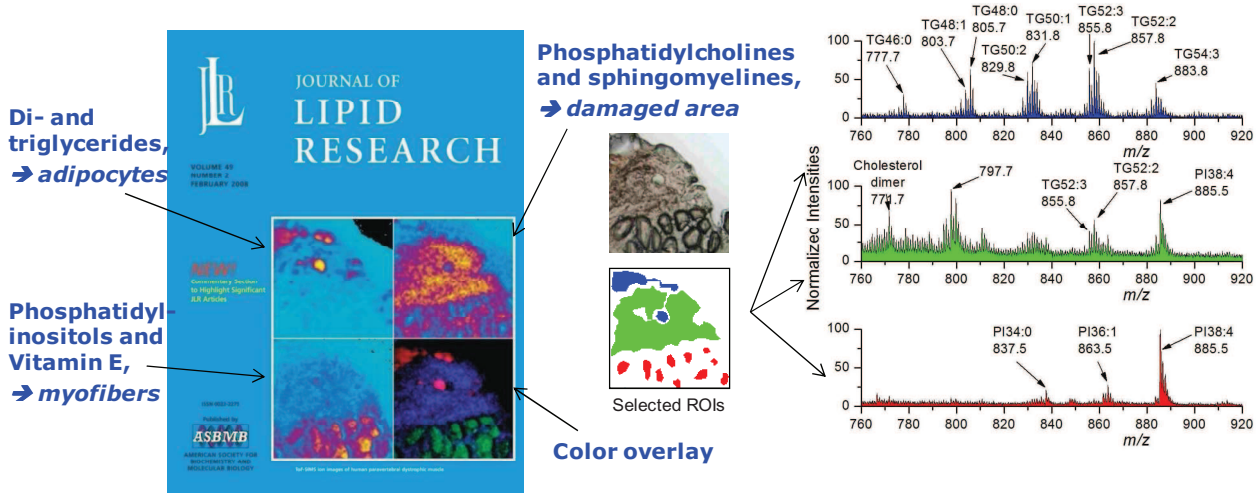

Figure 68: Some lipid biomarkers of the human Duchenne muscular dystrophy

Evaluation of gene therapy on MDX model

Gene therapy consists in the restoration of Dystrophin expression by exon skipping. In mdx mice, there is a premature stop codon in the exon 23 of the dystrophin gene. mRNA coded the mutated gene is unstable and there is no dystropin production in the cells. Intramuscular injection of an AAV vector coding for the small RNAs, complementary to the splicing branching point in the intron 22 and to the U1 binding region in the intron 23 leads to the deletion of the mutated exon 23 and restoration of the open reading frame. This approach permits to skip out the defective exon and thus reconstitute a shorter but functional modification of dystrophin. Expression of dystrophin is completely restored in two weeks followed by normalisation of the muscle morphology.

In this study we compared muscles from wild type, mdx and mdx mice after gene therapy by imaging SIMS mass spectrometry. Mdx model shows a complex time course of muscle degeneration/regeneration. In young and adult mice (up to 52 weeks old), muscle fibre necrosis is compensated by a vigorous regeneration, with a maximum of regeneration observed at 4 weeks. In order to perform a comprehensive comparison of muscle from wild type, mdx and mdx mice after gene therapy, we compared muscles of mice from 3 to 15 weeks old. Figure 69 presents the analyses performed on the muscle sections.

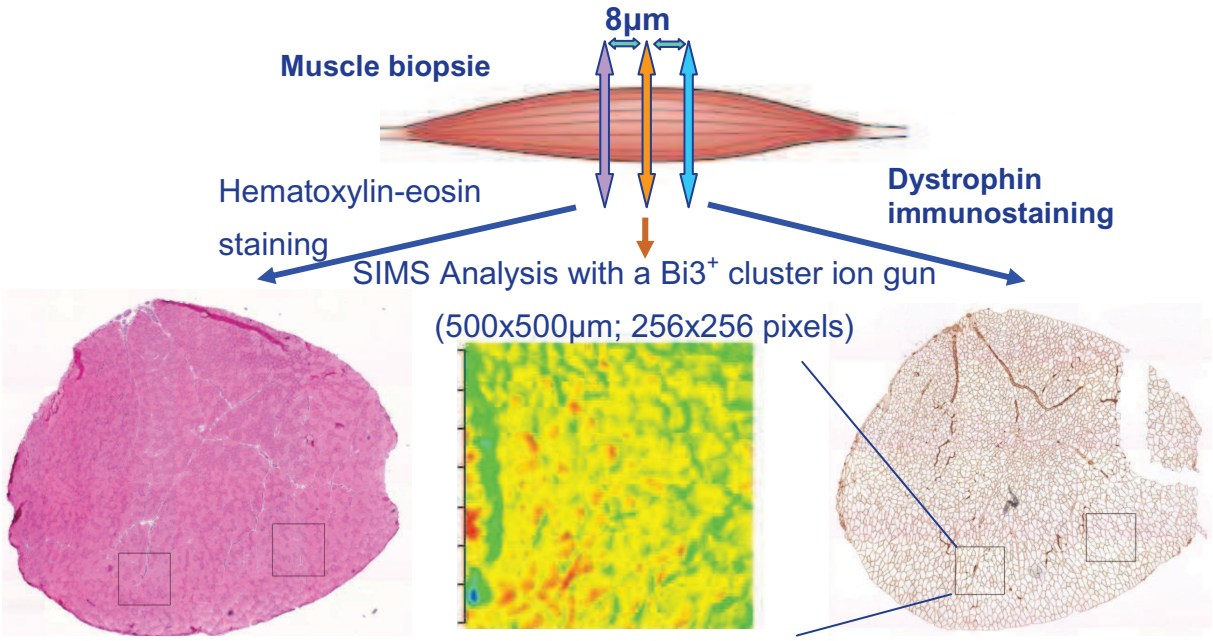

Figure 69: Repartition of muscle slices for analyses

Statistical analysis of the data was performed by using SpectViewer software developed by CEA and in collaboration with CEA. The following modules were explored: data conversion; statistical analysis; temporal and spatial correlation (PCA and K-means clustering). Data analysis was organized in the following data flow(Figure 70):

(1) Data pre-processing (conversion of .RAW data (Ion-ToF) into .FXS (FXS spectviewer) followed by data binning;

(2) statistical analysis (Wilcoxon signed rank test);

(3) temporal and spatial correlation (K-means clustering, PCA and hierarchical clustering).

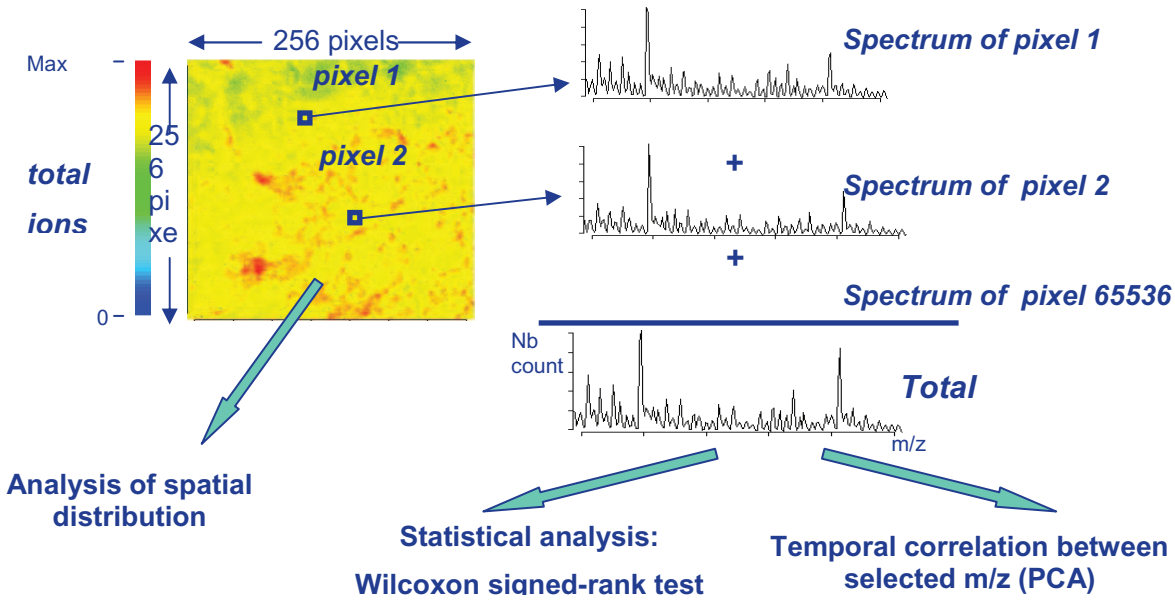

Figure 70: Data analysis workflow

1.3.6.6 Round-robin experiment with biological tissue

A wide variety of methodologies and instrumentation can be used for mass spectrometry imaging. As part of WP6, the scope and limitations of the imaging approaches at the different partner laboratories were evaluated in a systematic study. The results can serve as a guideline which method/instrument is most suitable for specific applications in order to assist scientists in choosing the best approach for their application. Standard biological tissue samples (mouse brain sections) were distributed to the partners for MIMS experiments. MALDI as swell as SIMS sources were included in this study. The instrumentation included quadrupole, time of flight and Fourier transform mass analyzers. SIMS measurements were able to resolve features in the range of 1 micrometer for small molecules such as fatty acids. The fastest measurements were acquired in the selected reaction monitoring mode (SRM) on a triple quad instrument. An overlay of selected measurements is shown in Figure 71.

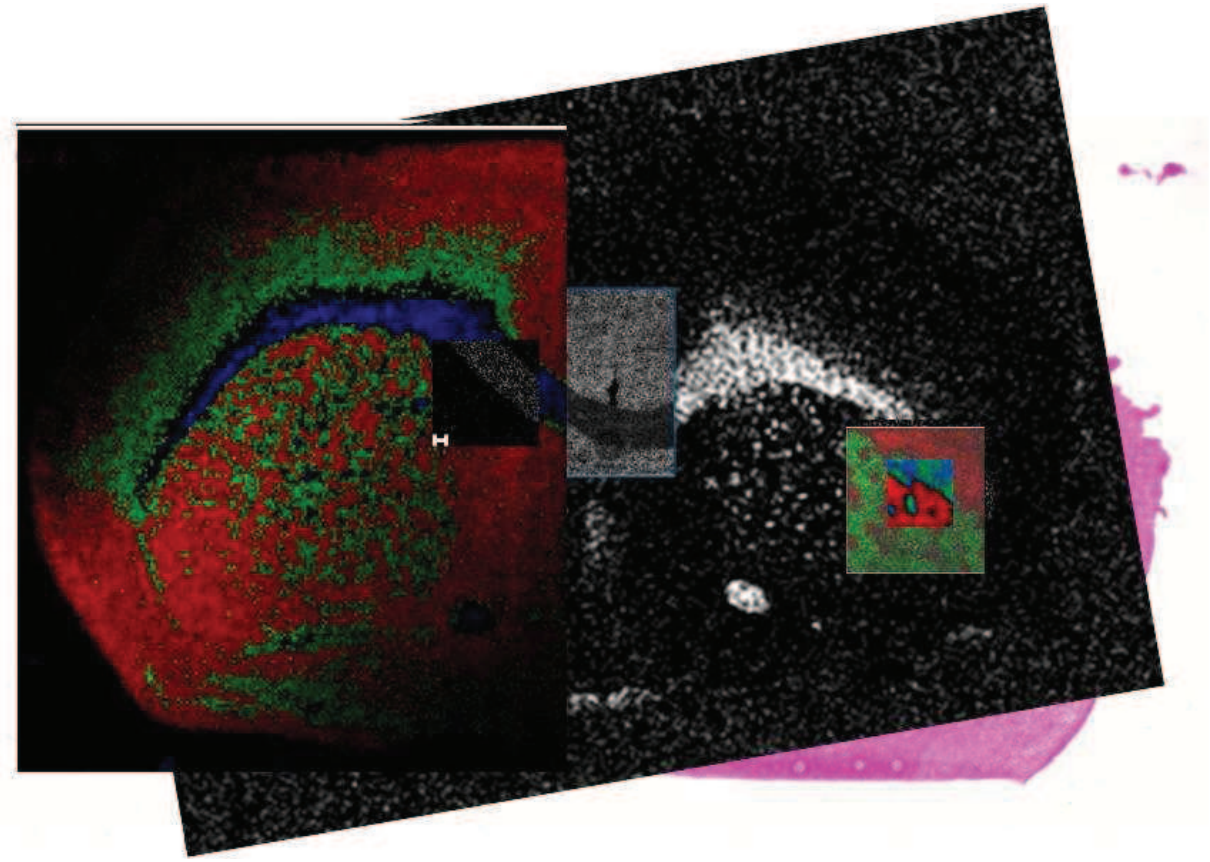

Figure 71: Overlay of selected MS images of a mouse brain section acquired by different methods

As expected each of the measurement techniques had specific advantages. The highest spatial resolution (down to 2 µm) was achieved with the SIMS-TOF measurements. The usage of cluster ion source allows detection of intact lipids with SIMS, which would not be possible otherwise. SIMS measurements of FOM can be resampled and thus areas of interest can be analyzed in more detail. The highest resolving power and mass accuracy was achieved by Fourier transform mass spectrometers (both ion cyclotron resonance and orbital trapping analyzers). A newly developed ion source allowed the combination of accurate mass measurements and spatial resolution in the micrometer range for phospholipids. The fastest measurements were acquired in the selected reaction monitoring mode (SRM) on a triple quad instrument utilizing continuous raster mode. This technique allows high through-put

screening of drug compounds and their metabolites. MALDI-TOF measurements were best suited for the analysis of proteins.

The best combination of sample preparation, ionization type and mass analyzer is highly dependent on analyte and sample properties and has to be chosen carefully. A more detailed description of the results will be published in a scientific journal in order to make them available to the MS imaging community.

1.3.6.7 Achievements of the project compared to the objectives and the state-of-the-art

Work package 6 is the final experimental work package of the COMPUTIS project. It integrates the developments of the other work packages which include sample preparation (WP1), instrumental developments (WP2), measurements techniques (WP3) and software tools (WP4&5).

The specific objectives of WP6 are:

- To investigate samples of tissue that is affected by disease
- To perform a functional exploration of muscles in normal, Duchenne muscular dystrophic (DMD) and gene therapy restored mouse models
- To validate differential analysis of molecular signatures from normal and diseased targets
- To determine changes in expression patterns and identify metabolites and biomarkers for different states of the progressive diseases adequate for treatment evaluation

Standardization and reference

The first part of WP6 focuses on protocols to analyze infected tissue and to provide cellular reference objects. Two different diseases were tackled within this task. First, tissue samples infected with a parasite were analyzed at Novartis and JLU. The results show that the combination of several different techniques is necessary in order to obtain useful results. Second, tuberculosis infected material was measured at Novartis. Methods were optimized to localize the distribution of several tuberculosis drugs (Rifampicin, Isoniazid and Pyrazinamide) in the tissue sections. Several lipid species were observed and the optimized methods have been applied to the subsequent analysis of a range of tuberculosis drugs in the rabbit lung and granuloma tissues.

Two different cell lines were labeled with a membrane and a cytoskeleton marker, respectively, in order to provide reference for the comparison of MIMS and non-MS imaging techniques. MS imaging of individual cells was achieved showing excellent agreement with fluorescent images of stained cells. These results confirm that the developed sample preparation protocols are suitable for the analysis of biological samples and the spatial integrity of the sample is not disturbed. They also represent the first measurements of single cells with the high accuracy of FT mass spectrometers.

Biomarkers from images

A number of diseases including tuberculosis, Duchenne muscular dystrophy and breast cancer were investigated by MS imaging. The investigation of tuberculosis granulomas by Novartis provided significant new information on the effectiveness of the drug compound moxifloxacin. The combination with SIMS imaging of various lipid species at AMOLF enabled significant advancements to the study of tuberculosis and therapies. Highly accurate measurements of drug compounds and neuropeptides in mouse model were obtained at JLU. These measurements are unique in terms of spatial resolution and mass accuracy. These measurements allow confident compound identification at the cellular level.

FOM investigated lipid markers for breast cancer and identified a series of possible marker compounds by principle component analysis. These measurements provided more reliable and specific information than classical histological techniques. FOM investigated tumor-specific proteins by high resolution mass spectrometry coupled to liquid chromatography.

A major focus of this report was Duchenne muscular dystrophy (DMD) which was investigated by multiple techniques. CNRS identified lipid markers compounds for DMD in humans by SIMS measurements. They also studied markers and the regeneration process of DMD in mouse model. A gene therapy for DMD was tested successfully in mice at Généthon.

The combination of different imaging techniques across partner laboratories resulted in complementary information which turned out to be essential for many biological applications. The results presented in this report are of high biological and medical relevance. They also represent major methodological improvements in MS imaging, which in many cases is a direct result of new technologies developed in work packages 1 to 5.

Method integration on tissue standards

A wide variety of methodologies and instrumentation can be used for mass spectrometry imaging. As part of WP6, the scope and limitations of the imaging approaches at the different partner laboratories were evaluated in a systematic study. Standard biological tissue samples (mouse brain sections) were distributed to the partners for MIMS experiments. SIMS measurements were able to resolve features in the range of 1 micrometer for small molecules such as fatty acids.

The fastest measurements were acquired in the selected reaction monitoring mode (SRM) on a triple quad instrument. The highest resolving power and mass accuracy was achieved by Fourier transform mass spectrometers (both ion cyclotron resonance and orbital trapping analyzers). A newly developed ion source allowed the combination of accurate mass measurements and spatial resolution in the micrometer range for phospholipids.

MALDI-TOF measurements were best suited for the analysis of proteins. In general the imaged compound distribution was in good agreement for all methods. The best combination of sample preparation, ionization type and mass analyzer is highly dependent on analyte and sample properties and has to be chosen carefully. It is planned to publish the results of this study in a scientific journal in order to make them available to the MS imaging community.

This study is the most comprehensive comparison of different MS imaging techniques based on the same tissue sample. The results can serve as a guideline which method/instrument is most suitable for specific applications in order to assist scientists in choosing the best approach for their application.

2 DISSEMINATION OF THE KNOWLEDGE

The dissemination of the knowledge to the scientific and industrial communities was addressed by work package 8 “Dissemination and Exploitation”. The dissemination beyond the consortium addresses the scientific community, the users / professionals / industrials potentially interested by the results reached during the project, as well as the public in general.

Communication towards the scientific community is essentially performed by means of publications in scientific journals, communications and posters presented in conferences. Information on the theme of the project and the main results can be found by the public on the public website of the project, with a booklet synthesizing the main results of the project.

A workshop presenting the main Computis results was organised as a satellite meeting of the International Mass Spectrometry Conference in Bremen in August 2009. A final Computis seminar was organised in Paris on 19 November 2009 to present main achievements of the Computis project and state of the art of internationally renowned experts in Mass Spectrometry.

2.1 Communications in journals and conferences

The knowledge acquired during the Computis project has been broadly disseminated through the scientific and industrial communities by means of papers published in scientific journals, communications and posters in scientific and technical meetings, invited lectures and even a book. The audience is international and addresses both the mass spectrometry community and more largely the scientific and industrial community.

If the first papers dealt with the objectives and specifications of the project and the work program, the majority of papers is largely presenting the results and achievements of all the partners relative to experimental technologies with applications, as well as the standard data format imzML developed by Computis for the images issued from mass spectrometry tools.

Table 6 summarizes the means of communication per year.

	Book	Publications in journals	Oral presentations in conferences with papers	Posters in conferences	Invited lectures	Total
2006			4	10	1	15
2007	1	8	12	10	4	35
2008		12	15	24	16	67
2009		15	15	34	46	110
2010	1	7			1	9
Total	2	42	46	78	68	236

Table 6: Synthesis of the paper and oral communications towards the scientific and industrial communities

The main publications are listed below:

Accurate Mass as a Bioinformatic Parameter in data-to-knowledge conversion: FT ICR for peptide de novo sequencing, B. Spengler. *European Journal of Mass Spectrometry* **13** (January 2007) 83-87.

Attempts for molecular depth profiling directly on a rat brain tissue section using fullerene and bismuth cluster ion beams. Delphine Debois, Alain Brunelle, Olivier Laprévote. *International Journal of Mass Spectrometry* **260** (February 2007) 115–120.

High-resolution MALDI imaging mass spectrometry allows localization of peptide distributions at cellular length scales in pituitary tissue sections, A.F. Maarten Altelaar, Ioana M. Taban, Liam A. McDonnell, Robert P.J. de Lange, Roger A.H. Adan, Wolter J. Mooi, Ron M.A. Heeren, Sander R. Piersma. *International Journal of Mass Spectrometry* **260** (February 2007) 203-211.

Microprobing and Imaging MALDI for Biomarker Detection, B. Spengler, book chapter of "MALDI-MS", F. Hillenkamp, J. Peter-Katalinic, eds., Wiley-VCH, Weinheim (February 2007) 109-130.

Identification of leptomeningeal metastasis-related proteins in cerebrospinal fluid of patients with breast cancer by a combination of MALDI-TOF, MALDI-FTICR and nanoLC-FTICR mass spectrometry, A. Roempp, L. Dekker, I. Taban, G. Jenster, W. Boogerd, H. Bonfrer, B. Spengler, R. Heeren, P. Sillevis Smitt, T. Luider. Proteomics **7** (February 2007) 474-481.

Tools and strategies for visualization of large image datasets in high-resolution imaging mass spectrometry, I. Klinkert, L.A. McDonnell, S.L. Luxembourg, A.F. Maarten Altelaar, E.R. Amstalden, S.R. Piersma, M. Konijnenburg, R.M.A. Heeren. Review of Scientific Instruments **78** (May 2007) 053716

Imaging Mass Spectrometry, L.A. McDonnell, R.M.A. Heeren. *Mass Spectrometry Reviews* **26** (July/August 2007) 606-643.

Mass Microscopy, M. Setou, R.M.A Heeren, M. Stoeckli, S. Simma. M. Matsumoto. *Seikagaku* **79** (September 2007) 874-879.

Artefacts of MALDI sample preparation investigated by high-resolution Scanning Microprobe MALDI Imaging mass spectrometry (SMALDI-MS), W. Bouschen, B. Spengler. *International Journal of Mass Spectrometry* **266** (October 2007) 129-137.

Lipid mapping in human dystrophic muscle by cluster-time-of-flight secondary ion mass spectrometry imaging. N. Tahallah, A. Brunelle, S. De La Porte, O. Laprévote. *Journal of Lipid Research* **49** (February 2008) 438-454.

A novel workflow control system for Fourier transform ion cyclotron resonance mass spectrometry allows for unique on-the-fly data-dependent decisions, I.M. Taban, Y.E.M. van der Burgt, M. Duursma, Z. Takáts, M. Seynen, M. Konijnenburg, A. Vijftigschild, I. Attema, R.M.A. Heeren. *Rapid Communications in Mass Spectrometry* **22 (8)** (April 2008) 1245-1256.

Automated, feature-based image alignment for high resolution imaging mass spectrometry of large biological samples, A. Broersen, R. van Liere, A.F. Maarten Altelaar, R.M.A. Heeren, L.A. McDonnell. *Journal of the American Society for Mass Spectrometry* **19** (6) (June 2008) 823-832.

In situ localization and quantification of surfactins in a Bacillus subtilis swarming community by imaging mass spectrometry, D. Debois, K. Hamze, V. Guérineau, J.-P. Le Caër, I. B. Holland, P. Lopes, J. Ouazzani, S. J. Séror, A. Brunelle, O. Laprévote. *Proteomics* **8** (August 2008) 3682-3691.

A high-resolution scanning microprobe MALDI ion source for imaging analysis on an ion trap / Fourier transform ion cyclotron resonance mass spectrometer, M. Koestler, D. Kirsch,

A. Hester, A. Leisner, S. Guenther, B. Spengler. *Rapid Comm. Mass Spectrom.*, **22** (September 2008) 3275-3285.

The patinas of the Dogon-Tellem statuary: A new vision through physic-chemical analyses, V. Mazel, P. Richardin, D. Debois, D. Touboul, M. Cotte, A. Brunelle, P. Walter, O. Laprévote. *J. Cultural Heritage* **9** (September 2008) 347-353.

Tissue analysis with high resolution imaging mass spectrometry, A.F. Maarten Altelaar, R.M.A. Heeren. Book: Mass spectrometry of proteins and peptides : Methods and protocols, Series: Methods in Molecular Biology **492**, edited by M.S. Lipton and Ljiljana Pasa-Tolic. - [New York;NJ]: Humana Press (24 October 2008) 295-308.

Mass-based classification (MBC) of peptides: Highly accurate precursor ion mass values can be used to directly recognize peptide phosphorylation. B. Spengler, A. Hester, *J. Am. Soc. Mass Spectrom.* **19** (December 2008) 1808-1812.

Quality of surface: The influence of sample preparation on MS based biomolecular tissue imaging with MALDI-MS and (ME-)SIMS, R.M.A. Heeren, B. Kürker-Kaletas, I.M. Taban, L.P. MacAleese, L.A. McDonnell. *Applied Surface Science* **255** (4) (December 2008) 1289-1297.

Concise representation of mass spectrometry images by probabilistic latent semantic analysis, M. Hanselmann, M. Kirchner, B.Y. Renard, E.R. Amstalden, K. Glunde, R.M.A. Heeren, F.A. Hamprecht: *Analytical Chemistry* 80 (24) (December 2008) 9649-9658.

Lipid Imaging with Cluster Time-of-Flight Secondary Ion Mass Spectrometry, A. Brunelle, O. Laprévote. *Analytical & Bioanalytical Chemistry* **393** (January 2009) 31-35.

Perspectives for Imaging Mass Spectrometry in the Proteomics Landscape, L. McAleese, E. R. Amstalden van Hove, J. Stauber; R.M.A. Heeren. *Proteomics* **9** (4) (February 2009) 819-834.

Nitromatrix provides improved LC-MALDI signals and more protein identifications, P. Kouvonen, L.A. McDonnell, R.M.A. Heeren, G. Corthals. *Proteomics* **9** (6) (March 2009) 1662-1671.

In situ lipidomic analysis of non-alcoholic fatty liver by cluster TOF-SIMS imaging, D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote. *Analytical Chemistry* **81** (April 2009) 2823-2831.

Sample preparation issues for tissue imaging by imaging mass spectrometry, B. Kükrer Kaletas, I.M. van der Wiel, J. Stauber, L.J. Dekker, C. Güzel, M. Kros, T. Luider, R.M.A. Heeren. *Proteomics* (Wiley) **9** (10) (May 2009) 2622-2633.

Imaging mass spectrometry: hype or hope? R.M.A. Heeren, D.F. Smith, J. Stauber, B. Kükrer-Kaletas, L. MacAleese. *Journal of the American Society for Mass Spectrometry* **20** (6) (June 2009) 1006-1014.

In-situ Primary Metabolite Localization on Rat Brain Section by Chemical Mass Spectrometry Imaging, F. Benabdellah, D. Touboul, A. Brunelle, O. Laprévote. *Analytical Chemistry* **81** (July 2009) 5557-5560.

Toward digital staining using imaging mass spectrometry and random forests, M. Hanselmann, U. Köthe, M. Kirchner, B.Y. Renard, E.R. Amstalden, K. Glunde, R.M.A. Heeren, F.A. Hamprecht. *Journal of Proteome Research* **8** (7) (July 2009) 3558–3567.

Mass spectrometry of art and cultural heritage, E.S.B. Ferreiraa, R.M.A. Heeren, K.J. van den Berg, C. Maines, K. Sutherland, C. Higgitt. International *Journal of Mass Spectrometry* **284** (1-3) (July 2009) 1-1.

Fast and automated large-area imaging MALDI mass spectrometry in microprobe and microscope mode, L.A. Klerk, A.F. Maarten Altelaar, M. Froesch, L.A. McDonnell, R.M.A. Heeren. *International Journal of Mass Spectrometry* **285** (1-2) (August 2009) 19-25.

MALDI reveals membrane lipid profile reversion in MDX mice, F. Benabdellah, H. Yu, A. Brunelle, O. Laprévote, S. De La Porte. *Neurobiology of Dis*ease **36** (November 2009) 252-258.

Mass Spectrometry Imaging of rat brain sections: nanomolar sensitivity with MALDI versus nanometre resolution by TOF-SIMS, F. Benabdellah, A. Seyer, L. Quinton, D. Touboul, A. Brunelle, O. Laprévote. *Analytical and Bioanalytical Chemistry* **396** (January 2010) 151-162.

Imaging mass spectrometry using a delay line detector, M. Froesch, S.L. Luxembourg, D. Verheijde, R.M. A. Heeren. *European Journal of Mass Spectrometry* **16** (1) (January 2010) 35-45.

C60+ Secondary ion microscopy using a delay-line detector, L.A. Klerk, N.P. Lockyer, A. Kharchenko, L. MacAleese, P.Y.W. Dankers, J.C. Vickerman, R.M.A Heeren. *Analytical Chemistry* **82** (3) (February 2010) 801-807.

Autoradiography, MALDI-MS, and SIMS-MS Imaging in Pharmaceutical Discovery and Development, E. Solon, A. Schweitzer, M. Stoeckli, B. Prideaux, *The AAPS Journal* **12** (1) (March 2010) 11-26.

imzML: Imaging Mass Spectrometry Markup Language – a common data format for mass spectrometry imaging in "Data Mining in Proteomics" edited by Michael Hamacher, Christian Stephan and Martin Eisenacher, Humana Press, New York. in press.

Characterization of cellular proteins by nanoLC-FTICR-MS/MS supporting the evaluation of imaging MS data, Y. Schober, M. Koestler, M. Walther-Schmidt, B. Spengler, A. Römpp. *Analytical and Bioanalytical Chemistry,* paper submitted.

2.2 Public websites

A public website was launched by CEA http://www.computis.org in April 2006, to host general information of the project, organization and contact information. As more information becomes available, the site will be updated accordingly. Responsibility for the public website was transferred to Novartis in 2007.

The public website http://www.computis.org/ was updated every trimester. Its final version includes a brief description of the main goals of the project, presentations of the results of the technical work packages (WP1 to WP6), the booklet synthesizing the main results of the project, a news chapter with summary of the Computis workshop at the IMSC conference in Bremen on 30 August 2009 and the final Computis seminar in Paris on 19 November 2009, the imzML data format specifications, a list of publications/communications, an updated list of the partners, and a contact address.

To promote the imzML standard format for imaging mass spectrometry, imzML was presented during several HUPO meetings and its adoption by the mass spectrometry manufacturers as a standard for data delivery is in progress. Specifications and examples files of imzML are available on http://www.maldi-msi.org/, as well as a free converter from LTQ-based ThermoFisher raw files into imzML files. The visualization tool Data Cube Explorer can also be downloaded freely on this website.

2.3 Workshop at the 18th IMSC conference in Bremen on 30 August 2009

A workshop entitled “Imaging mass spectrometry and data management strategies”, highlighting major results of the COMPUTIS project, was organised under the responsibility of Prof. Bernhard Spengler from JLU during the 18th International Mass Spectrometry Conference that took place in Bremen, Germany between August 30 and September 4, 2009. The conference gathered a record attendance of 2670 participants from 45 countries from all over the world.

http://www.imsc-bremen-2009.de/

Figure 72: Location of the IMSC conference in Bremen

The workshop was scheduled on Monday, August 30 from 6:00 pm to 8:00 pm in Borgward lecture room. About 60 participants attended the workshop.

The description associated with the workshop in the conference program was:

"Imaging mass spectrometry is about to become a platform technique of paramount importance and applicability. Technology and methodology for this field is still developing and is expressing huge progress constantly. New developments and fundamental research, as well as recent biomedical applications are presented in this workshop for being discussed on an international level. Topics presented include SIMS imaging, MALDI microprobe imaging, stigmatic (microscope mode) imaging, imaging software, data exchange and applications."

The programme of the workshop was the following:

- 6:00 pm Mass Spectrometry Imaging in the EU consortium COMPUTIS
Olivier Gal, CEA, Saclay, France
- 6.05 pm High spatial resolution imaging using Orbitrap FTMS
Bernhard Spengler, Justus Liebig University, Giessen, Germany
- 6:20 pm Multimodal MSI in breast cancer research
Ron M.A. Heeren, FOM Inst. Atomic/Molecular Physics, Amsterdam, Netherlands
- 6:35 pm Lipid mapping by cluster SIMS
Olivier Laprévote, ICSN-CNRS, Gif Sur Yvette, France
- 6:50 pm Biomarker detection for Duchenne dystrophy
Fedor Svinartchouk, Généthon, France
- 7:05 pm "imzML" - a common data format for imaging mass spectrometry
Andreas Römpp, Justus Liebig University, Giessen, Germany
- 7:20 pm Algorithms to process MSI data
Vincent Picaud, CEA LIST, Saclay, France
- 7:35 pm Impact of MSI research
Brendan Prideaux, Novartis Institute for BioMedical Research, Basel, Switzerland

A booklet describing the progress and results of the Computis project from January 2006 until May 2009 was compiled by all partners of the project, edited by FOM and distributed to all participants of the workshop. This booklet can be downloaded on the public website of the Computis project. http://www.computis.org/

2.4 Final COMPUTIS seminar in Paris on 19 November 2009

CEA organised with the help of CNRS, on 19th November 2009, a final COMPUTIS seminar entitled "Mass Spectrometry Imaging Workshop" in the premises of the Centre of Research and Restoration for Museums of France (C2RMF) located within the Louvre building in Paris.

The seminar aimed at presenting the major results of the COMPUTIS project by the partners of the project, as well as up to date work and results from 3 European experts and 1 Canadian expert in mass spectrometry imaging.

The programme of the workshop was the following:

Mass Spectrometry Imaging Workshop – Paris – November 19th, 2009

Programme

09:00	**Coffee and Registration**
09:30	**Introduction to the Workshop:** **Welcome in C2RMF** – Pascale Richardin (C2RMF, Paris) **The Computis Project** – Marie-France Robbe (CEA, LIST, Saclay) ***Chairman***: Olivier Laprévote (CNRS, ICSN, Gif-sur-Yvette & Univ. Paris 5, Paris)
10:00	**Imaging mass spectrometry, principle and potentials: Ten years of collective efforts** Pierre Chaurand (Université de Montréal, Canada)
10:30	**Lipid imaging by mass spectrometry: MALDI-TOF and TOF-SIMS** Alain Brunelle (CNRS, ICSN, Gif-sur-Yvette)
10:55	**Developments in high resolution imaging mass spectrometry** Ron M.A. Heeren (FOM, AMOLF, Amsterdam)
11:20	**ToF-SIMS imaging of organic materials in cultural heritage: From their identification to their spatial organisation** Pascale Richardin (C2RMF, Paris)
11:50	**Lunch**
	Chairman: Olivier Gal (CEA, LIST, Saclay)
12:50	**MS manufacturers presentations** Applied Biosystems, Bruker Daltoniks, Leap Technologies, Thermo Fisher Scientific, Waters
13:40	**A close-up view of biological samples: High-resolution MALDI imaging and image analysis** Bernhard Spengler (Justus Liebig University, Giessen)
14:05	**Visualization, denoising and structuration of data for MSI** Jean-Pierre Both (CEA, LIST, Saclay)
14:30	**Specificity in MALDI-MSI – What are we imaging?** Malcolm Clench (Hallam University, Sheffield)
15:00	**Pause**
	Chairman: Andreas Römpp (Justus Liebig University, Giessen)
15:15	**MALDI-MSI for clinical proteomics** Isabelle Fournier (Université Lille 1)
15:45	**MIMS studies in evaluation of gene therapy on dystrophic mice (mdx) model** Fédor Svinartchouk (Genethon, Evry)
16:10	**The future of MS imaging in biomedical research** Markus Stoeckli (Novartis, Basel)
16:35	**Conclusions – round table**
17:00	**End**

The seminar was sponsored by the Ile de France district, the French Society of Mass Spectrometry SFSM and 5 manufacturers of mass spectrometry: Applied Biosystems, Bruker Daltoniks, Leap Technologies, Thermo Fisher Scientific and Waters.

The C2RMF lent freely an amphitheatre for the conference presentations, and a room for the posters and catering.

115 participants registered to the seminar and more than 100 people participated freely in the seminar. The distribution of participants was (Figure 73): 81 from France, 9 from the United Kingdom, 7 from Switzerland, 6 from Germany, 6 from the Netherlands, 2 from Belgium, 2 from Italy, 1 from Canada, and 1 from the United States.

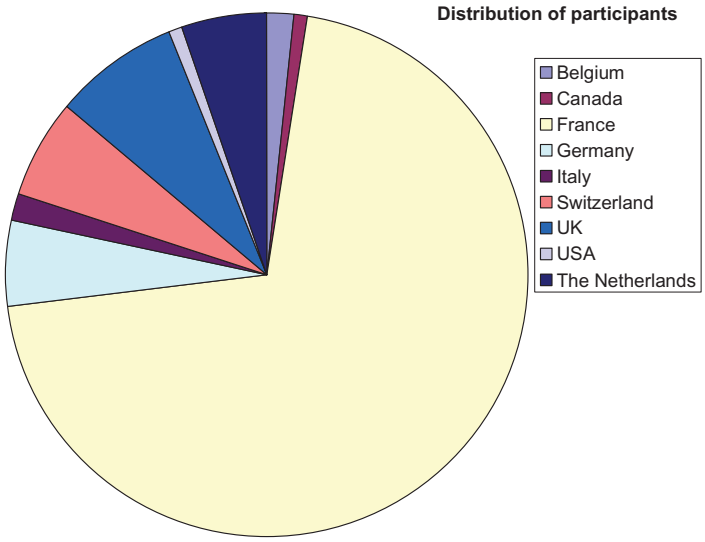

Figure 73: Distribution of participants to the final Computis seminar

Figure 74: Photos of the Final Computis seminar

2.5 Booklet

A booklet presenting the major results of the Computis project was edited in May 2009. It presents the work and results of the project up to this date by means of 16 thematic posters dealing with the organization and objectives of the project, sample preparation, C60 imaging of tissue and cluster ion sources for SIMS, Duchenne muscular dystrophy, high mass resolution and accuracy FT-ICR MSI, from signal to data, from data to knowledge, imzML,

biological applications (cell cultures, breast cancer, study of a dermal uptake of a novel acne compound, distribution of tuberculosis drugs in rabbit lungs), development of a delay-line detector for C60 SIMS MSI. The booklet was distributed at both Computis workshop during the IMSC conference in Bremen in August 2009 and the final Computis seminar in Paris in November 2009.

The booklet was distributed during the workshop at the IMSC conference and the final COMPUTIS seminar; this booklet can also be downloaded on the public website of the project www.computis.org.