

3 PLAN FOR USING AND DISSEMINATING THE EXPLOITABLE RESULTS

Work package 7 concerns the development of industrial concepts for the technologies and software comprised in the Computis project. This was addressed by first defining the needs in biomedical and industrial pharmaceutical research where MIMS could contribute unique and valuable information. Information was collected via a survey with pharmaceutical representatives (Novartis) and reviews of the biomedical needs (FOM). Descriptions and cost information were collected and compiled for all the systems and sub-systems developed through the project.

Both MALDI (Novartis) and SIMS (CNRS) MSI technologies were applied to the localization of a topically-applied anti-acne development compound in skin. The dual approach was utilized to assess the effectiveness of several drug delivery formulations at delivering the compound across the stratum corneum and into the sebaceous gland target organs. High spatial resolution MALDI-MSI of Novartis development compounds and endogenous lipids were performed on whole body rat sections by JLU.

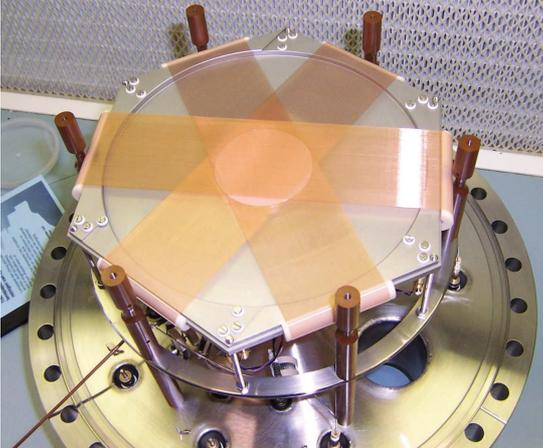
The imzML new standard format for imaging mass spectrometry data was heavily promoted at multiple conferences and seminars. Dissemination occurred through oral and poster presentations and interactive discussions held in MSI workshops at major Mass Spectrometry conferences. JLU held discussions with all leading MSI instrument manufacturers resulting in the data format being adopted by the companies.

To identify and gain a greater understanding of potential applications and users of MSI as well current research being conducted, a survey was performed by CEA comprising selected existing MS and MSI experts and instrument manufacturers. Information regarding software and hardware developed within the framework was disseminated at national and international conferences in the form of presentations and meetings. Contacts with manufacturers and potential users were conducted during seminars and conferences throughout the activity period.

At the end of the Computis project, the exploitable results are:

- Delay-line detector
- Sputnik robot spotter
- Post Source Decay-like method
- imzML data standard format for imaging mass spectrometry data
- imzML converter for Thermo LTQ-based raw files
- Easy MSI software
- Data Cube Explorer software
- VBrowser software
- SIMS lipid reference database

Tables 7 to 15 present the information relative to the main results of the COMPUTIS project.

Title	Delay-line detector
Abstract	<p>A hexanode delay-line detector was mounted on a MALDI ToF mass spectrometer. These detectors, originally designed for photo-emission electron microscopy, can provide down to 80 μm resolution. When combined with an ion optical system that provides a magnification of 100x this will yield a resolution in mass microscopy of 800 nm.</p> <p>The position information is calculated from the runtime difference of an electron cloud from the MCP that hits the detector wires at a certain position.</p> <p>The outcoupling of the signals is realised via capacitors, one preamplifier per line and an ADC card. The detector is equipped with a phosphorscreen to provide ion optical tuning capabilities during microscope mode imaging mass spectrometry.</p> <p>The delay-line detector was assembled and mounted on a non-imaging MALDI-ToF mass spectrometer to test the data acquisition, control (and optimization) and processing facilities of the existing software. Such a detector has the potential to essentially improve the spatial resolving power and is adaptable to many common imaging mass spectrometers.</p>  <p>Figure 75: Delay-line detector</p>
Innovative aspects	High resolution simultaneous arrival time and position detection with multi hit capability
Collaboration details	<p>ULVAC-PHI, 370 Enzo, Chigasaki, Kanagawa, 253-8522 Japan to develop a new detector design suitable for their commercial instruments for use in a direct imaging mass microscope.</p> <p>RoentDek Handels GmbH, c/o Institut für Kernphysik, Max-von-Laue-Strasse 1, D-60438 Frankfurt am Main, Germany, the company delivering the DLD components.</p>
Collaboration sought	Conception of an industrial product and manufacturing agreement with ULVAC-PHI
Market applications	High resolution mass spectrometry

Subject descriptors	High resolution system to detect the position and arrival time of ions
Organization	FOM/AMOLF Kruislaan 407, 1098 SJ, Amsterdam, The Netherlands
Organization type	Academic institute
Property rights	Roentdek holds IP on hexanode detector FOM holds copyright on deconvolution software
Sources of support	Partial EU supporting
Stage of development	Laboratory prototype

Table 7: Description of the delay-line detector characteristics

Title	Sputnik robot spotter
Abstract	<p>In MALDI mass spectrometry, the precise and reproducible deposition of small matrix droplets, followed by the acquisition of spectra at the position of these droplets, is found to be in some cases more sensitive and easy to manage than coating and imaging an entire surface. This simple method, which is called <i>profiling</i>, needs a system in which the location of the droplet deposition and the droplet deposition are automated.</p> <p>The spotting robot so-called “Sputnik” is dedicated to organic matrix droplets deposition with a regular deposition step. The droplets are delivered by a patented piezoelectric ejector, having different nozzle diameters (80 & 150 μm), with different ejected droplet volume (1 & 6 nl, respectively). The ejector is connected to a syringe pump and all the sucking, ejection and rinsing steps are fully automated and computer controlled. The precise positioning of the droplet on the sample is performed by a computer controlled plate. This system is presently able to deposit several droplets of $\sim 400 \mu\text{m}$ diameter (depending on the tissue, the sample support, etc) at a given position.</p>  <p>Figure 76 : Sputnik spotter</p>
Innovative aspects	<p>Coordinates of the deposited droplets can be transferred to the MALDI spectrometer as a “spot set”</p> <p>Droplets and sample surface can be controlled by an optical microscope</p>

	fitted with a video camera, with no parallax
Collaboration details	The Sputnik prototype was developed in collaboration with the French company Siliflow SAS, 26, rue Barthélemy de Laffemas, 26000 Valence, France, http://www.siliflow.com
Collaboration sought	Manufacturing agreement with Siliflow
Market applications	Profiling in mass spectrometry
Subject descriptors	Deposition of liquid droplets needing a precise and reproducible positioning
Organization	Centre National de Recherche Scientifique-Institut de Chimie des Substances Naturelles, 1 avenue de la Terrasse, 91198 Gif sur Yvette cedex, France
Organization type	Academic institute
Property rights	Siliflow
Sources of support	Partial EU supporting
Stage of development	Laboratory prototype

Table 8: Description of the Sputnik robot spotter

Title	Post-Source Decay-like method
Abstract	<p>A Post Source Decay-like (PSD-like) MS/MS mode was developed for a SIMS-TOF mass spectrometer. The time-of-flight mass analyzer fitted with an electrostatic mirror can be used to separate stable ions from those named metastable ions which decay in flight.</p> <p>These latter fragment ions keep in the field free spaces the same velocity as their precursors but spend less time in the reflectron because turning back at a smaller depth in it, due to their lower kinetic energy. The analysis of the in-flight decays of metastable ions is called Post-Source Decay (PSD). The precursor ions are selected using a coincidence method derived from the electronics and data acquisition of nuclear physics; this method was possible due to the recording of single-ion events.</p> <p>The performances of the method on molecules currently observed in TOF-SIMS imaging was studied: mammalian tissue sections and well known fragmentation pathways (phosphocholine, a phosphatidylcholine (PC34:1) and cholesterol). Pure samples were first analyzed, their structure verified, then compared to analyses of tissue sections. The possibility to acquire PSD images on a tissue section was demonstrated.</p>
Innovative aspects	Enables MS/MS analysis for SIMS imaging

Collaboration details	The Post-Source Decay like method was proposed to ION-TOF company, without answer from this company.
Collaboration sought	None
Market applications	None
Subject descriptors	Partial structural analysis of lipids by TOF-SIMS
Organization	Centre National de Recherche Scientifique-Institut de Chimie des Substances Naturelles, 1 avenue de la Terrasse, 91198 Gif sur Yvette cedex, France
Organization type	Academic institute
Property rights	CNRS. Not patented. Method published in Rapid Communications in Mass Spectrometry.
Sources of support	Partial EU supporting
Stage of development	Proof of concept

Table 9: Description of the Post-Source Decay-like detector for ToF-SIMS

Title	imzML data standard format
Abstract	<p>The rapid increase in MS imaging applications has lead to a large number of different data formats. This complicates the comparison and exchange of MS imaging data. Existing common data formats are not sufficient to describe an MS imaging experiment completely. MS imaging data sets consist of a large number of mass spectra which are usually acquired with identical settings. The data format imzML was developed to allow the flexible and efficient exchange of MS imaging data between different instruments and data analysis software.</p> <p>For this purpose, the MS imaging data is divided in two separate files. The mass spectral data is stored in a binary file to ensure efficient storage. All metadata (e.g. instrumental parameters, sample details) are stored in an XML file which is based on the standard data format mzML developed by HUPO-PSI. The original mzML controlled vocabulary was extended to include specific parameters of imaging mass spectrometry (such as x/y position, spatial resolution). The two files (XML and binary) are connected by offset values in the XML file and are unambiguously linked by a universal unique identifier. The resulting datasets are comparable in size to the raw data and the separate metadata file allows flexible handling of large datasets.</p> <p>Several imaging MS software tools already support imzML. This allows choosing from a (growing) number of processing tools. One is no longer limited to proprietary software, but is able to use the processing software</p>

	<p>which is best suited for a specific question or application. On the other hand, measurements from different instruments can be compared within one software application using identical settings for data processing. All necessary information for evaluating and implementing imzML can be found at http://www.maldi-msi.org/</p> <p>There has been an increasing interest by scientists within the MS imaging community. imzML has the potential to become the universal data format for MS imaging worldwide.</p>
Innovative aspects	imzML enables the flexible and efficient exchange of complete MS imaging data sets. This was not possible with previous data formats such as mzML or DICHOM.
Collaboration details	The data format has already been adopted by all major companies that manufacture MS imaging instrumentation: Thermo Fisher Scientific (San Jose, CA, USA), Bruker Daltonik GmbH (Bremen, Germany), Waters (Manchester, UK), AB Sciex (Concord, Ontario, Canada). Thermo and Bruker will implement filters for imzML in their MS imaging software tools. Waters plans to develop a new MS imaging software suit that is based on imzML AB Sciex software is based on Biomap which already support imzML.
Collaboration sought	Information exchange with HUPO, MSI manufacturers and MS community
Market applications	Mass spectrometry sector, free use and dissemination of the standard format
Subject descriptors	Standard format for the storage/exchange of mass spectrometry imaging data
Organization	Justus Liebig University, Schubertstrasse 60, Building 16, 35392 Giessen, Germany
Organization type	Academic institute
Property rights	Computis partners
Sources of support	Partial EU supporting
Stage of development	Version 1.1.0 was released in September 2009. This version is functional and stable. It is used on an everyday basis in Computis labs and a growing number of other institutes as well.

Table 10: Description of the imzML data standard format characteristics

Title	imzML converter for Thermo LTQ-based raw files
Abstract	A conversion module was developed to proprietary data (Thermo LTQ-based *.RAW files) into imzML data format. This module provides a flexible basis to integrate additional data formats. It can be downloaded freely on the imzML website http://www.maldi-msi.org/
Innovative aspects	First converter towards a proprietary data format to the standard imzML format
Collaboration details	The converter can be (and is) used by academic institutions and industrial companies. It is applicable for all modes of MS imaging including biomarker identification, drug compound monitoring, histological tissue analysis and many more.
Collaboration sought	Information diffusion
Market applications	The converter is freely available on www.imzml.org .
Subject descriptors	Converter from Thermo LTQ raw data to imzML format
Organization	Justus Liebig University, Schubertstrasse 60, Building 16, 35392 Giessen, Germany
Organization type	Academic institute
Property rights	Property rights belong to Justus Liebig University.
Sources of support	Partial EU supporting
Stage of development	Demonstrator

Table 11: Description of the imzML converter for Thermo LTQ-based raw files

Title	Easy MSI software
Abstract	<p>Easy MSI is a user-friendly software in C++ to view and interpret mass spectrometry images (MS). It runs under Linux and Windows platforms. Easy MSI provides basic functionalities for data display and spectral/spatial exploration, and a user interface for some more specialized treatments such as denoising spectra or structure analysis. The main functionalities of the visualization module are zooming, peak or pixel picking, interactive tool to define polygonal regions of interest and display of the resulting spectrum, display of weighted total image, correlation matrix between peaks, and dump in SVG or postscript formats.</p> <p>Easy MSI offers various tools to bring out automatically the important information lying in MS images: indicators to detect interesting peaks or</p>

peaks correlated to a given one. It can perform elementary transformations of data (image cropping and image binning) and spectrum filtering (wavelet denoising for MALDI data, background suppression).

Easy MSI offers clustering tools to evaluate the degree of structuration of images and to partition the dataset into subsets. Pixels can be classified according to the similarity of the pixel spectrum (pixel clustering) or mass m/z according to the similarity of the corresponding images (m/z clustering). The module proposes a choice between several clustering algorithms with hierarchical capabilities.

The “Diffusion map” module performs clustering analysis on reduced data. The advantages of this method come from the use of compressed data that can be stored, then offering the possibility to carry out quickly as many clustering operations as wished, as well as the presence of an adjustable denoising parameter during clustering operations thus offering a possibility of denoising without time consumption.

Moreover Easy MSI is able to perform multimodal 2D image registration. It can be used, for instance, for registering microscopy images with images extracted (clusters, total current...) from the spectral data. In order to offer the multimodal registration capability, the chosen criterion for registration is the mutual information between the two images.

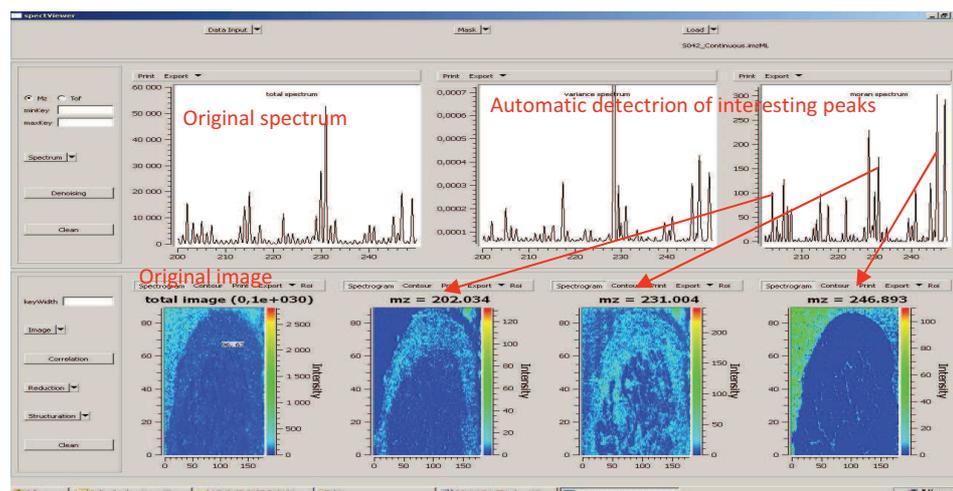


Figure 77: Easy MSI screenshot

Innovative aspects

Easy MSI offers a time efficient solution compared to manual interpretation, with a high quality and accuracy of the automated treatment compared to basic visualization tools. Easy MSI handles the imzML data format standard, thus it is compatible with any MSI-TOF equipment. In addition, it includes several clustering methods to bring out the different zones of the image and it offers assistance to the diagnosis: registration of images from complementary imaging technologies, and interrogation of biological databases in a near-future.

Collaboration details

CEA searches a partner for the commercialisation of Easy MSI. This partner can be a mass spectrometry manufacturer that can propose for sell the software with its equipment or include some modules into its own software, or a software editor to distribute the software. CEA are searches partners to continue the development of the software via a direct financial

	support or the participation to research national or European funded projects.
Collaboration sought	Distribution agreement with a mass spectrometry manufacturer or a software editor. Collaboration with scientific teams to continue the development of Easy MSI.
Market applications	Mass spectrometry in the fields of health/biology research and pharmaceuticals industry for biomarker discovery, disease diagnosis or clinical tests, as well as semiconductor and material industry.
Subject descriptors	Software to view and process mass spectrometry images with assistance for data interpretation
Organization	CEA-LIST, Saclay Research Center, Building 516, 91191 Gif sur Yvette cedex, France
Organization type	Academic institute
Property rights	Property of CEA. No patent.
Sources of support	Partial EU supporting
Stage of development	Demonstrator

Table 12: Description of Easy MSI software characteristics

Title	Data Cube Explorer software
Abstract	DataCube Explorer is a lightweight visualization tool for analyzing MSI datasets. It offers both an image-based as a spectrum-based view of the data, with an easy way to dynamically scroll through the masses in a dataset. Some features are on-the-fly spectral binning, region-of-interest spectral analysis, and image smoothing. It also includes a self-organizing map feature for image classification.

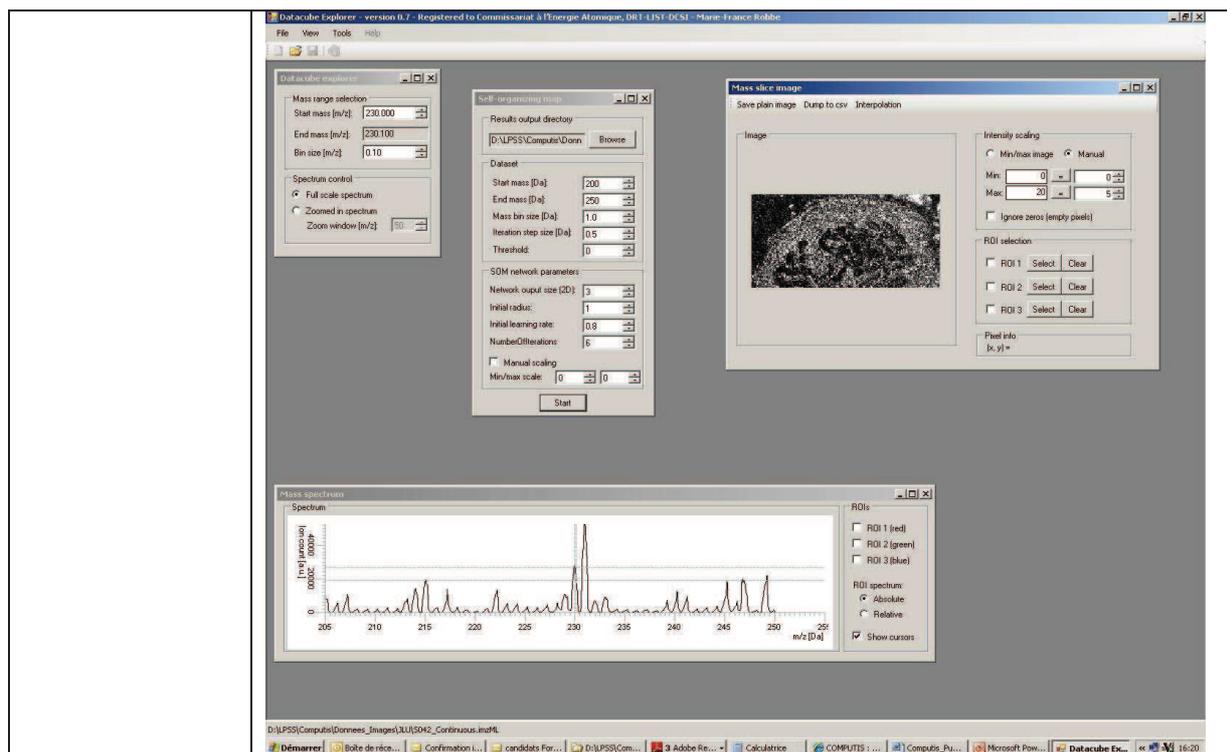
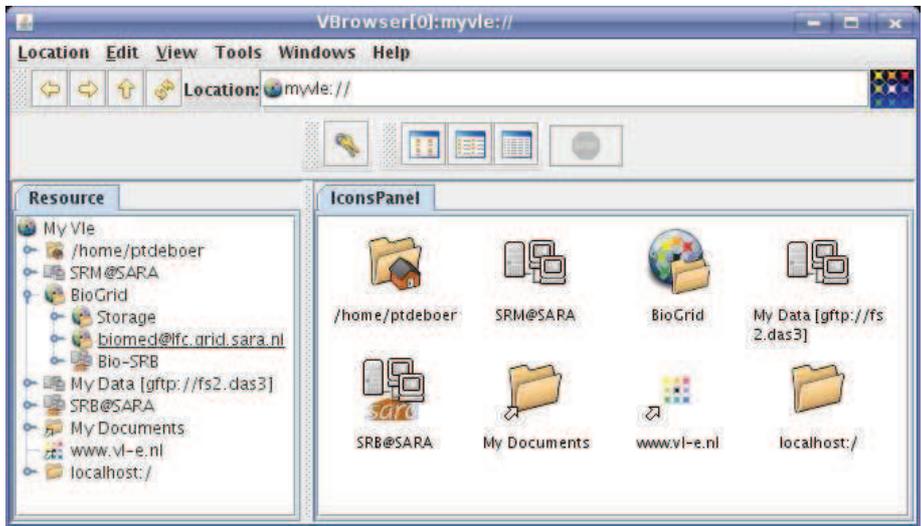


Figure 78: Data Cube Explorer screenshot

Innovative aspects	Quick open access tool for imaging MS data exploration and processing. Image reduction possibilities on-the-fly
Collaboration details	Data Cube Explorer can be downloaded freely on http://www.maldi-msi.org/
Collaboration sought	Information exchange.
Market applications	Data Cube Explorer is made free available in the technological domain at no cost to the MSI research community. As a result there is limited economic market.
Subject descriptors	Software tool for imaging MS data exploration and processing
Organization	FOM/AMOLF Kruislaan 407, 1098 SJ, Amsterdam, The Netherlands
Organization type	Academic institute
Property rights	FOM holds copyright, licence freely available
Sources of support	Partial EU supporting
Stage of development	Demonstrator

Table 13: Description of Data Cube Explorer software

Title	VBrowser software
Abstract	<p>VBrowser is a uniform frontend interface to file-based resources. It provides a familiar Graphical User Interface (GUI) for accessing networked resources, similar to an explorer-like browser. It process both local file systems and Grid-based file structures, including Grid-FTP, SSH-FTP, SRM, LFC, SRB, among other structures, and presents them to the user in a homogeneous and familiar tree.</p> <p>The interface of VBrowser can be further customized by the user, by creating virtual folders and links to remote (online) resources. As such, the user can group relevant files and other resources together into a single (virtual) browsable environment.</p> <p>The imzML Metadata Browser is a plug-in within the VBrowser framework. It provides advanced features while browsing imzML data files. It renders both tree-like navigation and text-based XML navigation of imzML metadata. It is even possible to associate the imzML resources to specific mass spectrometry applications.</p> <p>The imzML Metadata Browser holds also an internal metadata repository of imzML resources so that the user does not have to check the files again. The plug-in further expands the repository by simple navigation of imzML files. This mechanism allows the scientists to easily locate and select the proper imzML resource without the hassle of check all the resources manually. As part of keep information about the files, the internal repository also keeps the references to specific imaging analysis applications which can be used with the imzML files.</p>  <p>Figure 79: Main interface of vBrowser</p>
Innovative aspects	<p>Among others, the innovative aspects of VBrowser + imzML Metadata Browser features include the following:</p> <ul style="list-style-type: none"> • Easy to use • Cross-platform. Runs on Windows, Linux, Mac OS X and more • Supports metadata descriptions of transfer of large files

	<ul style="list-style-type: none"> • Tree-based and tabbed user interface • Resource Manager and configuration wizard (connections to local and remote sources) • Drag & drop support • Filename filters • Synchronized directory browsing • Seamlessly transfer of files between remote sources • imzML metadata catalogue descriptions • Single sign-on authentication to all (remote) Grid resources using Grid proxy authentication. • Easy "single click" viewing of remote data for both standard and application specific data. • Extensible framework for application development using simple applet style plugins to view custom data. • Multi client framework for Grid file protocols, OGSA and WSRF compliant web- and Gridservices, supporting Grid-FTP, SRM, LFC, SRB, SSL/TLS (FTPS) and SSH File Transfer Protocol (SFTP)
Collaboration details	<p>VBrowser and the imzML Metadata Browser have been announced as available within the BiG Grid context. The BiG Grid is a joint project of the Netherlands Bioinformatics Centre (NBIC), the National Computing Facilities Foundation (NCF) and the National Institute for Subatomic Physics (Nikhef). BiG Grid aims to set up an advanced ICT infrastructure for scientific research, in order to support e-science. The Big Grid project facilitates access to advanced hardware installations, middleware solutions and supporting manpower.</p>
Collaboration sought	<p>The BiG Grid is a joint project of the Netherlands Bioinformatics Centre (NBIC), the National Computing Facilities Foundation (NCF) and the National Institute for Subatomic Physics (Nikhef).</p>
Market applications	<p>VBrowser + imzML Metadata Browser is made available to anybody who wishes to download it. PCC plans to get financial benefits from its support and technical assistance.</p> <p>Rebuilds of these two products are free but do not get any commercial support or consulting services from PCC. PCC may also gain benefits by providing extensions or specific rebuilds of the products.</p>
Subject descriptors	<p>Software Engineering: Distribution, Maintenance, and Enhancement portability; Information Management: Data Structure, File Management Metadata Languages; Information Systems Applications: File Manager (client)</p>
Organization	<p>Power Computing & Communications UvA BV, PO Box 2535, Kruislaan 403, 1000 CM, Amsterdam, The Netherlands</p>
Organization type	<p>SME</p>
Property rights	<p>Both VBrowser + imzML Metadata Browser are developed as open source software. The software can be distributed in both binary and</p>

	source code without restrictions.
Sources of support	EU funding, Dutella project of the VL-e laboratory
Stage of development	Demonstrator

Table 14: Description of vBrowser software

Title	SIMS lipid reference database
Abstract	A first database of reference spectra of components for lipids was acquired in TOF-SIMS with bismuth aggregates. Today, this database is composed of 24 lipids in 12 classes. In a near future, this catalogue could be included in a digital database in order to be used through analysis software.
Innovative aspects	First database of reference spectra for lipids in TOF-SIMS
Collaboration details	Work entirely done by CNRS
Collaboration sought	Any company or lab interested by TOF-SIMS lipid analysis.
Market applications	Biomedical research, biomarker discovery
Subject descriptors	Database of lipid reference spectra acquired in TOF-SIMS with bismuth aggregates
Organization	Centre National de Recherche Scientifique-Institut de Chimie des Substances Naturelles, 1 avenue de la Terrasse, 91198 Gif sur Yvette cedex, France
Organization type	Academic institute
Property rights	Public
Sources of support	Partial EU funding
Stage of development	Laboratory prototype

Table 15: Description of the lipid reference database for SIMS

4 CONCLUSIONS

The Computis project concerns imaging mass spectrometry and its application to many biological questions. The goal of the Computis project was to develop, optimize, combine, correlate and apply methods of mass spectrometric molecular imaging, especially various specialized methods of Secondary Ion Mass Spectrometry (SIMS) and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS), and to merge the results with well known pathological / histological pictures of cells and tissues into a common image.

The Computis project included work about sample selection and preparation protocols, instrumentation developments, analytical studies and benchmark on biological samples, development of viewing and processing software tools, and application to the study of biological diseases.

The partners of the project were Commissariat à l'Energie Atomique (France), Justus Liebig University (Germany), Dutch Foundation for Fundamental Research on Matter (The Netherlands), Centre National de la Recherche Scientifique (France), Power Computing & Communications (The Netherlands), Généthon (France) and Novartis (Switzerland).

The work performed all along the 4-year-duration of the project was broadly disseminated through massive communications and publications in conferences and scientific journals, the organisation of two workshops with the distribution of a booklet synthesizing the main achievements of the project, and a public website <http://www.computis.org/>

Several major results were obtained at the end of the project. Some of them have been freely distributed on <http://www.maldi-msi.org/>. A market study was carried out to define a strategy for the distribution of software components. For new high-performance equipments, some partnerships are in progress, while collaborations are still opened for others.