NO3- in sections of radish and marigold leaf are presented as overlays on optical images of the section (as used in the programme).Subsequent to broadcast of the programme further sections of radish have been analysed in negative ion mode using 9-aminoacridine as matrix on a Waters Synapt G2 mass spectrometer in high resolution mode. Metabolites containing 15N have been identified using METLIN and these data are presented.

# O21. We Can See - Mass Makes the Difference

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## Abstract

Molecular imaging is broadly applied in biomedical research to depict the distribution of analyte molecules of interest. MALDI mass spectrometric imaging (MSI) in a newer member in the family of available technologies, which has the distinct advantage of allowing completely label free imaging of multiple analytes simultaneously. This makes it an ideal tool for research, where either labels are not available, or one searches for unknown molecules with a distinct spatial distribution. The MALDI MSI acquisition starts with a flat sample, typically a biological tissue section, which is mounted on a surface and dehydrated. The sample is coated with some organic matrix and introduced in a mass spectrometer, where a laser is used to raster over the sample, ablating and ionizing molecules by assistance of the deposited matrix. These ions are measured using an analyzer appropriate for the molecular class to be detected, e.g. quadrupole instruments for small molecules or TOF instruments for biopolymers. From the acquired data set, we can obtain intensity images for each species of interest. Simplified, one can describe a MSI analyzer as a digital camera, which has instead of 3 color channels a large number of mass channels.

This technology has been successfully applied to a wide range of molecules and analytical problems in academic and industry. In this presentation an overview is given on the technology applied to pharmaceutical research, including examples where MALDI MSI was applied to drug discovery projects adding unique value. Capabilities in spatial resolution, molecular classes, sensitivity and quantification are shown and discussed.

### References

http://maldi.ms

## Acknowledgements

I like to thank Dieter Staab, Nicole Ehrhard, Brendan Prideaux and Gregory Morandi for their contribution to the data shown in this presentation.

# O22. Chemical Imaging of the Sphingolipid and Cholesterol Distribution in the Plasma *Membranes of Fibroblast Cells*

Mary L. Kraft[1], Jessica F. Frisz[1], Haley A. Klitzing[1], Kaiyan Lou[1], William P. Hanafin[1], Robert L. Wilson[1], Vladimir Lizunov[2], Kevin J. Carpenter[3], Joshua Zimmerberg[2], Peter K. Weber[4]

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## Abstract

The cellular plasma membrane is organized into domains of differing protein and lipid composition in order to coordinate cellular function. Changes in the abundances of cholesterol and sphingolipids influence many cellular processes. Yet, the distributions of cholesterol and sphingolipids within the plasma membrane have not been established. The inability to directly visualize lipids and cholesterol without the use of labels that may induce lipid clustering renders characterizing the precise distribution of cholesterol and sphingolipids within the plasma membrane especially challenging. High resolution secondary ion mass spectrometry (SIMS) performed with a Cameca NanoSIMS 50 offers a direct approach to chemically image the distributions of metabolically labeled lipids within the plasma membranes of intact and fixed cells.[1] In this work, we used high resolution SIMS to image the distributions of metabolically incorporated $^{15}$N-sphingolipids and $^{18}$O-cholesterol within the cellular plasma membrane (Figure 1). Specifically, the $^{15}$N- and $^{18}$O-enrichment NanoSIMS images revealed the local abundances of $^{15}$N-sphingolipids and $^{18}$O-cholesterol within the plasma membrane with ~100 nm lateral resolution. Computational statistical approaches were used to assess whether the sphingolipids and cholesterol were heterogeneously distributed and co-localized within the plasma membrane. Our results show that lipids are spatially organized on length scales that range from two hundred nanometers to a few micrometers within the plasma membrane.

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Acknowledgements

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Figures

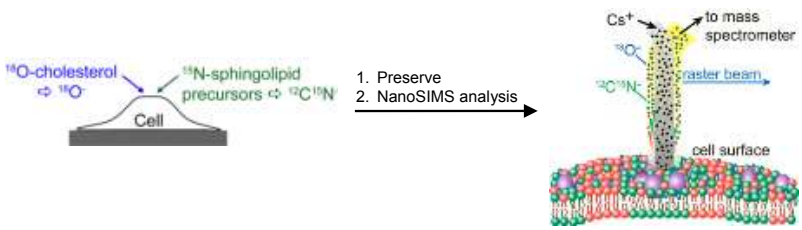


Figure 1. Rare stable isotopes are metabolically incorporated into the cholesterol and sphingolipids in living cells. The metabolically labeled cells are then chemically fixed, and NanoSIMS is employed to chemically image the isotope enrichment that is characteristic of the $^{18}$O-cholesterol and $^{15}$N-sphingolipids in the plasma membrane.

# O23. Intracellular distribution of Phosphatidylcholine molecular species in neuron revealed by imaging mass spectrometry

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## Abstract

Phosphatidylcholine (PC) is the most abundant component of lipid bilayers and exists in various molecular forms, through combinations of two acylated fatty acids. Arachidonic acid (AA)-containing PC (AA-PC) can be a source of AA, which is a crucial mediator of synaptic transmission and intracellular signaling. However, the distribution of AA-PC within neurons has not been indicated. In the present study, we used imaging mass spectrometry to characterize the distribution of PC species in cultured neurons of superior cervical ganglia. Intriguingly, PC species exhibited a unique distribution that was dependent on the acyl chains at the sn-2 position. In particular, we found that AA-PC is enriched within the axon and is distributed across a proximal-to-distal gradient. Inhibitors of actin dynamics (cytochalasin D and phallacidin) disrupted this gradient. This is the first report of the gradual distribution of AA-PC along the axon and its association with actin dynamics. Further analysis of single cells will be presented and discussed.

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Yang HJ et al, *JBC* (2012) 287(8), p5290-300

# O24. Group Independent Component Analysis (GICA) as a Tool for Discovering Dissimilarities between Tissues in Mass Spectral Imaging

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## Abstract

### Introduction

Automated pattern discovery techniques, such as Principal Component Analysis [1], have gained great popularity as processing tools for mass spectral imaging (MSI) data in recent years. Up to now, the majority of work on these techniques has been focused on pattern discovery within a single tissue section. In this work, we extend these techniques to automated discovery of patterns across multiple tissue sections. This enables us to highlight which biomolecules at which locations differentiate one tissue slice from another. We propose the use of Group Independent Component Analysis (GICA) [2] as a means to achieve this goal.

### Methods

When presented with the MSI measurements of several tissue samples (e.g. diseased versus control tissue), Group Independent Component Analysis (GICA) uses a combination of Principal Component Analysis and Independent Component Analysis to discern the various patterns present in the tissues. It then assesses the extent to which these patterns are observed in each of the tissue sections under study. As a result, the technique reports the patterns that are common across all tissue sections as well as the patterns that only show up differentially in a particular tissue section. These patterns are characterized by both their mass spectral signature as well as their spatial distribution throughout the tissue.

### Results

We first verify the operation of our method on a computer-generated artificial MSI dataset. The artificial dataset consists of 2 simulated tissues that contain both common and differential mixtures of mass spectra. We then demonstrate our method on a case study of 12 μm thick transversal sections of mouse spinal cord (lumbar region), comparing an amyotrophic lateral sclerosis (ALS) mouse model to a wild type (WT) mouse. The spinal cord sections were mounted on ITO glass slides and coated with alpha-CHCA matrix using Labcyte's Portrait P630 acoustic spotter. The MSI measurement was done on an ABI 4800 MALDI TOF/TOF mass spectrometer. The acquired mass range extends from m/z 3500 to 20000 divided into 15691 m/z-bins. Grids of 62 by 44 (ALS) and 56 by 43 (WT) pixels were measured with an interspot distance of 50 μm in both the x and y directions.

The results of the GICA algorithm show several clearly defined biomolecular patterns that are common to both tissue sections, correlating to anatomically relevant distributions in these types of tissues. However, more important is the discovery of distinct biomolecular patterns that are differentially expressed between the tissue sections. For example, we show patterns that are only expressed in the diseased spinal cord tissue, as well as others that are only present in the healthy tissue.

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# O25. Quantification of drugs, small molecules and neuropeptides directly in tissue sections

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## Abstract

MALDI mass spectrometry imaging has been used to produce qualitative distribution maps of peptides, proteins, lipids, and drugs and their metabolites directly in biological tissue sections. There is a great need to quantify the distribution of target compounds and their metabolites, as well as endogenous compounds in the tissue sections from different organs. In the present study we present novel mass spectrometry imaging software and protocols for the quantification of drugs after in vivo administration and, for the first time, an endogenous neuropeptide directly in tissue sections. The software imports raw data in different formats and the data is processed by several available user-defined baseline corrections, subtractions, denoising, smoothing and normalization methods. Drug or endogenous neuropeptide concentrations are determined using either external standard curves, or by using labeled compounds, i.e., deuterated analogues as internal standards. After selecting regions of interest on the tissue section the software automatically calculates the concentration based on the standard curve. Employing the described protocol we demonstrate that it is feasible to quantify in vivo administered drugs and an endogenous neuropeptide. We quantified the distribution of imipramine and tiotropium in the brain and lung, respectively, of dosed rats. The neuropeptide substance P was quantified in different mouse brain structures, which correlated well with previously reported concentrations. Our approach facilitates data processing and provides better reproducibility and may be considered as an effective tool to quantify drugs and endogenous compounds in tissue regions of interest.

## Acknowledgements

This work is financially supported by Swedish Research Council under grant numbers: 2008-5597, 2009-6050, 2010-5421, 2010-0881, and the Knut and Alice Wallenberg Foundation.

# O26. Observing directly the distribution of an inhaled Domain Antibody in healthy and infected mice by MALDI MS Imaging

Laura Tomlinson[1]; Claire Scott[1]; Peter Francis[1]; Neil Freeman[1]; Andy West[1]; Laura Slack[2]; Ruud De-Wildt [2]; Peter Morley[2]; Peter S. Marshall [1]

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## Abstract

Less than a tenth of the size of a full antibody, Domain antibodies (dAbs) are the smallest known functional binding units of antibodies. Currently being developed as therapeutics, dAbs correspond to the variable domains of either the heavy or light chains of human antibodies.

MALDI MS imaging is a technique which allows direct detection of the location of compounds in tissue slices. To date, pharmaceutical drug distribution studies using MALDI MS Imaging have focussed on detecting the distribution of small molecule drugs (and their metabolites). Now for the first time we have conclusively shown the distribution in mice of an intranasally dosed domain Antibody by MALDI imaging.
This study was to investigate the efficacy of an intranasally administered dAb in mice infected with the A/NWS/33-Tisdale virus lung homogenate. Histology has been performed on uninfected and infected mice lungs to determine what extent tissue inflammation/damage have been reduced by the dAb. MALDI MS Imaging, LESA MS and histology have also been performed on lung sections from uninfected and infected mice to determine the presence, targeted distribution and retention of the dAb at low or high dose in the lung over a 24 hour time period.

Excised lungs from dAb treated and untreated mice were collected at time zero, 1, 2, 3, 4, 8 and 24 hours after dosing with the dAb intranasally at either low or high dose and frozen. 16um thick sections were prepared from each lung onto indium tin oxide (ITO) coated glass slides and analysed by MALDI MS Imaging. Consecutive sections were prepared on conventional glass slides and used for LESA-MS analysis. H&E staining was performed on the sections used in MALDI MS Imaging.

Ahead of performing experiments to investigate the efficacy of an intranasally administered dAb in diseased mouse lungs, it was necessary to determine the biodistribution of domain antibodies in healthy mice as this has not been studied. For example, it is not known whether domain antibodies distribute evenly to both the upper and lower respiratory tract, and it is not known whether the specificity of the dAb affects this distribution.
The sequence of the dAb used in this study is shown below:

STDIQMTQSPSSLSASVGDRVTITCRASQYIHTSVQWYQQKPGKAPKLLIYGSSRLHSGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQQNHYSPFTYGQGTKVEIKR

Data acquired to-date indicates the presence of the intact dAb at all time points except the 24 hour animal. The distribution of the dAb within the lungs was widespread and included the airways and tissue regions.
These findings were confirmed with lower spatial resolution using Liquid Extraction Surface Analysis and nanoelectrospray MS analysis on two sections from each time point. Ions matching those produced from the analysis of the dAb standard were present in the sections from the dosed animals and absent from the Control. The assignment was confirmed by fragmenting the ion consistent with $[M+8H]^{8+}$ of the dAb. This yielded a series of ions corresponding to the losses of the first nine amino acids from the N-term of the protein.

Further confirmation of the presence and distribution of the dAb in the lungs was afforded by manual aqueous protein extraction from the lung sections followed by gel electrophoresis, enzymatic digestion and mass spectrometry.

Efforts continue to move to a more quantitative assessment of the distribution.

"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."

# O27. Exploring the potential of phospholipids for histological classification of clinical samples by MALDI imaging MS

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## Abstract

Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is a technology that provides information on the localization of molecules in a sample. IMS has become a powerful way to profile and characterize the spatial distribution of biocompounds (including peptides, proteins, lipids and metabolites) through the direct analysis of thin tissue sections. This technique is distinctive by its capability to preserve the spatial localization of diagnostic molecules and biomarkers – information that is typically lost when tissue extracts or homogenates are used.

Recent application of this technology to the analysis of thin tissue sections clearly shows retention of spatial and anatomic relationships permitting for example the complex interaction between diseased cells and their environment to be studied at the molecular level. From the systematic analysis of a single tissue section, molecular-specific maps directly correlated with tissue architecture may be simultaneously obtained for several hundreds of different endogenous compounds.

To date, all the significant proof of concept studies demonstrating the power and potential of IMS in the clinical setting have been performed through the profiling and imaging of proteins. However, other classes of biocompounds such as phospholipids are readily analyzable by MALDI IMS. We propose to systematically explore these expression differences by IMS and ultimately establish if diagnosis, prognosis and response to therapy can be accurately predicted.

Recently introduced as a novel approach for matrix application, sublimation has shown to be very powerful for the analysis of low-molecular-weight molecules such as phosphoplipids by IMS. Within few minutes, this process forms a very thin and exceptionally homogeneous film of matrix on thinly cut tissue sections. We have recently demonstrated that 1,5-diaminonapthalene as matrix (DAN) is of high efficiency providing rich lipid signatures in both positive and negative polarities compared to other matrices, with high vacuum stability and sub-20 μm spatial resolution capacities. The sequential acquisition of ion images using the same section in both polarities yields a significant amount of information.

We have used this IMS approach to investigate the potential of using phospholipid expression patterns to differentiate histologies within clinical samples using small cohorts of prostate cancer and colorectal cancer liver metastasis. In all cases, specific phospholipid profiles from normal and cancerous areas could be identified. Further, several signals were seen with clear expression differences when considering the different cell populations constituents of the tumors.

The potential for this type of analysis in which the spatial distribution of specific phospholipid species can be mapped throughout a tissue section is particularly exciting for the study of disease. Ultimately, we hope to demonstrate that this information could be used to better define diagnosis, prognosis and significantly impact the course of therapy.

# O28. MALDI MS imaging for pharma: A multi-platform multi-assay approach multiples MSI successes

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## Abstract

MS imaging (MSI) technologies and techniques are now becoming robust enough to move out of the research lab and be integrated into the drug discovery and development pipeline. However, for them to be fully implemented a number of crucial constraints have still to be fully overcome. Such areas include the cross validation of MSI data to established imaging techniques, monitoring for and mitigating the risks of sample instability, performing whole body analysis to allow delicate and hard to dissect tissues to be analyzed *in situ* and crucially how to move forward if parent compound and metabolites fail to be detected by MSI.

We will present a plethora of examples on why a strategy of combining multiple MALDI MSI platforms enables us to most effectively identify and characterize drug and metabolite abundances and distributions within a DMPK research environment. Primary focus will be on presenting data from a number of neurological PET ligands, while examples of other small molecule studies will be presented.

Distribution and quantitation of PET ligands has been achieved and validated using MSI (with on-tissue quantitation) with abundance and distributions compared to QWBA and μPET imaging data. Multiple PET ligands have been analyzed and for example, by using MSMS MSI analysis, the distribution of raclopride has been possible at single i.v doses <0.001mg/kg using solvent-free dry matrix, though no detection was possible using standard wet matrix applications even when dosed at >10mg/kg. Using whole animal tissue sections has enabled inter-organ bio-distributions to determined, through the use of adhesive carbon tape to allow simple and successful sample transfer of sections for analysis of small/delicate tissues/organs and enabled accurate mass identification of many endogenous and pharmaceutical compounds by MALDI FT-ICR-MSI. Determination of novel PET compounds ability to cross BBB has been achieved by mapping MSI distribution of heme to distributions of PET ligands prior to and following BBB disruption by surgical intervention. By using liquid extraction surface analysis (LESA) coupled to FTICR MS analysis we have been able to detect and monitor the distribution of metabolites that we were unsuccessful in detecting by MALDI and hence by using a MS profiling approach we are still able to drive research forward when MSI analysis fails

MSI has roles within pharmaceutical research beyond DMPK bio-distribution monitoring. Examples of such areas will be touch upon, where the use of untargeted MSI analysis can be demonstrated to be superior to established imaging and analytical assays.

In summary, MSI is playing a vital and expanding role within pharmaceutical research presented here example of solutions to a number of current technical obstacles, as well as a taste of the challenges still to be overcome.

# O29. Protein and Lipid Profiling of Recurrent and Non-Recurrent Renal Cell Carcinoma Tissues

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## Abstract

The incidence and mortality rates for renal cell carcinoma (RCC) have risen steadily for more than 30 years, with a poor 5-year survival rate and a characteristically unpredictable clinical course for the most common clear cell form (ccRCC). The primary treatment for patients with localized ccRCC is surgical excision, which can be highly effective for early stage cancers. However, due to lack of any early detection strategies, approximately 35-40% of patients with no evidence of metastasis at the time of surgery will subsequently experience metastatic progression. Two key clinical issues are the need to 1) identify ways of more accurately predicting which patients will experience metastatic progression following surgery for localized ccRCC and 2) develop new treatments that can be used in combination with surgical excision to reduce progression. The overall goal of the study was to improve understanding of the underlying mechanisms of ccRCC progression and enhance the ability to accurately predict which patients are at greatest risk of progression following surgery. We hypothesize that identification of specific tumor-associated proteins and lipids directly in histopathological specimens can be linked with an existing panel of biomarkers of ccRCC aggressiveness to develop a novel biomarker-based prognostic nomogram/scoring system that can significantly improve the ability to accurately identify individuals most at risk of ccRCC progression following surgery. To accomplish this, fresh-frozen tissue samples from 25 intermediate risk ccRCC patients who experienced progression to metastasis within 3 years of surgery and 25 intermediate risk ccRCC patients who remained progression free after 5 years of follow-up were evaluated by MALDI mass spectrometry based tissue imaging. By current histopathology criteria, each sample was roughly equivalent except for classification based on clinical outcome. Tissue protein profiles were obtained on an UltraFlex III or AutoFlex III MALDI-TOF/TOF mass spectrometer, and lipid profiles were obtained on a 7T Dual-Source Solarix FT-ICR mass spectrometer (Bruker Daltonics). Specific regions of interest in each tissue sample were evaluated and identified by a pathologist. Initial proteomic profiling results indicated 38 candidate m./z protein peaks that were differentially expressed in recurrent ccRCC or non-recurrent ccRCC tissues. Furthermore, tentative sequencing has been done for a subset of these candidates, identifying them as thymosin beta family peptides and S100 family member proteins over-expressed in recurrent ccRCC. Lipid analysis by FT-ICR has focused on identifying the differences in the degrees of saturation and unsaturation in common phospholipids and bioactive sphingosine and ceramide species. Linkage of differentially expressed protein and lipid species with clinical data and other molecular markers of ccRCC is ongoing. This approach has the potential to not only improve prognostic assessment and enhance post-operative surveillance, but also to inform on the underlying biology of ccRCC aggressiveness and new rational targets for therapeutic intervention.

### Acknowledgements

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# O30. Breaking boundaries in Imaging MS

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## Abstract

Biomolecular imaging mass spectrometry offers the possibility to perform an in-depth study of biomolecular distributions on complex surfaces [1,2]. Applications if this innovative technology can be found in various disciplines ranging from biomedicine to semiconductor technology. Applications in clinical research are currently a major driving force for the rapid development of this field of science. A direct insight in molecular signaling pathways in diseases and related metabolic studies generates a significant amount of interest in the technology. The level of molecular detail that can be visualized directly from tissue with this label-free molecular imaging technique is increasing rapidly. In this lecture the latest technological multi-modal biomolecular imaging MS developments towards high resolution, sensitive and detailed molecular evaluation of individual cells and tissue surfaces will be discussed.

Unraveling the complexity of molecular profiles at biological surfaces with imaging MS is severely hampered by the achievable mass resolution, sensitivity, dynamic range and spatial resolution of conventional ToF based mass spectrometric systems. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry offers unique capabilities is this respect and has already demonstrated to allow the resolution of new spatial features from complex surfaces. Secondary Ion Mass Spectrometry is one technique that could greatly benefit from the mass spectral capabilities of FTICR-MS. We have combined, for the first time, the advantages of a C60 primary ion source with the ultrahigh mass resolving power and high mass measurement accuracy of FT-ICRMS [6]. SIMS-FTICRMS with a mass resolving power in excess of 100 000 (m/Δm50%) is demonstrated, with a root-mean-square mass measurement accuracy below 1 part-per-million. Imaging of mouse brain tissue at 40 μm pixel size demonstrated the benefit of a high resolution approach. Tandem mass spectrometry of ions from biological tissue is employed to assign molecular formulas of through the determination of fragment ion elemental compositions.

A new horizon in imaging is offered by three dimensional molecular imaging analyses. The generation of a 3-dimensional dataset requires an additional z-dimension on top of the routine 2D (x,y) images generated. This added dimension can be achieved in a number of manners. Successive tissue sectioning with well defined and measured spatial intervals is one approach that is beginning to be adopted by imaging MS researchers. Alternate high resolution approaches and detection techniques will be described and discussed and demonstrate how boundaries in imaging MS are continuously being broken and shifted.

The innovative imaging MS approaches described above have been employed to elucidate several molecular signaling pathways in a variety of diseases. They have been combined with several optical and other hyperspectral technologies in a true high performance multi-modal imaging approach. It demonstrates how integrative imaging MS has evolved to a problem solving tool that spans several scientific disciplines and provides fundamental insight into complex tumor biology.

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### Acknowledgements

The entire BIMS group, past and present, is acknowledged for their contributions to the work desicussed and described in this lecture. Ljiljana Pasa-Tolic and Jean Futrell are gratefully acknowledge for being instrumental in the realization of the AMOLF – PNNL/EMSL partnership a framework in which part of this work was conducted.

# O31. Statistical tools for MALDI imaging: Which technique for which question?

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## Abstract

With the rise of imaging mass spectrometry in recent years, also the development of techniques for the interpretation has increased. Imaging mass spectrometry data are quite unique because they combine highly multivariate information with up to thousands of mass signals in the spectra with spatial information. Often times, there is also a significant technical variability from pixel-to-pixel (noise). The wealth of information in these datasets calls for statistical methods to make the data manageable. Because of the data, both spatial and spectral correlations can be used to process the imaging data. Spectral correlations can e.g. be used to generate concise representations of MALDI imaging data by techniques such as principal component analysis (PCA), probabilistic latent semantic analysis (pLSA) or various clustering techniques. Spatial correlations can be used for de-noising the imaging data.

Usually, a MALDI imaging study is not confined to the analysis of individual imaging dataset, but is rather performed on many samples, such as biological replicates or patient cohorts. For those analyses various statistical tools are available, ranging from univariate comparisons of the intensities of single peaks over multivariate analyses such as PCA or discriminant analysis to multivariate classification algorithms. While all these techniques are powerful tools for the analysis of imaging datasets, they can also lead to wrong conclusions if they are not applied correctly. E.g. statistical tools that are perfectly applicable in analyzing data from animal models may lead to wrong conclusions if applied to a clinical study on patients.

The aim of this presentation is to put these statistical tools into context, with a focus on the applicability and limitations of those tools.

# O32. Understanding The Fate Of Administered Drugs

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## Abstract

Requirements for patient safety and drug efficacy are steadily increasing in modern healthcare and are key drivers in modern drug development. While the science of drug development is busy identifying new substances for treatment, the culture of developing drugs is also under paradigms shifts led by our increasing need for understanding the fate (destinations and distributions) of administered drugs. These efforts are highly technology driven and platforms such as matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) play a central role in providing evidence of the specificity and selectivity of drugs at their sites of action within tissue. For example, choosing drug candidates based upon their signature patterns of uptake into targeted and off-target site provides a very powerful tool in understanding drug efficacy. We have recently studied the uptake of an inhaled bronchodilator, the muscarinic receptor antagonist ipratropium, in human COPD patients using MALDI-MSI in biopsies obtained by fiber-optic bronchoscopy shortly after dosing exposure. The study showed that inhaled ipratropium parent ion (*m/z* 332.332) and daughter ions (*m/z* 166.2 and 290.2) were coincidently partitioned within the sub mucosal spaces containing targeted M3 receptor rich smooth muscle bundle compartments in 4/5 subjects. Together with studies using inhaled tiotropium conducted in vivo in experimental models we have now demonstrated that drug distributions can be mapped within either biopsies or in whole organs using MALDI-MSI. As the biology of complex diseases becomes better understood and new targeted pathways are identified, MALDI-MSI will provide much sought after knowledge defining the patterns of drug uptake at theses sites of desired intervention.

# O33. MALDI Imaging Mass Spectrometry: moving towards high molecular weight proteins detection

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## Abstract

MALDI Imaging Mass Spectrometry (IMS) is a powerful and versatile tool allowing the direct detection and localization of drugs, lipids, peptides and proteins on thin tissue sections [1]. Although showing a relevant biological activity, proteins larger than 20 kDa are not routinely and directly detected (*e.g.* cytokines, enzymes, receptors and neuropeptide precursors). At the moment, the most common approach to detect high molecular weight proteins through IMS is based on proteolytic digestion of the tissue. However, this is a "bottom-up" strategy, generating many tryptic peptides with identical nominal mass, undermining the identification of potential biomarkers. Moreover, it discards all the information related to specific isoforms unless the appropriate peptide is detected in the analysis [2]. Conversely, direct MALDI IMS of high mass proteins would provide the advantage of detecting different isoforms of biological relevant molecules.

In this work we have developed an alternative approach for tissue preparation and we have evaluated its potential use for the on tissue analysis of high molecular weight proteins.
Imaging mass spectrometry of high molecular weight proteins was performed on different tissues both with the traditional preparation with Sinapinic Acid and with our alternative approach.
Our results demonstrate that this alternative strategy generates a significant improvement of protein detection sensitivity in the mass range of 20000 to 100000 *m/z*.
These data show the possibility of enhancing high masses detection for protein imaging mass spectrometry analysis on tissue sections.

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## Acknowledgements

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# O34. Imaging lipids by FT-ICR MS: Focus to MALDI, NALDI, DESI and DAPPI

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## Abstract

We have developed sample preparation protocols for problematic tissues, e.g. collapsing lungs [1] or globular eye lens [2]. The cookbook tricks have involved the application of an intact polymer generating low background ion signal and soft tissue landing using ethanol [3, 4], respectively. We have built a special apparatus for ambient ionizations implemented on FTICR [5], e.g. desorption electrospray (DESI), desorption atmospheric pressure chemical ionization (DAPCI) and desorption_atmospheric pressure photoionization (DAPPI). Such an armoury has enabled the analysis of a broad polarity range of analytes including fragile gangliosides (DESI) or nonpolar cholesterol derivatives (DAPPI). The positive effect of globosylceramide cationization process in MALDI has been utilized in interesting lipid distribution determination in kidney of knock-out mice suffering from Fabry disease. Semiquantitative and distribution qualitative analysis of glycerophospholipids and sphingolipids in porcine eye lens was achieved by joint application of accurate mass spectrometry and in-house developed software tool mMass [6]. An imprinting method called NALDI (Nanostructure-assisted laser desorption ionization) MSI was developed for tissue imaging analysis without application of any matrix [7]. Catalytic properties of nanostructures in NALDI analyses were disclosed for peptides and lipids. Oxidation processes in lipids have been utilized for multiple double bond position localization [8]. Initial results in MSI of tissues of mice suffering from cystic fibrosis will also be disclosed.

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## Acknowledgements

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# O35. Visualization of acetylcholine distribution in central nervous system tissue sections by tandem imaging mass spectrometry

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## Abstract

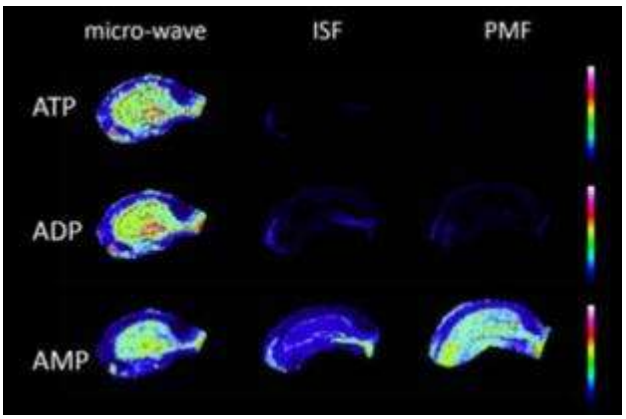
Previous studies have shown that MALDI-Imaging MS (IMS) can visualize distributions of diverse metabolic molecules in the biological tissues. On the other hand, a highly comprehensive/quantitative analysis of metabolites by capillary electrophoresis MS (CE-MS) can efficiently complements the IMS technology. However, optimization of animal organ sampling protocol for these metabolomic analyses has remained as a quite critical issue, because major degradation of the metabolites occurs in tissues within a couple of ten seconds after death, especially of high energy phosphate-metabolites. In this study, we have evaluated several sample preparation techniques, namely, a focused-microwave irradiation, in situ freezing (ISF) method, and a postmortem freezing fixation (PMF) with decapitation.

As results, we found that the PMF caused unacceptable autolytic reduction in ATP and increases in ADP and AMP; absolute quantification by CE-MS revealed that 90% of ATP was broken into downstream metabolites, and therefore, AMP level represents a ten times increase. Furthermore, we found that the micro-wave fixation as the best way for our purpose; extents of the ATP reduction, AMP elevation, lactate elevation as a results of enhanced anerobic respiration, were much lower in the micro-waved samples compared to in-situ frozen brains. Finally, we revealed that the microwave irradiation of brain samples improved IMS data quality in terms of the number of effective pixels and the image contrast (i.e., the sensitivity and dynamic range) (Figure).

### Acknowledgements

This study is supported by Research Grants for PRESTO from JST.

Figure




MALDI imaging results of adenosine nucleotides, by use of 9-aminoacridine as a matrix. As can be seen, IMS data quality in terms of the number of effective pixels and the image contrast (i.e., the sensitivity and dynamic range).

# O36. Clinical Testing of Rapid Evaporative Ionization Mass Spectrometry-based Intraoperative Tissue Identification

Zoltán Takáts

## Abstract

The recently introduced Rapid Evaporative Ionization Mass Spectrometry (REIMS) technique allows the in-situ, in-vivo chemical characterization of biological tissues. Chief application area of the technique is cancer surgery, where it can be used to establish surgical margins. Following exploratory studies, we have performed the first large scale study aimed at the validation of the tissue identification capability of the method in surgical environment.

REIMS-based tissue identification was established by using standard electrosurgical handpieces. Mass spectrometer (LTQ, ThermoScientific) was enclosed into special chassis. The study involved 250 patients undergoing bowel resection or liver metastasis resection. REIMS data was collected throughout interventions when electrosurgery was used. Data was identified using a database of authentic REIMS data and a multivariate statistical approach. Data analysis is performed in 0.5-0.9 s, giving real-time identification capability. Results of MS-based identification were compared to corresponding histopathology results for 3340 individual data points. Mass spectrometric identification results were in agreement with histopathology in 97.84% of the cases, identification failed in 1.35% of the cases while in 0.81 % of the cases the two identification results were different. Results indicate that REIMS is a solution for rapid intraoperative tissue identification yielding results superior to the current gold-standard intraoperative histology.

Nevertheless, identification of tissues is only feasible if a large collection of authentic spectra and appropriate search engine are available. Due to the nature of pattern-level tissue specificity, multivariate statistical tools (PCA, LDA, OPLS-DA, etc.) were proven to be the appropriate approach for spectral comparison and identification. However, regarding the construction of spectral library, the REIMS technology is not the ideal tool, since electrosurgical dissection does not feature cellular-level accuracy and also requires large amounts of human tissue samples. At this level, imaging mass spectrometry methods gain importance, especially due to the high similarity between REIMS, LDI or DESI spectra. Data pre-processing can eliminate the method-dependent features and create a unified data platform where identification of in-vivo data using imaging data as reference becomes feasible.

# O37. Spatial Imaging of Retinoids and bis-Retinoid Adducts in the Eye

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## Abstract

Age-related macular degeneration (AMD), the most prevalent cause of vision loss in the elderly populations of the industrialized countries today[1], has been associated with lipofuscin accumulation and subsequent toxicity.[2] Lipofuscin is the yellow-orange fluorescing age-pigment of the retinal pigment epithelium (RPE) lysosomes.[3] The best characterized component of lipofuscin is A2E, a side-product of normal vision.[4] However, a direct correlation of A2E with lipofuscin has not been possible before because fluorescence is not specific enough to differentiate chemically similar compounds. In this study, we utilized MALDI-IMS to spatially identify A2E and other *bis*-retinoids in the RPE.

After the removing the retina under dim red light, eyecups from *Abca4*$^{-/-}$ mice, Sv129 controls (*wt*), and from human donor specimens (fetal eyes to age 80) were flattened onto ITO-coated slides. Lipofuscin fluorescence was imaged with a color camera on a fluorescent microscope and spectra determined in a laser scanning confocal microscope. To quantitate tissue fluorescence, a Xenogen IVIS 200 imaging system was used. The same tissues were covered with 10 mg/mL DHA in 80% ethanol using spot- or spray-deposition and analyzed in a Bruker Autoflex III TOF-TOF mass spectrometer (50-250 μm steps, 250-2600 m/z range). Synthetic retinoids were used as standards.

*Abca4*$^{-/-}$ mice had more A2E (m/z 592) than age-matched *wt* controls. While limited central accumulation was seen in young mice, high levels of accumulation across the entire RPE were seen in old mice. The distribution of lipofuscin fluorescence was in good agreement with that of A2E detected by MALDI imaging. In human eyes, lipofuscin fluorescence also increased towards the center of the eye and became more abundant with age. However, the A2E levels only increased until the age of 65 and the distribution of A2E and its oxides was peripheral rather than central or uniform. Neither the fluorescence nor the A2E overall profile changed significantly with age or disease (dry- or wet AMD) conditions.

Lipofuscin fluorescence was a good indicator of A2E accumulation in the animal experiments. However, in contrast to current thought, A2E is not in high concentrations in areas of the human eye where lipofuscin is found in either normal or diseased conditions. Consequently, targeting A2E accumulation may have little effect on lipofuscin levels in the human eye. These observations will be essential for developing new non-invasive diagnostic methods and treatment approaches that target only the toxic components of lipofuscin instead of inhibiting normal vision and causing night blindness.

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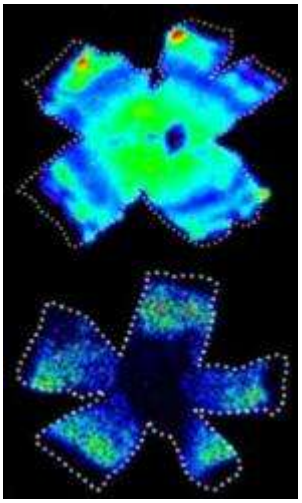
## Figure



The topographies of lipofuscin (top) and A2E (bottom) in eyes from 62 year human donors.

# *Poster Presentations*

# P1. The distribution of Anti-Cancer drug in solid tumours studied by MALDI-MSI

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## Abstract

Background: Vascular disrupting agents (VDAs) are involved in the treatment of many cancers. They work by selectively destroying pre-existing blood vessels causing a rapid shutdown of tumor blood supply. DMXAA (5, 6-dimethylxanthenone-4-acetic acid) has an anti-vascular effect in experimental tumors. These effect leads to endothelial cell apoptosis and activation of cytokines in the tumor microenvironment. The aim of this work is to investigate tumor response to DMXAA treatment by mass spectrometric methods. Method: limit of detection study on tissue was performed by preparation of serial concentration of 250 to 10 ng/ml from stock solution. The human colon adenocarcinoma cell line LS174T was used to develop a xenograft model in nude mice. Tumors were treated with DMXAA, and then removed for cryosectioning. Tissue sections were spray-coated with matrix and the analysis was performed using QStar Quadrupole Time-of-Flight Mass Spectrometer. Result: A strong peak of DMXAA was observed at protonated molecular ion [M+H$^+$] m/z 283. The MS/MS spectrum of DMXAA shows a protonated ion [M+H$^+$] at m/z 283 and major product ions at m/z 237 and 209. In limit of detection study, the drug was observed on tissue down to 100 pg. Optimum signal was obtained when using 70% EtOH / Water + 0.1TFA. The MALDI image of 4h treated xenograft tumor at m/z 283.2 has shown the drug distributed mainly in the centre of tumour. While, MALDI image of 24h treated xenograft tumor has shown drug distributed in the periphery. Hence, the drug will tend to migrate to the periphery of the tumor after vascular damage. Another marker of necrosis was determined by imaging the lipid at m/z 700-800.

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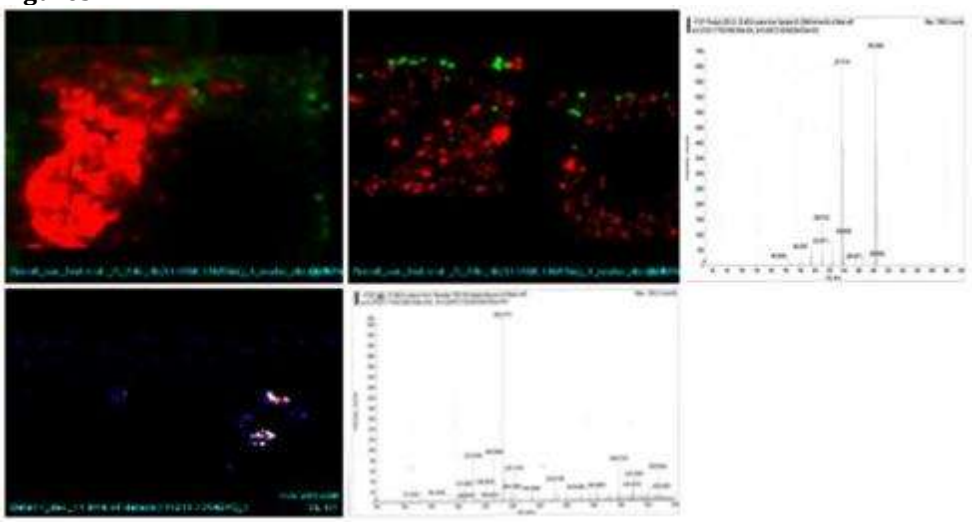
Special thanks to Institute of cancer therapeutic, university of Bradford for supplying us with xenograft tumor.

### Figures



# P2. Drug Localization in Different Lung Cancer Phenotypes by MALDI-MS Imaging

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## Abstract

Lung cancer is a common cause of cancer mortality in the world, largely due to the risk factor of tobacco smoking. The drug therapy at the molecular level includes targeting the epidermal growth factor receptor (EGFR) tyrosine kinase activity by using inhibitors, such as Tarceva and Iressa. The heterogeneity of disease phenotypes and the somatic mutations presented in patient populations have a great impact on the efficacy of treatments using targeted personalized medicine.

In this study, we report on basic physical and chemical properties of Tarceva and Iressa in three different lung cancer tumor phenotypes using MALDI-MS imaging, providing spatial localization of drugs without chemical labeling. Tarceva and Iressa were analyzed in i) squamous cell lung carcinoma, ii) adenocarcinoma and iii) large cell lung carcinoma following their deposition on the tissue surfaces by piezo-dispensing, using a controlled procedure: the compounds were deposited in small volumes with even amounts in each position of the array. This experimental arrangement forms the basis for in rodent models, characterizing drug distribution kinetics.

Reduced drug compound signals could be associated with tumor regions, particularly in the squamous cell phenotype due to the large size areas of tumor cells, compared with the other two lung cancers investigated. We found that our MALDI LTQ Orbitrap XL instrument set up could collect imaging data with high image resolution, which excludes the possibility of interpreting these results as artifacts. The importance of high-resolution sampling was crucial in order to accurately localize the EGFR tyrosine-kinase inhibitors deposited in heterogeneous cancer tissue compartments.

We present data showing that the sample preparation of the tissue prior to analysis is of importance. Pre-solvatization of the respective lung tumor tissue is recommended, and in our experience is specific for each and every regional part of the pulmonary tract. The specific experimental conditions are needed to be optimized for any given lung cancer tissue type.

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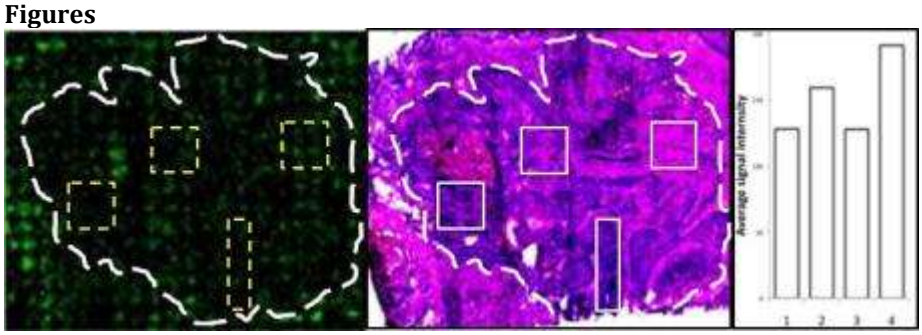
**Figures**



# P3. Memory Efficient Principal Component Analysis of Large Mass Spectrometry Imaging Data Sets

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## Abstract

Mass spectrometry imaging produces large data sets that typically cannot fit into memory. The common approaches to solving this issue is to reduce the data by either binning or only retaining peaks of interest. Multivariate analysis techniques such as principal component analysis (PCA) are then employed on the reduced data set to futher reduce the data while retaining the variance. However, standard implementations of PCA require data on the peaks of interest to be in memory, and this limites the amount of data that can be processed. As such a compromise must be made between the number of pixels (or samples) to include and the number of peaks to retain. This work describes a method of performing PCA without a limitation on the number of pixels and greatly increases the limit on the number of peaks that can be retained using summarisation matricies [1].

Data from MALDI imaging and LESA (liquid extraction surface analysis) experiments were converted to an open mass spectrometry (mzML) using msconvert (ProteoWizard). The mzML data were then converted to the open mass spectrometry imaging format (imzML) developed by the COMPUTIS project using custom in-house software. MATLAB was used for implementation of the memory efficient PCA algorithm for use on the imzML data.

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### Acknowledgements

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### Figures

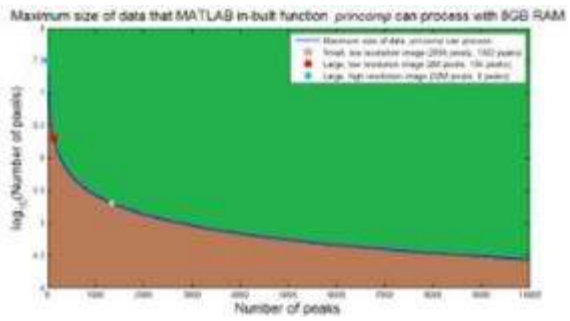


**Figure 1**. Data size in pixels and retained peaks that can be processed with 8GB RAM using MATLAB *princomp* (brown) and the proposed method (green and brown).

# P4. Laser-desorption-ionization assisted imaging of plants and insects surfaces

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## Abstract

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has recently found frequent application in mapping the distribution of small molecules in numerous biological samples or tissues. The data obtained using MSI have been used to follow or explain important biological processes or to rationalize previous biological observations. But because many experiments have been performed on a qualitative level, no data have been obtained regarding the concentration of imaged compounds in the samples. Here we offer a reasonably simple method to fill this gap.

We developed and optimized protocols for sample preparation for MSI experiments either using laser desorption/ionization (LDI) of matrix-assisted desorption/ionization (MALDI) for ionization of metabolites from surfaces. In one case quantification of glucosinolates on *Arabidopsis thaliana* leaves was successfully implemented.

Using developed set of methods we can perform qualitative and quantitative MSI of surface – accruing metabolites ranging from neutral and polar lipids, phenolics, phytoallexins. Obtained distribution maps were correlated with biological activity of visualized compounds and in many cases these MSI maps strongly support proposed biological hypothesis.

**Modern MSI methods are ready to contribute to surface chemistry analysis and addressing pending biological questions on spatial distributions of chemical signals.**

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**Acknowledgements**

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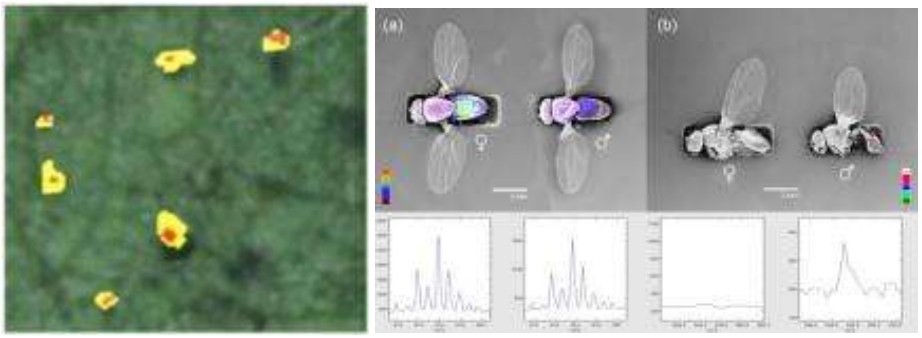
Figures



**LDI-MSI of cotton leaves and fruit flies surfaces.** Pigment gland of leaves are co-localized with areas of gossypol high concentrations (in red/yellow color). TGA and cVA (male) were detected on the fly body.

# P5. Bioimaging in metal based anticancer drug development and therapy

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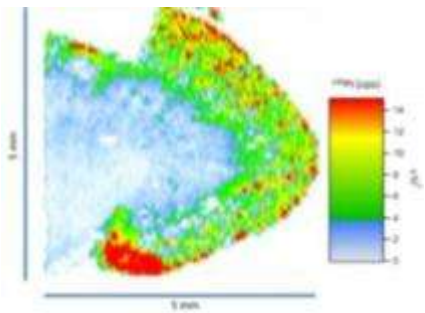
## Abstract

Platinum-based complexes (cisplatin, carboplatin and oxaliplatin) are worldwide approved as potent anticancer drugs. Despite their success, severe side effects (such as nephrotoxicity in case of cisplatin) or resistance against the drug are limitations in their application. Strategies to overcome these restrictions include variation of the leaving ligands or substitution of the central atom by other transition metals such as ruthenium. One of the emerging Ru-complexes is sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)], which is effective in preclinical colorectal tumor model in vivo. Prerequisites for clinical drug development are knowledge of toxicity, pharmacokinetics and pharmacodynamics of investigational compounds in various animal models. In the field of metal-based chemotherapeutic drug development, pharmacokinetics is usually determined by microwave assisted digestion of viscera originating from mice treated with a drug for a certain period of time. It is obvious that this technique is not suited for determination of the drug within various histological structures of an organ (e.g. cortex and medulla of the kidney) and only leads to an average concentration over the whole sample tissue. Hence we hyphenated laser ablation (LA) with an inductively coupled plasma-mass spectrometer (ICP-MS) and applied this technique on histological slides originating from mice treated with investigational anticancer compounds. Correlation of the resulting metal distribution pattern with histological structures allows improved metal tracking throughout the sample.

### Acknowledgements

The authors acknowledge financial support of the Austrian Science Fund (FWF, project I496-B11).

**Figures**



Raw counts within a kidney originating from a cisplatin treated mouse.

# P6. Heme compounds in a protohistoric vessel unearthed at the galician-portuguese border (San Millan, Ourense, Spain). Dating (TL) and identification of apo and holo forms of Heme b by LC-ESI-MS$^n$ and ESI-FTICR-MS

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## Abstract

Unearthed at the galician-portuguese border (San Millan, Ourense, Spain) the vessel was preliminary dated by Optical Stimulated Luminescence (OSL) and Neutron Activation Analysis of natural radioactive elements (K, Th, U) as being from a pré-roman date, that can said to be comprehended within the I Millennium B.C.. Crushed to a fine powder, the grey adherent material was extracted with methanol/water/formic acid and the products separated by HPLC in the gradient mode of methanol/water, in a C18 column. ESI-MS$^n$ mass spectra were obtained in a LCQ Fleet Thermo Finnigan ion-trap mass spectrometer. Accurate mass determinations were performed in an ApexQe FTICR Mass Spectrometer from Bruker Daltonics equipped with an electrospray ion source and a 7 T actively shielded magnet from Magnex Scientific.

Multiple fragmentation of the ion at *m/z* 616.2 was achieved till the MS$^5$ level and compared with standards. From the product-ions formed at MS$^2$ and MS$^3$ it was possible to propose a fragmentation scheme that accounts for the structural information obtained. Such information and its isotopic cluster on the FullMS scan are entirely compatible with the results published for Heme *b* in the literature. This shows the presence of this moiety, the most abundant of the nuclear moieties of Hemoglobins, in the vessel extract. Furthermore combined with the chromatographic behaviour of Hemoglobin standards in the HPLC-ESI-MS system, the results still show the presence of Heme *b* in its *holo* form, which seems to imply the presence of Heme-bound compounds or complexes in the sample extract.

Despite recent successes, the recovery of proteins from archaeological residues had a history of controversy in the specialized literature, successful detection of Hemoglobin in archeological samples remaining, so far, confined to lithic or painted artifacts.

The methodological difficulties and the scientific implications of these findings are analyzed and discussed.

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# P7. Evaluation of a novel Laser Ablation Electrospray Ionization Source for the Imaging of Bacteria from High Salt Content Liquid Medium

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## Abstract

The chemotaxis of different bacterial species is among the most interesting questions of microbiology. Imaging mass spectrometry is a tool capable of simultaneously mapping the spatial distribution of multiple different molecular species, thus making it a perfect tool to study the different metabolite gradients bacteria create in an initially uniform nutrient medium. These studies are often hindered by two factors: the high salt content of the medium that is needed for the proper bacterial growth and the vacuum incompatibility of the soft agar plates. Here we present the evaluation of a new Laser Ablation Electrospray Ionization (LAESI) source for studying chemical diffusion in semi-solid surfaces as well as bacterial metabolic processes in a liquid medium with high salt content.

A LAESI DP-1000 source ionization system (Protea Biosciences) was mounted on an LTQ-XL mass spectrometer (Thermo Scientific)onto several mass spectrometers, including an LTQ-XL (Thermo Scientific) and Synapt G2 (Waters Corp.). A number of subsystems, including a mid-IR laser (2.940 μm), x,y,z translational stages with an integrated Peltier cooling system, sample imaging cameras, and an electrospray ionization emitter with onboard high voltage power supply, were integrated into the LAESI source and controlled with custom software. Agar gel with a growth medium for bacteria was spiked with various standards ranging from small molecules to peptides and lipids. The resultant complex data sets were compiled and analyzed using custom imaging software (ProteaPlot) for generation of 2D digital images for select compounds of interest

Agar gel prepared with and without H1 bacterial growth medium was spiked using a set of standard solutions containing either a mixture of small molecules, peptides or phosphatidylcholines to assess the sensitivity of the LAESI-MS setup at different salt concentrations. In the case of peptides, the doubly charged ion proved to be the most abundant. The limit of detection was around 10 pmol/μL concentration for most of the studied compounds. The diffusion of molecules in the gel was also studied by spiking a spot in the agar gel with a cresyl violet solution. This procedure provided a method to visually follow the spreading of the spot. We also measured the sample at different time points using line scans across the spot what gives an insight into the the process of molecular diffusion. Finally, we grew *E-coli* on an agar streak plate. We measured the resulting bacterial colonies in both positive and negative ion mode after cutting them out of the agar plate. We could detect several bacterial metabolites and map their localization in the colony.

# P8. Mass Spectrometry-Based Identification of a Tumor Sialoglycoprotein Antigen involved in Cancer Progression.

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## Abstract

The UN1 monoclonal antibody (mAb) initially selected for high reactivity with human immature thymocytes (1), recognize the UN1 antigen as a heavily sialylated and O-glycosylated protein with the apparent molecular weight of 100-120 kDa; The UN1 antigen was immunodetected on the membrane of human thymocytes, a subpopulation of peripheral blood T-lymphocytes and leukemic T-cell lines (1, 2). In addition, UN1 was expressed at early stages of development in foetal tissues, and was down regulated in ontogeny (3). UN1 was also expressed in a variety of solid tumors, including breast, colon, gastric and squamous cell lung carcinomas, while undetected in normal tissues and benign lesions (3). In particular, a direct correlation was observed between the expression level of UN1 and the stage of malignancy in breast tissue (4). The expression pattern in primary cells suggested that UN1 behaved as an oncofetal antigen with a potential value for cancer immunophenotyping and clinical applications; however, the role of UN1 in tumorigenesis was not further addressed due to the lack of knowledge of its primary structure.

In this study, we have identified the UN1 antigen as CD43, a transmembrane sialoglycoprotein involved in cell adhesion, differentiation and apoptosis (5). Indeed, mass spectrometry detected two tryptic peptides of the membrane-purified UN1 antigen that matched the amino acidic sequence of the CD43 intracellular domain. Immunological cross-reactivity, migration pattern in mono- and bi-dimensional electrophoresis, and CD43 gene-dependent expression proved the CD43 identity of the UN1 antigen (6).

Glycosylation of CD43 generates different epitope that are recognized by distinct monoclonal antibodies (7). By glycosidase digestion, we have shown that the UN1 mAb recognizes a CD43 epitope that includes the monosaccharide GalNAc-O-linked to the peptide core. CD43 molecules harbouring the UN1 epitope are peculiarly expressed in breast, colon and sigmoid colon carcinomas, whereas undetected in normal tissues from the same patients. Our results confirmed the cancer-association of the UN1 epitope, highlighting UN1 mAb as a suitable tool for cancer immunophenotyping and analysis of CD43 glycosylation in tumorigenesis.

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# P9. Subcellular Imaging of 15N-labelled Cisplatin in Biological Samples Using Nano-scale Secondary Ion Mass Spectrometry (NanoSIMS)

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## Abstract

The role of platinum-based drugs in the treatment of cancer was established more than 30 years ago and is still growing. The insight into the subcellular localization of platinum-based drugs is of great importance to determine action and resistance mechanisms of these clinically indispensable chemotherapeutics[1]. Modern mass-spectrometry techniques allow highly sensitive and multielemental analysis on a subcellular scale. NanoSIMS offers high spatial and mass resolution together with a capability to measure up to 7 different elements simultaneously and provides a powerful tool for subcellular imaging of metals in biological samples.

NanoSIMS was implemented to study the distribution of $^{15}$N-labelled cisplatin (*cis*-dichloridodiammineplatinum(II)) in the human colon cancer cell line SW480. Using the positively charged Cs primary ion beam, we obtained $^{12}C^{14}N^-$, $^{31}P^-$, $^{34/32}S^-$ ion maps reflecting the cellular and nuclear (phosphorus rich structures) morphology with a remarkable spatial resolution up to 50 nm (Fig. 1). $^{195}$Pt or the combination of $^{194/198}$Pt and $^{12}C^{15}N/^{12}C^{14}N$ ratio ($^{14}N/^{15}N$ ratio) were used for cisplatin imaging. We were able to detect Pt in the cells after 24 h exposure to different concentrations of cisplatin (10–150 μM). In parallel we have quantified the amount of platinum associated with the cells by means of inductively coupled plasma mass spectrometry (ICPMS) to assess the NanoSIMS detection limit for platinum. The strongest correlation was shown between the Pt and S distribution both in the cytoplasm and nuclear regions, consistent with binding of Pt-compounds to thiol-containing molecules (thioredoxin system; thiol-rich proteins of lysosomes and nucleolus; metallothionines and glutathione). DNA is supposed to be the crucial but not necessarily the only target for the platinum-based drug[2]. The colocalization of Pt with P was observed in the nucleolus compartment, which is reach in S as well. The investigation of cisplatin on the subcellular scale using NanoSIMS might shed some light on its mode of action and resistance/detoxification pathways of the cell.

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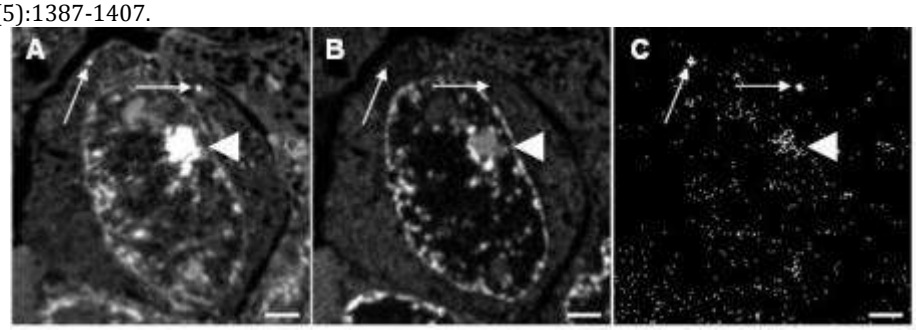


**Fig.1** Secondary ion maps of SW480 cell treated with cisplatin (24 h, 150 μM): $^{32}S^-$ (A), $^{31}P^-$ (B) and $^{194}Pt^-$ (C). Thin arrows show the colocalisation of Pt with S rich structures in the cytoplasm. Thick arrows show the colocalisation of Pt with S and P in the nucleolus. Scale bars = 2 μm.

# P10. Detection of Different Drugs and Metabolites by MALDI Imaging Mass Spectrometry

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## Abstract

The development of the MALDI Mass Spectrometry (MALDI-MS) during the last few years is allowing the detection of different types of biomolecules such as lipids and peptides. Moreover, Imaging Mass Spectrometry (IMS) methods localize these molecules directly in tissue slices. The improvement of the IMS technique is achieving also the detection of molecules with a low molecular mass, like drugs and metabolites.

In the present study we have assayed the identification by MALDI-MS of different representative drugs with affinity for receptors of amines (dopamine, fluoxetine, bromoxidine, carbachol), neuropeptides (DAMGO, galanin) and neurolipids (WIN55.212-2). The detection of other biomolecules involved in neurotransmitter signaling was also analyzed, comprising metabolites (arachidonic acid), second messengers (adenosin-3'-5' cyclic monophosphate or AMPc), endogenous endocannabinoids (anandamide, and 2-arachidonoylglycerol) and compounds selective for neurotransmitter transporters (hemicholinium-3).

The above-mentioned molecules have different chemical structures (derivatives of biogenic amines or small peptides and lipids), but all of them are biologically active compounds at the central nervous system (CNS). We have tested six commonly employed MALDI matrices: 2,5-dihydroxybenzoic acid (DHB), 2-mercaptobenzothiazole (MBT), 9-aminoacridine (9-AA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA) and 6-aza-2-thiothymine (AZA) in a LTQ Orbitrap XL MALDI Mass Spectrometer (Thermo Fisher), both in positive and negative detection. The obtained spectra allowed the detection of most of the assayed molecules using MBT and CHCA matrices. Actually, some of the drugs such as bromoxidine and carbachol were detected in the absence of chemical matrix.

Then, the limit of detection is calculated for the assayed matrices that yielded the best s/n ratio. The previous screening of each type of biomolecule for different ionization and detection conditions is a necessary step to localize them within biological samples and tissue slices by IMS. The identification of drugs and metabolites by IMS will facilitate the identification of therapeutic targets to develop drug treatments for neuropsychiatric and neurodegenerative diseases.

## Acknowledgements

Financial support from Diputación Foral de Bizkaia (6-12-TK-2011-025), the Basque Government (Saiotek, S-PE11UN091 and IT440-10) and the Spanish Government (ISCIII PI10/01202 and Innpacto, ONCOSLIDE) is acknowledged.

# P11. MALDI-imaging as a powerful tool for comparing various human skin states

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## Abstract

Since the introduction of matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) by Caprioli et al. in 1997 [**1**] there has been an increasing number of publications in the last couple of years. However, only a few groups have dealt with *ex-vivo* human skin and subsequent MALDI-MSI measurements so far [**2**]. The localization of endogenous and exogenous compounds within intact *ex-vivo* tissue itself, including the investigation of biological processes within tissue, is a challenging task in the field of skin research.

In this manuscript, we report opportunities offered by MALDI-MSI for comparing various skin states, based on the analysis of tryptic peptides (tryptic peptides MSI). Unfortunately, the identification of proteins *via* their tryptic peptides by use of MS/MS measurements directly on tissue is not trivial due to high biological background, resulting in fragment spectra, which are often difficult to interpret. By use of the spatial proteomics approach, MALDI-MSI measurements are used to generate two-dimensional maps of proteins *via* their tryptic peptides and eluates of tryptic peptides obtained from adjacent tissue sections are used to enable accurate identification of the corresponding amino acid sequences by liquid chromatography mass spectrometry investigations (LC-MS/MS). Strikingly, results of both workflows are correlated.
The specific distribution of several proteins, identified *via* their tryptic peptides, is presented as follows: In **Fig. 1 a** and **c** *m/z* 1015 is localized specifically in the area of eccrine sweat glands for both skin states. Furthermore, *m/z* 1354 is distributed only in the lower dermis [**Fig. 1 b**] for skin state 1, whereas for skin state 2 it is localized in the entire dermis [**Fig. 1 d**]. The identified proteins and the data on the correlation with LC-MS/MS results will be provided.

To summarize, two-dimensional maps of several proteins, identified by use of LC-MS/MS, were generated. Our work reports for the first time the opportunities of spatial proteomics in generating protein maps directly in skin tissue sections. The spatial proteomics approach presented here shows new insights into localizing and identifying endogenous proteins in skin tissue sections simultaneously without the need of labeling. Thus, MALDI-imaging is a powerful tool for comparing various skin states and for biomarker discovery.

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**Figure 1**

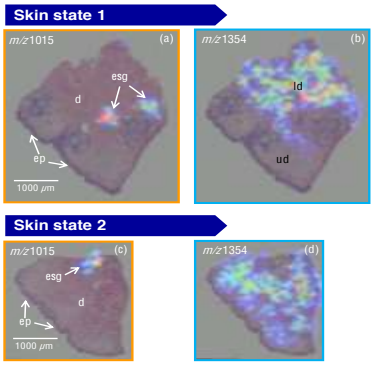


Fig. 1 Overlays of ion density maps and corresponding HE staining images. Overlays of the ion density maps of *m/z* 1015 and *m/z* 1354 and the corresponding HE staining image for skin state 1 is shown in a and b, respectively. Overlays for skin state 2 are depicted in c and d.

Abbreviations:
d – dermis
ep – epidermis
esg – eccrine sweat glands
HE – hematoxylin and eosin
ld – lower dermis
ud – upper dermis

Intensity scale:

# P12. Imaging mass spectrometry reveals a different composition and distribution of molecules in human normal and osteoarthritic cartilage

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## Abstract

Osteoarthritis (OA) is a pathology strongly linked with obesity. Many lipid mediators have been described as contributors to the OA development. In addition, cell death and the imbalance in the ion pumps and channels across membranes, contribute to the cartilage degradation. Currently, we do not yet completely understand all the factors that are responsible for initiating the degradation and loss of joint tissues. Time of flight-Secondary ion mass spectrometry (TOF-SIMS) and Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) allow us to study the detailed spatial distribution of many molecules at high spatial resolution in healthy and OA tissues sections.

In this study, we have studied and compared the distribution of lipids and other molecules in healthy and OA human cartilage by TOF-SIMS and MALDI-IMS.

The preliminary results obtained by TOF-SIMS in the positive mode, showed that the masses m/z 368.99, m/z 339.24, m/z 351.00 m/z 370.95, m/z 381.02 and m/z 383.26 were specific to the OA condition. We also detected cholesterol m/z 369 $[M\text{-}OH]^+$, m/z 385 $[M\text{-}H]^+$ as well as cationized species like m/z 970 $[2M+Au]^{2+}$. The spatial distribution of these cholesterol related peaks exhibited a remarkable difference between normal and OA cartilages. In OA samples, we observed a co-localization of cholesterol and other lipids in big droplets in the superficial area as the first function of principal component analyses shows in the figure. The first function in the healthy samples didn't show those lipid depositions. In the negative mode we found a higher abundance of oleic acid in the OA cartilages and with a specific localization again in those big droplets of lipids. These results were validated by oil-red staining. MALDI-IMS also revealed a high presence of phospholipids like phosphocholine, phosphatidylserine and sphingomyelin and their sodium and potassium adducts in the superficial area of the OA cartilage. Finally, we also studied the localization of electrolytes like sodium, potassium, calcium and phosphate ions in healthy versus OA cartilage. The accumulation of calcium and phosphates in areas surrounding only the cells from the OA cartilage showed the importance of these molecules in the tissue remodeling characteristic of this pathology.

In summary, the combination of TOF-SIMS and MALDI-IMS could help to understand the changes in the distribution and in the abundance of lipids, calcium and phosphates in the OA cartilage.

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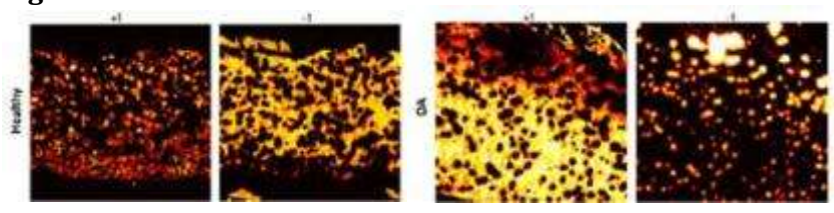
## Acknowledgements

Authors acknowledge the research program of the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)" (The Netherlands) and funding through Xunta de Galicia (Spain).

## Figure



# P13. Quantification of pharmaceuticals in tissue sections by MALDI-MSI

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## Abstract

Quantification of pharmaceutical compounds in tissues routinely involves homogenisation of the tissue followed by extraction of the compounds and subsequent analysis by LC-MS/MS. Whilst this method enables quantification of compounds in various tissues, the ability to monitor the spatial distribution is lost during sample preparation [1].

Matrix-assisted laser desorption/ionisation-mass spectrometry imaging (MALDI-MSI) can be used to monitor the distribution of pharmaceutical compounds in tissues but is widely considered a qualitative technique. However the ability to quantify compound present would be a distinct advantage.

Several quantitative methods have recently been reported that demonstrate quantitative analysis of pharmaceutical compounds in tissue section by MALDI-MSI [2] [3].

Two Wistar Han rats were dosed with Tiotropium Bromide at a nominal dose level of 200 µg/kg, to deliver a 50 µg topical dose of compound to the lung. Individual rats were then euthanized immediately after the administration of the dose or 15 minutes post dose. The lungs were then harvested from the animals and cryosectioned to produce 16 µm thick sections. The sections were thaw-mounted onto indium tin oxide coated glass slides. Serial dilutions of Tiotropium Bromide were prepared (100 – 0.1 ng/µL), mixed with control rat lung homogenate, embedded in pre-frozen gelatine and frozen to -20°C. The embedded array of homogenate standards were then sectioned at a temperature of -20°C using a cryostat to obtain 12 µm sections, which were thaw mounted onto glass slides.

Analysis of Tiotropium Bromide on a MALDI target plate revealed a strong peak at m/z 392 due to the positively charged nitrogen in the compounds structure and MS/MS showed the presence of two product ions at m/z 152 and m/z 170. The MALDI-MS and MS/MS images of the homogenate standard sections showed the distribution of Tiotropium ($[M]^+$) at m/z 392 and the product ions at m/z 152 and 170. The images show that visually the limit of detection is approximately 0.1 ng/µL. Utilising the region of interest tool of the BioMap 3.7.5.5 software, the average intensity of each standard was determined and plotted against the standards respective concentration. The graph showed a linear response ($R^2$=0.970) ranging from 0.1 – 10 ng/µL. MALDI-MS images of the dosed tissue showed the distribution of Tiotropium ($[M]^+$) at m/z 392 to be within the trachea and major airways immediately after dosing.

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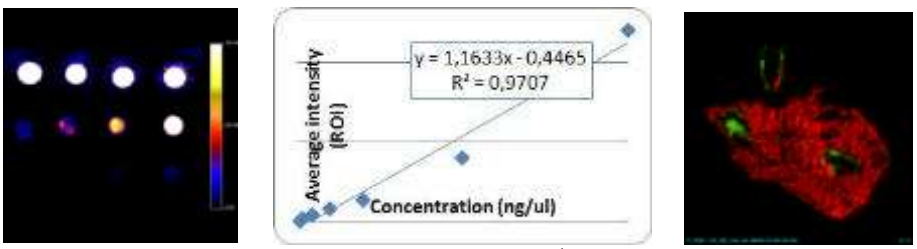
Figures




*MALDI-MS image showing the distribution of Tiotropium ($[M]^+$) at m/z 392 within the array of homogenate standards, the resulting calibration curve and a MALDI-MS image showing the distribution of Tiotropium (green) overlaid onto the distribution of the phospholid head group (red) at m/z 184.*

# P14. Ischemia in neonatal rat brains analyzed by Desorption Electrospray Ionization Imaging mass spectrometry

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## Abstract

Desorption Electrospray Ionization (DESI) Imaging was used for imaging of neonatal rat brains. The rat pups were subjected to surgery which induced ischemia by cerebral artery electrocoagulation combined with a transient and concomitant occlusion of both common carotid arteries. The pups were sacrificed 24 and 48 hours after the surgery, and the brains were removed and frozen in liquid nitrogen.

The images show a remarkable increase in the abundance of different species of *N*-acyl-phosphatidoylethanolamines (NAPE), appearing in the range of *m/z* 950-1060 in negative ion mode, in the entire ischemic area. Also, an increase in the abundance of free fatty acids is seen in the periphery of the ischemic area. Imaging in positive ion mode showed different species of phosphatidylcholine (PC) with increased abundance of the sodium adducts and decrease abundance of the potassium adducts in the ischemic area, indicating a break-down of the $Na^+/K^+$ pump.

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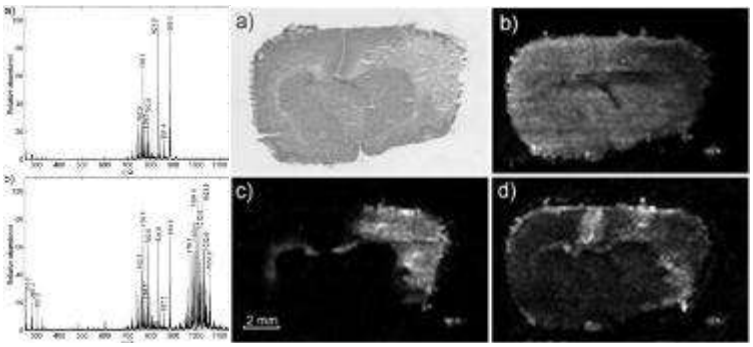
### Acknowledgements

Support from the Novo Nordisk Foundation, The Carlsberg Foundation and the Danish Council for Independent Research, Natural Sciences, is gratefully acknowledged.

**Figures**




**Left:** DESI mass spectra in negative ion mode. a) Healthy tissue; b) Ischemic tissue.
**Above:** DESI-MS images (negative ion mode) of the brain of a neonatal rat, sacrificed 24 hours after ischemia-reperfusion. a) microscope image of H&E stained brain section; b) Phosphatidylethanolamine(38:4) (*m/z* 766.5); c) N-Acylphosphatidylethanolamine(54:4) (*m/z* 1004.8); d) Arachidonic acid (*m/z* 303.3).

# P15. Examination of Treatments for Chronic Skin Diseases by MALDI-MS Imaging and Profiling

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## Abstract

Acitretin an aromatic retinoid used in the treatment of Psoriasis is currently administered orally. Topical administration of Acitretin may increase the local bioavailability at the site of action (i.e., diseased skin) whilst minimizing potential systemic exposure and teratogenic side effects [1]. Cornified-envelope structures have a pivotal position in the limit to which drugs cross the Stratum Corneum, facilitating their pharmacodynamic effects [2]; and skin lipid and filaggrin responses underpinning the integrity of the permeability barrier [3]. MALDI-MS has been employed as a sensitive technique for measuring the spatial distribution of pharmaceutical compounds and responses within skin [4]. The analysis of Acitretin and Acitretin treated synthetic skin equivalents via MALDI-MS imaging and profiling is described.

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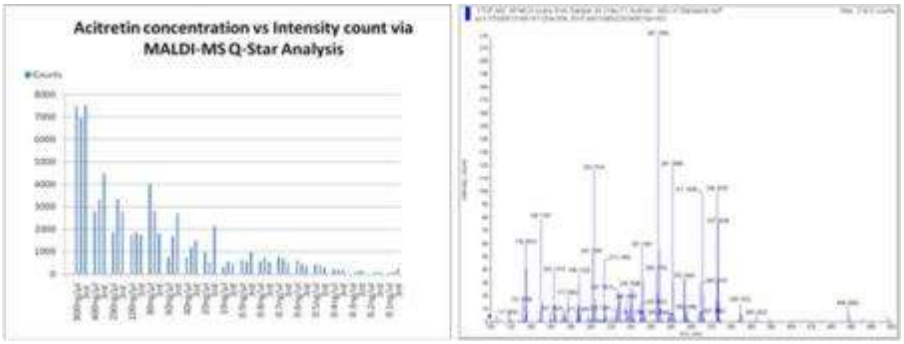
**Acknowledgements:** Stiefel UK Ltd (A GSK Company) Michael Donaldson; and Malcolm R. Clench

**Figures:** Acitretin profiling for prospective imaging in skin.

The initial studies reveal that the limit of detection for Acitretin within standard solution resides within the lower picogram scale (100pg/µl). Intriguingly, its ultraviolet light absorbing properties enable the compound to be profiled even in the absence of matrix according to our study within the nano scale range. Trace intensities of compound within lower epidermal skin sections were detected originating from outer-surface application with concentrations initially as low as 100ng/µl. A further tandem MS fragmentation verified the compound identity. Within our team, lipid species have been previously imaged and identified through lipid database sources for *ex-vivo* skin [5].



# P16. Papillary tumor grading in bladder cancer: MALDI imaging as diagnostic tool

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## Abstract

**Objectives:** Among frequent transitional cell carcinoma (TCC) the most common phenotype (70%) is a (single) papillary non-invasive neoplasia (pTa), which is generally both genetically stable and well differentiated. In contrast, its more poorly differentiated counterpart tends to occur relatively less frequently. The histological grading of TCCs reveals significant prognostic information, especially with regard to tumor progression.

In 2004, a novel grading system for papillary non-invasive bladder cancer was introduced, low grade (LG) and high grade (HG) in lieu of the former G1, G2 and G3. This change allowed for increased reproducibility as well as diminished interobserver variability in histopathological grading among individual pathologists. The major focus of our analysis was the verification of the histological diagnosis, which is especially challenging for the former G2 grade.

**Design and Methods:** In the presented work, tissue Matrix Assisted Laser Desorption/Ionization Time Of Flight (MALDI TOF) Imaging Mass Spectrometry (IMS) was used to generate peaks specific for histologically defined tissue areas. Initially, we identified differential peaks for G1 pTa tumors to represent the LG group (n=27) as well as for G3 pTa tumors to represent the HG group (n=21). Thereafter, we classified G2 specimens (n=31) automatically, based on their peak profile, into either the LG or HG group, and compared the proteomic grading result of each tumor with the histopathological re-classification of the same sample.

**Results:** G1 (LG) and G3 (HG) tumors were separated with an overall cross validation of 97.18%. G2 tumors indicated a true positive rate of 78.3% for LG and 87.5% for HG, in comparison to the pathological grading. Implicating the follow up data of the patient, MALDI imaging showed even better results.

**Conclusion:** MALDI TOF IMS is a powerful tool to ascertain and support pathological diagnosis/grading.

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## Acknowledgements

The authors are grateful for technical assistance by Nadine Reulen and Sonja Gostek, University Hospital Aachen. The work was supported by P.U.R.E. (Protein Unit Ruhr within Europe).

# P17. An integrated in situ proteomic and metabolomic MALDI-MS imaging using a single tissue section

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## Abstract

We have recently reported that a MALDI-MS system with 9-AA as a matrix achieved much improvement for a sensitive analysis of low-molecular-weight metabolites for metabolome MSI[1, 2]. The MALDI-MSI technique enabled a spatially resolved detection of more than 30 identified metabolites including nucleotides, cofactors, phosphorylated sugars, amino acids, lipids, and carboxylic acids in normal mouse brain tissue with their unique distributions. Furthermore, more than 100 unidentified metabolites were found and utilized for MSI. Further identification is in progress.

Proteomics is believed to provide important insights into the hierarchical regulation of metabolic behaviors. For visualizing spatial distribution of proteins, a new method based on direct enzymatic digestion of proteins on the tissue sections (*in situ* digestion) has been proposed. By using this approach, we attempted to spatially visualize proteome information on the same tissue section toward more profound understanding of biological systems. After metabolome analysis, the same brain tissue section was subjected to *in situ* digestion after washing off the matrix for metabolites where some tryptic peptide peaks were observed by MALDI-MS. These peaks were sequenced by MS/MS to identify. Detection of a peptide fragment from β-Tubulin (*m/z* 1620) indicated the occurrence of protein digestion on tissue. In addition, localization of several peptides could be visualized by this MALDI-MSI technique.

Identification of these peptide peaks is now underway. Experiments towards optimization for better peptide ionization and effective trypsin digestion are also required for more comprehensive data. Taken together, the present MSI technique enabled to serially visualize both metabolomic and proteomic localization on the same brain tissue. Although it is very primitive, MSI for metabolites and proteins was achieved using a single mouse brain section for the first time.

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### Acknowledgements

This research was supported by the Science and Technology Incubation Program in Advanced Region from the funding program “Creation of Innovation Centers for Advanced Interdisciplinary Research Areas” from Japan Science and Technology Agency, commissioned by the Ministry of Education, Culture, Sports, Science and Technology. We would like to specially thank Shimadzu Co. Ltd. (Kyoto, Japan) for their full cooperation.

# P18. Uranium imaging in cells by Secondary Ion mass spectrometry: Chemical or cryo preparations?

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## Abstract

Radionuclides microdistribution in the biological structures after internal contamination is studied by Secondary Ion Mass spectrometry (SIMS). These cartographies are an important contribution to explain and interpret the mechanisms of radioactive contaminants transport in different organs of living beings and different cells compartments.

This work presents *in vitro* experiments (human hepatic cells model) to study uranium bioaccumulation kinetic at different concentrations of radioelement (10, 50, 100 and 300 µM).
For each area analysed, mass spectra at around 238 uranium isotope and ionic images have been obtained with a SIMS CAMECA 4F-E7. $^{40}Ca^+$ and $^{23}Na^+$ images give the histological structure of cells and $^{238}U^+$ images show uranium accumulation within different cell compartments. More, images obtained with two different preparation methods of these biological samples are presented. Classic chemical fixation procedure and cryo-preparation are compared.

Classic chemical fixation procedure and cryo-preparation are compared to investigate a possible redistribution of labile ionic elements ($Na^+$, $Ca^+$) during the chemical preparation which would alter *in vivo* microscopic distribution.

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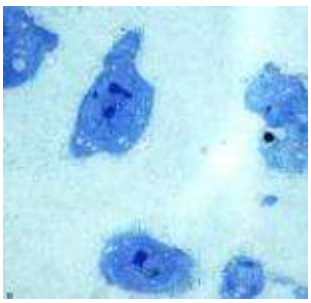
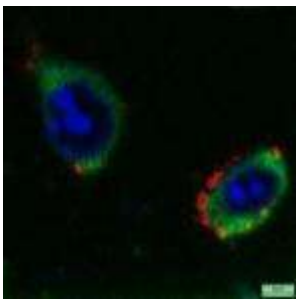
a)   b)

Figure 1 a) optical cells view b) superposed ionic images $^{238}U^+$ (red), $^{40}Ca^+$ (blue) and $^{23}Na^+$ (green)

# P19. Human SOD1-G93A specific distribution in murine brain of a transgenic model for Amiotrophic Lateral Sclerosis, as determined by MALDI Imaging Mass Spectrometry

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## Abstract

Amyotrophic Lateral Sclerosis (ALS) is a progressive, fatal neurodegenerative disease caused by the degeneration of motor neurons. The transgenic mouse model carrying the human SOD1G93A mutant gene (hSOD1G93A mouse) represents one of the most reliable and widely used model of this pathology. In the present work, the innovative technique of matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) was applied in the study of pathological alterations at the level of small brain regions such as facial and trigeminal nuclei, which in rodents are extremely small and that would be difficult to analyze with classical proteomics approaches.

Comparing slices from three mice groups (transgenic hSOD1G93A, transgenic hSOD1WT and non-transgenic, Ntg), this technique allowed to evidence the accumulation of hSOD1G93A in the facial and trigeminal nuclei, where it generates aggregates: they are likely to be correlated to the degeneration observed in this region for this ALS model. These results were confirmed by immunoistochemistry. Moreover, a statistical analysis allowed to highlight other peaks which contributed to discriminate the three mice groups analyzed. Some of them were identified by HPLC fractionation of extracted proteins and mass spectrometric analysis after trypsin digestion.

## Acknowledgements

This work was supported by "I-TECHPLAT Project" ("Docup 2000-2006, Misura 3.4" from Regione Piemonte local government) and "Lagrange-Fondazione CRT Project" grants.

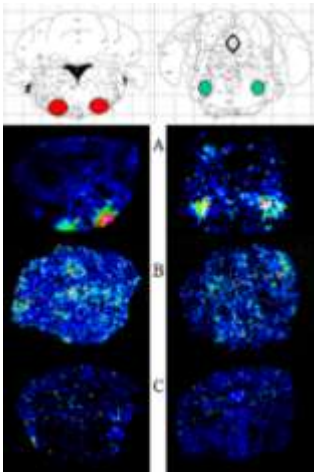


**Figure 1:** SOD1 peak distribution by MALDI-IMS in coronal brain sections from 24-weeks old hSOD1G93A (panel A), hSOD1WT (panel B) and Ntg mice (panel C) in the facial (left) and trigeminal (right) nuclei region. The corresponding map representations (from Paxinos & Franklin Mouse Brain Atlas, 2nd Edition) are shown on the top, where facial nuclei are highlighted in red and trigeminal nuclei in green.

# P20. Localisation of adenine nucleotides in mouse brain using ion-mobility enabled MALDI Imaging

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## Abstract

Information regarding the energetic state of tissue is important in a wide range of experimental studies, particularly in the study of metabolic stress, such as hypoxia or ischemia. Metabolic stress is known to lead to the degradation of adenine nucleotides in brain tissue. The energetic state of tissues or cells is often assessed by determination of adenine nucleotide (ATP, ADP and AMP) levels[1]. This is commonly achieved using ultraviolet (UV)-based high-performance liquid chromatography (HPLC), which provides quantitative data, but does not give specific localisation information. MALDI imaging can provide specific localisation of adenine nucleotides in tissue sections[2]. The applicability of ion mobility enabled MALDI imaging to detect differences between control and stressed brain tissue sections has been assessed.

Optimisation experiments using adenine nucleotide standards on and off tissue have allowed the determination of a selective and sensitive method for the detection of all three adenine nucleotides in brain tissue sections. The addition of an ion mobility separation step after MS/MS fragmentation introduces extra selectivity in the analysis, allowing the detection and localisation of ATP in tissue. This has not previously been shown.

A comparison of the intensity ratios of ATP: ADP: AMP levels in tissue revealed a lower ratio of ATP: AMP than expected in a normal mouse brain. A previous HPLC study comparing nucleotide levels between fresh, frozen and freeze-thawed brain sections indicated that ATP hydrolysis may occur very quickly as frozen tissue sections are brought to room temperature[1].

In order to prevent ATP hydrolysis during sample preparation, further development of tissue handling techniques was required. A sequential ethanol wash step was included to dehydrate frozen tissue sections prior to tissue thawing. This washing step has been shown to reduce ATP hydrolysis in tissue during sample preparation, thereby minimising experimental interferences. This method development has allowed the comparison of ATP: ADP: AMP levels between control and a stress-induced mouse brain, alongside localisation images of adenine nucleotides in brain (Figure 1). This approach offers complementary data to quantitative HPLC methods traditionally used for determining the energetic state of tissue.

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## Figures

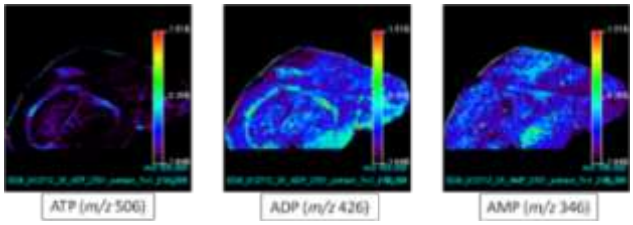


Figure 2. MALDI-ion mobility-imaging ion intensity images of ATP, ADP and AMP in mouse brain.

# P21. Drug and metabolites study in whole body animal by High Definition MALDI imaging

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[2]Lundbeck A/S, Denmark

## Abstract

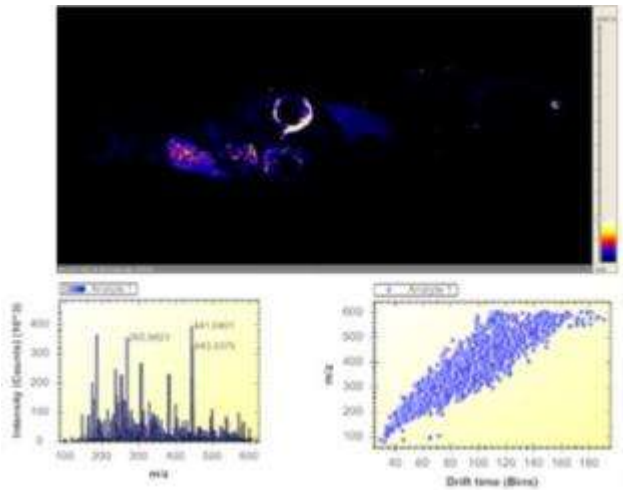
Mass spectrometry Imaging (MSI) is increasingly used in pharmacokinetic studies during preclinical studies. It has been recognized as a complementary technique to Whole-Body Autoradiography (WBA), The two main advantages of MSI are cost savings compared to radio-labeling of the drug and the absolute confirmation that the drug and its possibly produced metabolites are indeed visualized.

Here, we present the results from a study where two MS based imaging approaches were used to illustrate the spatial distributions in rat whole-body sagittal tissue sections of olanzapine and its metabolites (N-desmethyl and 2-hydroxymethyl), along with untargeted analysis of endogenous molecules in a whole body section.

Data were acquired using a MALDI SYNAPT G2 mass spectrometer with tri-wave ion guide optics to separate ions according to their ionic mobility in the gas phase. The obtained data sets were subsequently processed using High Definition Imaging (HDI) MALDI software for detailed image analysis.

A first experiment was carried out on the 2 h post-dose tissue section in a typical targeted multiplex targeted MS/MS, approach where the drug and the two known metabolites were MALDI imaged from a single tissue section. A second experiment was performed on the 6 h post-dose tissue section in an untargeted MS approach where all molecular species were separated by ion mobility prior to their mass measurement. Also in this instance, both the drug and the two main metabolites were imaged. However, here a vast amount of information is also generated by the ionisation of the endogenous species present in the whole body tissue section. The final part of the study involved the performance evaluation of isobaric species distinguished by the orthogonal separation capability (IMS) from the time of flight of the mass spectrometer.

Figures



# P22. Photochemical Harpoons: Covalent labels for multi-protein complexes

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## Abstract

The $K_{ATP}$ channel opener (diazoxide) displays marked cardioprotective effects and is reported to bind to mitochondrial $K_{ATP}$ channels[1]. However, the molecular structure of these channels is still largely unknown. Chemical proteomics allows the interrogation of protein function by identifying biomolecules involved in processes initiated by an external stimulus. This work describes the design of a chemical tool to covalently fluorescently label proteins which will bind this "selective" mitochondrial $K_{ATP}$ channel opener.

The model for the chemical tool consists of four components: (i) a photochemically activated reactive 'barb' component, which upon irradiation will covalently bond to an adjacent protein backbone; (ii) a fluorescent tag attached to the photoreactive 'barb' component; (iii) and a small biologically active 'bait' molecule (diazoxide) which selectively binds to a specific protein target[2]; (iv) a flexible linker between the bait and reactive barb/tag components.

In order to identify which functionalised diazoxide component 'bait' is appropriate for the attachment with the other components, biological testing assays of lactate dehydrogenase activity (LDH) and mitochondria function (MTT)[3] have been applied on variety of diazoxide analogues already prepared.

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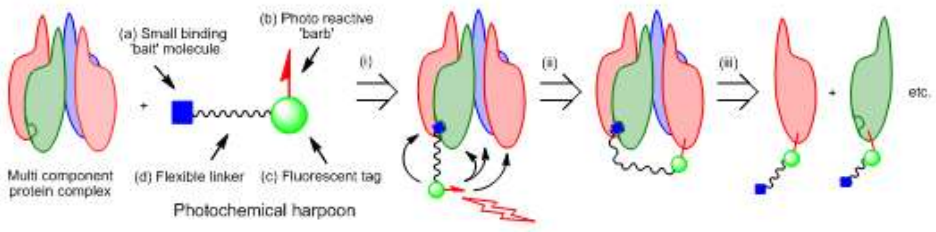
Figures



# P23. Spatial functional proteomic analysis of pleomorphic adenoma by MALDI-imaging segmentation

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## Abstract

Pleomorphic adenoma is the most common neoplasm of the salivary glands. These benign tumors are composed of epithelial and myoepithelial cells that are arranged in various morphological patterns which make them ideal for a detailed MALDI imaging (MALDI-MSI).

After surgery pleomorphic adenomas were snap frozen in liquid nitrogen and stored at -80 °C. For the MALDI-MSI experiments 10 µm thick sections were cut on a cryotome (Leica CM 1850) and thaw mounted on ITO glass slides (Bruker Daltonik). After drying in a vacuum desiccator, sinapinic acid (10 mg/mL in acetonitrile/water/TFA 60:40:0.2, v/v/v) as matrix was applied with the vibrational vaporization technology (ImagePrep, Bruker Daltonik).

Mass spectra were acquired on a MALDI-TOF instrument (autoflex speed LRF, Bruker Daltonik) equipped with a 1 kHz smartbeam II laser. The instrument was operated in linear mode and positive polarity. Spectra were acquired at a mass range from m/z 2,000 to 20,000 at a sampling rate of 0.13 GS/s. The MALDI-imaging raster width for the MALDI-MSI experiment was set to 50 µm. A total of 300 laser shots were summarized per position. Acquisition and result visualization was carried out using the flexImaging 3.0 software (Bruker Daltonik).

For each data set, we have performed a spatial segmentation as we have described it in detail recently [1]. Ten clusters were generated showing different functional areas of the pleomorphic adenoma and the normal salivary gland (Fig 1). The dark blue area depicts the central and the orange area the peripheral part of the adenoma. The red and the light blue cluster define the pseudocapsule. The other colors on the left side of the tissue section can be correlated to functional structures in the normal salivary gland tissue.
In conclusion it could be demonstrated that segmentation provides a unique way to depict the complex functional proteomic heterogeneity of a tissue in one image.

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**Figure**

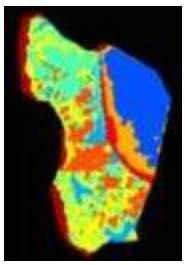

Fig. 1: Spatial segmentation of MALDI-MSI data of a tissue section containing pleomorphic adenoma and normal salivary gland. The pleomorphic adenoma is defined by the dark blue and orange area on the right side, the pseudocapsule by the red area and the surrounding normal salivary

# P24. Analysis of Nonaminoglycoside Distribution in Tissue via MALDI-Imaging Mass Spectrometry (MALDI-IMS)

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## Abstract

Drug discovery is a major area of research in the medical field, often complicated by the development of drug-resistant diseases, potential toxicity, and unwanted metabolites. Drug development is further complicated by the reliance of drug characterization on complicated procedures to tag compounds of interest. Radioactive isotope labeling and fluorescent tagging are two popular methods for biodistribution studies; however, imaging mass spectrometry (IMS) can be employed more effectively because of its tag-free methodology. In this study, we used IMS to track compounds proposed to treat a single nucleotide polymorphism (SNP)-driven genetic disorder: Ataxia- Telangiectasia (AT), caused by the insertion of a premature termination codon (PTC). Unable to produce functional ATM protein, patients exhibit symptoms such as radiosensitivity, impaired cerebral development, and susceptibility to cancer as ATM is involved in the repairing of double-stranded DNA breaks.1 Aminoglycoside compounds have been used to treat PTC disorders in the past; however, these compounds exhibit toxicity in mammals. Recently, a class of compounds termed nonaminoglycosides has been show to restore functional ATM protein *in vitro*.2 These compounds show distinct advantages over minoglycosides, and *in vivo* mouse models were used to study biodistribution of these novel drugs. Two read-through compounds, BZ16 and BZ6, were ionized off-tissue to develop a spectral profile. Dihydroxybenzoic acid (DHB), *a*-cyano-4-hydroxycinnamic acid (HCCA), and lack of matrix were used as test conditions. Optimization experiments were conducted to determine the effect of an ethanol wash step on compound delocalization. BZ16 and BZ6 were spotted on top of control mouse brain tissues and imaged. These slides were subsequently washed for 30 s in 70% ethanol and imaged again. For animal models, 24 severe compromised immunodeficiency (SCID) mice and 24 C57BL/6 mice were treated over the course of two months with one of the following: DMSO, BZ16, or BZ6. All injections were administered intraperitoneally at a dosage of 30 mg/kg for a total of 6 treatments. After sacrifice, organs were frozen in -80oC isopentane and sliced on a Leica cryostat (Wetzlar, Germany) at -21$^{o}$C and a thickness of 16 µm. Imaging mass spectrometry runs were conducted using a Bruker AutoFlex Speed MALDI-TOF/TOF (Bremen, Germany). Data were analyzed using Bruker FlexAnalysis and FlexImaging software. Both BZ16 and BZ6 were efficiently ionized without the need for MALDI matrix. Imaging experiments indicated that BZ16 may cross the blood-brain barrier, an imperative characteristic for an effective treatment of AT. Experiments also revealed that BZ6 does not appear to cross the blood-brain barrier. Further inquiries revealed that BZ6 accumulates in the liver.

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## Acknowledgements

This work was supported by the Ruth L. Kirschstein National Research Service Award (GM007185, UCLA Cellular and Molecular Biology Training Grant; to C. Ferguson) and the National Institutes of Health National Center for Research Resources (S10 RR025600; to J. Loo).

# P25. Comparison of different on-tissue digestion protocols to improve MALDI-MSI Experiments

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## Abstract

Objective: Matrix-assisted laser desorption ionization (MALDI)-Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection[1] and drug development. But in some cases exclusive visualization of the spatial distribution of potential markers or areas of interest is not a sufficient and satisfying result. Hence, over the last years, increased efforts have been made to develop and improve methods to identity unknown components.

Current on –tissue protein identification techniques in MALDI-IMS are either done via spray coating or droplet deposition of trypsin solution[1,2] and/or matrix solution respectively. Both can be performed in an either manual or automated manner. Latter allows greater confidence of inter-run reproducibility. Still the entire process from sample collection to data analysis is influenced by a number of more or less controllable and uncontrollable factors. The overall goal of this study was to test and compare various protocols of tissue digest in order to improve the quality of our MSI data.

Design and Method: In this work the MALDI-ImagePrep device from Bruker Daltonics is used for automated spraying of trypsin and matrix on mammalian tissue samples. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics). Different experiments were carried out varying 1) the protease, 2) the trypsin incubation time / drying phase, 3) the matrix, 4) the raster width of the laser and 4) the setting of the laser attenuator.

Results: Preliminary results reveal interesting differences concerning the spatial resolution. But due to the fact that the experiments are still ongoing at the time of abstract submission final conclusion could not be drawn.

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## Acknowledgements

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# P26. Tissue-proteomics characterization of the oncogenic epidermal transformation in presenilin-deficient mice

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## Abstract

A new model of oncogenic epidermal transformation induced by inactivation of presenilins (PS)/ϒ-secretase was recently described in mice [1]. In this mouse model, conditional genetic inactivation of presenilins in epidermis enhances EGFR signalling resulting in cell transformation. In recent years, several mass spectrometry-based proteomic methodologies have been developed that now make it possible to identify, characterize, and comparatively quantify the relative level of expression of proteins that are differentialy expressed in a given cell type or tissue [2]. In this work we present a tissue proteomics approach using 2D-GE (figure 1) combined with MALDI-TOF/TOF MS for the identification of differentially expressed proteins in epidermis that act as molecular signatures in order to characterize the phenotype of epidermis from normal to hyperplasia and oncogenic transformation. Differential protein expression was also investigated using the software SameSpot. The MS/MS analysis revealed 70 proteins, including, translationally-controlled tumour protein, 14-3-3 protein zeta/delta, 14-3-3 protein sigma, and vimentin, that were differentially expressed during the epidermal transformation in PS-deficient mice. The identification of specific molecular changes by means of tissue proteomics can be particularly interesting for future therapeutic interventions in this skin disease.

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## Acknowledgements

H. M. Santos acknowledges the post-doctoral grant SRFH/BPD/73997/2010 provided by the FCT-MCTES (Fundação para a Ciência e a Tecnologia – Ministério da Ciência, Tecnologia e Ensino Superior, Portugal. J. L. Capelo acknowledges the financial support provided by Xunta de Galicia (Spain) throughout Isidro Parga Pondal research program and the research project 09CSA043383PR (Biomedicine).

Figures

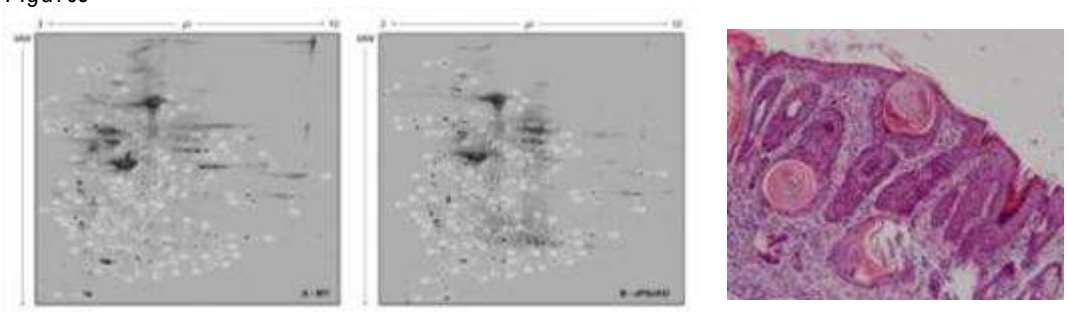

Figure 1. Representative gels of the soluble proteins extracted from skin of control (A) and epidermal presenilin-deficient mice (B)-. IPG pH 3-10,24cm and SDS-PAGE 10% acrylamide/bis. Eosin/haematoxylin staining of section of epidermal presenilin-deficient mice.

# P27. Drug dosed brain tissue imaging using nanospray desorption electrospray ionization, nano-DESI

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## Abstract

The ability to visualize molecules and their localization in brain tissue can lead to a greater understanding of the chemistry in the brain. Up to date there is still much to be learned about the molecular interactions taking place both in healthy and diseased brains. Besides lipids and proteins there are important signaling molecules such as neurotransmitters that are essential for the function of the brain. The brain function can however be altered by drugs that effect different neurotransmitter receptors. The behavioral alterations after drug administration have been thoroughly studied but the actual chemical interactions are still largely unknown. Mass spectrometry imaging provides a great tool to further understand the brain chemistry since the localization of specific ions within the tissue can be mapped. With mass spectrometry imaging it is also possible to visualize the distribution of administered drugs in the brain. Nicotine, for example, is a well-known compound that quickly enters the brain via the blood-brain barrier. In the brain nicotine acts as a stimulant, but in too high doses nicotine becomes toxic. Another example is the insecticide chlorpyrifos which can cause development disorders after chronical exposure. Because neurotransmitters are often present at fairly low concentrations, highly sensitive imaging techniques are necessary for mapping their spatial distribution in the brain.

Here we present the use of nanospray desorption electrospray ionization, nano-DESI, for imaging of drugs, metabolites and lipids in rat brain tissue. Nano-DESI is a recently developed mass spectrometry imaging technique (Roach et al.) that employs localized on-line liquid extraction and enables imaging of molecules on the sample in atmospheric pressure and without any sample pretreatment. The lateral resolution that can be obtained using nano-DESI can be altered between 10 and 150 μm (Laskin et al). Visualization and data treatment, such as principal component analysis (PCA), is made possible by in-house written software specifically aimed at nano-DESI analysis. Nano-DESI has been successfully used for detection of trace amounts of neurotransmitters in the brain along with highly abundant lipids and other brain metabolites. PCA enabled clustering of mass spectral features into classes based on their spatial distribution in the sample.

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## Acknowledgements

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### Figures

Figure 1. The spatial distribution of PC (16:0/18:1) imaged using nano-DESI.

# P28. Technical improvements for the optimization of 3D MALDI imaging mass Spectrometry

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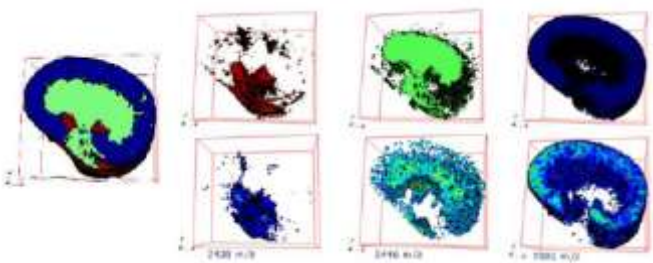
## Abstract

MALDI imaging mass spectrometry (MALDI-IMS) provides information on the distribution of (bio)molecules of tissue sections in a spatially resolved manner. Through registration and stacking of several consecutive slices this information can be extended to the third dimension. This is important for the understanding of the interplay of different cell- and tissue types in their 3D-formation particularly in cancer research and for analyzing the distribution and mode of action of compounds in 3D-drug imaging studies.
3D MALDI-IMS requires measuring tens of serial sections, normally over several days. Unfortunately, so far it is not known whether reproducible results can be obtained for single 2D MALDI imaging datasets. This deficiency is probably due to the lack of adequate statistical methods or limited availability of data to statisticians. Since reproducibility is a major prerequisite for 3D MALDI-IMS in our opinion this questions should be addressed before starting 3D experiments.

Another requirement specific for 3D MALDI-IMS is that for interpretation of results it is essential to correlate the information with other imaging modalities like magnetic resonance imaging and to compare the data with the annotation of classical histological staining results. For the reconstruction of the 3D model it is critical to maintain the integrity of the tissue section. While classical formalin fixation and paraffin embedding procedures are inappropriate for this purpose due to their cross-linking properties, alternative methods have to be applied.

Here we want to address the specific technical requirements which are inherent to 3D MALDI-IMS. Results of a reproducibility study will be presented where we compare and evaluate 2D MALDI imaging datasets from three different laboratories using instruments from the same fabrication. In addition, we introduce a complete and robust 3D MALDI-IMS workflow focusing in particular on the issue of sample preparation and of data collection. The experimental pipeline will be demonstrated using a mouse kidney sample as a model system involving (1) PAXgene fixation and paraffin embedding to allow for sample integrity, (2) MALDI imaging mass spectrometry to analyze the protein distribution, (3) registration of the MALDI imaging data, (4) data analysis using spectral clustering, co-registration with (5) the magnetic resonance image of the sample and (6) images of hematoxylin and eosin stainings of the MALDI measured slices.

**Figures**



# P29. Internal calibrants allow high accuracy peptide matching between MALDI imaging MS and LC-MS/MS

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## Abstract

One of the continuing challenges for MALDI imaging is the unambiguous identification of analytes for which a spatial distribution has been obtained **[1,2]**. This is of particular relevance to tryptic peptide MALDI-TOF/TOF based imaging, where *in situ* MS/MS typically provides less than ideal data quality **[1]**. Furthermore, *m/z* errors introduced by sample height, sample composition and sample specific ionization artefacts preclude confident matching of LC-MS/MS and imaging data.
To address this challenge, the work presented here details the application of a set of internal calibrants for addition to tryptic peptide MALDI imaging experiments on formalin-fixed paraffin-embedded (FFPE) tissue. The introduced calibrant set was able to improve mass error by at least one order of magnitude (to below 10 ppm in some cases), as confirmed by matching of *in situ* MS/MS to LC-MS/MS **[3]**. As a result, tryptic peptides mapped *in situ* by MALDI-TOF/TOF can now be assigned an identity with greater confidence. The work presented supports the inclusion of internal calibrants as standard for initial MALDI imaging experiments seeking to characterize tissue composition.

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# P30. Identification of lipids in synovial tissue by MALDI MS

Mark Kriegsmann[1], Rita Casadonte[1], Jörg Kriegsmann[1], Friederike Zweynert[2], Mike Otto[1], Erin Sheeley[2], Richard Caprioli[2].

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## Abstract

Rheumatoid arthritis (RA) is a common systemic autoimmune disease with a heterogeneous clinical presentation and disease course. The currently available biological tests are not accurate enough, leading often to delayed diagnosis of the disease and irreversible joint damage. New approaches are needed to transcend the limitations of current technologies. Lipidomics is an emerging field in the last years and has attracted growing attention among researchers. Matrix-assisted laser desorption/ionisation mass spectrometry (MALDI MS) has been shown to be a useful tool in the investigation of proteins but has also been used in the investigation of lipid profiles mainly on solid tumor or animal tissue specimens. Recent studies suggested sphingolipids to play a role in the pathogenesis of RA. Furthermore, phospholipid compositions may be altered in serum and synovial fluid from patients with RA. Using MALDI MS we were able to generate a lipid profile from a patient with RA on synovial tissue underlining the power of this technology and its potential for the investigation of biomarkers.

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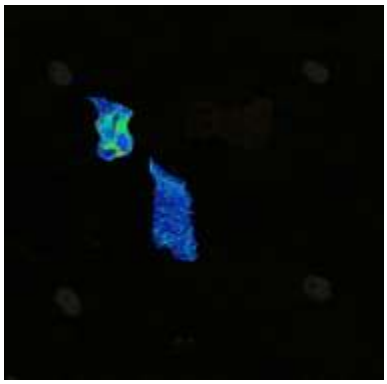
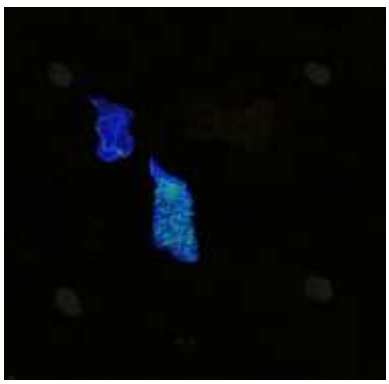
Kriegsmann, M. et al. Scand. J. Rheumatol. 2012 April (in press)

### Figures

Lipid images from synovial membrane sections of patients with RA and OA.





# P31. Identification of proteins in synovial tissue by MALDI MS

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## Abstract

Objective: To identify and image protein biomarkers in the synovial tissue of patients with rheumatoid arthritis (RA) and osteoarthritis (OA).
Methods: MALDI MS imaging technology was applied to the analysis of frozen synovial tissue. Patients were classified according to the ACR criteria for RA. Imaging mass spectrometry was used in a "profiling" mode to detect discrete spots for rapid evaluation of proteomic pattern in various tissue compartments. Data generated were then subjected to computed analysis for biomarker discovery.

Results: Several peaks (m/z) consistent in mass with calgranulins, defensins and thymosins were detected and their distribution in various synovial compartments (synovial lining and sublining layer) were demonstrated.

Conclusion: MALDI MS imaging technologies is a powerful tool for rapid detection of proteins (in situ proteomics) in synovial tissue. Most promising proteins are calgranulins, defensins and thymosins and may play a role in RA physiopathology.

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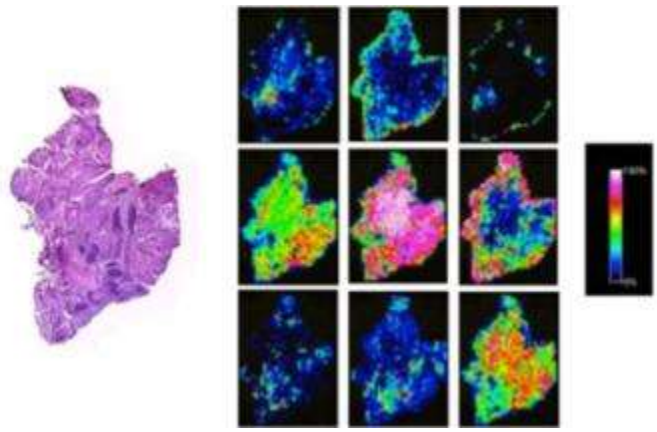
### Figures

H&E-stained section and mass spectral images from a single synovial membrane section of a patient with RA.



# P32. Liquid Extraction Surface Analysis Imaging of in-situ Lipids and Proteins from Human Liver Analysed by High Resolution Mass Spectrometry

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## Abstract

A number of MS ion sources and sample interrogation methods have been introduced for direct tissue analysis and imaging, such as laser desorption, desorption electrospray, primary ion beams, high energy laser ablation and more recently liquid extraction surface analysis (LESA) using the Advion Triversa Nanomate.

In LESA, a liquid micro-junction between a sample surface and conductive pipette tip allows extraction of molecules from the sample. The droplet is retracted into the pipette tip and delivered to the back of the ESI Chip for infusion nano-electrospray high-resolution mass spectrometry and tandem mass spectrometry (MS/MS). Recent reports describe use of LESA for analysis of small and large molecules in complex samples such as dried blood spots [1] and tissue sections [2-4]. Here we present the use of LESA for analysis of lipids and proteins in human liver samples. 0.5μL droplets of 70%$MeOH_{(aq)}$+0.1% Formic Acid were aspirated and a liquid micro-junction between the pipette tip and tissue section was maintained for 5 seconds. The re-aspirated solution was injected using nano-electrospray into a Thermo Fisher Orbitrap mass analyser with a tip voltage of 1.75kV, gas pressure 0.5psi and capillary temperature of 250°C. The process was repeated at defined intervals (1mm) across the tissue. Data for each location was collected for 2 min in the form of a series of 5 co-added microscans. Images were constructed using in-house software. Results demonstrate direct analysis of lipids and proteins from human liver and the opportunities for using this technique to provide spatially resolved maps of multiple analyte classes in tissue.

## Acknowledgements:

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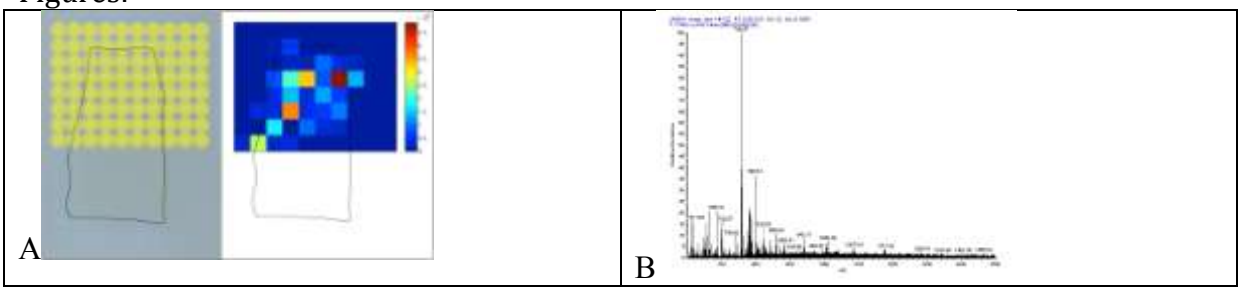
Figures:




A) An example of a selected ion map from imaged tissue. B) A typical mass spectrum from imaged tissue.

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# P33. Mass Spectrometry Images the Lipidome of Breast Tumor Xenograft Tissue

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## Abstract

Lipid distributions in breast tumors are largely unknown due to limitations of lipid imaging techniques. Lipids are crucial components of cellular membranes and play important roles in cancer proliferation and metastasis. Recent developments in MALDI mass spectrometry imaging enabled detection and visualization of lipids directly from thin tissue sections. MS-based imaging does not require any labeling and can simultaneously localize different lipid classes. MSI is also a perfect tool for analysis of complex samples, such as tumor tissue sections, because it can be easily integrated with other imaging modalities such as optical microscopy (1). In this study, we performed a multimodal imaging of lipids from different microenvironments of breast tumor xenograft models. These genetically engineered tumors express a red fluorescent protein inside the hypoxic (low oxygen tension) tissue regions. The MSI molecular lipid images revealed the heterogeneous lipid distributions within tumor tissue. Two of the most common lipid species, namely PC(16:0/16:0) and PC(16:0/18:1) were localized in the viable tumor regions, while PC(18:0/20:1) was detected from the necrotic tumor regions. Secondary ion mass spectrometry imaging of small molecules revealed the presence of phosphocholine signal in the necrotic tumor tissue as well as showed the distributions of elements, salts and cholesterol. The incorporation of MSI combined with ion mobility separation of isobaric lipid species into a multimodal imaging approach revealed tumor tissue complexity at the level not detectable for any other imaging technique.

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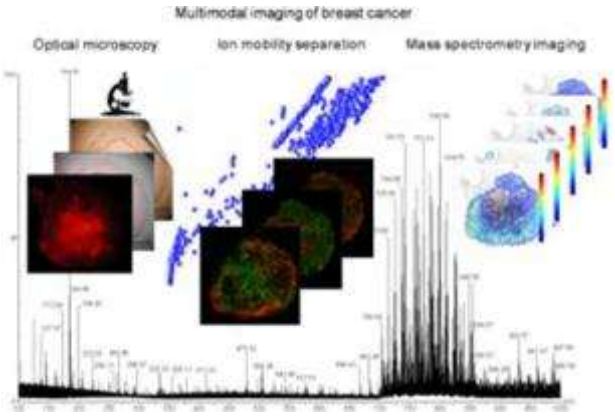
## Acknowledgements

This work is part of the research program of the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)", which is financially supported by the "Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)". We gratefully acknowledge financial support from NIH grant R01 CA134695. The authors also gratefully acknowledge continued support from the Netherlands Proteomics Centre (NPC).

## Figures



# P34. MALDI Imaging of Drug Compounds in Animal Tissues

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**Abstract**

The combination of a MALDI ion source with a time-of-flight mass spectrometer allows investigating small molecules as well as proteins or polymers. This versatility is utilized by Merck KGaA to monitor drug candidate compounds in animal tissues as a function of time by MALDI imaging. We can visualize and evaluate the spatial distribution of those analytes. Correlating these images with H&E stain of the same body section we learn about the substance spreading and its interactions within the tissue.

# P35. MALDI-FTMS Imaging: a powerful tool for imaging plant tissue slices

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## Abstract

Recently MALDI mass spectrometry imaging (MSI) has been a powerful tool to map spatial distribution of molecules on the surface of biological materials. Though MSI is frequently applied to animal tissue slices to map various bioactive molecules on the slice, it's still been difficult to be applied to plant tissue because of the difficulties in sample preparation and the existence of secondary or tertiary metabolites. In general, plant tissue is highly water rich and cell wall exits between plant cells, so special sample preparation technique was required to make thin slice of it. Ultra-high-resolution and ultra-high-accuracy in mass spectrometry are also necessary to identify metabolites contained in plant tissue.

We have developed in-house MALDI MSI ion-source, which was originally developed as a microscopic MALDI ion-source, and it was equipped with Bruker-Daltonics commercial FTICR-MS (Apex-Qe-94T). In our ion-source, small transparent ITO-coated conductive slide glass was inserted into the original ion-funnel horizontally and tightly focused UV-LASER beam was irradiated vertically from upper long working distance Cassegrain mirror objective, while sample specimen can be observed by lower zooming video microscope in real-time. High-precision sample stage was also equipped inside the vacuum chamber and the exact position was able to be controlled by in-house software. All of the MSI experiment sequence, including sample specimen observation with video-microscope, sample stage motion control and triggering MALDI-FTMS run, was also controlled by in-house-build software written with LabView.

We tried to make thin (40 micrometer) slice of quick frozen young leaf of Arabidopsis thaliana by Kawamoto's method [1] and the frozen slice was vacuum dried under freezing temperature followed by matrix application using sublimation [2], before dedicated for MALDI MSI experiments.

Here, we tried to use matrix substance 9-amino-acridine (9-AA), instead of 2,5-dihydroxy-benzoic acid (DHBA), because 9-AA was reported to work as a MALDI matrix of small acidic compounds in negative-ion mode [3], including typical plant hormones such as Auxin. We performed a lot of try-and-error experiments varying thickness of matrix layer on the dried slices, to find optimized experimental parameters to map small molecules, such as plant hormones or acidic compounds contained in vacuole, on the plant tissue slices.

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## Acknowledgements

This work was supported by MEXT(Japan) Grant-in-Aid for Scientific Research, Perceptive Plants.

**Figures**

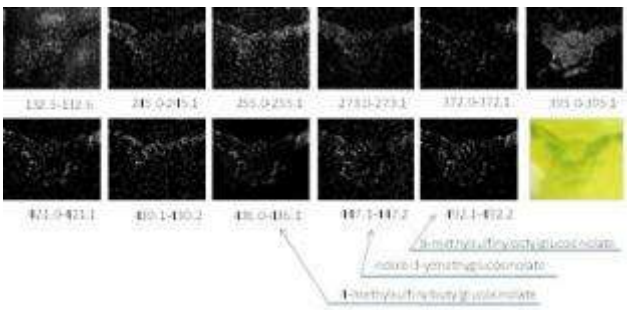

Figure 1: MS images of various ions from thin slice of young leaf of Arabidopsis thaliana.

# P36. A new image of the heart failure

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## Abstract

Background: The prognosis for patients diagnosed with heart failure has significantly improved over the past three decades; however, the disease still confers a high degree of morbidity and mortality. Given that is clear that the information is still lacking as to myocardial substrate and genetic and proteomic profiling that might characterize heart failure [1]. The spatial and temporal aspects of molecular processes ( as myosin α, myosin β , PPARα, cardiac ankyrin repeat protein and calpain) as in cells and tissues play an enormous part in cardiovascular disease. New high-resolution molecular and structural imaging strategies are needed to visualize heart tissue. Objective: The aim of our study is to provide a molecular basis of the human heart failure through the local identification of biomolecules by Mass spectrometry imaging (MSI). Methods: We have investigated an MSI approach using SINAPT on 10 human heart . Human failing left-ventricular free wall heart explants were obtained from the heart transplant collection at Padua University. Sample (left ventricle) from explanted heart (heart failure) were frozen and stored at -80 °C until sectioning. 12 µm thick heart tissue transverse sections were cut using a cryomicrotome and thaw mounted onto an ITO slide. A sample of the left ventricle was further divided into manageable blocks for paraffin embedded. Adjacent (serial) sections were then stained with one of the three following techniques to correlate heart's molecular profile with morphological features: a) *Hematoxylin and Eosin* (H &E) staining. b) Sirius red-collagen-staining for connective tissue. c) Heidenhain's Azan stained for connective tissue. Before MSI analysis, tissue sections were briefly washed by immersion in 70% and 90% ethanol and dried in a vacuum desiccator for 10 min. Trypsin was resuspended in water at a concentration of 0.05 µg/µL, and a 5 nL per spot in a 150 µm × 150 µm raster was deposited by CHIP (Shimadzu, Japan). CHCA matrix was prepared at a concentration of 10 mg/mL in 1:1 ACN:$H_2O$/0.1% TFA and was applied by an ImagePrep (Bruker, Germany) application system. Samples were analyzed on a MALDI-Q-TOF (Synapt HDMS, Waters, U.K.) instrument in time-of-flight (TOF) mode detecting the positive ions [2]. The images were acquired with 150 µm × 150 µm spatial resolution. To reduce the dimensionality of the dataset by the creation of a new set of variables, the principal component analysis (PCA) was used. Preliminary results:Surface rastering of heart tissue sections generated a plethora of ions and peptide with a range of 300-600 m/z, 800-1000 m/z, 1100-1200 m/z, 2400-2500 m/z, 3200-3300 m/z, 3400-3500 m/z . We used Peptide Mass Fingerprinting (PMF) to identify proteins (myosin α, myosin β , PPARα, cardiac ankyrin repeat protein and calpain) by matching their constituent fragment masses (peptide masses) to the theoretical peptide masses generated from a protein database. Using MS/MS, peptide sequencing will be performed to identify protein fragments directly from the tissue section. Conclusions: The identification of proteins and their spatial and temporal aspects of observation may help explain the pathophysiology of heart failure and may suggest new avenues for diagnostic and therapeutic intervention.

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# P37. Studies on progressive proteinuric nephropathy by MALDI imaging on kidney tissues: identification of immunogenic Albumin peptide.

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2- Dept. of Molecular Medicine, Mario Negri Institute for Pharmacological Research Centro Anna Maria Astori, Science and Technology Park Kilometro Rosso, Bergamo, Italy
3- BECS, Dept. of Biomedical Engineering and Computational Science, Aalto University, Helsinki, Finland

## Abstract

**Background:** Previous studies have documented that excess of an autologous albumin is proteolytically cleaved by proximal tubular cells into N- terminal fragments that can be further processed *in vitro* into antigenic peptides, triggering an immune response. The most abundant albumin peptide ($Alb_{1-24}$) was taken up by dendritic cells (DC) and digested by a proteasome-dependent pathway to antigenic peptides that bore binding motifs for MHC class I and activated syngeneic $CD8^+$ T cells. The functional role of $Alb_{1-24}$ processing in inducing immune response was further demonstrated in a rat model of proteinuria secondary to renal mass reduction (RMR) by 5/6 nephrectomy, where T cell activation was prevented by treatment with the proteasome inhibitor bortezomib (JASN 20: 223, 2009). **Aim:** To evaluate the presence and localization of $Alb_{1-24}$ in the kidney of RMR rats by MALDI imaging technology (MALDI-IMS).

**Material and Methods:** Kidney sections from Sprague Dawley rats with RMR (n=3) were studied at 4 weeks after surgery and compared with age-matched sham operated controls (n=3). RMR rats developed high levels of proteinuria as compared to controls (229 ± 64 *vs* 25 ± 5 mg/day). At sacrifice, kidneys were harvested and midcoronal sections frozen in liquid nitrogen. The kidney tissues were cut to a thickness of 10-12 μm and placed on conductive indium tin oxide- coated glass slides (*ITO*, Bruker Daltonics, Germany). A homogenous matrix preparation of sinapinic acid (SA) was applied using *ImagePrep* (Bruker Daltonics™). Matrix-coated slides were imaged on the same day by MALDI-TOF MS using an Autoflex III™ imager mass spectrometer, equipped with Smartbeam™ (Bruker Daltonics, Germany) in linear, positive mode. The lateral resolution for MALDI imaging was set to 200μm for initial tissue screening and 50 μm for more detailed distribution analysis of MS peaks of interest. FlexImaging™ 2.1 software (Bruker Daltonics, Germany) was used for assembling of the spectra and visualization. On average, 60 sections from each kidney region were scanned. Following MALDI-IMS analyses, the matrix was washed off the slides with 70% ethanol and sections were stained with Hematoxylin & Eosin, scanned and co-registered with the MALDI-IMS results.

**Results:** MALDI IMS allowed identification of a peptide with an average mass $[M_{av}+H]^+$ of 2791.0 ± 1 Da, which corresponded well with the predicted $[M_{av}+H]^+$ of $Alb_{1-24}$, in focal areas of the remnant kidneys of RMR rats (n=3), on several consecutively cut tissue sections. Higher density images (50μm x 50μm) from the signal-rich regions and matching regions from hematoxylin-eosin Y, post-analysis stained sections demonstrated a peptide localizing to the cortex, within and in proximity of tubular cells. Due to very low abundance of the endogenous peptide, that prevents its sequence identification we spiked the tissue sections with low amounts ($10^{-4}$μg) of synthetic $Alb_{1-24,}$ in the vicinity of areas where the endogenous signal was detected and rescanned the regions of interest. The detected m/z of the endogenous peptide matched well with the observed and predicted average mass of synthetic $Alb_{1-24}$ peptide (2791.185±1Da).

**Conclusions:** MALDI-IMS can be successfully utilized to identify fast metabolized, low abundant endogenous peptides in the diseased tissues.

# P38. Obtaining lipidomic based distinction and localization of kidney microstructures on ultrathin tissue sections (100 nm to 2 µm)

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## Abstract

The health status from cellular to individual level is often related to lipid-related modifications within the organism. Within the renal microsystem the lipidomic status concerning cholesterol proportion, alkyl chain length and saturation as well as oxidation status provides a huge variety of information relating to pathological modifications. Lipid associated abnormalities are therefore seen as modulators of progressive renal diseases, e.g. lipoprotein accumulations are crucial indicators for glomerular injury or malfunction. Combining Imaging mass spectrometry (IMS) and histopathology represents a comprehensive approach for finding histological, structural and molecular answers to pathological issues on cellular levels.

Formalin-fixed rat kidney tissue, embedded in sucrose, from previous medical studies, stored over the past years, was sliced into tissue sections between 100 nm and 2 µm using cryo-microtomy (Thermo Fisher Scientific), and mounted onto indium-tin oxide (ITO) coated glass slides (Sigma Aldrich). α-cyano-4-hydroxy-cinnamic acid, 2,5-dihydroxy benzoic acid, 2-(4'-hydroxybenzeneazo)benzoic acid and graphene were chosen as matrices. A ChIP-1000 piezo-printer (Shimadzu) was used for matrix application. Imaging experiments were performed by means of a high-vacuum MALDI-TOF/RTOF (Shimadzu Biotech Kratos Analytical) and a Synapt MALDI-q-ion mobility TOF instrument (Waters) in positive and negative ion detection mode. Data were processed using BioMap (3x, Novartis) and MATLAB 7.13 (Mathworks).

Rat kidney microstructure differentiation was investigated by comparingg lipid structure and localization pattern. Pattern comparison of phosphatidylcholine species of different alkyl chain length e.g. 1-tetradecanoyl-2-sn-glycero-3-phosphocholine, 1-hexadecanoyl-2-sn-glycero-3-phosphocholine and 1-octadecanoyl-2-sn-glycero-3-phosphocholine revealed differences in macrostructural distribution between medulla and cortex. Similar localization differences were observed for 1-hexadecanoyl-sn-glycero-3-phosphocholine and 1-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine. Distinctive molecular distribution was further observed for diacylglycerophosphoethanolamine, cholesterol, N-(tetracosanoyl)-sphing-4-enine-1-phosphocholine, 1-O-(1Z-tetradecenyl)-2-(9Z-octadecenoyl)-sn-glycerol and it's oxidized species. 16 lipid species and their modified co-species could be localized and identified within this approach. On cellular level, lipidomic distribution pattern comparison possibly reveals early stage pathological modifications within the glomerular membrane.

The presented data show that the study of long-term stored ultra-thin histopathology samples (fixed and stained) can be feasible opening therefore the door for comprehensive pathological answers by investigating material available in biobanks.

## Acknowledgements

Parts of this project were funded by the Vienna University of Technology (Innovative Projects) and the Federal Ministery for Science and Research (UniInfrastruktur IV).

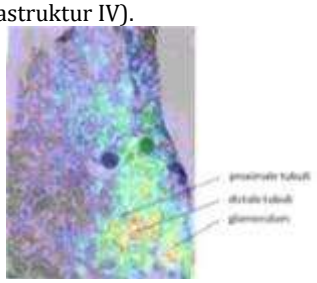

**Figure 1:** Histologically stained (Toluidine) formalin-fixed rat kidney tissue (0.5 µm) embedded in sucrose of approx 2x2 mm size. Overlay: Biomap visualiziation of Cholesterol (m/z 369) intensities. Histologically relevant microstrucutres correlate very nicely with molecular lipid pattern.

# P39. Identifying the differentiation stage of hematopoietic cells from mouse bone marrow by multivariate analysis of TOF-SIMS data

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## Abstract

Identification of the conditions that permit the control of stem cell renewal and differentiation is a major goal of tissue engineering research. Combinatorial biomaterials that exhibit spatial variations in mechanical and biochemical properties have been developed to permit systematically screening the effects of multiple microenvironments on stem cell fate using a minimal number of cells.[1-3] To utilize such combinatorial systems, the differentiation stage of individual cells at specific locations in the culture must be identified. Currently, differentiation stage is most commonly assessed via fluorescence microscopy using cocktails of differentiation stage specific antibodies;[4] but single-cell fluorescence analysis of small cell populations can yield ambiguous results.[5] As a complementary approach, we have combined multivariate analysis with time-of-flight secondary ion mass spectrometry (TOF-SIMS) to identify the differentiation state of individual hematopoietic cells (HCs) isolated from mouse bone marrow. We show that this approach permits identifying the differentiation state of primary HCs from three distinct stages of B cell lymphopoiesis at the single cell level: HSPCs, common lymphoid progenitors, and mature B cells. A partial least squares discriminant analysis (PLS-DA) model was constructed using calibration spectra from primary HCs at three distinct stages of B cell lymphopoiesis: HSPCs, common lymphoid progenitors, and mature B cells. The PLS-DA model was then used to identify the differentiation state of the individual HCs in a separate test set. We found that the PLS-DA predictions were most accurate when the mice from which the cells in the calibration and test sets were harvested were similar in age. This approach to identifying the differentiation stage of individual cells complements the traditional immunolabeling methods and functional assays that are used to identify HC differentiation stage in experimental culture systems.

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# P40. MALDI imaging revealed mitochondrial dysfunction in Barrett´s adenocarcinoma patients as predisposition to response on neoadjuvant chemotherapy.

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## Abstract

In advanced stages of adenocarcinoma of the esophagus, Barrett´s adenocarcinoma, neoadjuvant chemotherapy can improve patient outcome. However, only a subgroup of patients responds to neoadjuvant chemotherapy. So far, there are no reliable biomarkers available that predict therapy response before treatment. Therefore, in the present study, we evaluated in pretreatment tumor biopsies if the response to neoadjuvant chemotherapy can be predicted on a proteomic level.

Pretherapeutic Barrett`s adenocarcinoma biopsies were analyzed by MALDI Imaging Mass Spectrometry. The gained proteomic profiles were correlated with response data of the patients. We revealed 22 m/z species correlating with drug sensitivity in Barrett's adenocarcinoma. Hierarchical clustering showed that this proteomic profile could be used to accurately define drug sensitive from drug resistant patients. By LC-MS/MS, four mitochondrial proteins were identified, showing a reduced expression in drug sensitive patients. The most discriminating protein was COX7A2, a subunit of complex IV of the mitochondrial respiratory. In a validation set of patient samples, immunohistochemistry of COX7A2 confirmed the MALDI Imaging results and revealed the predictive impact of COX7A2. Electron microscopy on patient samples revealed that the loss of COX7A2 is strongly associated with severe mitochondrial alterations. Cell culture experiments showed that mitochondrial damage based on down regulation of COX7A2 predisposes cancer cells to neoadjuvant chemotherapy.

MALDI Imaging patterns could be used to distinguish drug sensitive from drug resistant patients and provided new insights in the mechanisms of response. For the first time we showed that mitochondrial damage is a predisposition for response to neoadjuvant chemotherapy in Barrett's adenocarcinoma and COX7A2 plays a key role in this mechanisms.

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### Acknowledgements

**Grant support:** The authors gratefully acknowledge the financial support of the BMBF (grants no. 01EZ0803, no. 0315508A and no. 01IB10004E) and the Deutsche Forschungsgemeinschaft (SFB 824 TP B1, SFB 824 TP Z2, and WA 1656/3-1).

# P41. Mass Spectrometry Imaging of Tissue Microarrays to Access Breast Cancer Heterogeneity

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## Abstract

The capability of mass spectrometry imaging (MSI) to simultaneously detect numerous different compounds while preserving their spatial distribution makes it well suited to study breast cancer tissues. As breast cancer is a highly heterogeneous disease, it still remains often unclear which molecular mechanisms contribute to the ineffectiveness of certain therapies.

In this study, we used matrix-assisted laser desorption ionization MS imaging and secondary ion mass spectrometry imaging to assess the peptide and small molecule content of formalin-fixed paraffin-embedded (PFFE) breast cancer tissue microarrays (TMAs). The use of TMAs, which typically consist of several hundreds of tissue cores arranged in an array on a glass slide, facilitates high-throughput and multiplex analysis of tumor tissues. An optimized sample preparation protocol, which includes deparaffinization, antigen retrieval and on-tissue tryptic digestion, was employed to unlock the proteome of PFFE tissues for MSI analysis.

Principal component analysis in combination with correlation clustering allows for the identification of tissue cores with correlated mass spectrometric profiles. Using this approach, we were able to detect and visualize breast cancer heterogeneity solely based on the mass spectrometric profiles generated by MSI.
The correlation of MSI data obtained from TMAs with clinical data using statistical analysis opens up possibilities for improved diagnosis, prognosis and the investigation of mechanisms underlying therapy response.

# P42. Use of Matrix-Assisted Laser Desorption Ionization to Investigate the Hypothesis of Protein Radical Acetylation

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## Abstract

Acetyltransferase-catalyzed protein acetylations are key post-translational events of the cell cycle. Recently we raised the hypothesis that protein acetylation may also be promoted by acetyl radical generated during the reaction of peroxynitrite with α-dicarbonyl catabolites such as methylglyoxal and diacetyl. Diacetyl is a food and cigarette electrophilic contaminant and peroxynitrite is a powerful nucleophile originated in organisms by the diffusion-controlled reaction of nitric oxide ($NO^{\bullet}$) with the superoxide ion radical ($O_2^{\bullet-}$). NO synthases are reportedly activated up to $10^3$ resulting in $10^6$ fold increased formation of peroxynitrite in pro-inflammatory conditions such as those implicated in myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders. Aiming at demonstrate the feasibility of radical acetylation of RNAse *in vitro* by the peroxynitrite/diacetyl reaction, we investigated the reaction of RNAse (68.4 μM/L) with diacetyl and peroxynitrite (5.0 mM/L and 1.3 mM/L, respectively) in 200 mM/L phosphate buffer, pH 7.2, room temperature. The spent reaction mixture was submitted to an ultrafiltration device (Vivaspin molecular cut-off of 3000 MWCO), lyophilized, and the product rehydrated with TFA 0.1%. Product analysis was performed with a Reflex MALDI-TOF mass spectrometer (Bruker-Franzen Analytic GmbH) on an alpha-cyano-4-hydroxycinnamic acid matrix. Figure 1 shows the mass spectra patterns of RNase in the absence (A) and presence of either peroxynitrite alone (B), diacetyl alone (C), and the diacetyl/peroxynitrite system (D), generator of acetyl radical. The trace D describes a broad mass increase of RNAse, consistent with previous data obtained with isolated amino acids and L-Lys-containing peptides, showing that the protein acetylation reaction is fast, competitive, and takes place randomly in the nitrogen atoms of several basic amino acid residues such as L-Lys and L-His. The mass increases observed in our model studies with RNAse support the proposal that non-enzymatic, radical acetylation of proteins might contribute to epigenetic protein modifications, under conditions of oxidative stress and carbonyl stress, including aging and metabolic disorders in which oxygen and nitrogen reactive species are putatively involved.

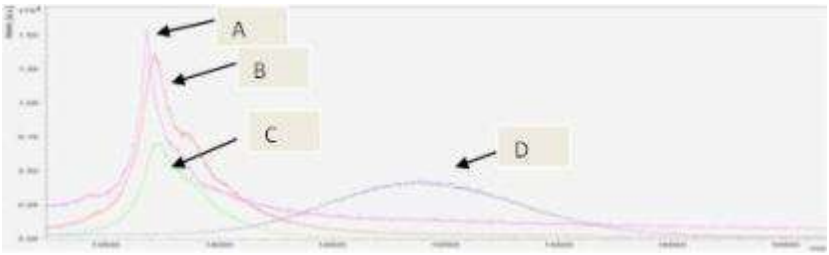


Figure 1- MALDI studies of RNAse acetylation by the diacetyl/peroxynitrite system in phosphate buffer, pH 7.2. RNAse alone (A) and treated with either peroxynitrite (B), diacetyl (C), or both reagents (D).

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# P43. Construction and application of a Time-Of-Flight spectrometer for MeV SIMS

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## Abstract

Cellular biochemistry has been a subject of high interest in biological and medical research in the last decade. By using chemical imaging we get a better understanding of cells biological functions and metabolic processes. Smaller molecules have specific functions and play an important role in regulating intra- and inter-cellular processes. These intracellular processes are usually localized to specific regions of the cell. For chemical imaging of these molecules different analytical imaging techniques have been used. To ensure the intactness of biological tissue and other material, time-of -flight secondary ion mass spectrometry (ToF-SIMS) is one of the methods of choice. It provides both chemical speciation and imaging capability for visualizing biochemistry processes on a sub-cellular level[1].

For this purpose we decided to develop and construct a linear ToF SIMS at Jozef Stefan Institute. This type of SIMS uses same principles as Plasma desorption mass spectrometry (PDMS) in which the high energy focused ion beam desorbs secondary molecular fragments from a solid sample surface. As reported by *Macfarlane and Torgerson* in *1976* the ion beam in MeV energy range can desorb large molecular fragments[2]. By induced electronic sputtering mechanism it is possible to produce molecular images of large molecules (above 1000 u) with submicron lateral resolution[3]. These desorbed molecules are usually the key for understanding the cells chemical and biological processes.

The probing high-energy ion beam is formed in a Cesium sputtering ion source and accelerated in the tandem accelerator of Jožef Stefan Institute (JSI). The beam is focused by magnetic quadrupole triplet lens to the dimension of 1μm[2]. The ion beam of these dimensions enables the analysis of the selected areas of the tissue, including intercellular details. In this way, the the MeV SIMS spectrometer under construction is expected to provide important data to understand biomolecular processes in living oranisms. Raster-scanning of the beam over the sample results in two-dimensional molecular distribution maps, which will be combined with the quantitative elemental maps of the tissue obtained by micro-PIXE technique[4].

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# P44. **The Application of MALDI IMS: Making a difference in advancing drug development**

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**Abstract**

Imaging mass spectrometry (IMS) has emerged as a powerful technology for examining the distribution of drugs and metabolites directly from tissue. The ability to differentiate parent and metabolites in the same experiment without the need for labeling sets it apart from other methods. By combining high mass resolution with high spatial resolution in an IMS experiment it is possible to accurately correlate ion images for drugs and metabolites with the underlying tissue histology.

We will present data that demonstrates the ability of IMS to link chemistry and biology and provide examples of how this is permitting us to closely examine the basis of drug toxicity and pharmacology and refine our understanding of pharmacokinetics and drug transport. Examples in this presentation will include the distribution analysis of a drug and its metabolites in liver tissue and the correlation with observed hepatotoxicity, assessment of the PK/PD relationship for a drug and key metabolites in tissues as well as the comparative CNS penetration and metabolism of a compound and its metabolites in different preclinical models.

# P45. The use of internal calibration and new software tools to enhance Peptide MALDI imaging MS and identification via correlation with LC-MS/MS data.

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## Abstract

One of the important challenges for MALDI imaging mass spectrometry (MALDI-IMS) is the unambiguous identification of measured analytes. One way to do this is to match tryptic peptide MALDI-IMS *m/z* values with LC-MS/MS identified *m/z* values. Matching using current MALDI-TOF/TOF MS instruments is difficult due to the variability of *in situ* time-of-flight (TOF) *m/z* measurements. This variability is currently addressed using external calibration, which limits achievable mass accuracy for MALDI-IMS and makes it difficult to match this data to downstream LC-MS/MS results. To overcome this challenge, the work presented here details a method for internally calibrating peptide MALDI-IMS datasets including the use of new software tools to present and check the data in a more convenient way and enhance the ability to calibrate large datasets. By calibrating all spectra to internal peak features the *m/z* error for matches made between MALDI-IMS *m/z* values and LC-MS/MS identified peptide *m/z* values was significantly reduced. This improvement was confirmed by follow up matching of LC-MS/MS spectra to *in situ* MS/MS spectra from the same *m/z* peak features. The sum of the data presented here indicates that internal calibrants should be a standard component of tryptic peptide MALDI-IMS experiments.

# P46. Complimentary High Mass Resolution measurements applied todrug distribution analysis in tissue

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## Abstract

MALDI MS Imaging has the potential to provide a better understanding of the influence of drug disposition on respiratory pharmacology and toxicology findings, and to aid in the design of the next generation of inhaled molecules.

In this study sequential Ultra High Mass Resolution MALDI MS Imaging and Liquid Extraction Surface Analysis (LESA) nanospray MS measurements on a 12T Fourier Transform ion cyclotron resonance (FT-ICR) mass spectrometer have successfully visualised drug distribution in lung tissue.

With a mass resolving power of typically >300K, the FT ICR mass spectrometer has the ability to resolve drug (and metabolites) from endogenous compounds in the tissue with the same nominal mass.

Sequential Ultra High Mass Resolution MALDI MS Imaging and Liquid Extraction Surface Analysis (LESA) nanospray MS on a FT-ICR, coupled with histology have been performed on rat lung sections to determine the distribution of a pharmaceutical compound following dry powder administration.

Excised lungs from rats dosed with the compound, 3-{8-(2,6-difluorophenyl)-2-[(1H-imidazol-2-ylmethyl)amino]-7-oxo-7,8-dihydropyrido[2,3-D]pyrimidin-4-yl}-4-methyl-N-2-thiazolylbenzamide

were collected at selected time points and frozen. 16um thick sections were prepared and thaw mounted onto indium tin oxide (ITO) coated glass slides and analysed by MALDI MS Imaging. Consecutive sections were prepared on conventional glass slides and used for LESA-MS analysis. H&E staining was performed on the sections used in MALDI MS Imaging.

Conventionally the only assessment of compound measurement for respiratory targets has been the generation of total lung concentration in tissue homogenate but this data provides no distribution information within the lung. MALDI MS Imaging has the potential to not only elucidate the deposition and distribution within the lung but to also discriminate between compound distribution in the airways and surrounding tissue. In addition, histology can also be incorporated to correctly assign the distribution of the compound.

To assess the utility of the technique MALDI MS Imaging data was acquired from rat lungs collected after dosing with a dry powder administration of the compound of interest. The data obtained showed that the compound was distributed throughout the lung.

To further evaluate if the variation in signal intensity across the tissue reflects the drug distribution rather than the effects of ion suppression, LESA nanospray MS experiments were performed on the same FT ICR MS instrument. As the instrument is fitted with a dual source operating in ESI and MALDI mode, a single platform could be used to switch ionisation modes rapidly and analyse the consecutive lung section.

**"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."**

# P47. Innovative bionformatic tools for the analysis of MS based imaging dataset in plant metabolomics

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## Abstract

The investigation of the spatial distribution of metabolites and bioactive compounds in tissues is an asset to increase our understanding of metabolic and biological processes occurring in plants. In the specific case of fruits this can have important technological, nutritional and economical implications.

MS based techniques represent an excellent tool to study the distribution of small molecules in tissue, but in view of possible high-throughput applications it is necessary to develop innovative bioinformatic tools for data analysis and interpretation.

Among the different critical aspects, metabolite identification is particularly challenging because with direct ionization techniques it has to be based only on (high resolution) mass to-charge ratios. Single mass-to-charge values are not sufficient for chemical identification, but the co-localization of characteristic molecular fragments can be used to overcome such limitation. Image segmentation and signal clustering is another promising research field in view of an automatic mining of MS imaging datasets.

In this communication we will present how advanced image analysis tools can be used to to increase the selectivity of MS imaging experiments [1] and to visualize the asymmetric distribution of relevant metabolites in Golden Delicious apples.

Preliminary results on the automatic segmentation of DESI and MALDI datasets will be also discussed.

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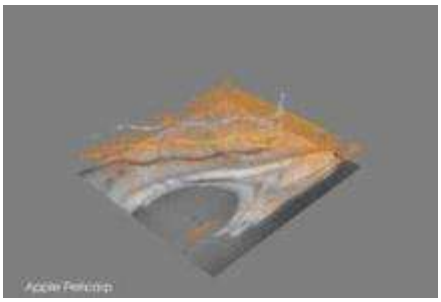

# P48. Mapping the Lipid composition in mouse brain by micro extraction analyses

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## Abstract

Lipids can perform structural and functional roles within the body and are known to be important mediators of cell signaling. One of the most lipid-rich organs is the brain, and lipids account for approximately 50% dry weight. Deregulation in lipid metabolism in the brain is commonly associated with many disorders and diseases. Mass Spectrometry Imaging techniques permit the direct scanning of tissue slices without losing the precise anatomical localization, which is of importance to understand the physiopathology of the different lipid species in central nervous system. Here we demonstrate the analysis of mouse brain tissue by Liquid Extraction Surface Analyses (LESA) using a LTQ Orbitrap XL mass spectrometer equipped with a TriVersa Nanomate ion source.

Lipids were directly extracted with 0.5ul Chloroform/Methanol/Isopropanol (1/2/4), 1mM Ammonium Acetate spiked with Lipid standards, and infused at a flow rate of 40nL/min. MS Spectra were acquired by multiple overlapping segment range acquisition (200-550,500-1000,950-2000m/z) with a target mass resolution of R = 100,000 at m/z 400 in both polarity modes. MSMS fragmentation was performed by data-dependent selection of the 10 most intense peaks in the segment range using dynamic exclusion. The total acquisition cycle was 8min, where each extract was acquired for 3.75min in positive Ion mode and 3.75min in negative Ion mode. Different regions of mice brain hippocampus like the dentate gyrus, the CA1 region or the layers 2/3 of the entorhinal cortex where analyzed and compose of mainly saturated and mono unsaturated Phosphatidylcholines (PC) and Phosphoethanolamines (PE). Diacylglycerols (DAG), Phosphatidylserines (PS) and Phosphatidylinositol (PI) were detected in the negative Ion mode. Spingolipids like Ceramides (Cer), Hexocylceramides and Sulfatide species could also be monitored as well as Phosphatidylinositol phosphate (PIP), which plays an important role in lipid and cell signaling and membrane trafficking. We currently working on optimizing further the extraction efficiency using multiple step lipid micro extraction for Lipid class specific extraction based on their polarity including also Glycolipids like Gangliosides. Furthermore we are applying this approach to profile lipid species from a complete mouse brain from several horizontally sliced mouse brain tissues to be able to create and reconstruct a 3 dimensional brain image based on Lipid analyte signals.

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## Figures

# P49.Matrix Additives for enhanced Imaging of lipids by MALDI-MSI

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## Abstract

Lipids are readily detected as $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ in positive ion MALDI-MS experiments. Additives can be used to promote formation of a particular adduct, improve sensitivity, reduce spectral complexity and/or enhance structural characterization in CID (*MS/MS*) experiments. In situ analysis and imaging gives rise to dominant $[M+K]^+$ in fresh tissue and $[M+Na]^+$ in saline fixed systems. We have evaluated salts of $Li^+$, $Na^+$, $K^+$, $Cs^+$ and $NH_4^+$ as acetates, chlorides and nitrates (5-80mM) in MALDI-MS and *MS/MS* of lipids for dried droplet analysis[1]. Suitable systems identified were then studied in imaging. The importance of additive counter anion choice and concentration is demonstrated and nitrates are presented as particularly useful additives for lipid analysis of complex lipid samples. We show the use of select additives in MALDI-MSI with the aim of utilising additives for improved sensitivity and/or structural characterisation. A number of additives have been considered for imaging previously[2, 3]. Lithium adducts have been shown to be particularly useful for characterisation[2, 3]. We consider the use of lithium nitrate in imaging.

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### Figures

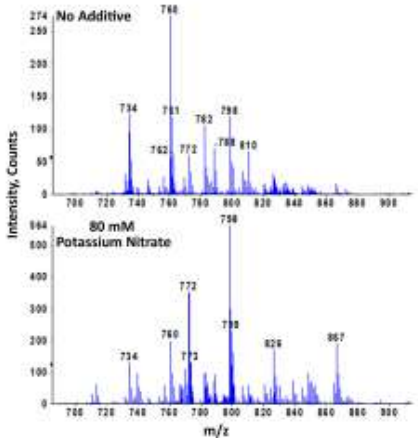


**Figure 1**. Adducts detected and improved ion counts afforded by addition of potassium nitrate additive to matrix in complex lipid extract analysis. $[M+H]^+$ *m/z* 734, 760, 782. $[M+Na]^+$ *m/z* 756, 782, 810. $[M+K]^+$ *m/z* 772, 798, 826. PC 32:0, 34:1 and 36:1 respectively.

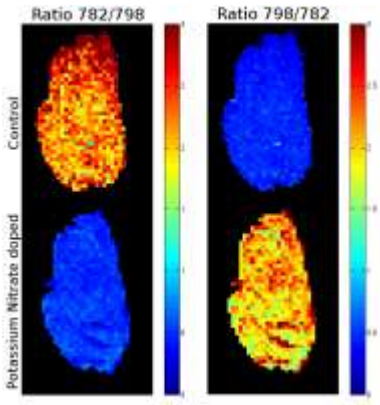


**Figure 2**. Ratio of sodium (*m/z* 782) and potassium (*m/z* 798) adduct ion counts of PC 34:1 in control saline fixed mouse brain tissue (CHCA matrix) and potassium nitrate (CHCA+40mM $KNO_3$) images.

# P50. Molecular Phenotyping of Migraine Using Imaging Mass Spectrometry

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## Abstract

Migraine is a paroxysmal brain disorder characterized by disabling attacks of headache and associated neurological symptoms. The migraine aura, which occurs in one-third of migraine patients and can lead to migraine headaches, is caused by cortical spreading depression (CSD), a self-propagating wave of neuronal and glial cell depolarization in the cerebral cortex.

Previous studies have established a number of metabolites, peptides and proteins that are associated with CSD, many of which are detectable by MALDI-MS. CSD is a transient disturbance that does not lead to detectable histological change. The ability of imaging MS to detect changes in a tissue's molecular composition, prior to or even without histological change, makes it a powerful method for determining the molecular changes following CSD.

Experimental and clinical evidence strongly suggests that CSD, when induced in one hemisphere, does not cross to the other hemisphere. This suggests that the non CSD side of the brain can be used as an internal control. 2D imaging MS datasets of metabolites, peptides and proteins have been recorded as proof of concept. The metabolite and peptide datasets revealed molecular signatures associated with CSD induction, similar changes were not observed in the protein datasets. The peptide datasets in particular revealed multiple peptides at increased levels at the CSD site, as well as others displaying wave like structures spreading away from the initiation site.
A 3D imaging MS analysis of peptides and proteins have been completed. The 3D imaging MS dataset of CSD clearly shows increased levels and, and spatial extent, of specific neuropeptides. The 3D datasets have also been analyzed with non-negative matrix factorization, to summarize the biomolecular changes associated with CSD and thereby extract its spatio-chemical signature.

### Acknowledgements

This work is financially supported by the ICT consortium COMMIT project 'e-biobanking with Imaging' and the Cyttron II project 'Imaging Mass Spectrometry.' RJC gratefully acknowledges Marie Curie Intra European Fellowship project 'ENIGMAS'.

# P51. MALDI imaging mass spectrometry for the analysis of formalin-fixed paraffin-embedded tissue (FFPE)

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## Abstract

The use of MALDI imaging mass spectrometry (IMS) is typically limited to fresh frozen tissue specimen. However there is substantial interest in the possibility to investigate FFPE specimens that have been archived together with the clinicopathological information over many decades. In terms of clinical applicability it is important to determine how effective the approach will be in diagnosing FFPE biopsies sample.We have used IMS to directly analyze protein from FFPE sections by adapting the heat-induced antigen retrieval method and following it with enzymatic hydrolysis.We have employed a vibrational vaporization device that allowed trypsin and matrix deposition onto tissue section under controlled condition, producing high-quality MALDI mass spectra and high-spatial-resolution ion images. We have first investigated the reproducibility of the method by comparing the peak distributions of 5 different biopsies that were taken from the same patient. We than performed an on-tissue digestion experiment on a fresh-frozen tissue and on a FFPE tissue taken from a patient affected by synovitis, to determine the comparability of these two types of samples. The resulting MS spectra showed remarkably similar peptide profiles between fresh-frozen and FFPE tissues. Our results highlight the use of this technology for the rapid detection of protein on FFPE samples with high accuracy and reproducibility, enhancing the ability to simultaneously identify and visualize biomolecules in tissue sections. Our approach will be of significant benefit to researcher and clinicians for diagnostic and prognostic purposes.

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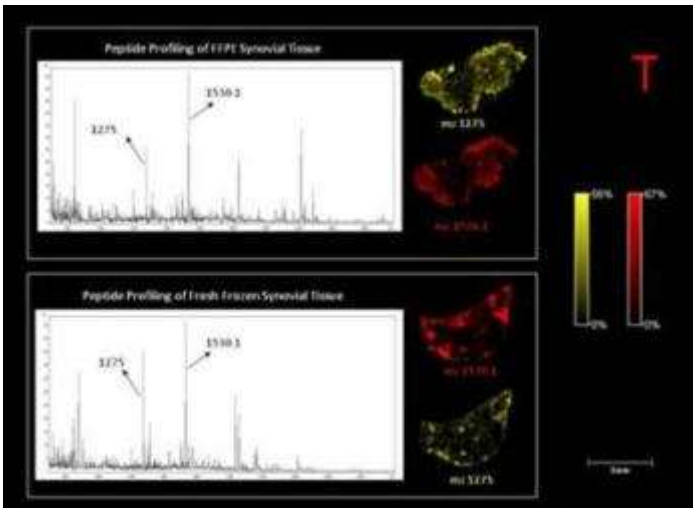
**Figures**

Maldi imaging of FFPE tissue generates peptide profiling similar to fresh frozen tissue.



# P52. Combining Imaging Mass Spectrometry and Cell Membrane Microarrays for Drug Screening

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## Abstract

Testing the affinity of drug candidates for different receptors is an important task during the drug-discovery process. Usually, it is performed using analysis of radioligand binding sites in both cell membranes homogenates and tissue slices by autoradiography. However, in addition to the inherent problems to the handling of radioisotopes, the labeling process itself may alter the original pharmacodynamic properties of the drug candidate (Kd and Bmax). In the present work we explore an alternative, quick and cost-effective procedure based on the combination of cell membrane microarrays and imaging mass spectrometry. The arrays are of the size of a microscope slide and can contain up to 512 microspots of up to 1 mm diameter, of cell membrane homogenates isolated from different types of tissue or genetically-modified cells to e.g. over express a specific neurotransmitter receptor. The arrays are incubated with the unlabelled drug following the standard autoradiographic procedures and covered with a suitable matrix preventing analyte migration, using either a sublimator or a hand sprayer. Then, they are introduced in the spectrometer (an LTQ Orbitrap XL, Thermo Fisher) where they are scanned at spatial resolutions of 100-150 □m, using the Orbitrap option and using an spectral window of no more than 150 Da. In this way the distribution of the drug over the array can be obtained. Such results are compared with those observed using the same radiolabelled compounds by radioligand autoradiography.

### Acknowledgements

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# P53. Repeat MALDI analysis of tissue sections provides additional information.

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## Abstract

MALDI-MS is an extremely powerful, label free technique for detection and imaging of the distribution of small molecules in tissue. One advantage of MALDI is the ability to detect a wide range of species without specific labelling. However, it has been shown that different matrices promote the ionization of different biomolecules which argueably limits its power as a truly label free technique. To help address this issue binary matrix systems have been suggested and has recently shown some promise.[1] It has previously been shown that a single tissue section may be analysed multiple times and still provide useful biochemical data.[2] In this study we investigate the information that can be obtained from a single tissue section undergoing MALDI analysis using sequential matrix coatings and whether sensitivity can be improved by aggregation of data from the repeat analysis of a single tissue section.

Initial studies were carried out on sagittal rat brain sections airspray coated with matrix. Repeat analysis data sets acquired using MALDI-QqTOF (QSTAR XL, Applied Biosystems) with matrix having been reapplied between each raster image collection. This study was conducted first using α-CHCA (15 mgmL$^{-1}$ in 80 % $CH_3OH$ / 0.1 % TFA) as the matrix for positive ion mode analysis.
Repeat analysis of a single tissue section after re-coating with the same matrix showed that high quality data could be collected on each occasion. The opportunity for improved sensitivity through the co-registration and aggregation of spectra from the same tissue position after repeat analysis is demonstrated for selected lipids.

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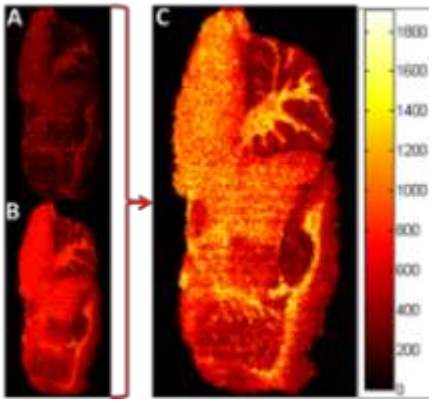


**Figure 1.** Ion images of *m/z* = 826.6 from reanalysis of single tissue section with reapplication of CHCA prior to second analysis. Potential for increased sensitivity is demonstrated by combining ion data from both acquisitions. Acquisition one – A; acquisition two – B; sum of acquisitions -

# P54. Quantification strategy including internal standardization for elemental imaging of biological tissues using LA-ICP-MS

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## Abstract

The use of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) for imaging of biological tissues offers a wide range of applications including cancer research, medical research and proteomics [1]. LA-ICP-MS is utilized as tool to study the distribution of metal based chemotherapeutics in tissues and to monitor disease-induced alterations of metals in inner organs due to its outstanding capability of spatial resolution power down to the micrometer scale. Quantification of trace elements in biological tissues by LA-ICP-MS is a crucial step as it requires the combination of appropriate calibration standards and internal standardization. As the availability of suitable certified reference materials for the analyte and matrix of interest is often limited, the use of matrix-matched calibration standards serves as alternative. A uniform approach for internal standardization has not been established yet. The isotope $^{13}C$ offers its use as internal standard due to its presence in biological samples but poses problems concerning its significantly different atomic mass and first ionization potential compared to many analytes [2].
In this study homogenized standards of tissues spiked with different amounts of trace elements are used for calibration. The concentration of the matrix matched standards is validated with microwave assisted acid digestion. Homogeneity of the standards is tested using LA-ICP-MS. In order to correct for matrix effects, variations during the ablation process and instrumental drift glass slides are coated with internal standard.

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### Acknowledgements

The authors acknowledge the Austrian Science Fund (FWF, project I496-B11) and the Johanna Mahlke geb. Obermann Foundation for financial support.

# P55. Imaging Mass Spectrometry Analysis of Soft Tissue Sarcomas

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## Abstract

Soft tissue sarcomas are a relatively rare and heterogeneous group found difficult by pathologist because of their rarity, and the fact that >50 entities are described with overlapping morphology. Distinction is crucial since they differ in clinical behaviour and outcome and require different treatment. Sarcomas can be subdivided in two main categories; sarcomas that are caused by a specific translocation or a specific gene mutation, and sarcomas that are characterized by complex karyotypes reflecting genetic instability.

High grade leiomyosarcoma (LMS), high grade osteosarcoma (OS), high grade myxofibrosarcoma (MFS) and pleomorphic rhabdomyosarcoma (PRMS) are the complex-karyotype soft-tissue sarcomas most frequently encountered in the clinic and are challenging because of their moderate response to chemo- and radiotherapy. By focusing on a group of high grade sarcomas that still contain a minimal amount of differentiation we intend to identify proteins that could serve as diagnostic tools as well, ultimately, as targets for treatment.
We have collected a large number of high grade LMS (46), MFS (66), PMRS (2) and OS (30) tumor tissue samples, reviewed the histology and constructed a patient series. MDM2 and CDK4 staining were performed to exclude dedifferentiated liposarcoma. Muscle markers were used to establish myogenic differentiation.

Peptide and protein imaging MS data acquisition is currently underway. Here we will report our initial findings, and compare the results of a histology-defined biomarker discovery data analysis strategy with an untargeted imaging MS based molecular histology analysis. The latter is essential as previous investigations have revealed substantial intratumor heterogeneity in these tumors [1, 2].

### Acknowledgements

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# P56. Discrimination of Overlapped Latent Fingermarks by MALDI MS Imaging

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## Abstract

Latent fingerprint image comparison and match in the Police Database is the most common forensic practice for suspect identification. However, there is a number of scenarios in which the retrieval of images from crime scene fingermarks is particularly challenging to the extent of preventing suspect identification; overlapping fingermarks can be particularly problematic as there might be an insufficient ridge detail for personal identification.

Fingermark residue can be of both endogenous and exogenous nature. In the former case these species are naturally present and include, amongst other, lipids, amino acids, peptides and proteins; in the latter case these species are present as "contaminants" upon fingertip contact and may include a variety of substances of forensic interest which may provide important investigative leads. Altogether, these components are hugely variable between individuals depending on their lifestyle and on what they have come into contact with.

MALDI MS Imaging has recently been used to show that fingermarks' ridge detail and chemical information of both endogenous and exogenous nature can be obtained simultaneously[1,2,3]. Therefore, the ability to generate molecular images of specific components which are differentially present in overlapping fingermarks can allow their separation thus enhancing the chances of suspect identification.

A wide scenario of overlapping fingermarks including groomed, ungroomed and artificially/accidentally contaminated marks was investigated with and without the application of multivariate analysis. In all of the cases, MALDI mass images were produced allowing overlapping fingermarks to be separated thus becoming viable to be used for interrogation of the Police Database (Fig 1). Molecular markers enabling separation were both of endogenous and exogenous nature.

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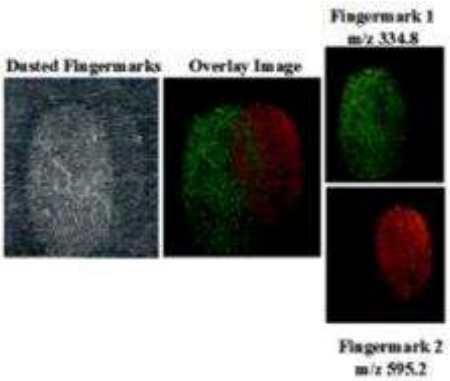
Figure 1. MALDI MS Imaging separation of overlapping fingermarks



# P57. Correlating UHMW-PE cross-linking with biomolecular induced material modification by means of MALDI-RTOF imaging mass spectrometry

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## Abstract

Ultra-high molecular weight polyethylene (UHMW-PE) is one of the most common materials used for artificial acetabulum replacement. Despite the considerable benefits of high biocompatibility, low friction coefficient and high wear resistance, patients often suffer from implant failure. Oxidation, known as one of the primary sources inducing the material aging process and further leading to implant loosening and infections, might be enhanced by biomolecular adsorption. Lubrication properties and oxidative characteristics of synovial components (proteins, lipids or polysaccharides) can be related to early implant failure. Recent developments show, that the polymers' degree of crosslinking is related to mechanical properties. Consequently, understanding implant failure mechanisms on the molecular level requires identification of adsorbed biomolecules and correlation with material modifications. MALDI imaging mass spectrometry (IMS) enables two-dimensional localization and identification of those components.
UHMW-PE samples (cross-linked, non cross-linked) were sliced into 15 µm sections using cryo-microtomy (Reichert-Jung), mounted with conductive tape (Shimadzu Biotech Kratos Analytical) on indium-tin oxide (ITO) glass slides (Sigma Aldrich) and incubated in bovine synovia for 1 h, 3 and 7 d. Common MALDI matrices (α-cyano-4-hydroxy-cinnamic acid, sinapinic acid, 2,5-dihydroxy benzoic acid and graphene) were chosen for IMS. Matrix and trypsin application were carried out by a ChIP-1000 piezo-printer (Shimadzu Biotech Kratos Analytical). IMS experiments were carried out on an AXIMA TOF$^2$ instrument (Shimadzu Biotech Kratos Analytical, UK), using a 20Hz nitrogen laser (337nm) and on an Synapt MALDI-q-ion mobility TOF instrument (Waters, UK), using a 200Hz Nd-YAG laser (355nm). Biomap (3x, Novartis) and MATLAB 7.13 (Mathworks) were used for investigating analyte distribution and performing statistical cluster analysis.
Within the simulation model it was possible to adsorb synovia related compound followed by IMS-based localization and identification. Major lubrication related phospholipid species, e.g. 1,2-dipalmitoyl-glycerophosphocholine, as well as cholesterol, squalene and glycosylceramides have been identified. Besides fibrinopeptide A fragments, t-cell receptor related antigens and IgG chain fragments several peptide species could be identified and localized. Hydroperoxide, the most relevant material-inherent degradation marker, was co-localized with adsorbed synovia compounds. Cross-linking of UHMW-PE proved to influence adsorption patterns and compound adsorption preference as well as diffusion dynamics. The *in vitro* simulation model was validated with results from explant material investigated after *in vivo* application (Fig. 1 and 2). Analytes found in the in vitro experiments were also detected on those samples confirming the hypothesis that biomolecules interact with the smooth UHMW-PE surface and are capable of inducing material failure.

### Acknowledgements

Parts of this project were funded by the Federal Ministery for Transport, Innovation and Technology (FemTech), the Vienna University of Technology (Innovative Projects) and the Federal Ministery for Science and Research (UniInfrastruktur IV).

**Figure 1:** UHMW-PE hip joint explant
after revision; thin slices including the contact layer edge are sampled and investigated by IMS;

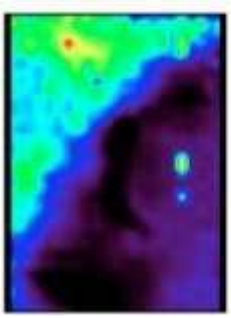

**Figure 2:** Adsorbed and diffused compounds, e.g. cholesterol, are identified and localized by Biomap based visualization representing the ion intensity values on 2-dimensional maps

# P58. Direct Analysis of Tissue Sections by Liquid Extraction Surface Analysis –Differential Ion Mobility Mass Spectrometry

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## Abstract

Direct surface analysis such as liquid extraction surface analysis (LESA) is particularly attractive to detect drugs and metabolites in tissues by maintaining spatial integrity of the sample. However this approach suffers from the lack of chromatographic separation prior to MS detection, preventing the distinction of isomers. Differential ion mobility spectrometry (DMS) is a powerful tool to detect and resolve isomers in the gas phase, based on their difference in ionic mobility at low and high electrical field when a varying separation field waveform is applied [1,2]. Specific compensation voltages (CoV) are required to allow transmission of ions toward the mass analyzer. The addition of organic modifiers in the transport gas significantly enhances its separation power [3,4]. The simultaneous generation of qualitative and quantitative (QUAL/QUAN) data is of prime importance in drug discovery and toxicology. To illustrate, QUAL/QUAN determination was performed on a kidney sample previously submitted to immunoassays analysis representative of positive case. Cocaine (COC) and its isobaric metabolites (i.e. benzoylecgonine BZE and norcocaine NCOC) could be clearly separated by DMS by applying a compensation voltage ramp (from -35 to -10V) and using acetone as organic modifier (Fig.1). The confirmation of BZE and NCOC was performed at specific CoV in MS/MS and MS3 acquisition modes from infusion of the liquid extract (Fig.2).

In addition, the applicability of LESA-DMS-SRM/MS for quantitative analysis has been investigated using various approaches. The system was found to be linear over three orders of magnitude but the main challenge for surface analysis is the preparation of the calibration standards. The analytes were spotted onto blank tissue sections at increasing concentration. The internal d3-standards were either spotted (at a fixed concentration) or added in the extraction solvent. The first approach was found to provide better results in terms of precision (0.2% - 11.0%, n=3) and accuracy (95.9% - 110.1%, n=3) for COC within the linearity range of 5 to 1’000 ng/mL. Further investigations are on-going to determine matrix effects and recovery using different extraction solvent compositions.

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# P59. Molecular characterization of the stromal compartment of triple negative breast cancers

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## Abstract

Triple negative breast cancers are breast cancers that lack expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). These tumors have a relatively poor prognosis and no targeted therapy. Previous reports from our group have shown that the amount of stroma in primary tumors is an independent prognostic factor in breast cancer and other solid tumors (1). This effect was strongest in triple-negative breast cancers, indicating that stromal expansion is an important biological phenomenon for this type of tumor. Additionally, the recent discovery of caveolin as a strong stromal-derived biomarker has increased interest in the metabolic relationship between the epithelial and stromal compartment of breast tumors(2). Down regulation of caveolin in the stromal compartment is thought to be influenced by the tumor cells and lead to stromal aerobic glycolysis (3).

Several markers for high risk stroma have been previously identified with immunohistochemistry (2,4,5). Immunohistochemistry is most often used to confirm a hypothesis because of the need for prior knowledge and the availability of highly specific antibodies. Furthermore similar agents are unavailable for tracing the distributions of specific lipids and metabolites. Imaging mass spectrometry is an emerging, but rapidly developing, technique in clinical biomolecular analysis that can simultaneously measure the distributions of hundreds of distinct biomolecular ion. Using essentially the same technique but different tissue preparation strategies imaging MS can be used to analyze peptides, proteins, lipids and metabolites. When combined with pathohistological analysis mass spectral profiles of specific cell types, including stroma and tumor interface zones, can be extracted and biomarkers identified.

We have started an imaging MS analysis to assess the molecular characterization of the stromal compartment of triple-negative breast cancers. Frozen sections of high-stromal and low-stromal invasive breast cancers have been analyzed for protein (26 patient tissues) and metabolite (14 patient tissues) content. Candidate MS peaks that are differentially expressed in high-risk stroma compared to low risk stroma have been highlighted by supervised and unsupervised data analysis. The tissue series is currently being increased in size to improve the statistical power of the analysis. Candidate biomarkers will then be identified and, if a suitable antibody is available, independently validated by immunohistochemistry. When completed, these research questions may lead to molecular markers for high-risk stroma (associated with disease relapse) for triple negative breast cancer.

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# P60. Mass spectrometry imaging for visualization of the green tea polyphenol EGCG in mammalian tissue micro-regions

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## Abstract

Epigallocatechin-3-*O*-gallate (EGCG), the major active polyphenol in green tea (*Camellia sinensis* L.), has been shown to possess various health promotion effects. Although the understanding of spatiotemporal distribution in tissues is indispensable for elucidating the detailed mechanism of EGCG action, such information still remains unclear. Mass spectrometry imaging (MSI) is a remarkable new technology that enables us to determine the distribution of endogenous or exogenous molecules present in tissue sections by direct ionization and detection with matrix-assisted laser desorption ionization (MALDI)-MS. Recently, we have developed the highly sensitive MSI technique for visualizing of spatiotemporal dynamics of the tissue metabolome[1]. In this study, we attempted to develop a MALDI-MSI technique for visualizing two-dimensional distribution of EGCG in tissue micro-regions.

We have recently reported that a MALDI-MS system with 9-aminoacridine (9-AA) achieved a great improvement for the sensitivity of low-molecular-weight metabolite analysis that is advantageous for the simultaneous detection of a variety of cellular and tissue metabolites[1-3]. Here we applied this technique for imaging the major green tea polyphenol EGCG with higher sensitivity. First of all, more than 40 kinds of chemical compounds, including 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), and 9-AA, were examined for screening the potential matrix against EGCG. Spots of mixed solutions of these chemicals and EGCG were measured by MALDI-TOF-MS in both positive and negative ionization modes. An EGCG peak was not observed by the major matrix (DHB, CHCA, SA, and 9-AA). On the other hand, β-carboline derivatives, CHCA analogues, and naphthalene derivatives were able to detect EGCG with higher sensitivity (femtomole order) in negative ionization mode. Almost all matrices capable of detecting polyphenols as previously reported had no ability to detect EGCG with the same sensitivity. To examine whether these chemicals can visualize EGCG on the tissue section, we were undertaken to detect an image of EGCG spotted on the normal mouse liver section. Although MSI experiment revealed that a peak intensity of EGCG was greatly reduced as compared to the case of the screening test on a stainless MALDI sample plate, two-dimensional image of EGCG spot was obtained with signal-to-noise ratio of more than 100.

These results suggest that our selected chemicals act as a matrix against EGCG on both the sample plate and the tissue section. This finding is the first report showing that MALDI-MSI technique enables to visualize two-dimensional distribution of EGCG. Now, we are trying to detect EGCG in the liver section derived from EGCG-administrated mouse.

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# P61. Improved Analysis of Mass Spectral Imaging data using Hierarchical PCA

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## Abstract

**Introduction**

Mass spectral imaging (MSI) is becoming an important tool for the molecular imaging of biological samples. This tool has the ability to reveal the spatial distribution of hundreds of molecular species throughout organic tissue in a single experiment. However, due to the high dimensionality of MSI data, dimensionality reduction has become a common pre-processing requirement to enable thorough analysis. Principal component analysis (PCA) is one of the best known techniques to perform this step. Using PCA, only the most prominent principal components (PCs), are retained for further analysis. These are characterized as mass spectral patterns that exhibit particular spatial distributions. In this study, we demonstrate how a hierarchical application of PCA is capable of retrieving patterns that are ignored by standard PCA implementations.

**Methods**

In this study, a workflow of a hierarchical application of PCA is described, in which the tissue is divided into subsections of homogeneous pixels to which PCA is applied as a separate procedure. In each iteration, the data is divided into spatial sub-areas with similar molecular content by means of the most important PCs, to which PCA is again applied. This results in less prominent patterns in the data being retrieved, because they would be considered noise by a globally applied PCA. Our objective is to better extract patterns that are only present in a relatively small spatial area, and are characterized by ion peaks of only modest peak height in the presence of substantial ion count noise.

**Results**

The efficiency of the proposed approach was demonstrated on a MALDI MSI dataset using a case study of human renal cell carcinoma. The 10µm thick sample was mounted on an ITO glass slide, then analysed by an ABI 4800 MALDI TOF/TOF mass spectrometer. The mass range extends from m/z 300 to 2000. The grid size was 44 (y axis) by 43 (x axis), resulting in 1892 data-points. Histological examination of the tissue sample using H&E staining clearly demonstrates two regions: normal renal cortex and an area with clear-cell renal cell carcinoma. We demonstrate how this approach allows the extraction of a third pattern that was unobservable from previous analyses using standard PCA. This pattern is located within the previously defined normal cortex. Upon close histological re-examination and alternative staining using Jones to stain basal membranes and Sirius Red for collagen staining, this third area is confirmed as a more fibrotic cortex.

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# P62. Comparison of different on-tissue digestion protocols to improve MALDI-IMS Experiments

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## Abstract

Objective: Matrix-assisted laser desorption ionization (MALDI)-Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection[1] and drug development. But in some cases exclusive visualization of the spatial distribution of potential markers or areas of interest is not a sufficient and satisfying result. Hence, over the last years, increased efforts have been made to develop and improve methods to identity unknown components.

Current on –tissue protein identification techniques in MALDI-IMS are either done via spray coating or droplet deposition of trypsin solution[1,2] and/or matrix solution respectively. Both can be performed in an either manual or automated manner. Latter allows greater confidence of inter-run reproducibility. Still the entire process from sample collection to data analysis is influenced by a number of more or less controllable and uncontrollable factors. The overall goal of this study was to test and compare various protocols of tissue digest in order to improve the quality of our MSI data.

Design and Method: In this work the MALDI-ImagePrep device from Bruker Daltonics is used for automated spraying of trypsin and matrix on mammalian tissue samples. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics). Different experiments were carried out varying 1) the trypsin incubation time, 2) the drying phase, 3) the matrix and 4) the raster width of the laser.

Results: Initial results reveal interesting differences concerning the overall spatial resolution. According to our findings the overnight digestion at 37°C yields a higher spatial resolution compared to attempts with two or four hour digestion time. Furthermore an additional drying phase right after digestion proved to be useful in order to avoid possible "overwetting" of the tissue during the subsequent spray process of the matrix. Contrary to the experiences of other scientists, tissues sprayed with HCCA matrix did not lead to increased resolution compared to DHB matrix. So far the analyzed data showed no significant change of spectra intensities between the various experiments. All measurements carried out with a raster width of 100 µm provided satisfying results. However the comparison tests using 50 and 25 µm raster width are not yet fully completed. Even though individual images showed in parts the desired gain of resolution, a final conclusion cannot be drawn at this moment.

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## Acknowledgements

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# P63. Differential proteomic analysis using MALDI imaging in a mice model of liver inflammation

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## Abstract

Amongst the techniques to detect and characterize markers in biological specimen, mass spectrometry and especially Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is nowadays well established. MALDI MSI is a powerful label-free technique for studying the spatio-temporal distribution of a large range of biomolecules directly in tissue sections[1]. Due to the high incidence and poor prognosis of hepatocellular carcinomas (HCC)[2], there is a need to search for early HCC diagnosis markers related to the lymphocytes infiltrating liver, and MALDI MSI may represent an interesting alternative to conventional omics approaches. However, due to the genetic diversity and the difficulty to access to longitudinal samples, it is difficult to set-up with patient liver. Therefore, we established a chronic or acute hepatitis model by injecting respectively the glycosphingolipid GalCer intraperitoneally or the lectin from Jack bean seeds concanavalin A (Con A) intravenously into mice vein tails[3], then develop a reproducible sample preparation protocol which can be translate from animal models to human tissues in order to identify the proteome changes in response to liver inflammation. In addition, we compared the protein profiles of liver proteins between Con A treated and untreated mice. We found a set of proteins with a differential intensity level that discriminate Con A treated mice from control, which reveal the possibility to explore and understand the cause of inflammation in human HCC.

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## Acknowledgements

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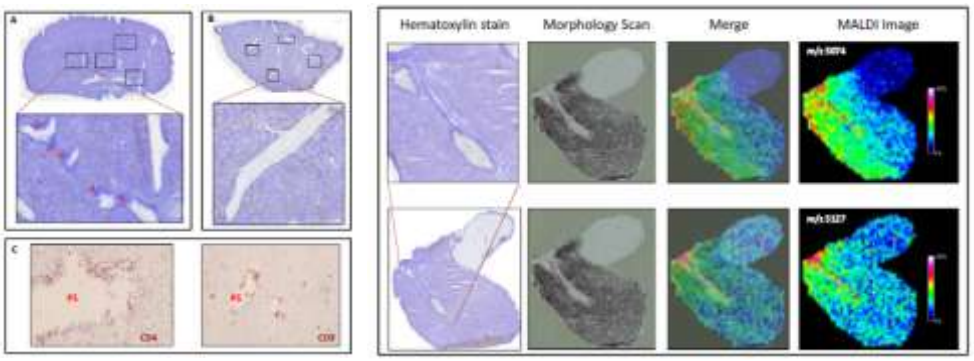
### Figures



# *Sponsors Talks*

# State-of-the-art tissue preparation for MALDI imaging

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## Abstract

During the last years, MALDI mass spectrometry has been subject to considerable changes. Whereas the focus of MALDI before has mostly been on the analysis of peptides and proteins (proteomics), the development and progress of MALDI imaging have clearly expanded its options. The aim of MALDI imaging is to provide localized information on diverse analytes in various materials. Predominantly, the examined samples are thin tissue slices of different origins.

The advantage of mass spectrometry is its ability to identify a wide diversity of molecules. Those may be small molecules such as drug candidates and their metabolites, as well as larger biomolecules like peptides and lipids, or even intact proteins.

Due to these general applicability, MALDI imaging now plays an important role in many scientific fields. Under those, pharmaceutical research is certainly one major area.
Pharmacokinetics in laboratory animals is an important step during the development of new pharmaceutical compounds. Their successful release is highly dependent on the investigation of the pathways of drugs and their metabolites throughout the living body. In contrast to earlier studies, which mainly displayed the distribution of compounds over the organism by radioactive markers, today it is also possible to solve this via MALDI imaging.

MALDI imaging has also been successfully employed for biomarker identification and detection. In this field, special emphasis is laid on biomarkers involved in cancer research. The differentiation of healthy and ill tissue based on the occurrence of specific substances is a highly interesting method which is, despite its successes, still in its early stages.

MALDI imaging measurements critically depend on the preceding sample preparation. Here, the essential point is the deposition of matrix solution, which has to fulfill two tasks. The first one is the extraction of analyte molecules from the tissue and their incorporation into co-crystals. The second task is the ionization of analyte molecules during the MALDI process.

Currently, the Suncollect spotter/imager is the most suitable instrument for this challenge. It successfully performs the act of balance during the matrix-spray process: on the one hand, a highly efficient extraction process is needed to achieve good sensitivity when measuring the tissue sample. On the other hand, a rapid drying of the matrix solution is required in order to achieve both homogeneous crystallization and high spatial resolution due to small crystal size and low dispersion of small molecules.

The advanced micro spray technology, combined with a software tool that allows the user to optimize the parameters of the spraying procedure individually for each sample, makes this instrument superior to other methods and instruments.

# Heat stabilization as a technique to increase biomarker detection when using MALDI-MS Imaging

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## Abstract

Proteases and other protein-modifying enzymes rapidly change the proteome composition after sampling. This can lead to variation between samples or even misleading data conclusions since a combination of the *in vivo* proteome and *in vitro* changes of the same proteome is seen in the sample. Losing vital information about the 'pre-sampling' state will distinctly affect the final analysis result.

To avoid the problem of *ex vivo* protein degradation, rapid heat inactivation of protein modifying enzymes can be used. In this study, the Stabilizor T1 system (Denator AB) has been utilized to ensure complete inactivation and stabilization of proteins in the sample to prevent degradation and change. This technology can be applied to all solid tissues. Heat inactivation in combination with MALDI-MS Imaging has been used to detect specific biomarkers and to monitor time dependent changes in mouse brain tissue samples post-sampling. Directly after excision mouse brains were bisected and one of the hemispheres was snap frozen in liquid nitrogen from fresh and the other hemisphere was heat treated followed by snap freezing in liquid nitrogen. The snap frozen samples were used as standard control. Analysis was performed on all sections which were kept for 0, 1, 2, 3, 4 and 5 minutes in room temperature and time dependent changes was evaluated.

Inactivation of protein modifying enzymes using the Stabilizor T1 system prior to MALDI-MS Imaging results in preventing degradation at the time of sample handling, tissue sectioning and analysis. This provided less bias in the quantitative measurement of specific molecule levels and a more reproducible biomarker identification.

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Heat stabilization of the Tissue Proteome: A New Technology for Improved Proteomics, Svensson et al., *Journal of Proteome Research*, 2009, 8 (2), pp 974–981

Stopping the clock on proteomic degradation by heat-treatment at the point of tissue excision, Goodwin et al., *Proteomics*, 2010, 10(9), pp1751-61

# MULTIPLEXED LABEL-FREE BIO-AFFINITY ANALYSIS AND MALDI-MS CHARACTERISATION OF BOUND ANALYTE

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## Abstract

Surface Plasmon Resonance (SPR) is an optical technique offering label-free analyses of molecular interactions in real time. It provides information on kinetic processes (association and dissociation), binding affinity and molecule detection.
SPR imaging (SPRi) uses SPR in a micro-array approach. It is the ideal solution for rapid and multiplexed investigations. It allows:

- the rapid quantification and monitoring of biomolecular interactions
- the study of up to several hundreds of label-free bindings simultaneously

The coupling of Surface Plasmon Resonance imaging (SPRi) and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is an innovative approach for biomarker discovery in biological fluids. Multiplexed SPRi analysis allows the direct visualization and thermodynamic analysis of molecular avidity, and is advantageously used for ligand fishing of captured biomolecules on multiple immobilized receptors on a SPRi-Biochip surface. MALDI-MS is a powerful tool for the identification and characterization of molecules by their molecular weight and peptide sequence. Therefore, the combination of SPRi and MS into one concerted procedure, using a unique dedicated surface, is of great interest for functional and structural analysis of bound molecules. Results will be shown using the Lymphocyte Activation Gene 3 (LAG3) protein, a potential biomarker of breast cancer and tuberculosis. LAG3 was captured in human plasma by SPRi down to several femtomoles/mm$^2$. Then, after MS pre-processing, LAG3 was successfully identified by MALDI-MS directly on the SPRi biochip. The coupling of SPRi to MS analysis is possible and is a valuable tool for biomarker identification.

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