COMPUTIS

Innovation in Imaging Mass Spectrometry

Scientific highlights from the COMPUTIS project

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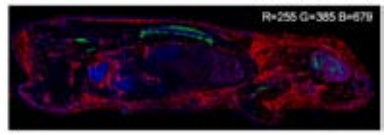
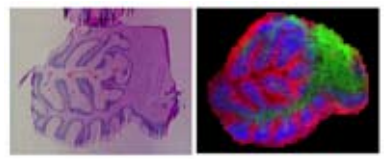
Introduction of scientific highlights from the COMPUTIS project

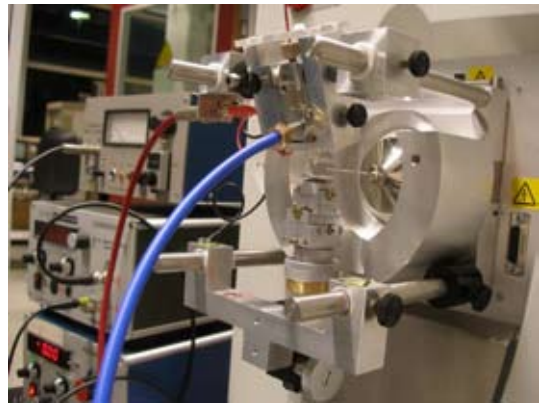
COMPUTIS is a FP6 project that has been launched at the beginning of 2006, with a total duration of 4 years. It aims at developing technologies and methods for mass spectrometry imaging, from instrumental developments and sample preparation to data processing and analysis to the biological applications.

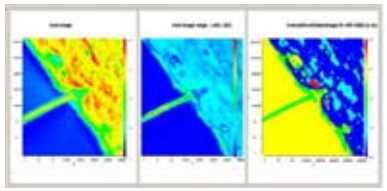
The whole team of the COMPUTIS project is pleased to give in this booklet a general overview of the developments carried out in the framework of COMPUTIS, through brief highlights on various realizations and results.

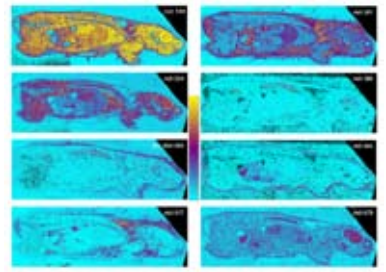
More information is available at the COMPUTIS website: http://www.computis.org.

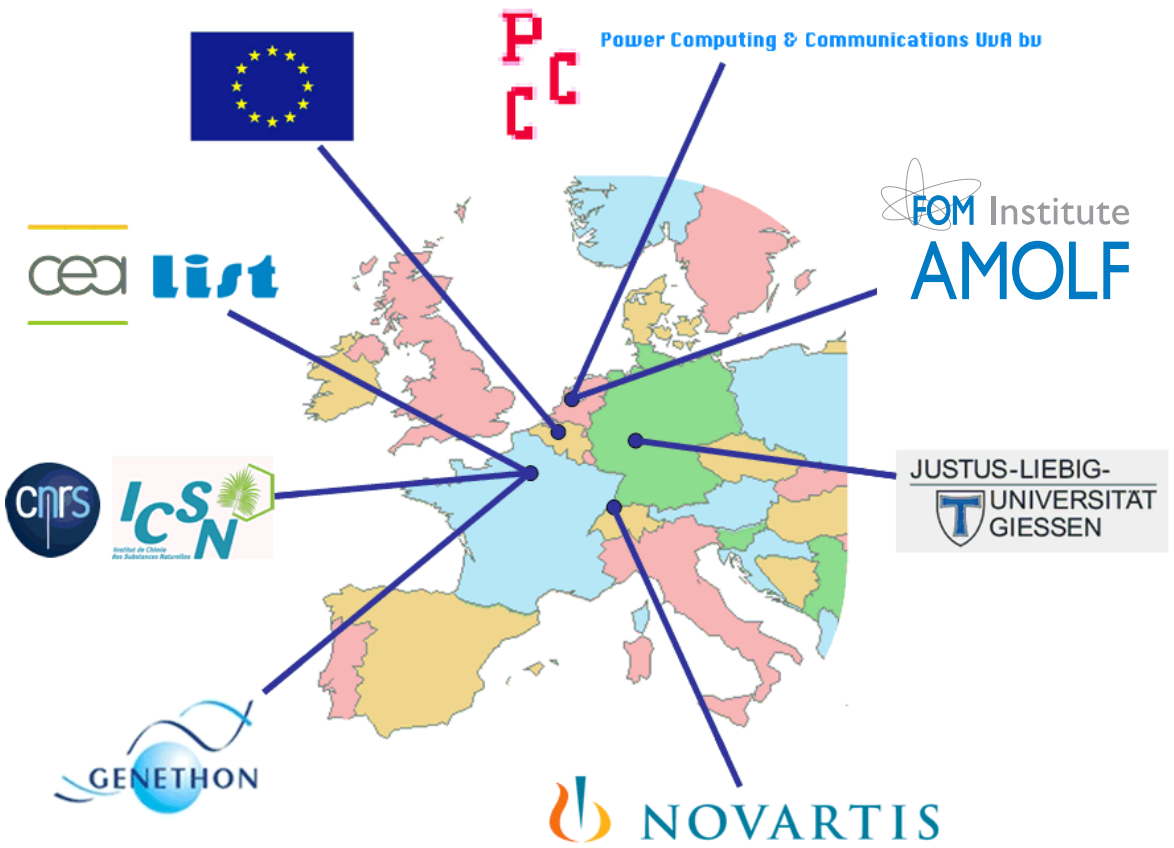












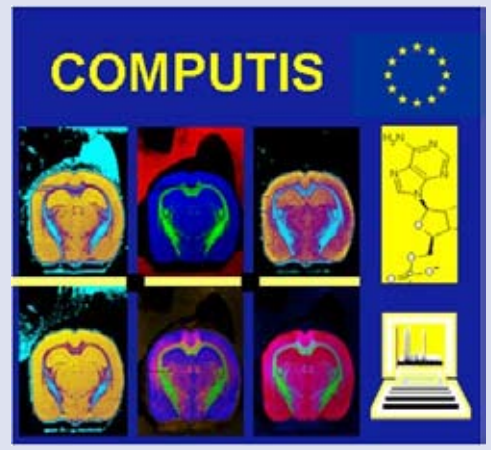
Introduction of scientific highlights from the COMPUTIS project

COMPUTIS Partners

The COMPUTIS project is developed by a consortium of 7 partners:

- CEA LIST, Commissariat à l'Énergie Atomique, Saclay, France (project coordinator)
- Justus Liebig University, Giessen, Germany
- FOM Institute / AMOLF, Dutch Foundation for Fundamental Research on Matter, Amsterdam, Netherlands
- Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France
- Power Computing and Communications, UvA BV, Amsterdam, Netherlands
- Généthon, Évry, France
- Novartis Pharma AG, Basel, Switzerland

COMPUTIS is a Specific Targeted Research Project of the 6th Framework Program Priority 1: Life Sciences, Genomics and Biotechnology for Health – Contract n° 518194.

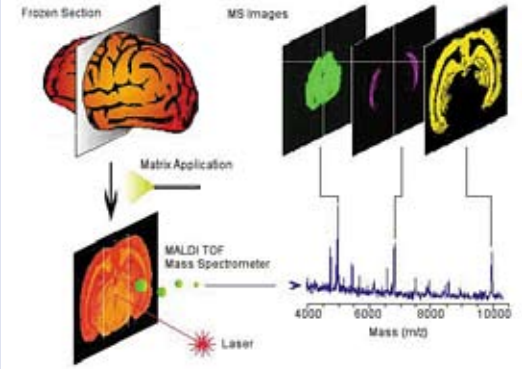


Motivation and Objectives

Mass Spectrometry Imaging (MSI) is a rapidly developing technology that provides information-rich data on various classes of biomolecules (proteins, lipids and metabolites) directly on tissue at the micrometer scale.

COMPUTIS is a 4-year European FP6 project (2006-2009) that aims at developing analytical and software technologies for MSI enabling innovative methods of investigation in structural genomics, proteomics and metabolomics, by combining various methods of SIMS and MALDI-MS.

The three principal objectives of the project are:
- innovating in MSI instrumentation,
- developing advanced diagnostic methods,
- monitoring therapeutic effects.



Principle of scanning microprobe MSI with the MALDI method (from M.Stoeckli et al., Nat. Med. 7, 493-496, 2001)

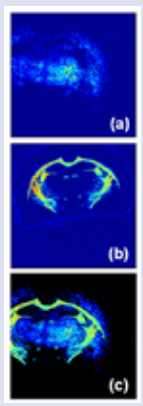


Illustration of the principle of combining SIMS and MALDI MSI: (a) MALDI image of a mouse brain section showing the distribution of a neuropeptide (m/z 6756); (b) SIMS image of the same mouse brain showing the distribution of cholesterol (m/z 385.38) in the corpus callosum area; (c) superimposed images after registration.

Project Organisation

The COMPUTIS project is organised in 8 technical work packages and one management work package. The decision-making is under the responsibility of the steering committee, which decides about the high-level management issues. The general organisation and management structure is shown on the chart below.

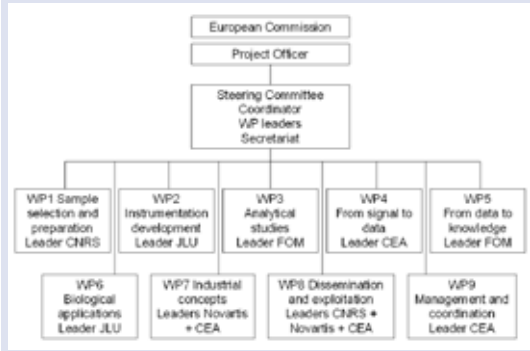


Chart of the general organisation and management structure of the COMPUTIS project.

The technical work packages correspond to the 3 principal objectives of the project, as previously described:

WP1 *Sample selection and preparation*,
WP2 *Instrumentation development* and
WP3 *Analytical studies* are devoted to the development of improved instrumentation and experimental protocols;

WP4 *From signal to data* and
WP5 *From data to knowledge* aim at developing methods, algorithms and software tools for processing and analysing data, including multi-modality and multi-sample data;

WP6 *Biological applications*,
WP7 *Industrial concepts* and
WP8 *Dissemination and exploitation* are focused on the valorisation efforts, from the scientific applications to the industrial exploitation.

WP9 management and coordination

The highlights presented in this booklet are often a joint effort between different workpackages.

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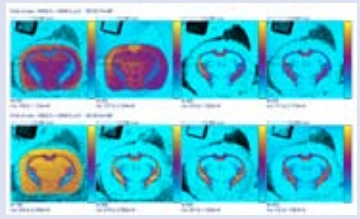
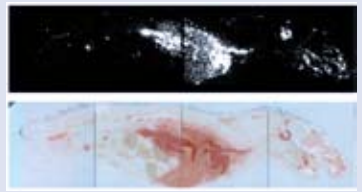
Standardized reference mass spectrometry images

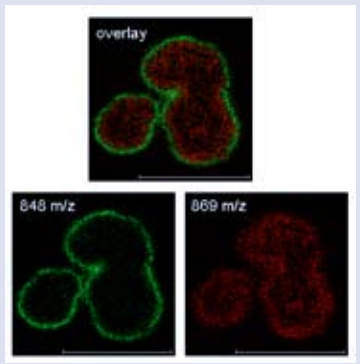
Standardized reference mass spectrometry images have been defined at the beginning of COMPUTIS in order to have reference data, for the improvements of sample preparation methods, for the improvements of analytical methods and to be utilized for the data analysis methods developped within the project.



TOF-SIMS positive and negative ion images of a rat brain tissue section



Top: MSI of the heme group (m/z 616); Bottom: Optical image of the same section before matrix coating. The blood distribution matches well with the MSI signal



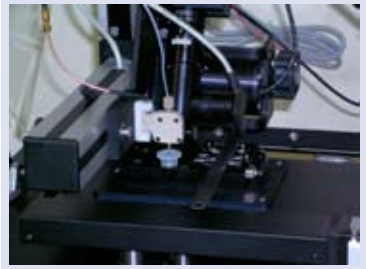
Metal assisted (Meta)-SIMS analysis of neuroblastoma cells

PROSPECTS

- Cluster-TOF-SIMS, MALDI, and MetA-SIMS images are available.
- These images show different sample sizes, cells, rat brains and whole body of animal.

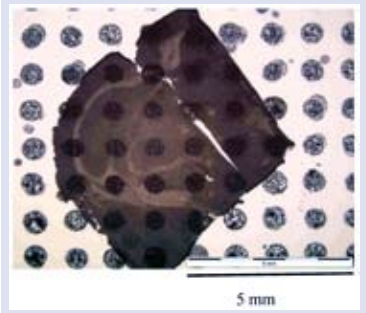
"Spoutnik": a piezoelectric matrix spotter for MALDI profiling

For some applications, the acquisition of a MALDI image is sometimes not necessary. This is the case when comparing destructured / structured areas on mouse muscle sections during the study of the Duchenne Muscular Dystrophy. In that case, a simple "profiling" is sufficient. Nevertheless, matrix droplets need to be deposited with always the same volume and with a precise positioning.



View of the piezoelectric robotic spotter

Functions of the piezoelectric spotter



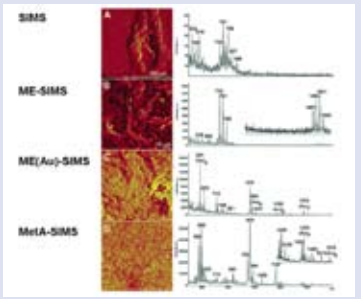
Sinapinic acid droplets deposited onto a rat brain tissue section

PROSPECTS

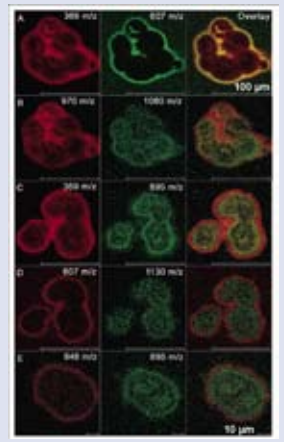
- A piezoelectric spotter has been developped for precise and reproducible MALDI profiling onto tissue samples
- Droplet diameter is ~250-300 µm

Gold Enhanced SIMS + MALDI Mass Spectrometry Imaging

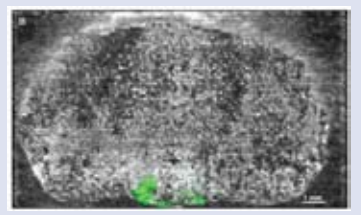
Surface metallization by plasma coating enhances desorption/ionization of lipids and sterols in SIMS imaging experiments (MetA-SIMS). In addition, gold deposited on top of matrix-coated rat brain sections enhances image quality and signal intensity for stigmatic imaging MALDI-TOF. Matrix-enhanced SIMS (ME-SIMS) enhances phospholipid signals.



Total Ion Current and Spectra After Different Surface Modification



MetA-SIMS Cellular Localization of Selected Ion Counts of Neuroblastoma Cells



Stigmatic Imaging MALDI-TOF of a Rat Brain Section: Vasopressin (m/z 1085, green) is overlaid with the TIC

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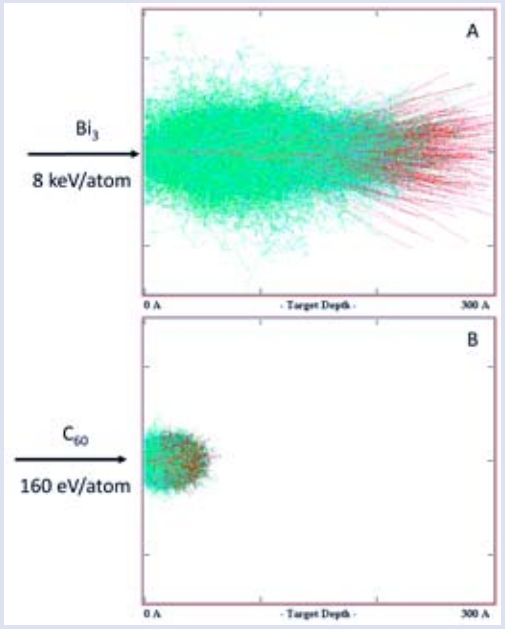
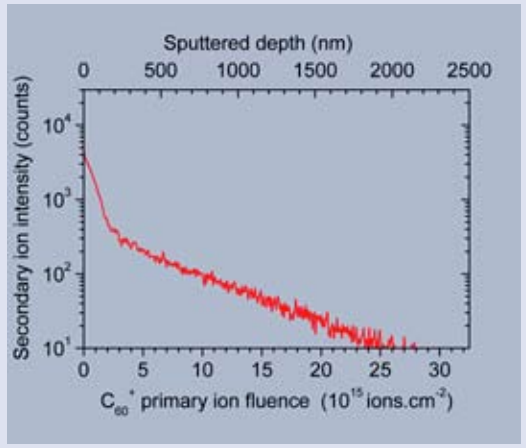
Evaluation of 3 dimentional imaging using a C60 ion source

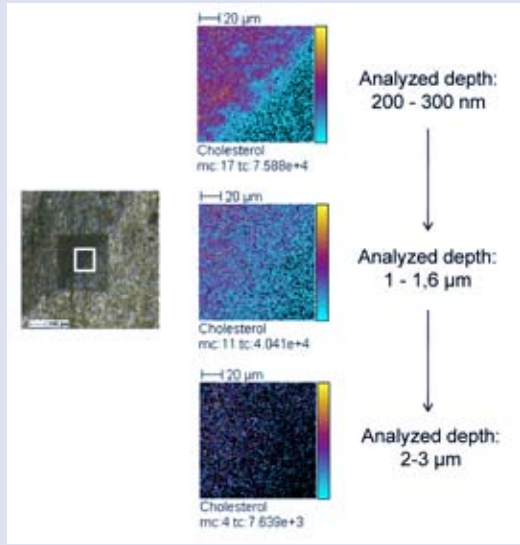
Fullerene ion beams have been used to sputter the surface of a rat brain tissue section, in order to test the possibility of depth profiling into a true biological sample.



Simulation of the depth of penetration in adipose tissue by bismuth ions and by C60 ions (SRIM)



Intensity as a function of C60 fluence, measured directly on a rat brain section



Cholesterol ion images recorded at the edge of the corpus callosum of a rat brain tissue section, and with different depths

PROSPECTS

* Although the present results are not in contradiction with the literature, larger ion doses have been reached in the present experiments, showing that the lipids are mainly concentrated in the first 200-300 nm below the sample surface.

* After having removed these layers, the concentration of the various lipids is not high enough and the damage induced by the sputter gun remains still too high, although much lower than with metallic heavy ions, to consider dual beam depth profiling experiments in biological samples with a good depth resolution of 1 µm or less. The success of depth profiling in tissue sections remains challenging.

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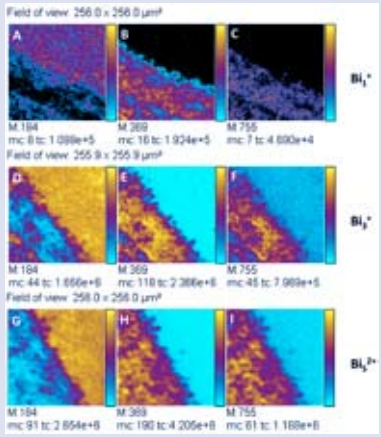
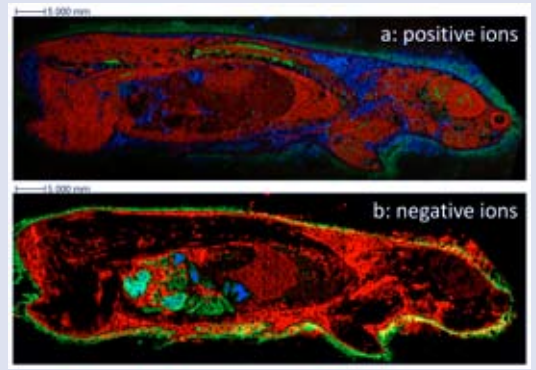
Cluster Ion Sources for SIMS

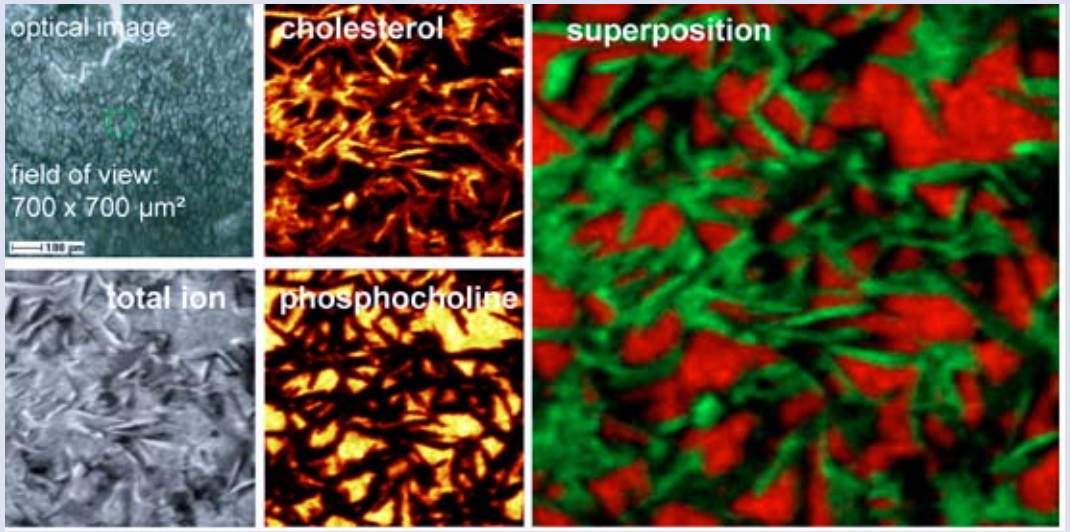
Newly developed cluster ion sources have been evaluated within COMPUTIS. Bismuth cluster liquid metal ion source appears to be particularly efficient, sensitive and resolutive for TOF-SIMS imaging.



Images recorded at the edge of the corpus callosum on a rat brain tissue section illustrating the sensitivity enhancement provided by cluster ion in SIMS



TOF-SIMS images (three-color overlays) of a whole mouse section. a: positive secondary ions. Red: phosphatidylcholine fragment (m/z 224); Green: cholesterol (m/z 369 and 385); Blue: diacylglycerol (m/z 577). b: negative secondary ions. Red: sum of stearic (m/z 255) and oleic (m/z 281) fatty acid carboxylates; Green: cholesterol sulfate (m/z 465); Blue: taurocholic acid carboxylate (m/z 514).



TOF-SIMS image recorded with a field of view of 55 µm x 55 µm and a pixel size of 215 nm, inside the corpus callosum of a rat brain tissue section.

PROSPECTS

- Bismuth cluster ion sources make possible acquisition of TOF-SIMS images with high sensitivity, high spatial resolution .
- The intensity of the ion source enables the acquisition of large images such as whole body mouse sections, as well as images with very small pixel sizes, well below 1 µm.

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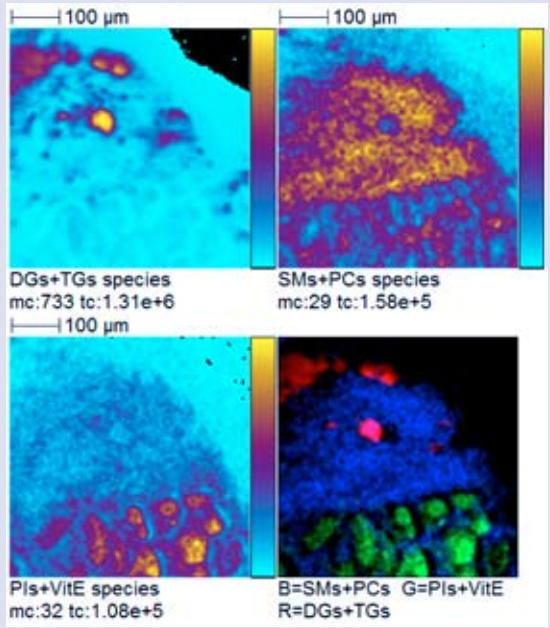
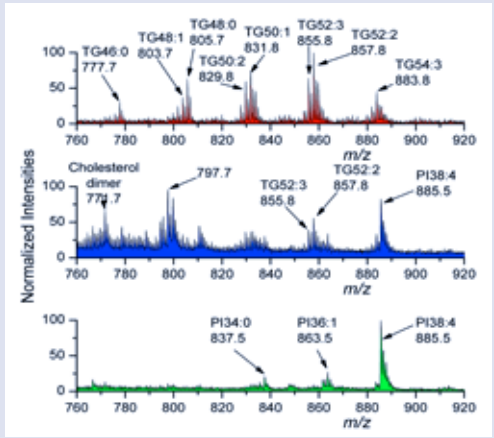
A. Brunelle, O. Laprévote, Curr. Pharm. Design. 2007, 13, 3335-3343
A. Brunelle, O. Laprévote. Anal. Bioanal. Chem., 2009, 393, 31-35

TOF-SIMS imaging of Human samples of Duchenne Muscular Dystrophy

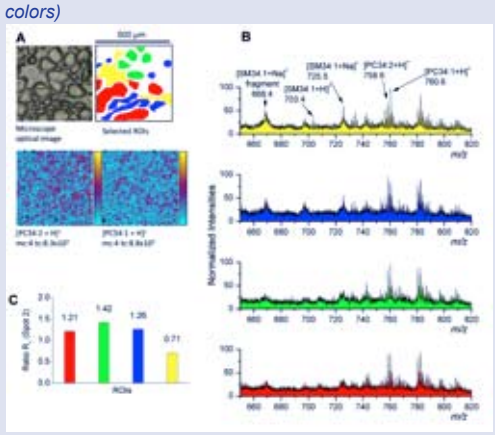
Human striated muscle samples, from male control and Duchenne Muscular Dystrophy (DMD)-affected children, have been subjected to cluster-time-of-flight secondary ion mass spectrometry (cluster-ToF-SIMS) imaging, using a 25 keV Bi3+ liquid metal ion gun. Characteristic distributions of various lipids have been observed. Vitamin E and phosphatidylinositols have been found to concentrate within the cells, whereas intact phosphocholines accumulated over the most damaged areas of the dystrophic muscles, together with cholesterol and sphingomyelin species. Fatty acyl chains composition varied depending on the region, allowing estimation of the local damage extent.



ToF-SIMS ion images of human paravertebral dystrophic muscle: Top left: di- and triglycerides (adipocytes); top right: phosphatidylcholines and sphingomyelins (damaged area); bottom left: phosphatidylinositols and vitamin E (myofibres); bottom right: three-color overlay. Field of view: 500 x 500 µm2, pixel size: 4 µm.



Mass spectra (m/z [760-920]) of each the three different areas evidenced in the dystrophic muscle tissue (same colors)



ToF-SIMS analysis of in positive ion mode. A) microscope image; selected regions of interest (ROI) (red, green, blue = cells, yellow = intercellular space), m/z 758 and m/z 760 ion images, B) m/z [650-820] enlargement of each ROI's spectrum, C) Intensity ratio (758/760) for each selected ROI.

PROSPECTS

- Cluster-ToF-SIMS imaging allowed to directly and relatively rapidly probe intact biological tissue sections with a complete preservation of the sample molecular and structural integrity, a simultaneous analysis of different morphological regions at a micrometer scale and a high molecular specificity and sensitivity without any prior preparation procedure.
- The specific localization of the different compounds in the positive ion mode imaging and its confirmation in the negative ion mode demonstrated the absence of any molecular delocalization at the micrometer scale on the samples. ToF-SIMS imaging thus gives a considerable amount of information on the local molecular composition within tissues or organs, and can be recognized as a powerful approach for localized lipidomics studies.

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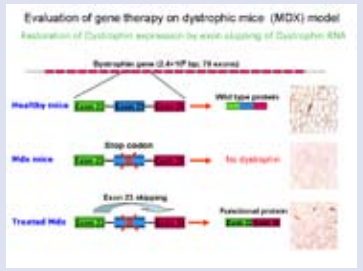
REFERENCES

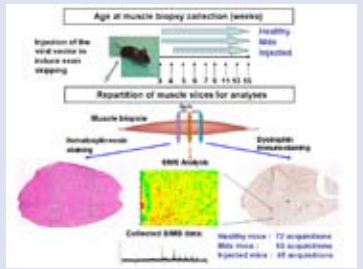
N. Tahallah, A. Brunelle, S. De La Porte, O. Laprévote, J. Lipid Res. 2008, 49, 438-454

DMD: approach gene therapy by exon skipping

Duchenne muscular dystrophy (DMD) is a severe recessive muscular dystrophy caused by a mutation in the gene coding for the protein dystrophin, an important structural component within muscle fibres. New promising approaches like exon skipping with oligonucleotides or AAV vectors are under preclinical and clinical trials. Mice with a mutation of the dystrophin gene (denoted mdx) are widely used animal model of DMD.



Exon skipping: How it works

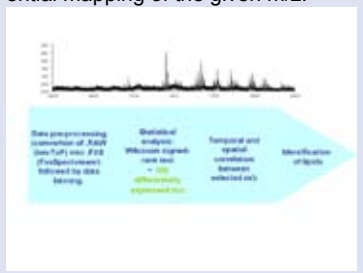


Data collection and repartition of muscle slices for analysis

✱ Gene therapy by exon skipping restores functional dystrophin in mdx mice

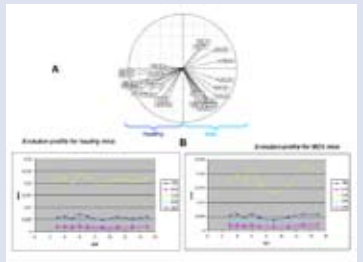
ToF-SIMS data analysis

The total ToF-SIMS spectra of the samples were analysed at first (With J-P Both , CEA de Saclay). We reasoned that differential ion expression found in the total spectra in mdx versus normal mice could reflect a differential mapping of the given m/z.



Data processing

Superposition of histological images with ToF-SIMS ion images. Ions found differentially expressed between mdx and healthy mice by the analysis of the total spectra (m/z 188 and 299) are differently distributed in the MDX and healthy muscles.

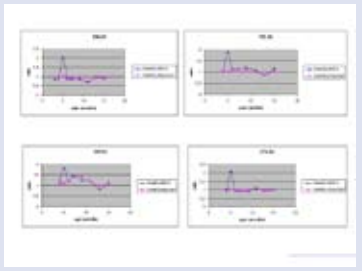


Two-dimensional Principal Component Analysis (PCA): clear separation of selected ions for mdx and healthy mice (A). Multi-dimensional PCA permitted us to establish temporal correlation between differentially expressed m/z (B) (With J-P Both CEA de Saclay).

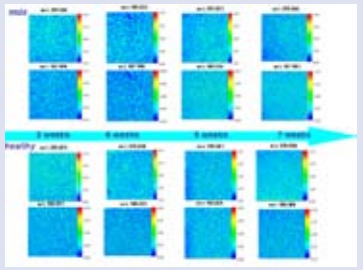
✱ Data analysis permits to find differentially expressed ions between MDX and healthy mice. Some of the differentially expressed ions are differently mapped on the MDX and healthy muscles.

Gene therapy restores expression of some ions in MDX muscles

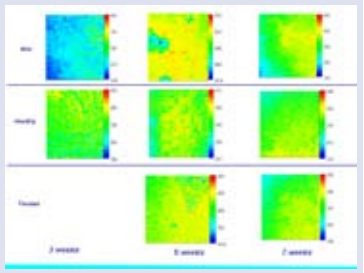
Comparison of the time course of the dystrophin restoration with the corresponding SIMS data showed reversion of some differentially expressed ions.



Destabilisation of MDX muscle at 5 weeks. Expression of some ions are normalised in MDX mice after gene therapy



Distribution time course of some differentially expressed ion in MDX and normal mice



Example of spatial ions clustering by using K-means algorithm. Distribution of ions co-clusterized with ions 188 and 299.

✱ SIMS analysis can be used to distinguish between healthy and MDX mice. It can be also used to follow therapeutic treatment by exon skipping.

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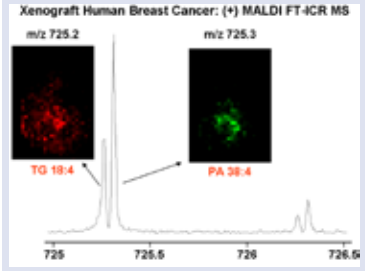
NOTES

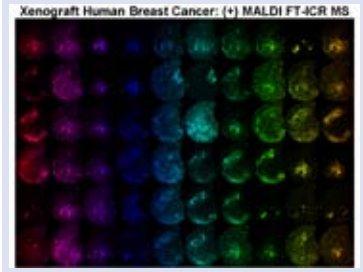
HIGH MASS RESOLVING POWER AND HIGH MASS ACCURACY FT-ICR MASS SPECTROMETRY IMAGING

MS Imaging of Lipids in Breast Cancer

The high mass resolving power and high mass accuracy of FT-ICR MS allows identification of a variety of lipids in human xenograft breast cancer tissues. FT-ICR MS is used complementary to MALDI-TOF and TOF-SIMS, on the same tissue sections. FT-ICR resolves isobaric species that are missed by TOF analyses.



High mass resolving power identifies two masses with different spatial localization.



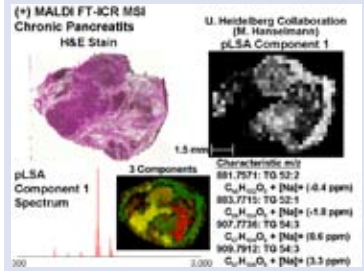
FT-ICR MS resolves hundreds of spatially localized species that can be identified by accurate mass and MS/MS.

PROSPECTS

- Localization and identification of lipids within breast cancer tumors. Delineation of tumor types based on spatial and chemical profiles.

Characterization of Human Pancreatic Disease by FT-ICR MSI

Pancreatic cancer is typically diagnosed at a late stage; thus the survival rate compared to other cancers is low. FT-ICR is used to identify lipids and peptides in healthy pancreas, chronic pancreatitis and pancreatic cancer. Statistical tools are used to identify regions of interest and ease data analysis.



FT-ICR MS and pLSA statistical analysis identify localization of different lipid species in chronic pancreatitis tissue.

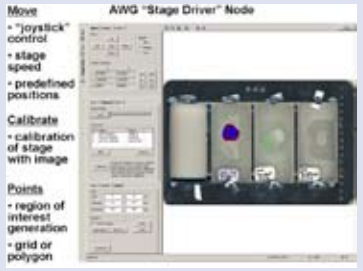
PROSPECTS

- Classification of pancreatic disease by supervised and upsupervised statistical analysis tools applied to FT-ICR MSI datasets.

Software Developments for FT-ICR MSI

The AMOLF flow-based instrument control software allows easy generation of flexible, data-dependent FT-ICR MS experiments. A new “stage driver” node has been added to the software that allows control of all aspects of an imaging MS experiment. This includes stage control, calibration, monitoring and region of interest

The AMOLF AWG instrument control software allows control of experimental parameters, visualization of the mass spectrum and on-the-fly image generation.



Details of the new “stage driver” node.

PROSPECTS

- Flexible experimental design and data-dependent experiments for FT-ICR MSI.

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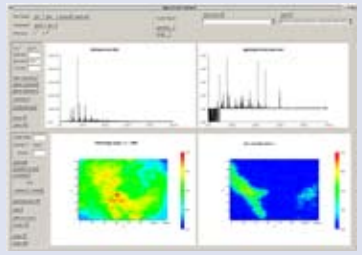
REFERENCES

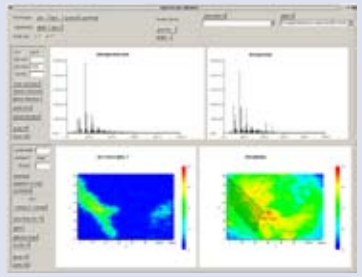
Taban LM, van der Burgt YEM, Duursma M, et al. Rapid com. in MS 2008, 22, 0951-4198.

Navigation and Correlation

We highlight a simple visualization tool to explore in a unified way Maldi and Sims mass spectrometry data.
The tool has various display modes highlighting peaks, ROI selection, pixel selection, zooming.



In the higher part of image we see here a total spectrum and a simple variance indicator signaling peaks with structured images. In the lower part we have total image and image of one of the signaled peaks.



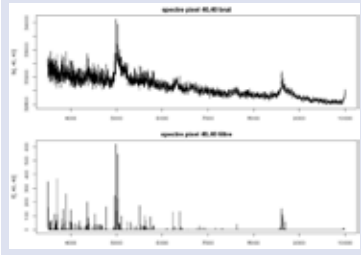
ROI extraction - Pixel spectrum display - Zooming - Dump in different formats (ps, svg)

PROSPECTS

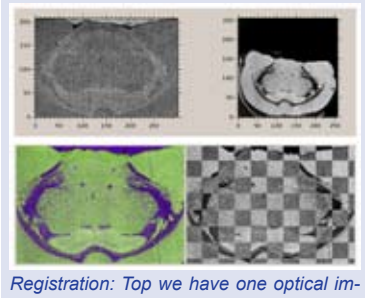
- New display modalities to highlight peaks expressed in few pixels.

Denoising and Registration

We illustrate here two items:
- Spectrumdenoising for Maldi (binned) data based on wavelet
- Image registration to superpose MALDI, SIMS, or artificial image produced by structural analysis using maximization of mutual information



Denoising: We have a raw spectrum and its denoised version. The algorithm uses translation invariant wavelet decomposition, thresholding and implement positivity constraint on reconstucted signal



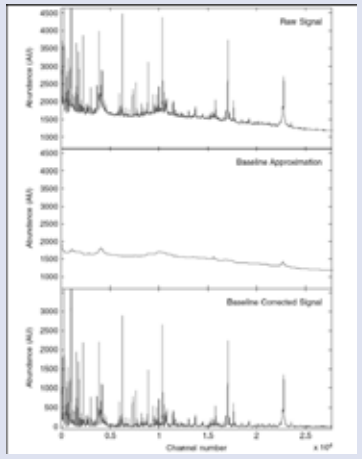
Registration: Top we have one optical image (left) and one cluster image, bottom the superposition of images

PROSPECTS

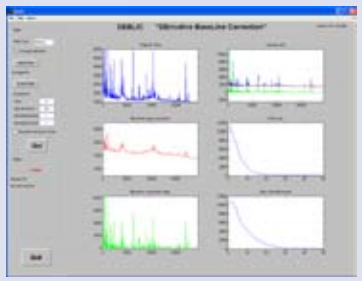
- Extended wavelet denoising tool adapted to SIMS data
- Multiscale representation of images to enhance registration

DEBLIC: Baseline Correction of MALDI MSI Data

We have developed a simple derivative based algorithm to correct baseline drift commonly encountered in MALDI mass spectrometry, DEBLIC (DErivative BaseLine Correction). This allows easy visualization of peaks that are not seen prior to baseline correction.



Baseline Correction for MALDI-TOF of a Human Cerebellum Section



User Interface for DEBLIC Baseline Correction

PROSPECTS

- Improvement of peak selection in MALDI MSI via baseline correction.

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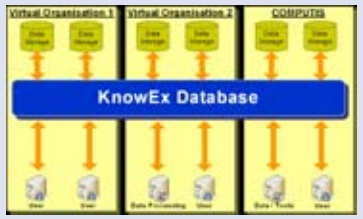
jean-pierre.both@cea.fr

REFERENCES

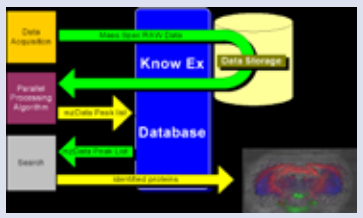
Eijkel, G.B. et al. Surf. Interface Anal. 2009, 41, 675-685.

KnowEx: Knowledge Exchange Database

KnowEx is a middleware that provides common tools to scientists for collaborations/sharing of data.



Sharing of Data/Tools via KnowEx: Various research groups and guests can access common experimental data. In addition, data anlysis tools can be run directly from KnowEx and the results stored in the database.



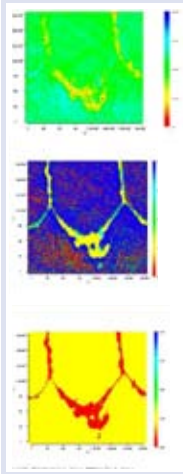
Workflow for Mass Spectrometry Imaging with KnowEx: Aquired MS imaging data can be accesed via KnowEx and processed quickly with parallel processing. A database search can be instituted directly from KnowEx for peptide/protein identification.

PROSPECTS

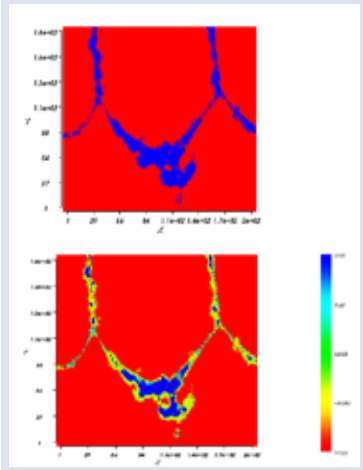
- Easy sharing of data, results and processing tools between collaborators.
- The data for this pamphlet was compiled via KnowEx!

Structuration

We use clusterization algorithms to group pixels with similar spectra or peaks giving similar images. Apart from Kmean (fast) and fuzzy clustering we use a non-linear embedding technique called diffusion maps before classical clustering, this provides data compression and a time denoising parameter.



Diffusion maps and denoising



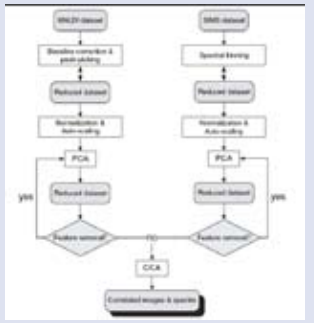
Hierarchical clustering

PROSPECTS

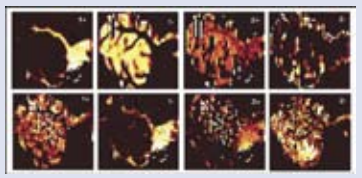
- Use new distances in clustering algorithms to classify spectra.

Analysis Software Tools

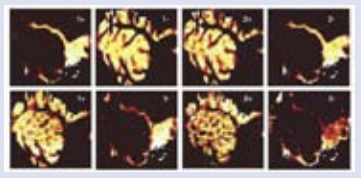
A protocol has been develped to gain the most information possible from a single tissue section by TOF-SIMS and MALDI-TOF mass spectrometry imaging experiments. A suite of software tools is used to analyze the SIMS and MALDI datasets. This suite includes tools for baseline correction (DEBLIC), normalization and autoscaling, peak picking (PEAPI), principal component analysis (PCA) and canonical correlation analysis (CCA).



Schematic of Computational Steps for MALDI and SIMS Imaging Datasets



PCA Score Images for SIMS (top) and MALDI (bottom) of Human Cerebellum Tissue



CCA Score Images for SIMS (top) and MALDI (bottom) of Human Cerebellum Tissue

PROSPECTS

- Improved data mining via baseline correction and peak picking algorithms
- Correlation of SIMS and MALDI data by CCA for improved individual results.

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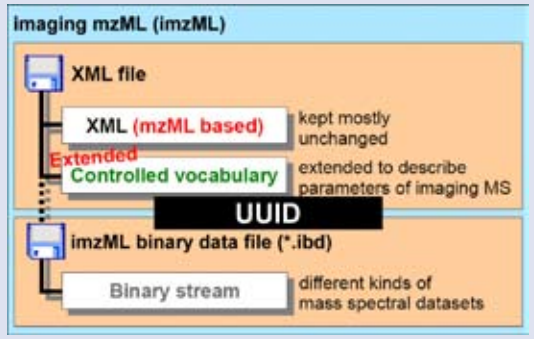
REFERENCES

LAFON S., KELLER Y., COIFMAN R.R., "Data Fusion and MultiCue Data Matching by Diffusion Maps", IEEE Trans. on Pattern Analysis and Machine Intelligence, Vol. 28, pp. 1784-1797, Nov. 2006

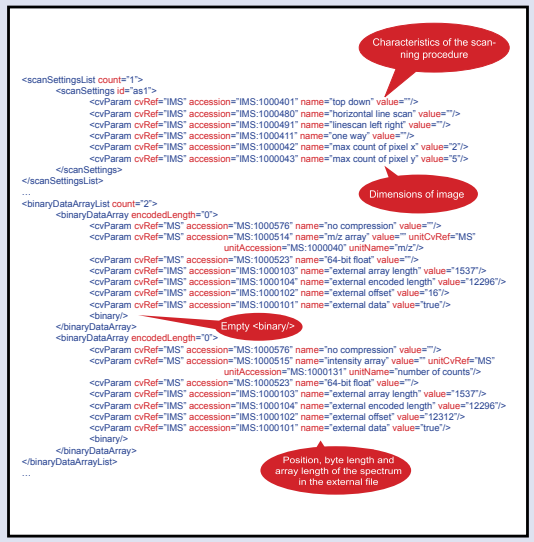
Eijkel, G.B. et al. Surf. Interface Anal. 2009, 41, 675-685.

Metadata: XML and controlled vocabulary

An important task within the COMPUTIS project [1] is the comparison of images generated by diverse types of mass spectrometers. Both the DICOM standard for in-vivo imaging data [2] and the mzML standard by HUPO-PSI [3, 4] are not able to completely represent an imaging MS experiment. Therefore a standardized data format was developed to simplify the exchange of imaging MS data between different instruments and data analysis software.



imzML consists of two separate files: one for the metadata and one for the MS data. The metadata is saved in an XML file. The mass spectral data is saved in a binary file. Both files are linked by a Universally Unique Identifier (UUID).



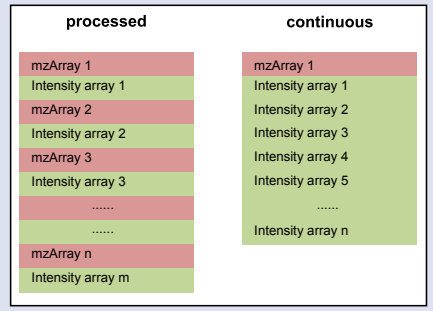
The XML file includes all relevant metadata. The format is based on mzML version 1.1 (HUPO-PSI).

PROSPECTS

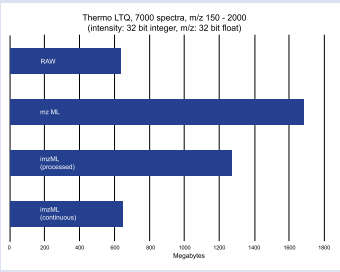
- imzML is easily converted to mzML which also allows access to additional tools

Binary data structure & software applications

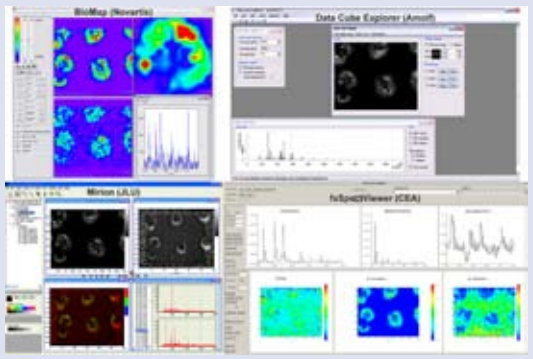
imzML is an efficient format for exchange and comparison of imaging MS data.



There are two different formats in which the MS data can be stored depending on the type of data: PROCESSED: different m/z and intensity array for each spectrum (e.g. after peak picking), CONTINUOUS: the same m/z array for all spectra (profile



Comparison of the file size: The imzML (processed) file is about 30% smaller than the mzML file. The imzML (continuous) file is almost four times smaller than the mzML file. The XML part containing the metadata is rather small compared to the size of the binary data files.



The imzML format is already supported by several software applications: Biomap (NOVARTIS) [6], Mirion (JLU), Datacube Explorer (AMOLF), fxSpectViewer (CEA)

PROSPECTS

- imzML allows flexible handling and fast processing of data
- imzML allows flexible choice of the software tool which is best suited for aspecific application

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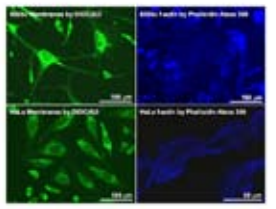
andreas.roempp@uni-giessen.de

REFERENCES

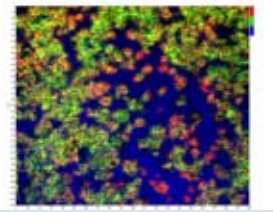
[1] www.computis.org [2] http://medical.nema.org [3] www.psidev.info
[4] http://psidev.info/index.hp?q=node/257
[5] www.wikipedia.org/wiki/Controlled_vocabulary
[6] www.maldi-msi.org
[7] http://code.google.com/p/jmzml/
Klinkert I, McDonnell LA, Luxembourg SL, et al. Review of scientific instr.2007, 78, 0034-6748.

Cell cultures as model systems for imaging MS studies

Cell cultures are highly homogeneous and are relatively easy to handle. Therefore they represent an interesting model system for biological samples.



Histological staining of HeLa cells



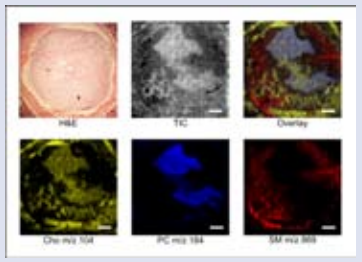
Imaging MS of adherent cells at 10 µm resolution

PROSPECTS

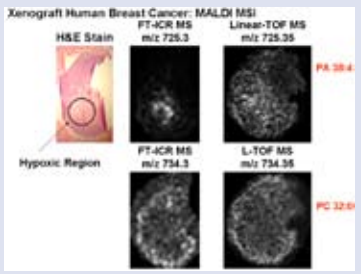
- Cell cultures are used to optimize the sample preparation and measurement protocols for biological samples. The comparison with histological staining can be used to validate the results of imaging mass spectrometry experiments.

Identification of Different Cellular Regions in Xenograft Breast Cancer Tumors

Human breast cancer tumors grown in mice are analyzed by SIMS and MALDI mass spectrometry imaging to identify different cancer regions. Phosphocholine has been found to localize in viable tumor regions of human breast cancer. Various lipids are distributed in different tumor regions.



ME-SIMS of Breast Cancer Xenograft Tumors W



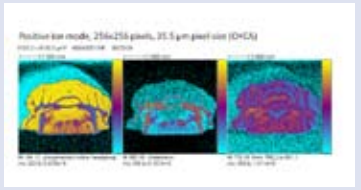
MALDI FT-ICR MS and MALDI TOF Images of Selected Lipids in Human Breast Cancer

PROSPECTS

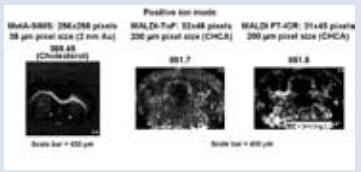
- Identification of cancer regions based on molecular signals measured by mass spectrometry imaging.

Comparison of imaging MS techniques

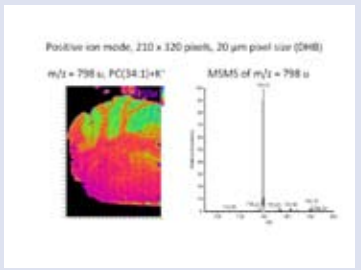
Adjacent mouse brain sections are distributed to the COMPUTIS partners for comparative measurements. First results of this round-robin study are shown below.



TOF-SIMS Imaging MS (CNRS)



MetA-SIMS, MALDI-ToF and MALDI FT-ICR Imaging MS (FOM)



MALDI-Ion Trap Imaging MS (JLU)

PROSPECTS

- The results will be analyzed in detail with respect to mass range, accessbile compound classes, mass accurcy and spatial resolution. The final goal is to provide guidelines on which technique (e.g. MALDI or SIMS) is best suited for a specific imaging MS application.

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MALDI-MSI of treated scalp biopsies

Acne occurs following increased production of sebum by the sebaceous glands and subsequent occlusion of the pilosebaceous orifices. The sites of action for the novel compound investigated are the sebaceous glands and hence the uptake of the drug into the gland is of paramount importance for successful treatment. MALDI-MS and MS/MS imaging technologies have been utilized to acquire images showing penetration of the acne compound through the stratum corneum and into the dermis.

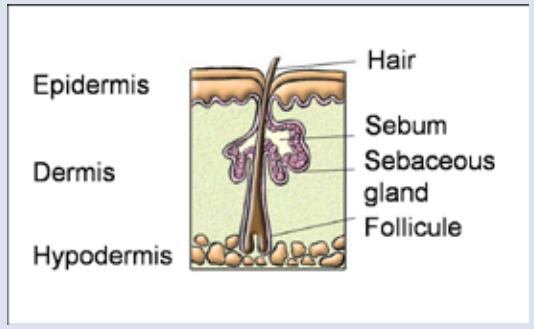
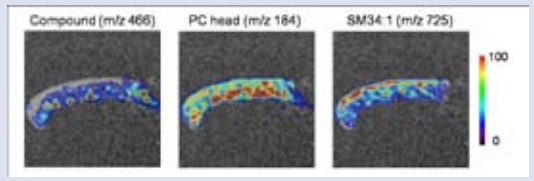


Figure showing the structure of the skin as observed through a horizontal cross section. The sebaceous gland target organ for the compound of interest is located within the dermis.



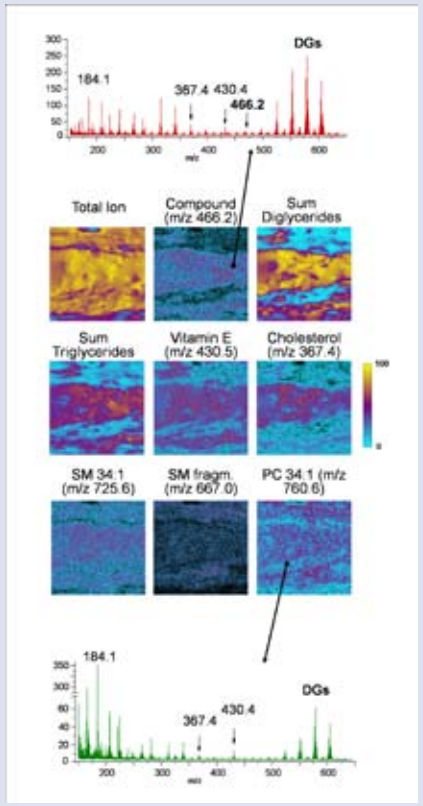
MALDI-MS images showing the distribution of the compound, phosphotidyl choline head group and sphingomyelin 34:1 in the treated scalp skin section. The compound has readily penetrated through the stratum corneum and the signal is located within the dermis where the sebaceous gland and hair follicule regions of interest are located.

PROSPECTS

- MALDI-MSI has been utilized to determine the successful penetration of a novel compound across the stratum corneum and into the dermis.
- MALDI-MSI is a highly sensitive method of imaging whole skin tissue sections. Detailed localization information can be determined with the complementary use of SIMS-MSI.

High resolution TOF-SIMS MSI of compound distribution

The application of TOF-SIMS MSI to treated skin tissue sections enabled the acquisition of high spatial resolution images showing the sebaceous gland structure and presence of compound within the glands and hair follicules. All TOF-SIMS analyses were conducted at the Institut de Chimie des Substances Naturelles (CNRS).



TOF-SIMS MS images showing the co-localization of the topically applied compound with diglycerides, triglycerides, vitamin E and cholesterol acquired from treated scalp tissue sections (pixel size = 2 micron x 2 micron).

PROSPECTS

- TOF-SIMS MSI produces valuable, high resolution images enabling small skin structures to be imaged that may not be resolved by MALDI-MSI. Therefore, additional valuable information is provided to the drug discovery field.
- The combination of MALDI and TOF-SIMS MSI methods enable important compound and lipid distribution information to be determined at whole tissue and discreet gland levels.

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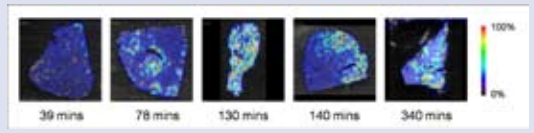
NOTES

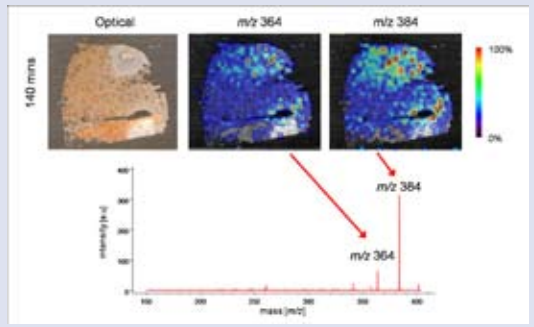
MALDI-MSI APPLIED TO THE ANALYSES OF THE DISTRIBUTION OF TB DRUGS AND DISTRIBUTION IN INFECTED RABBIT LUNG

MALDI-MS/MS imaging of Moxifloxacin distributions in treated rabbit lung sections

Tuberculosis is a common infectious bacterial disease predominantly caused by mycobycterium tuberculosis. Drugs may take between 6-24 months to treat TB patients due to potential difficulties in reaching the bacteria which are located within the TB-induced granulomas. The application of MS imaging technologies allows the dermination of the localization of TB drugs at set time points post-dose. The example shown here is for the drug Moxifloxacin which has been shown to penetrate readily into the granuloma.



MALDI MS/MS Image of Moxifloxacin distribution (main product ion m/z 384). Moxifloxacin signals were frequently observed at higher intensities inside the granuloma than in the surrounding lung tissue, from 90 minutes up to and including the latest time point measured.



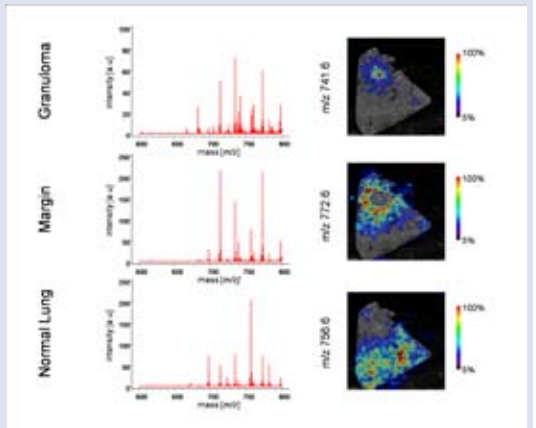
MS/MS image of product ions m/z 364 and 384 in treated lung tissue biopsy. The granuloma (white tissue) can be clearly distinguished and the drug is clearly localized within this region at the time point shown (140 mins post-dose)

PROSPECTS

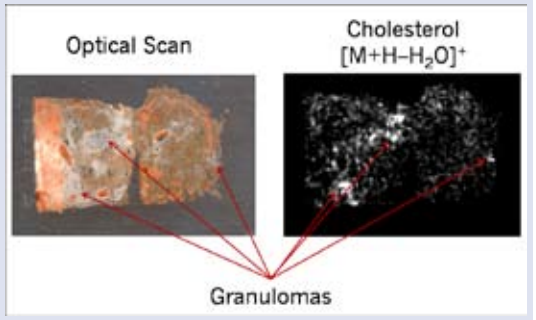
- High sensitivity MALDI MS and MS/MS imaging successfully applied to the localization of TB drugs in treated, infected tissue.
- Vast potential of MSI for the imaging of drugs in biological tissue for which alternative methods do not exist. The technology is of high value to the drug discovery process.

MALDI-MSI for localization of potential markers and lipids

After M. tuberculosis enter the lung, a complex immune response is triggered resulting in the formation of the tuberculosis granulomas. Understanding granuloma structure and formation process is of vital importance in both the deveopment phase of novel drugs and the optimization of the performance and delivery of existing compounds. MALDI-MSI has enabled tissue-specific profiles to be determined for granuloma, granuloma margin and healthy tissue regions.



MALDI-MS images for several potential diglyceride markers. Distinct profiles are observed for the granuloma, granuloma margin and healthy lung tissue.



MALDI MS image demonstrating the clear co-localization of cholesterol signal (m/z 369.3) with the granuloma regions of the lung tissue section.

PROSPECTS

- MALDI-MSI has been used to determine a range of tissue-specific markers for granulomas and surrounding regions.
- Identified markers have the potential to be used to monitor the therapeutic effects of novel and existing TB drugs.

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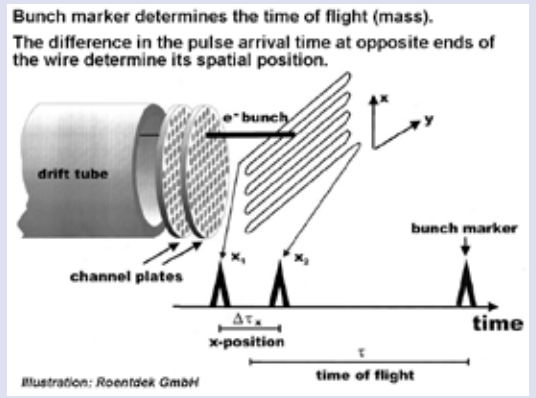
NOTES

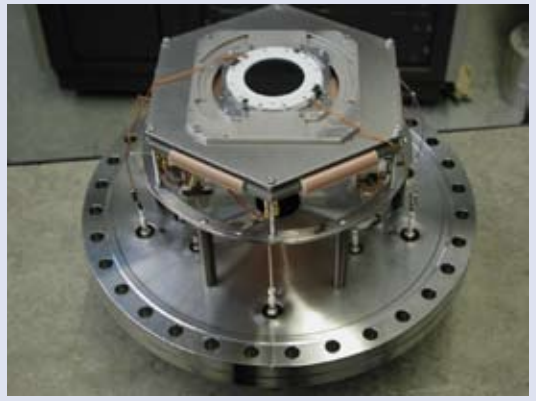
DEVELOPMENT OF A DELAY LINE DETECTOR FOR C60 SIMS MASS SPECTROMETRY IMAGING

Motivation and Theory

C60 SIMS allows higher molecular weight species to be analyzed compared to smaller metal-cluster beams. It is inefficient to focus the beam to small areas for high spatial resolution. Thus, a position sensitive delay line detector has been developed to allow full ion-current for microscope-mode C60 SIMS.



Theory of Operation



Delay Line Detector Assembly: Spatial Position is Measured on Three Delay Lines

PROSPECTS

* High spatial resolution C60 SIMS imaging at full ion current.

Performance and Application

The image resolving power of the C60 delay line detector combination is ~4 um. A section of rat kidney implanted with polymer has been imaged with the new apparatus.

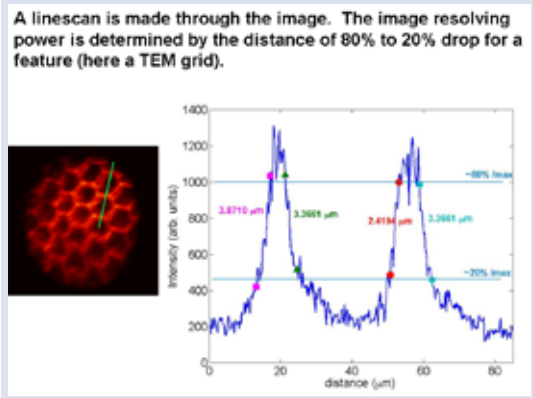
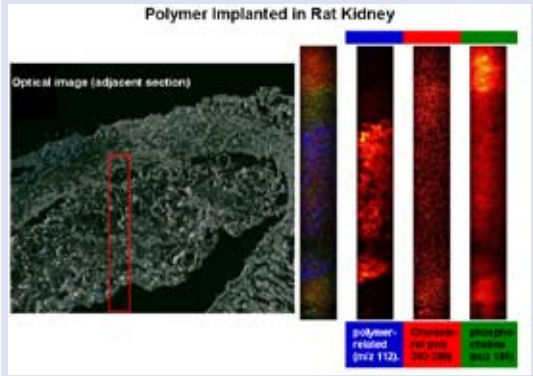


Image Resolving Power



Tissue Imaging of Implanted Polymer in Rat Kidney

PROSPECTS

* High spatial resolution microscope-mode C60 SIMS mass spectrometry imaging.

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REFERENCES

Klerk, L.A.; Kharchenko, A.; MacAleese, L.; Lockyer, N.P.; Vickerman, J.; Heeren, R.M.A. High resolution C-60 SIMS and MALDI microscopy using a Delay Line Detector, 57th ASMS Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, June 1-4, 2009.

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