European Cooperation in Science and Technology
- COST -

Secretariat

Brussels, 9 June 2011

COST 4117/11

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1104**:** Mass Spectrometry Imaging: New Tools for Healthcare Research

Delegations will find attached the Memorandum of Understanding for COST Action BM1104 as approved by the COST Committee of Senior Officials (CSO) at its 182nd meeting on 17 May 2011.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action BM1104

MASS SPECTROMETRY IMAGING: NEW TOOLS FOR HEALTHCARE RESEARCH

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 Rules and Procedures for Implementing COST Actions, or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to establish imaging mass spectrometry and related translational technologies in clinical research. It will lead to standardized protocols for describing tissues by their molecular content and distribution, which will then be exploited to develop new molecular histological signatures for improved disease diagnosis as well as new methods for quantitative imaging of lead formulations for pharmaceutical development

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 56 million in 2011 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter IV of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Mass spectrometry imaging is a rapidly developing technique that uses spatially resolved proteomic and metabolomic techniques to simultaneously trace the distributions of hundreds of biomolecules directly from patient tissue samples. Using essentially the same technology peptides, proteins, pharmaceuticals and metabolites can be analyzed, without a label and without prior knowledge. The driving force behind the high and increasing popularity of imaging mass spectrometry is its demonstrated potential for the determination of new diagnostic/prognostic biomarkers, across several chemical domains, including pathologies of overlapping/identical morphology that cannot be distinguished using established histopathological methods.

All major mass spectrometry vendors now supply instruments capable of imaging experiments, and imaging mass spectrometry is now being implemented in a number of European clinics and pharmaceutical companies. Investigations utilizing mass spectrometry imaging for healthcare research would benefit enormously from standardized, best practice protocols developed by the integrated application and comparison of the formidable array of approaches already developed within individual European laboratories. The concerted research plan enabled by this Action will investigate the full potential of integrated mass spectrometry imaging to develop new molecular diagnostic and prognostic tools for a variety of diseases as well as providing new tools aiding pharmaceutical development.

Keywords: imaging mass spectrometry, biomarker discovery, cancer, pharmaceuticals, diagnosis

B. BACKGROUND

B.1 General background

The ability of modern proteomic techniques to identify and quantify the levels of thousands of proteins from a tissue or biofluid sample has transformed medical and biological research. In addition to global protein profiling, methods have been developed that target protein isoforms,

specific pathological entities or subcellular components. It has been increasingly recognized how the rich molecular information provided by this array of approaches offers new possibilities in the clinical field. This ranges from new insights into the molecular changes associated with pathogenesis, the identification of new therapeutic targets, to improved diagnosis and prognosis through the determination of biomarker proteins and protein profiles associated with a disease and its progression, respectively.

Many European clinical institutions have initiated research programs for the identification of biomarker protein and protein profiles, mostly in easily accessible body fluids such as plasma, serum and urine. Difficulties associated with dilution of the biomarkers in these fluids and the intrinsic variability of the protein content of body fluids have led to the alternative strategy of searching for biomarkers in the affected tissue. Recent notable examples include a combination of tissue microdissection, protein extraction, and extensive peptide/protein separation to quantitatively investigate the changes in protein content associated with the different stages of pancreatic intraepithelial neoplasia and multiple sclerosis.

Several groups have demonstrated how mass spectrometry (MS) based methods can be directly applied to tissue. Rapid progress in the field now allows the parallel analysis of hundreds of biomolecules, high sensitivity and selectivity, and the ability to distinguish between close variants. Using essentially the same technique but modified sample preparation strategies imaging MS can be used to analyze peptides, proteins, metabolites and pharmaceutical, without labelling and without prior knowledge. When combined with a histological analysis of clinical tissues MS can be used to identify differentially expressed biomarkers spanning multiple molecular domains and provides an example of a general observation in biomarker research, namely that a single biomarker is not sufficient to annotate the complexity of real biological systems.

Imaging MS can be considered spatially resolved direct tissue analysis, and uses the same tools to simultaneously analyze the distribution of hundreds of biomolecules in tissue. Several studies have already shown how imaging MS can be used to chart biomolecular variation in clinical tissue samples: many candidate peptide/protein/metabolite biomarkers have been identified, including markers that discriminate between clinically challenging pathologies. This spatio-chemical direct tissue analysis has the potential to bring modern biomolecular techniques into the clinic. In many regards it is the aim of imaging MS to become a biomolecular screening addition to histopathological analysis.

The potential of imaging MS for clinical research is reflected in its increasing use in European clinical institutions and the availability of imaging mass spectrometers, tissue preparation stations and data analysis solutions from European manufacturers. European research groups have now developed a formidable array of capabilities: high spatial resolution, high mass resolution, multiple molecular classes, high sensitivity and specificity, in-tissue identification and quantitation, high throughput analyses, and integration with established pathological and ‘omics methodologies.

European investigations utilizing imaging MS for healthcare research would benefit enormously from the integrated application of many of the above tools. The network provided by this COST action will enable multiple synergies to be developed between its members, enabling new diagnostic tools to be developed as well as providing new tools for pharmaceutical development. Importantly it will provide a testing ground for applicability of the technology to a range of pathologies, help develop standardized methodologies for its wider implementation and improve the accessibility of European research infrastructure. The involvement of all major European MS manufacturers will aid economic and R&D competitiveness.

B.2 Current state of knowledge

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

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

Arrays of methods have been developed to achieve these goals:

1. Sample preparation strategies to enable the analysis of peptides, proteins, lipids, pharmaceuticals and metabolites;
2. Mass analysis methods optimized for sensitivity, spatial resolution, mass resolution, high mass molecules, or combined with an additional ion-separation dimension;
3. Combined imaging MS - histological analysis of tissues for the identification of candidate biomarkers;
4. Imaging MS based molecular histology for distinguishing between pathologies with overlapping/identical morphologies.

Imaging MS is now beginning to show its ability for disease detection and pharmaceutical development. For example demographic changes and the increasing clinical use of in-vivo imaging techniques are leading to the detection of an increasing number of soft tissue sarcomas. Several of the most common soft-tissue sarcomas have overlapping/identical morphologies but different clinical behaviour and different treatment targets. It was recently demonstrated that imaging MS could discriminate between such clinically challenging tumors. Furthermore, it also revealed substantial biomolecular intratumor heterogeneity within the tumors, the resolution of which was crucial to the correct diagnosis. Many tumors can exhibit intratumor heterogeneity, imaging MS based analysis could thus aid in the development of more robust diagnostic tests.

Despite the increasing use of imaging MS and its commercial availability the majority of studies are independent research projects. The recent training schools provided by Nordic Translational Imaging have strongly indicated that the lack of an effective collaborative vehicle (that enables information and methodological exchange) has held back the potential impact of imaging MS in healthcare and pharmacological research: an explicit comparison of the capabilities developed in individual laboratories, for multiple pathologies, would enable standardized best-practice guidelines to be ascertained, thus propelling the impact of European researchers and companies in biomolecular research.

B.3 Reasons for the Action

The COST Action will enable scientific exchange between imaging MS experts and healthcare researchers, to establish it among a broader scientific community in healthcare and pharmacological research. Central to this purpose is the dissemination and integration of the complementary techniques and expertise throughout the different groups and their sustained interaction. Interaction between imaging MS researchers is crucial for devising best practice guidelines and web-based experimental resources, while interaction with healthcare researchers is essential in order to ensure the imaging MS efforts target real needs in healthcare research, e.g. differentiation between clinically challenging tumors, more cost-effective methods for differentiation of lead compounds in pharmaceutical development. The strictly collaborative nature of this Action is ideally suited to the COST framework and builds upon several of the tools previously developed within COMPUTIS and Nordic Translational Imaging. COMPUTIS is a 6th framework project targeting improved technology for high spatial resolution imaging mass spectrometry and Nordic Translational Imaging is a Nordforsk funded consortium to advance imaging in health and disease through advanced researcher training.

The aim of the cooperation is to establish imaging mass spectrometry and related translational technologies in clinical research. General objectives include:

1. More reliable and cost effective diagnostics through imaging of multiple molecular classes;
2. Rapid application to topical diseases;

3. Improved efficacy of treatment for topical diseases by optimizing formulation based on quantitative compound imaging;
4. Transition to non-invasive quantitative high throughput screening (biopsies & body fluids).

The application of these methods to clinical samples and model systems will enable the potential of the technique for improved diagnosis/prognosis to be ascertained, as well as providing new tools aiding pharmaceutical development. In these regards the Action will further aid European scientific and economic needs by providing new methods that could lead to improved patient diagnosis and prognosis, as well as new methods that both speed-up and lower the cost of drug development.

Fast progress with regards to the clinical application of imaging MS revealed biomolecular intratumor heterogeneity in multiple patient samples that is consistent with the clonal development of soft-tissue sarcomas; revealed differential metabolic signatures in breast cancer model systems that exhibit different metastatic potential; allowed label-free drug metabolism and pharmacokinetics studies. The knowledge and expertise are available; the COST Action will enable them to be rapidly exploited throughout the European healthcare field thus aiding investment and competitiveness.

B.4 Complementarity with other research programmes

This Action builds upon the COMPUTIS and Nordic Translational Imaging projects. COMPUTIS is a recently completed 6th framework project that developed improved technology for imaging mass spectrometry while Nordic Translational Imaging is a Nordforsk funded consortium to advance imaging in health and disease through advanced researcher training. The Action also complements the FP7 project Proteomics Specification in Time and Space (PROSPECTS), which uses subcellular fractionation to analyse subcellular proteomes.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of the Action is to establish imaging mass spectrometry and related translational technologies in clinical research. It will lead to standardized protocols for describing tissues by their molecular content and distribution, which will then be exploited to develop new molecular histological signatures for improved disease diagnosis as well as new methods for quantitative imaging of lead formulations for pharmaceutical development.

C.2 Secondary objectives

Specific objectives designed to achieve these primary goals, deliverables and the expected scientific impact include:

i) Best practice guidelines for imaging MS data acquisition;

Deliverables: robust sample preparation and MS analysis methods for European healthcare researchers.
Impact: enable researchers to describe clinical tissues by their molecular content, spanning multiple molecular classes.

ii) Data analysis and data sharing;

Deliverables: proven strategies for biomarker discovery, molecular histology and quantitation.
Impact: enable researchers to efficiently analyze the highly complex data provided by multi-modality imaging mass spectrometry, and to efficiently interact with healthcare professionals.

iii) Application to topical diseases.

Deliverables: application of the standardized tools to topical diseases including breast cancer, prostate cancer, pancreatic cancer, ovarian cancer, myxoid sarcomas, chondrosarcoma, endometriosis, Parkinson's disease, atherosclerosis and chronic obstructive pulmonary disease.

Impact: Molecular profiles spanning multiple molecular classes associated with each disease will provide new insights into their molecular pathology as well as identify new biomarker signatures for improved diagnostic / prognostic performance.

C.3 How will the objectives be achieved?

An extensive programme of Short Term Scientific Missions between the different members of the COST Action, supplemented by Workshops and Training Courses, will be used to compare and contrast the methods that have developed in each individual laboratory.

Imaging MS requires the localized extraction of the molecules of interest followed by spatially correlated mass analysis. The sample preparation and mass analysis methods are critical factors that determine which molecules can be detected, and the sensitivity and resolution at which they can be detected. Imaging MS and healthcare researchers will visit member laboratories to test the performance of the imaging MS methods (sample preparation, mass analysis) developed in each laboratory. The explicit inclusion of multiple pathologies and multiple practitioners is essential in order to devise standardized methods for each molecular class and tissue.

Data analysis and data sharing routines will be shared between the different members of Action to investigate the performance of the different routines for a range of pathological test samples. This will establish context-dependent best-practice guidelines for analyzing these rich datasets. For example, if the goal of an experiment is to identify new biomarkers that discriminate between well-differentiated tissues then the tissue morphology can be used to class each region of the tissue (e.g. adenocarcinoma, stroma, non-small cell carcimoma). A statistical analysis of the biomolecular signatures of the different classes will provide the sensitivity and specificity of the biomarkers.

In this case the analysis specifically focuses on biomarkers consistently detected in the tumors. If the goal of the experiment is to discriminate between pathologies that can have overlapping/identical morphologies (chronic pancreatitis and pancreatic adenocarcinoma; central and peripheral chondrosarcoma; myxoid sarcomas) then morphology cannot be used to assign classes to the clinically challenging samples, and an unsupervised multivariate analysis is preferable to examine endogenous differences in their biomolecular profiles.

Once established the standardized methods will be used to ascertain the capabilities of imaging MS for healthcare and pharmacological research: the former encompasses molecular histological methods for improved diagnosis and biomarker discover, and the latter improved efficacy of treatment for topical diseases by optimizing formulation based on quantitative compound imaging.

C.4 Benefits of the Action

The scientific benefits of the Action span several domains: techniques for the integration of biomolecular information, additional insights into the biomolecular associations between different chemical classes in tissue, how these associations can be affected by disease or administration of a drug, and ultimately improved diagnostic and prognostic tools via the elucidation of validated biomolecular profiles. Benefits to society are two-fold: 1) the provision of new and specific tools (molecules and technology) to be used as novel assays (diagnosis/prognosis); and 2) further innovation of MS technology with currently unknown applications in biomedicine. Both of these aspects will impact on healthcare and provide extra impetus to the already significant role biotechnology plays within Europe. The inclusion of SME's, annual training courses, and a freely-available web-based resource providing standardized protocols will ensure the wider research and development community benefit from this COST action.

C.5 Target groups/end users

There are several target groups and end users that will benefit from this Action:

i) Healthcare researchers will benefit from improved and standardized methods for molecular histological analysis of pathological tissues. Annual training courses organized by the Action will provide the academic background and hands-on training to enable its rapid implementation ;

ii) Pharmacological researchers and industry will benefit from improved and standardized methods for analyzing pharmaceuticals and the metabolites in tissues. This will provide a more rapid and cost effective method for testing the efficacy of lead compounds during drug development;

iii) Mass spectrometry researchers and industry will benefit from an array of tools that have been designed for analyzing the large datasets generated by imaging mass spectrometry and data sharing platforms that enable efficient interaction with external collaborators;

iv) Imaging mass spectrometry researchers will benefit from the standardized protocols, training and tools generated by the Action. Improved sensitivity, integration of data from multiple molecular classes, efficient data analysis and data sharing methods, will provide the enabling technologies for the widespread application of these tools to a diverse array of pathological questions.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

i) Best practice guidelines for imaging MS data acquisition

Imaging mass spectrometry is able to analyze the distributions of peptides, proteins, metabolites and pharmaceuticals using essentially the same technology. The principal difference is how the tissues are prepared. For example tissue washes to remove salts have been found to improve protein analyses, increasing the number of proteins that can be detected and the sensitivity of the analysis. On the other hand neuropeptides are highly soluble and so the tissue washing techniques must be adapted to minimize lateral diffusion. The different research backgrounds and the availability of different hardware have led to the development of a range of imaging mass spectrometry methodologies. An extensive exchange program using Short Term Scientific Missions and Workshops will be used to establish standardized protocols for each molecular class in a range of tissues and which will then be made available in a publically accessible database on the Action's website.

The ability to analyze different molecular classes raises the possibility to describe the tissue by as complete a chemical representation as possible and examine the correlations between the different classes. Candidate metabolite or lipid biomarkers can indicate up-regulation of specific proteins. For example fusion protein generated by the genetic translocation characteristic of myxoid liposarcoma controls the expression of C/EBP, which affects the expression of peroxisome proliferator-activated receptor gamma (PPARγ), a key player in adipocytic differentiation. In addition to protein biomarkers, imaging MS could detect the lipid changes that occur during tumor progression. Standardized methods will be developed for aligning and integrating the imaging mass spectrometry datasets from multiple molecular classes, as well as with immunohistochemical (IHC) and in-situ hybridization analysis (ISH) of adjacent sections.

Deliverables: i) standardized and updated methods for imaging mass spectrometry of proteins, peptides, lipids, and metabolites (M24 and M48). ii) strategies for integrating imaging mass spectrometry datasets of different molecular classes into a single imaging dataset (M36). iii) tools for aligning datasets with ISH and IHC analysis (M24).
Milestone: Updated database of standardized methods for imaging mass spectrometry of biomolecules (M24 and 48).

ii) Data analysis and data sharing

Data analysis: Histology-defined analysis can be used for the identification of biomarkers specific to pathological entities, and histology-independent analyses examine and classify the tissue solely on the basis of its biomolecular content. Both of these approaches have the potential to generate new diagnostic tools and many data analysis techniques have been developed. However as most imaging MS experiments have been performed using commercial instruments using proprietary data formats many clinical users have been 'locked' into single data analysis packages and have not been able to exploit the new data analysis capabilities.

A new imaging data standard, *imzML*, was developed within the 6th framework program Computis. Substantial support from European instrument vendors has led to the *imzML* data standard being implemented as an export option on all commercially available instruments. The different data analysis capabilities developed in the partner laboratories will be made *imzML* compliant to enable widespread data sharing and an explicit comparison of the different imaging MS methods (Work Group i) and data analysis tools (this Work Group). Comparing the performance of the data analysis tools for a variety of pathologies will establish standardized tools and guidelines for the application of imaging MS in healthcare research.

Deliverables: i) Format conversion software (M12). ii) Methods for sharing data and metadata (M18). iii) Standardized methods for histology-directed tissue analysis (M36). iv) Standardized methods for histology-independent tissue analysis (M36).

Milestones: Format conversion software (M12); Standardized guidelines for data analysis (M36);

iii) Application to topical diseases

The impact of imaging MS is ultimately dependent on its ability to provide new tools that can either perform better, faster or cheaper than alternative technologies (*e.g.* label free DPMK analysis in drug formulation development in place of autoradiography) or provide new capabilities (identification of new biomarkers, differentiation of clinical challenging pathologies). To test the capabilities of imaging MS for healthcare and pharmacological research the standardized methodologies, data integration strategies and data analysis tools will be tested against a selection of present-day pathologies. These include breast cancer, prostate cancer, pancreatic cancer, ovarian cancer, myxoid sarcomas, chondrosarcoma, atherosclerosis, endometriosis, Parkinson's disease and chronic obstructive pulmonary disease. This will be performed by an extensive exchange program that utilizes the full and standardized capabilities of the formidable array of imaging MS techniques available within partner laboratories, and which includes the active participation of clinicians and industry (pharmaceutical and mass spectrometry). A comparison of the applications in which imaging mass spectrometry has proven successful will provide general applicability guidelines for the wider healthcare and pharmacological research fields.

Deliverables: Papers detailing the application of the standardized imaging MS tools to topical diseases such as breast cancer, prostate cancer, pancreatic cancer, ovarian cancer, myxoid sarcomas, chondrosarcoma, atherosclerosis, endometriosis, Parkinson's disease and chronic obstructive pulmonary disease. Guidelines for the application of imaging mass spectrometry in healthcare and pharmacological research (M48).

Milestones: Implementation of standardized data acquisition and data analysis protocols (M36).

D.2 Scientific work plan, methods and means

The scientific goals and objectives of this Action will be achieved by an extensive network of information exchange and explicit comparisons of the different methodologies developed within each laboratory for a range of healthcare and pharmacological applications. Researchers will undertake Short Term Scientific Missions in partner laboratories to learn the methodologies of their Action partners and to explicitly test the different methods on their own pathological and pharmacological research questions.

i) Best practice guidelines for imaging MS data acquisition

A wide ranging comparison of sample preparation methods will be made for each molecular class using an array of imaging mass spectrometry hardware for each pathological and pharmacological application. The resulting data will be compared on the basis of number of biomolecules detected, sensitivity, dynamic range, spatial resolution, mass resolution, speed, and reproducibility. The explicit use of an array of tissue samples and multiple practitioners from each member laboratory will provide the capacity and redundancy to conclude best practice guidelines.

ii) Data analysis and data sharing

Where necessary software will be created for converting the imaging MS datasets into the standardized imzML data format. An imzML data input will be created for each data analysis method , which will then be made available within the Action to establish their performance for each pathological and pharmacological research question, using the data generated in Work Group i). This will enable a range of classification algorithms (support vector machine, genetic algorithm, discriminant analysis, random forests), clustering algorithms (k-means, fuzzy c-means, hierarchical), multivariate analysis (principal component analysis, independent component analysis, probabilistic latent semantic analysis, non-negative matrix factorization), and smoothing algorithms (nearest neighbor, edge-preserving, wavelet, median filter, Markov random fields) to be used to analyze each imaging MS dataset.

Validation is crucial to all clinical investigations. The high dimensionality of imaging MS datasets and the availability of multiple data analysis routines requires great care to avoid over-fitting of the data to the available patient tissue samples. Validation of the results the imaging MS experiments will be required as a matter of policy, and a full statistical analysis will be provided for each data analysis routine. The Short Term Scientific Missions and Training Courses will ensure that users are trained to understand and how to use these data analysis algorithms.

iii) Application to topical diseases

The extensive network of imaging MS capabilities available within the Action will be exploited to investigate a number of healthcare and pharmacological questions. Once the standardized sample preparation and data analysis methods have been established, members of each of the participating labs will use the available infrastructure to analyze tissues from a number of pathologies of present-day concern. Imaging MS analysis of multiple molecular classes followed by histological analysis of the same tissue. These results will then be aligned and combined into a final *integrated* dataset. Histology-defined or histology-independent analyses will then be performed. Independent validation of the results will be expected, whether by quantitative MS analysis of tissue extracts or immunohistochemical analysis of specific proteins of interest.

The open nature of the data acquisition and data analysis process has been explicitly designed to highlight good practice and avoid unnecessary duplication, and is fully in-keeping with recommendations recently published in the Lancet (Sharing research data to improve public health, doi:10.1016/S0140-6736(10)62234-9). This within Action openness is crucial to establish best practice guidelines and thereby ascertain the ability of imaging MS to aid healthcare research.

E. ORGANISATION

E.1 Coordination and organisation

The COST Action will enable scientific exchange between experts that have significantly contributed to the development of mass spectrometry imaging, and to establish it among a broader scientific community. The tools already available and currently being developed within these labs provide complementary capabilities whose integration would provide the synergies underpinning the research.

The Action will be organized into a series of Working Groups that concentrate on different scientific goals of the project. The Management Committee will ensure the concerted action of each member institute's research, which will be coordinated through the COST Action and specifically through the different Working Groups. The Management Committee will allocate the coordination resources to each Work Group, such that the amount of resources dedicated to the work groups can evolve with the progress of the COST action. For example, the first year of the COST Action will focus on investigating the relative capabilities of the data acquisition methods and ensuring all data analysis routines are compatible with the imzML data standard. This will gradually change to data analysis followed by its application a number of pathological questions.

Integration and knowledge-transfer throughout the Action's consortium will be achieved by the extensive use of Short Term Scientific Placements and annual Workshops within each Work Group. Monitoring of the progress made will be achieved by requiring detailed progress reports from each Short Term Scientific Mission and Workshop to be submitted to each Work Group's Chair, who will then report to the Action's Management Committee. Specific attention will be placed on the Action's Milestones, and annual progress reports will be prepared by each Work Group and supplied to the Management Committee describing progress towards its realization and explanations for any deviation from the anticipated timeline. All progress reports will be contained on the Action website and will be available to all members of the Action.

An Action specific website will be created and maintained that will serve as a valuable web-based resource for the members of the Action and to ensure the dissemination of the Action's results such that they can benefit the wider mass spectrometry, healthcare and pharmacology research fields. The website will include a public area, a public password-protected area and an Action specific area. The two public areas will be used for helping to disseminate the research and results of the Action (see H.2 for more details), and the member's area for helping to coordinate the collaborative research of the Action.

Member's Area of the website will include an agenda detailing forthcoming Meetings, Training Courses, Workshops and Short Term Scientific Missions. The website will contain separate sections for management of the Action and for each Work Group, which will contain a separate agenda, a forum and common work area where data files and documents can be shared. In short the member's area will be designed to enable effective collaboration between the members of the Action.

E.2 Working Groups

Working Groups will be set up for each of the main objectives of the Action:-

1. Best practice guidelines for imaging MS data acquisition
2. Data analysis and data sharing
3. Application to topical diseases

Each Working Group will have a dedicated Chair and Co-Chair charged with organizing the activities within that Work Group. The Working Groups concern the Action's scientific output, the Chair and Co-Chair will be responsible for monitoring and evaluating the output of the Work Group and for delivering the Working Groups' Deliverables and Milestones. Annual Reports to the Action's Management Committee will document the performance of each Working Group. The Chair and Co-Chair will also be responsible for organizing Short Term Scientific Placements and an annual workshop for reporting and discussing the latest developments in each task. Several of these workshops will be held concomitantly, reflecting the interactivity between the different domains. For example, the different data analysis capabilities developed in the Working Group *Data analysis and data sharing* is crucial to the activities of the Working Group *Application to topical diseases.* Early Stage researchers and gender balance will be sought for every activity of each Work Group.

E.3 Liaison and interaction with other research programmes

Members of research programmes related to the activities of this Action as well as future programmes that could benefit from the Action will be invited to participate in Workshops, give seminars, exchange information and interact with the members of the Action. This includes bioanalytical development programmes (COMPUTIS, Nordic Translational Imaging, PROSPECTS), facility based programmes (BrainNet Europe) and application specific programmes concerning specific disease areas (EuroBoNet) or novel medicinal approaches (MediTrans).

E.4 Gender balance and involvement of Early-Stage Researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers.

Gender balance and early stage researchers will be explicitly included in the make-up of the Action's Management Committee, and upheld throughout all of the Action's activities by the creation of a designated equal-opportunities representative. The annual report from each Work Group will include a section detailing the gender balance and participation of early-stage researchers in its activities. The inclusion of early-stage researchers in all aspects of the organization of Training Schools, Workshops and Short Term Scientific Missions will provide ample training as well as the organizational capacity for this large collaborative research Action.

As a matter of policy all Training Courses and Workshops will seek gender balance by reserving half of the placements for female researchers until 3 weeks prior to the course. Thereafter the positions will be made available on a first come basis.

F. TIMETABLE

The Action is focused on the scientific exchange between experts in imaging mass spectrometry and its integration with high throughput genomics and proteomics analysis. The Action is estimated to last for 4 years.

Work Group 1: The establishment of standardized sample preparation and data acquisition protocols for each of the molecular classes and integrating the resulting datasets with each other and with pathohistological analyses will be concentrated within Years 1 and 2 but will be updated throughout the Action's duration.

Work Group 2. The development of format conversion software and methods for sharing data will be performed in Year 1. These will be exploited in years 2 and 3 to compare the data analysis routines using the test data generated in Work Groups 1 and 2.

Work Group 3: The investigation of the ability of imaging MS to provide improved diagnostic and biomarker discovery tools will be performed in Years 3 and 4 by exploiting the standardized and wide ranging methods to a number of pathologies.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CH, CZ, DE, EE, ES, FI, FR, HU, IT, NL, SE, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 56 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The results of this Action will be of prime importance for several different target audiences:

1. Other researchers working in the imaging mass spectrometry field – including spatially resolved proteomics, metabolomics and lipidomics. The improvements in sensitivity, quantitation, throughput, data analysis and data sharing will find many applications. The databases of sample preparation protocols and data analysis strategies together with tools for data sharing, all developed within this Action and demonstrated on a selection of pharmacological and pathological applications, will enable further investigations of this technique in healthcare research;

2. Researchers in biomolecular mass analysis – the standardized protocols resulting from the Action will enable effective imaging mass spectrometry experiments to be rapidly implemented in new laboratories and to combine these with established extraction-based biomolecular analyses;

3. Researchers in healthcare – proven techniques for the biomolecular analysis of tissue arrays will provide new methods to describe clinical tissues by their molecular content and distribution. This will provide new methods for biomarker discovery and new diagnostic tools to complement established histopathological analysis;

4. Researchers in pharmacological research – simultaneous imaging and quantitation of pharmaceuticals and their metabolites provide a rapid and cost effective method for the development of new drugs and in-vivo tracing agents (PET, MRI);

5. Standards Bodies – the data sharing methodologies that will be developed will provide new data standards and data analysis standards for imaging MS;

6. Industry – databases of effective protocols covering all aspects of the experiment, standardized data analysis tools and targeted training courses will enable the tools developed with the Action to be tested and implemented more rapidly and cost effectively than is currently possible. These tools can be directly applied to healthcare and pharmacological industries, and could be adapted to fields as diverse as food development and synthetic materials.

H.2 What?

An array of different methods will be used to disseminate the research of the Action and its results to the wider research communities.

• *Website – public area*

The website will describe the COST Action and its results. A comprehensive agenda of forthcoming Training Courses and Workshops, as well as specific contact details for the Chair and Co-Chair of the respective Work Group will enable external groups to apply to participate in the Training Courses, Workshops or the Action itself. The public area of the website will also provide a resource of teaching materials including lectures and self-learning exercises and will eventually include the databases of effective sample preparation and data analysis strategies, thus ensuring the wider research community benefits from the Action. A password-protected area of the website will be used to provide more access to external collaborators, for example sample preparation protocols and data analysis tools in development. A public library will contain all of the results of the Action.

• *Publications*

A variety of publications will be generated by the COST Action. A state of the art report (review) will first describe the current capabilities in the field of imaging MS within the fields of healthcare and pharmacological research. All interim reports, Short Term Scientific Mission progress reports and Workshop proceedings will be made available via the website. A series of guidelines and manuals detailing how to perform effective imaging mass spectrometry experiments for healthcare research will be complemented by lectures and self-learning exercises. These will be explicitly designed to enable the wider research community to exploit the findings of this Action. The new possibilities enabled by this Action will also form significant contributions to existing and new PhD theses.

• *Events*

A series of annual Training Courses will be organized for the wider research community that incorporates both lectures and hands-on practical experience. Where possible theses courses will be closely aligned with national and international conferences and symposia, for example as a pre-conference course.

Annual Workshops will be organized by each Work Group to compare and contrast the latest developments in each field and to provide focused, in-depth training in each sub-discipline. External speakers will be invited to these workshops, to provide additional context and insight to the Workshop and to disseminate the results of the Action to the wider research field.

In the final year of the Action a short conference will be organized that will present the results of the Action to the wider scientific community. Researchers from the healthcare, pharmacological and mass spectrometry communities will be invited to participate in order to disseminate the research to the widest possible community while at the same time placing the research in the wider context of biomolecular healthcare and pharmacological research.

• *Articles*

An array of articles in peer-reviewed scientific and technical journals will be produced by this Action. The earlier years of the Action will lead to a series of articles concerning method development, comparing and contrasting the different methods and establishing good-laboratory-practice protocols. For example the database of effective sample-preparation procedures will provide a valuable resource to the wider mass spectrometry and proteomics communities that is best disseminated through an article in a high impact proteomics journal. The later years will lead to a series of articles where imaging MS is used to investigate a variety of pathologies, the extensive training and an explicit demand of independent validation will help establish how imaging mass spectrometry can contribute to healthcare and pharmacology research.

H.3 How?

The explicit production of databases detailing effective experimental protocols for imaging MS analysis of tissue will provide a valuable resource to the mass spectrometry community that is currently lacking. These protocols and the standardized tools that enable efficient data analysis and data sharing with collaborators will provide highly valuable tools and thus help rapidly disseminate the results of this Action.

The demonstration of the capabilities of the improved tools for healthcare and pharmacological research will lead to academic research articles and conference presentations that will effectively communicate this Action. The members of the Action will present these results at appropriate conferences and publish in appropriate journals to ensure that the results are disseminated to the target audiences in mass spectrometry, healthcare and pharmacological research fields and their respective industries. In each case links will be provided to the Action's website to indicate where additional information can be obtained.

The training courses and workshops will be advertised on the websites of national and international organizations (such the European Proteomics Association) and advertised in academic presentations, posters and lectures. These courses are an integral part of the Action's program, they will provide highly effective dissemination tools that will ensure the results of the Action reach a wide audience and provide the target fields with the expertise required to further develop imaging mass spectrometry as a tool for healthcare and pharmacological research.