Going Forward: Increasing the Accessibility of Imaging MS

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Abstract

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 and its ability to simultaneously trace the distributions of pharmaceuticals and their metabolites in tissues without the need to develop expensive radioactively-labeled analogues. Both of these applications would benefit from standardized methods, for the development of novel MS-based molecular histology tests and FDA-approved MS-based assays for pharmaceutical development. In addition, the broader scientific community would benefit from the increased accessibility of the technique for new researchers.

Currently imaging MS studies are individual endeavors, utilizing the individual expertise and infrastructure of a single laboratory and their immediate collaborators. A wide array of tissue preparation, data acquisition and data analysis techniques have been developed but lack an international collaborative structure and data sharing capabilities. Such a collaborative framework would enable methodological exchange and detailed comparisons of analytical capabilities, to explore synergies between the different methods and result in the development of robust standardized methods. Here we describe a new European imaging MS network that will explicitly compare and contrast existing methods to provide best practice guidelines for the entire healthcare research community.

Introduction

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 the study of 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 international clinical institutions have initiated research programs for the identification of biomarker protein and protein profiles, mostly in easily accessible body fluids such as plasma or serum [1]. Difficulties associated with dilution of the biomarkers in these fluids and the intrinsic variability of the protein content of body fluids [2-4] have led to an alternative strategy: searching for biomarkers directly 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 [5] and multiple sclerosis [6].

Mass spectrometry (MS) based methods can be directly applied to tissue [7, 8]. 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 [9-11]. Rapid progress in the field now allows such multiplex imaging studies to be performed with high sensitivity and selectivity. Using complementary sample preparation strategies imaging MS can be used to analyze peptides, proteins, metabolites and pharmaceuticals, without labeling and without prior knowledge [12-15]. When combined with a histological analysis of clinical tissues imaging MS can be used to identify and visualize differentially expressed biomarkers [11, 16, 17], *e.g.* Walch and workers have demonstrated that imaging MS could identify features that differentiated between six different tumors [18].

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 [19, 20]. This spatio-chemical direct tissue analysis has the potential to bring modern biomolecular techniques into the clinic by providing a biomolecular screening that complements histopathological analysis.

The impact of imaging MS for clinical and pharmaceutical research is reflected in its increasing use throughout international clinical and pharmacological institutions and the availability of imaging mass spectrometers, tissue preparation stations and data analysis solutions from leading instrument manufacturers. A formidable array of capabilities has now been developed: high spatial resolution [14, 21-24], high mass resolution [25, 26], multiple molecular classes [12, 26, 27], high sensitivity and specificity, in-tissue identification[28-31] and quantitation [32, 33], high throughput analyses [34], and integration with established pathological [35, 36] and 'omics methodologies [37, 38]. Researchers utilizing imaging MS in healthcare research would benefit enormously from a detailed comparison of many of the above tools; the identification of synergies could provide new diagnostic tests for a range of pathologies as well as new tools for pharmaceutical development. Importantly, such a comparison would also provide a testing ground for the applicability of the technology for a range of pathologies and help develop standardized methodologies for its wider implementation and improve its accessibility.

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 and pharmaceutical analysis, specifically:-

- Analysis of multiple molecular classes.

- Parallel analysis of panels of biomolecules containing 100's of distinct species. For example the application of an automated peak detection routine to the imaging MS datasets of myxofibrosarcoma tissue samples identified 358 discrete peptide and protein peaks [39].
- Ability to distinguish between isoforms by exploiting the difference in their mass.
- Improved relative quantitation.
- Label free analysis – thus prior knowledge of the biomolecular content is not required.

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

- Sample preparation strategies to enable the analysis of peptides, proteins, lipids, pharmaceuticals and metabolites [40].
- Mass analysis methods optimized for sensitivity, spatial resolution [14, 22, 24], mass resolution [25, 26], high mass molecules [41], or combined with an additional ion-separation dimension [42, 43]. The review by Heeren and co-workers contained in this special issue contains a detailed overview of the technological developments that have been undertaken in one of Europe's principal MS technology development laboratories [44].
- A host of data reduction and data analysis capabilities (see review by Jones *et al.* in this special issue [45]).
- Combined imaging MS – histological/histochemical analysis of tissues for the identification of candidate biomarkers [8, 18, 35, 36, 46-51].
- Imaging MS based molecular histology for revealing molecular changes that occur prior-to/without morphological change [19, 20, 39, 52-55].
- Correlation with patient outcome for the identification of prognostic biomarkers [47, 56].
- Correlation with patient response-to-therapy, thereby potentially aiding personalized medicine [57].

The reviews contained in this special issue demonstrate the rapid progress being made in the field and provide ample evidence that imaging MS can complement established histological and histochemical methods and aid patient or disease stratification. Despite the increasing use of imaging MS and its widespread commercial availability the majority of studies are independent research projects, utilizing individual infrastructure and based on the individual expertise of the research group and their collaborators. The scattered nature of the expertise, the lack of methods for data sharing (beginning to be addressed by the *imzML* data standard [58]) and suitable training has severely limited the ease of knowledge transfer between laboratories, Figure 1. Currently, robust imaging MS experiments across multiple laboratories are conspicuous by their absence.

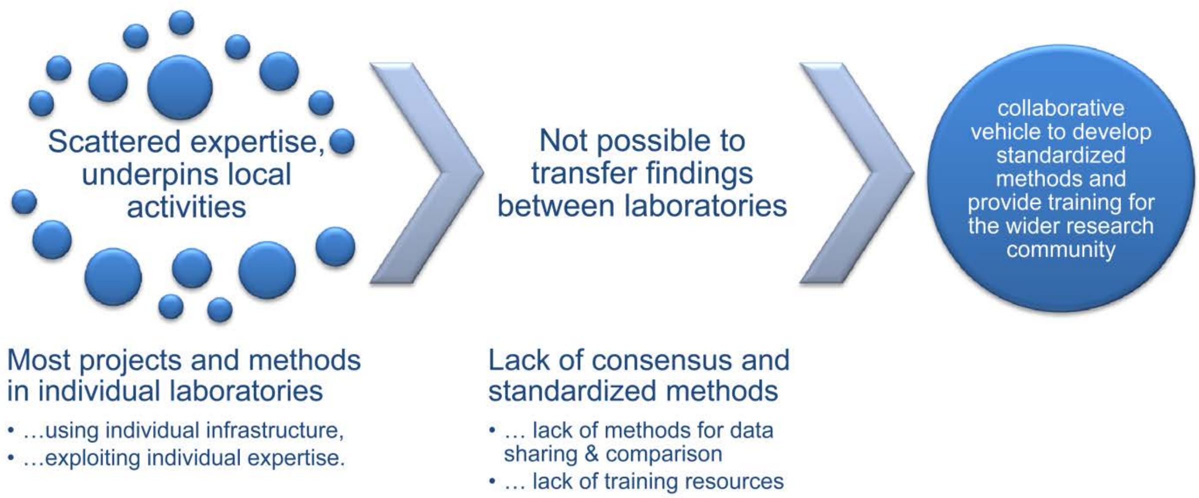

Figure 1. A collaborative vehicle is necessary to overcome the current individualist nature of imaging MS experiments and lack of data sharing/comparison tools.

The need for improved accessibility

Modern mass spectrometers provide exquisite mass analysis capabilities: high sensitivity, high resolution and high mass accuracy enable many biomolecules to be characterized directly from tissue. The review by Goodwin clearly communicates the importance of sample preparation to the success of an imaging MS experiment and the need to control as many variables as possible ("small mistakes can lead to big consequences" [59]). Many reviews have focused on sample preparation [13, 40], which reflects its critical role in determining the quality of imaging MS data. In 2007 a discussion began concerning the merits of a database containing sample preparation protocols to enable new researchers working in the healthcare community to exploit the technology. The problem of a lack of standardization and training in sample preparation was compellingly confirmed during the first Nordic Signals imaging MS training course (organized by Corthals, McDonnell and Heeren and held at the FOM Institute AMOLF in March 2009): despite most participants using the same commercial matrix deposition device its practical usage differed widely (and in most cases contrary to the manufacturer's recommendations). With suitable training all participants were able to generate near identical, high quality imaging MS datasets describing the molecular content of the tissue.

A similar situation was evident in the subsequent data analysis training course (organized by Corthals and McDonnell and held at Turku Centre for Biotechnology in December 2009). The majority of participants were restricted to the data analysis tools included in the software provided with their commercial instruments or the freely available Biomap software, an independent package developed for imaging MS by Markus Stoeckli (and which has been an essential element in the growth of the technique). Nevertheless the single biggest impediment to effective data analysis was a lack of training to fully utilize the statistical methods that were available. Following the training course the participants were able to generate robust classifiers and perform molecular histology much more effectively.

The restriction to data analysis packages supplied with the commercial mass spectrometers or Biomap means that most researchers have not been able to exploit the improved data analysis capabilities reported by data analysis specialists [45]. A lack of a data format standard is one, significant, reason most newly reported capabilities are not utilized as much as they may otherwise be; an open-source imaging MS data analysis platform that incorporates the latest algorithms would certainly help but would only be part of the answer: the researchers must understand the data analysis algorithms in order to use them correctly. The lengthy introduction to imaging MS data analysis [45] contained in this review is meant to begin to address this need.

Only with sufficient training and co-operation can the full potential of imaging MS be utilized to test the capabilities of these highly cross-disciplinary tools against an array of diseases of present day concern, both in terms of improved diagnosis and pharmacological development. Central to this purpose will be the dissemination of the complementary techniques and expertise and their sustained interaction. Interaction between imaging MS researchers is crucial for devising best practice guidelines and web-based experimental resources; the involvement of healthcare researchers is essential in order to ensure the imaging MS efforts target real needs in healthcare research, e.g. differentiation patient subgroups, and pharmaceutical development, e.g. more cost-effective methods for differentiation of lead compounds.

Increasing Accessibility of Imaging MS

A large European network was recently announced, COST Action BM1104, entitled Mass Spectrometry Imaging: New Tools for Healthcare Research, the sole aim of which is to establish imaging mass spectrometry and related translational technologies in clinical and pharmaceutical research. Best practice guidelines for data acquisition will be created 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. Importantly the network is also supported by the European Proteomics Association, which has made Imaging MS one of four special initiatives to be promoted. The central idea behind the COST Action is information exchange and training; for data acquisition, data analysis, and their application and to provide this knowledge as public resources, Figure 2. The generous hosting of the Action on the website www.maldi-msi.org, the de-facto online center for imaging MS news and resources, will further increase the networks visibility and impact.

Figure 2. The four elements of COST Action BM1104, designed to improve the accessibility and interlab reproducibility of imaging MS.

Data Acquisition – Best Practice Guidelines

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 are measured, and the sensitivity and resolution at which they can be detected. Imaging MS and healthcare researchers will visit each other's laboratories to test the performance of the imaging MS methods (sample preparation, mass analysis) that have been developed in each laboratory. The explicit inclusion of multiple pathologies and multiple practitioners provides the capacity and redundancy to begin devising best practice guidelines for multiple molecular classes and tissues.

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 their MS signatures [45]. 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 [58]. Substantial support from instrument vendors has led to *imzML* being implemented as an export option on most 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 data analysis tools, Figure 3. Comparing the performance of the data analysis tools for a variety of pathologies will establish standardized tools and *context-dependent* best-practice guidelines for analyzing such rich datasets.

Figure 3. The imaging MS data standard, *imzML*, will be adopted by European COST Action BM1104 to enable data sharing and a comparison of data analysis algorithms, both essential elements for the development of robust imaging MS data analysis strategies and improving the accessibility of the technique to new researchers.

The improvements in sensitivity, quantitation, data analysis and data sharing provided by this research network could benefit many application areas. A database of sample preparation protocols and data analysis strategies together with tools for data sharing, and demonstrated on a selection of pharmacological and pathological applications, will be made publicly available to enable further investigations in healthcare research.

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, which is crucial both for the wide exploitation of this up-and-coming technique but also for the development of robust inter-laboratory validated imaging-MS based assays.

Imaging MS Standardization and Method Validation

The primary bioanalytical goal of the COST Action is improved imaging MS analysis through an extensive comparison of the imaging MS methods that have been developed in European laboratories. No less important are the logical consequences of this goal:-

i) High performance imaging MS throughout member laboratories: the research network will help maintain optimum performance of the imaging MS infrastructure. Instead of the somewhat overly-reductivist question of 'which is the best instrument?', and which ignores the high expense of mass spectrometers, the training, best practice guidelines and open availability of high performance data analysis algorithms will mean that researchers can use their current infrastructure to its maximum capability. This aspect can be considered the first imaging MS multicentre standardization initiative, similar to the recently reported protein identification and quantitation inter-laboratory comparisons [60, 61], and will build on the substantial expertise of the Spanish Proteored organization [62].

ii) Inter-laboratory validation of imaging MS based assays. The development of clinical assays based on imaging MS needs to explicitly assess if the specific biomarkers or biomarker profiles can be detected in multiple laboratories (equipped with similar technologies) and using multiple tissue banks. The final aspect is crucial because of differences in tissue collection/storage may affect the MS profiles recorded by MALDI imaging MS. The COST research network provides the research capacity to begin to address this challenge:

a. Researchers from laboratory *A*, using tissues from tissue bank *A*, can visit partner laboratories to assess if the biomarkers determined by laboratory *A* are also effective when the imaging MS experiments are performed at partner laboratories *B*, *C* and *D*.

b. Researchers from laboratory *B*, *C* and *D*, using tissues from their own tissue banks can visit partner laboratory *A* to determine if the biomarkers determined in laboratory *A* are effective using independent tissue collections.

The separation of tissue source and imaging MS technique, two potentially significant sources of variation, is essential for the establishment of robust diagnostic tests based on imaging MS. Without standardization imaging MS will be limited to a biomarker discovery role in which the final validation, and any subsequent clinical assay, is performed using an alternative technique. Immunohistochemistry can, and will, be used for individual proteins if suitable antibodies are available. However mass spectrometry is much more sensitive to protein isoforms and so great care will need to be taken to ensure the antibody assays target the isoforms of interest. For example a recent report concerning prostate specific antigen [63] has highlighted that though multiple forms of PSA could be identified using MS methods, and which were demonstrated to be enzymatically active and could differentiate patient groups, an established antibody-based clinical method did not detect many of the isoforms and underestimated the PSA heterogeneity. Similarly whereas the change in mass associated with neuropeptide isoforms makes them readily distinguishable by imaging MS [64], high specificity antibodies for distinct isoforms may not be available. For metabolites and lipids, it is often the case that imaging MS provides the only method by which the distributions of specific species may be obtained [20, 38, 65]. In short the creation of standardized imaging MS methods, that demonstrate high reproducibility in multi-centre experiments, has enormous potential to offer new molecular histology capabilities.

Target groups

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 will provide the academic background and hands-on training to enable its rapid implementation. This will provide new methods for biomarker discovery and new diagnostic tools to complement established histopathological analysis.

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.

v) Standards Bodies: the data sharing methodologies that will be developed will provide new data standards and data analysis standards for imaging MS.

vi) 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.

The above list of benefits for specific groups of researchers underestimates the potential impact of the network. The network will be greater than the sum of its parts (groups of researchers) because of the high degree of complementary expertise; creating a community of researchers that actively share, discuss and generate new ideas will propel imaging MS into the healthcare field, as well as enrich the experiences and careers of the participants.

Concluding remarks

Imaging MS is now beginning to deliver its potential in the clinical and pharmaceutical fields. Multiple examples of semi-quantitative and quantitative imaging of drugs and tracing agents (PET, MRI) have been reported, and a number of histology-defined clinical imaging MS studies have reported biomarkers for improved diagnosis and prognosis. More slowly, imaging MS-based molecular histology tools are emerging to define tissues on the basis of their MS profiles, thereby potentially complementing established histological and histochemical methods.

Imaging MS is approaching a crossroad. The field may continue on its current path, in which most experiments utilize individual infrastructure, tissue resources and expertise – more candidate biomarkers will be reported, some proteins will be independently validated by applying immunohistochemistry to tissue-microarrays and metabolites/lipids/pharma will be validated using LC/CE-MS analysis of tissue extracts. The recent commercial availability of liquid extraction surface analysis provides a more routine method for localized sampling of tissues [66].

While such individual investigations will undoubtedly uncover a veritable trove of results the individual nature of the experiments will mean imaging MS is limited to a discovery role. Only by developing standardized and robust methods that can be *routinely implemented in a clinic*, with sufficient throughput to analyze tissue series (biomarker discovery) and speed (patient diagnosis) can the full potential of imaging MS begin to be harnessed. An essential element will be to ascertain which biomarkers, and biomarker profiles, can be internationalized. The other path in the cross-roads is to accept that such an investigation is better achieved as a community. The imaging MS community of researchers brought together within COST Action BM1104, in which each participant brings a willingness to compare and contrast methods that have been painstakingly developed in their laboratory, is hoped to act as a springboard for imaging MS to make the transition from an up-and-coming technique with high potential, to an established biomolecular histological tool with high utility in both the healthcare and pharmacological fields.

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Figure Captions

Figure 1. A collaborative vehicle is necessary to overcome the current individualist nature of imaging MS experiments and lack of data sharing/comparison tools.

Figure 2. The four tongs of COST Action BM1104, designed to improve the accessibility and interlab reproducibility of imaging MS.

Figure 3. A common data standard, *imzML*, enables data sharing and international comparison of data analysis algorithms, essential elements for the development of optimum and robust imaging MS data analysis strategies.

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