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Ambient Mass Spectrometry in Metabolomics

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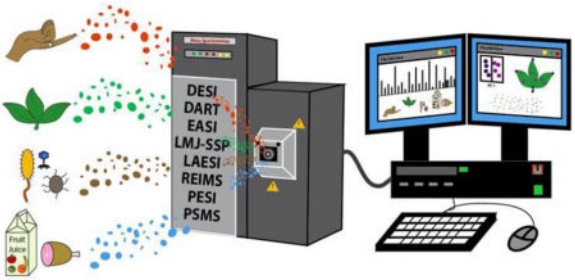
Abstract

Since the introduction of desorption electrospray ionization (DESI) mass spectrometry (MS), ambient MS methods have seen increased use in a variety of fields from health to food science. Increasing its popularity in metabolomics, ambient MS offers limited sample preparation, rapid and direct analysis of liquids, solids, and gases, *in situ* and *in vivo* analysis, and imaging. The metabolome consists of a constantly changing collection of small (<1.5KDa) molecules. These include endogenous molecules that are part of primary metabolism pathways, secondary metabolites with specific functions such as signaling, chemicals incorporated in the diet or resulting from environmental exposures, and metabolites associated with the microbiome. Characterization of the responsive changes of this molecule cohort is the principle goal in any metabolomics study. With adjustments to experimental parameters, metabolites across a range of chemical and physical properties can be selectively desorbed and ionized and subsequently analyzed with increased speed and sensitivity. This review covers the broad applications of a variety of ambient MS techniques in four primary fields in which metabolomics is commonly employed.

Graphical abstract

Ambient mass spectrometry continues to grow as a high-throughput alternative to more traditional hyphenated methods, playing an increasingly-relevant role in the growing field of metabolomics.



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Keywords

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Introduction

Metabolomics is the systems biology “omics” field that uses a holistic approach to characterize the small molecules (MW< 1500); i.e. the metabolome, in biological systems. It aims to characterize and quantify all the small molecules, including endogenous metabolites, lipids, and xenobiotics, in biological samples under a given set of conditions.[1, 2] The strength of metabolomics lies in its ability to connect observed changes in metabolites to biological responses. This produces biochemically-based fingerprints of diagnostic or classification value and assist in finding and identifying potential biomarkers that reflect actual biological processes. An additional strength is the fact that it is not necessary to have the genome sequence of the organisms involved in the study. Biofluids can be collected noninvasively, and time-dependent metabolic patterns of change in response to systemic perturbations such as disease, drug effects, or other stimuli of interest can be obtained. Despite the obvious benefits, challenges center on the fact that metabolomic studies require complex data interpretation methods and complex analytical workflows involving sample preparation.

The metabolome is estimated to be comprised of thousands to tens of thousands of chemically-diverse metabolites with a wide dynamic concentration range; and the number of observed metabolites is dependent on the analytical platform. This, therefore, makes it almost impossible to cover the whole metabolome with a single analytical method. The most commonly used analytical platforms in metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS). NMR is a non-destructive and highly reproducible platform that allows analysis of hundreds of compounds as well as provides structural information. Though destructive, MS allows rapid analysis of metabolites, and due to its higher sensitivity, provides a wider metabolome coverage compared to NMR.[3, 4] Tandem MS can provide limited structural information and when coupled to ion mobility, MS can also provide information on chirality and stereochemistry. It is often coupled with separation techniques such as liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE) and ion mobility (IM). Traditional hyphenated techniques such as LC or GC-MS offer an orthogonal dimension to mass-to-charge (*m/z*) in which metabolites can be separated, which also contributes to metabolite identification in discovery studies. These platforms allow the development of robust quantitation methods, at the expense of lengthy sample preparation times and the associated risks of metabolite alteration or loss during processing. Because the metabolome is constantly changing, many biological questions can only be addressed if samples are analyzed in real time, *in situ* and/or *in vivo*. Advances in ambient sampling/ionization mass spectrometry (ambient MS) have had a significant impact in metabolomics. Ambient MS allows for the direct soft ionization of metabolites, surface sampling, and in *in situ* analysis in real time in the ambient environment, with little to no sample preparation.[5, 6]

This review discusses the benefits and limitations of implementing ambient MS techniques in a variety of metabolomic studies. It provides a discussion of a selection of ambient MS applications in metabolomics reported until December 2016. Key examples of these applications have been grouped according to research areas in which the ambient MS field has had more impact in metabolomics to date, i.e.; health, plant and agriculture, microorganisms, and food. Literature searches performed independently by the authors using keywords such as “ambient mass spectrometry” or “mass spectrometry” and “metabolomics”, found, as anticipated,[5] that the number of publications in ambient MS-based metabolomics applications has sharply increased in the last 4 years. We have not attempted to make this review all-inclusive, but rather to select a number of interesting examples that illustrate capabilities and advantages of a family of ambient desorption/ionization techniques in the metabolomics field, and suggest trends according to the most promising applications. This is an applications review; we do not attempt to describe here the fundamental and mechanistic aspects or innovative instrumentation developments of ambient MS techniques since a number of review articles and tutorials are already available on this topic.[5–10] The goal of this review is to illustrate – with original publications – the large application coverage that ambient MS techniques have had in the field of metabolomics. We identify research areas that could benefit from the implementation of ambient MS techniques, and present ongoing challenges.

Common Ambient MS Techniques Used in Metabolomics

One-step techniques where desorption occurs by solid−liquid extraction processes dynamically followed by electrospray ionization (ESI) were introduced by Cooks et al.[11] with the development of desorption electrospray ionization MS (DESI-MS). DESI-MS essentially ionizes metabolites via an electrospray plume that is directed onto a sample surface.[12] A thin liquid film into which solid-phase analytes are extracted is formed on this surface. Primary droplets collide with the film producing secondary droplets that take up part of the analyte-containing liquid; ionization proceeding via ESI-like mechanisms. DESI-MS is a ‘soft’ ionization technique allowing for the study of many intact metabolites. The ratio of organic to aqueous solvent composition used in DESI-MS and the specific nature of the organic solvent have a large effect on the metabolites that can be desorbed from the surface. Both the pH and the ionic strength of the desorption solvent also have an effect on the ionization efficiency, and thus intensity and stability of the metabolite signal.[13] This makes quantitation difficult. Additional reagents in the solution used for desorption can allow for other species not normally ionized, to be ionized in a mode known as “reactive” DESI-MS. In addition, due to the chemical selectivity provided by these reagents added to the solvent spray, “reactive” DESI-MS allows for the detection of metabolites that are difficult to ionize.[14, 15]

First described by Haddad et al. and originally termed desorption sonic spray ionization (DeSSI), easy ambient sonic-spray ionization (EASI)-MS is a variant of DESI that uses a supersonic cloud of charged droplets for analyte desorption and ionization.[16] This voltage-free technique allows for simultaneous generation of both positive and negative ions, but suffers from low sensitivity and high gas flow needs. EASI-MS has been implemented

through the use of a permeable solid membrane that captures and traps analytes from a solution followed by direct sampling off the membrane surface.[17]

The liquid micro junction surface sampling probe (LMJ-SSP) is a different solid-liquid extraction-based ambient MS technique in which a continuous liquid micro junction is formed with the surface being sampled. Though there is a delay between extraction and ionization, this method is very sensitive and enables spot analysis of surfaces. By means of liquid junctions, separations are coupled with spray-based MS ionization techniques.[18] The smallest scale LMJ approach to ambient surface analysis has been called nanospray desorption electrospray ionization (nanoDESI).[19–21]

A variety of plasma-based ambient MS techniques have been developed since DESI was first described, but only some have been employed for metabolomics studies so far. An example of such techniques is direct analysis in real time (DART).[22–24] DART is an open air, direct sampling plasma ionization technique capable of high-throughput analysis of solids, liquids, or gases. The sample is exposed to a heated gas stream of metastable atomic or molecular species, typically He or N_2. Thermally desorbed analytes are then ionized through proton and charge transfer mechanisms, and subsequently suctioned into the mass spectrometer.[25–27]

Another technique that has been implemented in metabolomic studies, is probe electrospray Ionization (PESI), a two-step technique involving a solid-sampling electrospray probe.[28] An acupuncture needle, sometimes disposable, is used as the probe that is inserted into a sample, picking up or coating the exterior with material with a water-content in the picoliter range. This amount of water is sufficient for electrospray ionization when applying high voltage to the probe during analysis. The needle used in this method does not clog, however, needle sampling depth has a major effect on the reproducibility and cross contamination remains an issue. Despite the few drawbacks, this method inherently has a high salt tolerance and causes minimal ion suppression. Real time *in vivo* analysis using PESI is also possible.

Laser ablation electrospray ionization (LAESI), is a two-step laser-based desorption ablation technique coupled to ESI which was first introduced by Nemes and Vertes for *in situ* and *in vivo* experiments, allowing lateral mapping of metabolites on a sample surface.[29–32] In LAESI, the IR-ablated sample electrospray plume is merged with an electrospray droplet, and the analytes are ionized through ESI mechanisms. IR lasers are usually tuned for 2940 nm, with pulses of 5 ns duration at 2−20 Hz and pulse energy between 100 μJ and 2.5 mJ to focus on the excitation of OH vibrations from endogenous water molecules in the sample, which act as ionization matrix to facilitate desorption/ablation of neutral molecules from a surface.[33, 34] In metabolomic studies of biological samples using LAESI, desiccation has to be prevented and samples with low water content have to be wetted for analysis. The amount of water present correlates highly with signal intensity. This makes comparison between different sample types challenging.

Rapid evaporative ionization mass spectrometry (REIMS)[35] is a different ambient MS technique that has contributed to the metabolomics field with a particular role in the surgical

setting. It was developed by the Takáts research group and has been licensed with the name of i-Knife. During electrosurgery, high-frequency electric current is applied to surgical blades, with the pyrolized tissue plume producing aerosols and charged species that are removed by suction from the surgical site and transported to the mass spectrometer for remote analysis. REIMS is not as 'soft' as some of the other methods described here and thus some degree of fragmentation is to be expected.

Paper spray (PS) mass spectrometry (PSMS), first published by Wang et al. in 2010, is a simple method that allows for rapid quantitative and qualitative analysis of mixtures.[36] PSMS works by putting a sample such as serum onto a triangular paper. The triangular paper is positioned in front of the mass spectrometer and, after drying, analytes are eluted by a drop of liquid placed on the paper and simultaneously ionized at the paper triangle tip via the application of high voltage. Lin et al. have written a comprehensive review on PSMS, its various applications, and the many modifications that include leaf spray,[37] droplet monitoring,[38] and others.[39] Table 1 provides a summary of the ambient MS techniques discussed above; their benefits as well as their drawbacks. The operational details and physicochemical mechanisms of ambient techniques have been reviewed elsewhere[5–7, 40–43] and will not be discussed in depth in this review.

Targeted vs. Untargeted Metabolomics

Metabolomic studies are generally conducted either in a targeted or an untargeted manner. Targeted approaches deal with the measurement, detection, and quantitation of a defined set of known metabolites that may belong to one or more biochemical pathways.[44] Interactions and relations between predefined metabolites can be effectively established and placed in a biological context. In this approach, mass spectrometric methods are optimized to accurately detect a particular class of compounds, at the expense of narrower metabolome coverage. Therefore, data collected from a targeted study cannot be further retrospectively analyzed for testing alternative hypotheses concerning metabolites not present in the initial panel. Conversely, untargeted metabolomics, or metabolic profiling, is an impartial, all-inclusive top-down approach that attempts to characterize all detectable metabolites in a biological system.[45] Untargeted MS-based metabolomics has become a valuable exploratory tool capable of providing extensive chemical information for discovery and hypothesis-generating studies regarding biochemical processes; this enables the characterization of alterations across multiple pathways.[46, 47] Untargeted studies require the use of multivariate statistical tools capable of handling high dimensionality data, with usually one-fold more variables (metabolite features) than samples.

General Metabolomics Workflow

A general MS-based metabolomics workflow that includes both targeted and untargeted approaches is shown in Figure 1. The most important aspect of any metabolomic study is a careful definition of the scientific questions to be addressed, the experimental design, and the sample collection protocols. These factors will determine which metabolomic approach is best suited.[48] Examples of metabolomics studies comprise finding differences between complex samples, disease-related metabolic changes, the identification or validation of potential biomarkers, the investigation of a treatment effect, longitudinal studies, etc.

Variability of external conditions should be minimized from sample collection to data acquisition. The second part of the workflow illustrated in Figure 1 includes several steps common to both targeted and untargeted approaches, but with some notable differences. The sample preparation procedures determine which metabolites and pathways are more likely to be covered in the study. In relation to this step, ambient MS techniques offer capabilities that are different from traditional approaches in the sense that ambient ionization MS typically removes the need for extensive sample preparation. Minimal sample processing allows for reduced data collection time and *in situ* and *in vivo* analysis.

After data collection, data pre-processing involves metabolic feature extraction for analysis. There are no standardized procedures for data curation and normalization of untargeted datasets, which are multidimensional and often very large. The data pre-processing and pretreatment methods utilized influence the outcome of the data analysis. As mentioned above, orders of magnitude differences are expected between measured metabolite concentrations and metabolites present in high concentrations are not necessarily more important than those present at low concentrations. Different strategies and interesting discussions are provided by Van den Berg et al.[49] and De Livera et al. regarding this topic.[50]

Multivariate statistical analysis is used in metabolomics to reduce the dimensionality of the data, find underlying trends, and allow for the isolation of the features that are important in distinguishing between different groups. Unsupervised methods such as principal component analysis (PCA) are very useful for finding outliers, tracking data quality, and identifying the variables responsible for capturing the most data variance. Supervised methods such as partial least squares discriminant analysis (PLS-DA),[51] orthogonal PLS-DA (oPLS-DA),[52, 53] or support vector machines (SVM)[54] are better suited for identification of treatment differences, and for classification and prediction of class membership. Extensive literature addresses the various chemometric tools used in metabolomics[51–59], their detailed discussion is outside the scope of this review article.

Identification of metabolites following multivariate analysis remains the major challenge in untargeted metabolomics. High-resolution instrumentation is capable of providing accurate masses that can be searched against databases, such as HMDB,[60] METLIN,[61] and LIPID MAPS[62]. Despite the growth of metabolite databases over the years, limitations are associated with the information available since a large number of metabolites are still unknown. Tandem MS spectra can also be compared to databases such as METLIN, however, different instruments provide varying MS/MS product ion ratios, making tandem MS database matching a challenge. Additional strategies to the comparison of fragmentation patterns for confidently annotating metabolites include the matching of ion mobility drift time (or collision cross section) with chemical standards.[63] Ambient-ion mobility spectrometry (IMS)-MS/MS methods provide a post-ionization separation dimension that can be exploited for metabolite identification since more simplified spectra can be obtained with increased signal to noise ratio by eliminating chemical noise, cleaner product ion spectra even when precursor ion co-selection occurs, and separation of closely-related compounds such as isobars on a millisecond timescale.

Relative quantitation of the identified metabolites is usually performed with untargeted metabolomic studies by comparing compound abundance in the different sample classes. Ion suppression and differences in ionization efficiency make absolute quantitation difficult in untargeted MS-based approaches. Conversely, targeted approaches are best suited for absolute quantitation by combining the inclusion of an internal standard with tandem MS methods that compensate for matrix interferences and chemical noise. Even though ambient-MS approaches benefit from minimal sample processing leading to minimum loss or change in metabolite levels, absolute quantitation remains a challenge due to the matrix effects. Internal standards can be used, but such an approach leads to increased sample preparation, somewhat offsetting the intrinsic advantages of ambient MS.

The last step in the workflow illustrated in Figure 1 involves the investigation of the biological relevance of the validated metabolites by identifying, through databases such as KEGG and MetaCyc, the metabolic pathways in which they play a role and the upstream and downstream biological molecules to which they are interrelated. This last step attempts to address the initial scientific question by testing or developing hypothesis depending on the type of approach utilized, i.e., targeted or untargeted, respectively.

Applications of Ambient-MS in Metabolomics

Ambient MS has been utilized in many different areas of metabolomics; from health to food studies. There is a vast amount of publications that illustrate the trend of using ambient MS techniques to address different metabolite-related scientific questions – all of which cannot possibly be covered here. There are also a few review articles that have highlighted how MS techniques play a useful role in bioanalysis and metabolomics, with some focus on ambient MS.[64, 65] Complementing those, this review outlines some of the benefits and limitations of applying ambient MS in metabolomics studies by highlighting representative publications of the large swath of published applications.

Health

Health-related metabolomics research is one of the most important areas that have benefited from ambient-MS developments, taking advantage of the ability to analyze samples *in situ*, *in vivo*, and in real time with minimal preparation. Applications include fast diagnostics,[66–72] biomarker discovery,[68, 73] intraoperative tissue characterization,[74–79] molecular imaging,[14, 80–96] and drug metabolism.[97–102] Specific to cancer research are the capabilities offered by ambient MS techniques comprising the determination of boundaries between cancerous and non-cancerous tissues based on ion fingerprints, the discovery of potential biomarkers for diagnosis and prognosis, and the monitoring of drug metabolites to evaluate chemotherapy efficacy, as illustrated by some of the examples that follow.

REIMS

Since its first description in 2009,[35] REIMS has been proven to be a very clever application of ambient ionization/sampling during surgery as a real-time monitoring approach to identify and differentiate between tissue types through machine learning. Rapid evaporation of biological material with MS analysis has been performed *in vivo* for determining the

boundary between cancerous and healthy tissue in real time according to the output provided by multivariate statistical analysis.[77, 78, 103] Lipidomic spectral features were capable of differentiating between healthy layers of the intestinal wall, cancer, and adenomatous polyps based on the REIMS fingerprints of each tissue type that were clustered in a 3-D PCA space (Figure 2 and 3).[78] REIMS has also been implemented as an attractive platform for fast shotgun lipidomic profiling of human cancerous cell lines with minimal sample preparation.[104] These striking studies illustrate the capability of ambient-MS in assisting histological pathologists.

DESI-MS

DESI-MS has also demonstrated broad applicability in health metabolomics. Lipid patterns from lung tissue extracts of *Francisella tularensis* live vaccine strain-inoculated mice, were obtained by DESI-MS three-fold faster than by direct infusion ESI-MS.[105] Lung samples from mice inoculated via intranasal inoculation were discriminated by PCA from controls and intradermal inoculated samples, which were grouped within a single cluster in the scores plot, suggesting that the intradermal vaccination had no measurable effect on the lipid profiles of mouse lung tissues. The differences in the relative ratios of glycerophosphocholine and glycerophosphoinositol species, identified in the PCA loadings plot, were associated with immune response in lung tissues.[105]

Similar to REIMS, DESI-MS can provide reliable intraoperative tools not only for diagnosis but also for assisting surgeons with pathologic information for decision-making, mainly based on lipid profiling. The extent of microsurgical resection of brain tumors is associated with length of survival. In 2013, Eberlin et al.[75] reported a DESI-MS-based method to rapidly analyze and classify brain tumors based on lipid patterns. It was an exploratory study that demonstrated the potential value of the chemical information obtained directly from tissue samples and the reliability of the classification system based on SVM in providing diagnostic information. This study involved 36 glioma and 19 meningioma samples that were analyzed *ex-situ*. The machine learning model was tested and validated for intraoperative use by analyzing tissue sections from 32 surgical specimens obtained from 5 research subjects who underwent brain tumor resection. The molecular diagnosis derived from the ambient MS imaging (MSI)-based method corresponded to histopathology diagnosis with very few exceptions, illustrating the potential of the DESI-MSI to provide accurate histological information of brain tumors, and define tumor margins.[75] Following this work, Jarmusch et al. presented a highly sensitive strategy to guide the intraoperative resection of brain tumors on a timescale amenable to intraoperative analysis, based on phospholipid signals obtained from tissue smears by DESI-MS.[76] Unsupervised multivariate statistical analysis was successfully applied to detect the intrusion of gliomas into normal tissue.[76]

In a different study, DESI-MS, together with UPLC-MS, were used to detect similar glycerophospholipid profiles in esophageal cancer tissue, showing a potential combination of both platforms for simultaneous screening and quantitation.[106] The main differences between mass spectra from the two approaches were associated with the different adducts formed.[106] The repeatability and reproducibility of DESI-MS for the imaging analysis of

human esophageal cancer tissue was also evaluated using a set of optimal geometric and electrospray solvent parameters.[88] The results of the study provided acceptable levels of reproducibility for the analysis of lipids suggesting that DESI-MS can be suitable for clinical research and diagnostics.[88] An integrated bioinformatics platform was described for intuitive histology-directed interrogation of DESI-MS imaging datasets, based on optimization of data preprocessing steps, precise image co-registration, and efficient tissue-specific molecular ion feature extraction.[87] Multivariate molecular signatures were analyzed with direct correlation to morphological regions of interest, offering insights into how different tumor microenvironments interact with one another, leading to region-of-interest–specific biomarkers and therapeutic targets.[87] Additional examples illustrating the contributions and further potential of DESI-MSI in cancer research exist in the literature.[80, 81, 90, 93] A DESI-MSI-based method based on PCA of lipidomic profiles, for example, allowed identifying specific lymph node tissue types without the need for immunohistochemistry (IHC) experiments.[80] Identification of lymph node micro- and macrometastases was achieved with a sensitivity of 89.5%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 97.2% when compared with standard IHC.[80]

Ambient Mass Spectrometry Imaging

MSI coupled to ion generation techniques that operate under ambient conditions have also shown to be powerful screening tools in drug metabolism and drug discovery. For example, ambient-MSI techniques have become attractive alternatives to whole-body autoradiography (WBA), which is the industry standard for performing preclinical drug distribution studies on lab animals, and has been used in the drug development process for more than 60 years. WBA provides quantitative, spatial information (on the order of micrometers) on drug compounds in relation to dose and time. However, WBA cannot provide structural information and thus, species that result from drug metabolism cannot be distinguished from the labeled drug since it is just the radioactivity of the label that is measured. In addition, the process to include a radioactive isotope for detection makes the procedure time-consuming and costly. The main two ionization techniques that have dominated MS imaging, and that have been extensively utilized for whole body imaging, are secondary ion mass spectrometry (SIMS), and matrix assisted laser desorption/ionization (MALDI).MALDI MS-based methods remain limited for analyses in the low *m/z* region that corresponds to small drug metabolites due to matrix interference and SIMS produces only limited structural information due to the high amount of energy transferred to the substrate. The first attempt to use ambient-MS for whole body imaging to mitigate these issues was by Kertesz et al. using DESI-MS/MS with selected reaction monitoring (SRM) to image the drug propranolol in whole body sections of mice.[102] The authors found comparable levels of propranolol and associated metabolites between DESI-MS/MS and WBA in brain, lung and liver, but found differences between the techniques in the analysis of kidney. Despite the advantages provided by DESI-MS in terms of fast analysis with minimum sample preparation, the major drug metabolite, hydroxypropranolol glucuronide was not observed above noise level in this experiment, attributed to ion suppression or extraction deficiencies. In 2008, Wiseman et al.[101] reported the use of DESI-MS imaging in a targeted study to analyze the intensity and spatial distribution of the antipsychotic drug clozapine and its associated metabolites in histological sections of various untreated rat tissues. Many drug metabolites were

simultaneously determined in a spatially-resolved fashion, gaining relative quantitative information for each tissue type. Absolute quantitation of the drug metabolites, however, was only possible with LC-MS/MS. The authors found large amounts of clozapine in kidney and testis by DESI-MS/MS, in contrast with previous findings.[101] Liu et al.[98] also performed a whole body tissue distribution study of clozapine in neonate mice, obtaining consistent results with those reported by Wiseman et al. Relative quantitation and distribution information was obtained for the drug and two of its metabolites.[98] The authors also found that DESI solvents containing 90% acetonitrile were proven to be the least damaging to the tissue, and were able to monitor a metabolite that was not detected by LC-MS/MS since the homogenization process diluted the final concentration of the compound, highlighting the benefits of minimal sample preparation required by ambient-MS strategies.[98]

Different attempts to achieve quantitation with ambient-MSI techniques have been reported in the literature. Vismeh et al. developed a novel approach using DESI-MS for the analysis of brain tissue sections of rats treated with clozapine.[100] Lipid profiles across various tissue sections dramatically affected the detection of the metabolite of interest; leading to 80% reduction in the target ion intensity. The molecular and physical differences between tissues require tissue-specific calibration curves for any target compound. Loxapine, a close analog of clozapine, was used as internal standard and pipetted on top of the rat brain tissue sections where clozapine was present. A calibration curve for clozapine was constructed with simultaneous DESI-MSI analysis of clozapine and loxapine. Both molecules behaved similarly across the tissue and quantitation was confirmed by means of LC-MS/MS.[100] This quantitation approach can be applied for drugs and associated metabolites that are well characterized and when an internal standard that shares their properties is available. In a different MSI study, quantitative nano-DESI imaging was suggested as a promising tool for accurate localization and relative quantification of analytes within heterogeneous tissue sections.[95] Lanekoff et al. have shown in a proof of principle study that shotgun-like quantitation of endogenous phospholipids in different brain tissue sections can be possible by using 2 phosphatidylcholine standards in the nano-DESI solvent for simultaneous imaging and quantitation of 22 endogenous phosphatidylcholine species observed in MSI.[95] A different strategy that could improve quantitation in targeted studies by reducing matrix interferences is the coupling of an ambient-MSI technique with post ionization separation by IMS. This allows imaging contrast enhancement through improved selectivity and signal-to-noise ratio by separating isobaric interferences. Phosphatidylcholines were imaged by Bennett et al. on mouse brain tissue sections by coupling differential mobility spectrometry (DMS) to DESI-MSI using fixed DMS compensation voltages that selectively transferred one or a class of targeted compounds.[107] By reducing chemical noise, the signal-to-noise ratio was improved 10-fold and the image contrast was doubled, effectively increasing image quality. DESI-MSI may also assist in psychiatric studies. Reduced relative levels of two phophatidylcholines localized in the corpus callosum white matter of two patients with schizophrenia compared to two controls supports a previous hypothesis about phospholipid dysfunction in this disease.[86]

Air-flow-assisted DESI-MS

Modifications to DESI-MS have led to the development of air-flow-assisted DESI (AFADESI).[97] This technique was used to study the anti-insomnia drug candidate, N^6-(4-ydroxybenzyl)-adenosine (NHBA) and how it spatially and temporally distributes throughout the body following administration (Figure 4). PCA was performed to compare *in situ* metabolic analysis via AFADESI-MSI of control, low dose and high dose rats. Six endogenous metabolites whose abundances were temporally altered in the rats and identified as GABA, choline, glycerophosphocholine, adenosine, creatine, and valine were dubbed the 'mechanistic small molecules' of the drug action of NHBA. Also, using AFADESI-MSI, Li et al. spatially explored *in situ* the alteration of metabolites in 52 postoperative lung cancer tissue samples with high sensitivity using an untargeted approach.[68] They discovered potential biomarkers to achieve rapid and direct histopathological diagnosis of cancer, by analyzing not only lipids but also amino acids, choline, peptides and carnitine. Cancerous and adjacent normal tissues were successfully discriminated by this technique using oPLS-DA.[68]

PESI-MS

The intact analysis of biological tissues has also been investigated with PESI-MS. The non-invasive character of the probe and the rapid ambient analyses provided by this technique without any pretreatment makes it an attracting tool for analyzing cancerous tissue specimens for diagnoses. The boundaries of cancerous regions were determined through the analysis of triacylglycerol in clear cell renal cell carcinoma (ccRCC) human specimens[69] and in chemically induced hepatocellular carcinoma in mice.[72] Similarly, solid probe assisted nanoelectrospray ionization (SPA-nanoESI) —a technique where an acupuncture needle is used to capture biofluid followed by its insertion into a solvent-loaded nanoESI capillary— has also been proven useful for discriminating normal from ccRCC human specimens, and for different tissue types in living mice.[108]

Touch Spray MS

In a similar approach to PESI-MS, touch spray MS (TS-MS) used medical swabs to detect bacterial phospholipids from *Streptococcus pyogenes*, responsible for strep throat (Figure 5).[67, 109] Sampling probes consisted of sterile medical swabs possessing an aluminum handle and a rayon swab head. The principle of TS-MS is based on the application of solvent to the metallic probe and a high voltage that generates a strong electric field, resulting in the field emission of analyte-containing charged droplets that undergo evaporation and Coulombic fission by mechanisms similar to those of ESI. TS-MS was also performed on fresh kidney tissue samples within 1 h of resection, from 21 human subjects afflicted by renal cell carcinoma (RCC).[66] The spectral features obtained in the negative ion mode analyzed with both PCA and LDA provided the separation between RCC (16 samples) and healthy renal tissue (13 samples). Discrimination of tissue samples was also achieved successfully with DESI-MS to compare TS-MS results, although different ionic species were responsible for sample class separation.[66]

LAESI-MS

Similar to DESI-MS, LAESI is a commercially-available and routinely used technique in ambient-MSI. It allows the analysis of spatially distributed metabolite abundance information without the need of a matrix. Different classes of metabolites from lipids and fatty acids, to small acidic and basic molecules can be sampled at once with LAESI, both *in situ* and *in vivo*.[9] Because of its high water content, various biofluids, single cells, and brain tissue have been studied with this technique.[84, 110] The transformation of T-cells and kidney epithelial cells infected with human T-lymphotropic virus as well as Tax1 and Tax3-expressing cell lines were metabolically profiled and compared with LAESI-MS.[111] For the first time, multiple novel metabolites that may have implications in the viral transformation of host cells were identified. A different example that illustrates the capabilities of utilizing LAESI-MS for metabolite profiling is a qualitative study of the neuromuscular junction using the *Torpedo* electric organ, homologous to the mammalian neuromuscular junction. The authors found 24 metabolites associated with this organ, and though quantitation was difficult, they managed to use deuterated standards spiked on the tissue to analyze a few of the most important metabolites.[112] As mentioned above, IMS can be integrated with ambient ionization techniques for imaging experiments. LAESI-travelling wave IMS-quadrupole-time-of-flight-MSI enabled the construction of molecular images of selected ions that were distinguished by drift times in the sagittal sections of mouse brain tissue.[113] This integrated technique provided a new approach for mapping the distribution of isobaric compounds in tissue.

DART-MS

Another ambient MS technique that has been used for health metabolomics is DART-MS. DART combines the advantages of plasma-based ambient MS in terms of simplicity and sensitivity, with the robustness of commercial instrumentation. Several strategies for coupling DART-MS to multivariate statistical models for high-throughput metabolomics analysis of blood sera have been reported in the literature, including transmission mode sampling to increase reproducibility with lower risk of cross contamination.[24, 71, 114, 115]

The combination of DART- or DESI-MS with nuclear magnetic resonance (NMR) spectroscopy has proven to be a powerful platform in health metabolomics. DART-MS and NMR coupled to multivariate statistical strategies were successfully used for classifying serum samples from breast cancer patients and healthy controls.[116] This platform was also implemented in a biomarker discovery study for monitoring health status in whale sharks (*Rhincodon typus*) from an aquarium collection. PCA of spectral data showed that individual animals could be resolved based on the serum metabolite composition, and that two unhealthy individuals could be discriminated from the remaining healthy animals, based mainly on the concentration of the osmolyte homarine, suggesting that this metabolite may be a useful biomarker of health status in this species. NMR and DESI-MS have also enabled the differentiation between lung cancer and healthy mice based on the analysis of urine samples.[117] For DESI-MS and MS/MS analysis, samples were directly deposited onto paper with no additional preparation steps, allowing the tentative identification of a total of 80 metabolites.

Microorganisms

Before the concept of ambient ionization/sampling was widespread, the analysis of microorganisms required significant sample preparation steps. New opportunities are now available through the use of ambient MS. For example, DESI-MS and extractive electrospray ionization MS (EESI-MS), a technique involving the confluence of two electrospray streams, were used to analyze the central carbon metabolites in *E. coli* extracts.[118] By means of tandem MS experiments, 13 of 17 central carbon metabolites were identified from uniformly labeled and unlabeled metabolites in the quenched supernatant.[119] DESI-MS has also been used to generate highly reproducible mass spectra *in situ* from whole bacterial cells with essentially no sample preparation.[120] An untargeted study differentiated bacteria species and strains *in situ* using DESI-MS with high reproducibility.[120] DART has also been successfully applied to bacterial identification through fatty acid methyl ester profiles of membrane lipids.[121]

Other studies have used nanoDESI or LMJ-SSP to investigate microbial colonies in real time. Watrous et al. used nanoDESI-MS to investigate the spatiotemporal metabolic profile from living bacteria from Petri dishes without sample preparation (Figure 6).[122] The authors used MS/MS fingerprints to develop networks-based workflows to group tandem MS spectra based on the similarity of the fragmentation patterns obtained from related, but different, precursor ions. Using this strategy, the authors were able to build molecular networks of spectrally related molecules in live colonies over time, and analyze microbial interactions.[122] Imaging using nanoDESI-MS was also performed on live biological specimens to not only gain spatiotemporal information, but also profile locations within the same colony *in vivo*.[123] By means of LMJ-SSP, MS fingerprints were generated from multiple cultured fungal and bacterial species directly from a Petri dish.[124] The speed offered by these ambient MS-based methods was a significant advantage for microbial analysis. Other untargeted metabolomic studies of bacteria have been done using a data-independent MS/MS approach (DIA) with nanoDESI. Discrimination of *Bacillus subtilis* and *Pseudomonas aeruginosa* was achieved *in situ* based on their DIA profiles according to the presence/absence of specific ionic species. Day to day variation in the data was minimal, demonstrating the high reproducibility of this method.[125]

REIMS was first extended for bacterial analysis by the Takáts research group.[126] REIMS was able to classify bacterial species from intact living microorganisms without sample preparation. REIMS profiles, obtained in only 3 seconds, were not restricted to lipid species originating from the cell wall and cell membrane, but attributed to additional cellular components. The authors went further to show that REIMS, in conjunction with supervised and unsupervised statistical analysis was able to blindly differentiate different strains in vivo.[127]

Plant Biology and Agriculture

Many reviews have explored and documented the benefits of metabolomics for understanding plant biology.[128–132] One of the earlier studies using ambient ionization techniques for plant metabolomics focused on *in situ* analysis of alkaloids with DESI.[133]

The various parts (seeds, stems, leaves, roots and flowers) of the poisonous plants studied, poison hemlock (*Conium maculatum*), jimsonweed (*Datura stramonium*) and deadly nightshade (*Atropa belladonna*), were directly analyzed via DESI-MS and MS/MS without any treatment or extraction. The authors were not only able to distinguish between the various plant parts, but also they successfully identified the majority of known alkaloids present and their distribution with good reproducibility. Liu et al.[37] developed leaf spray MS (LS-MS) based on PSMS[134] for analysis of living and dehydrated plant tissues. Briefly, LS-MS works by applying a voltage directly to wetted or un-wetted tissue, in this case a leaf, to generate charged droplets that are analyzed by the mass spectrometer. Even if LS-MS is not strictly considered an ambient MS technique because it does no focus on surface analysis, it still allowed the rapid identification of a wide range of endogenous plant metabolites including many phytochemicals.[37]

Other approaches such as negative ion mode DART-MS coupled to PCA and linear discriminant analysis were successfully applied to discriminate between samples of Red Oak (*Quercus rubra*) and White Oak (*Quercus alba*) under conditions that favored pyrolysis, based on 11 ionic species. The strengths of the method were mainly its speed (a few seconds per sample) and the tiny quantities of wood that are consumed for the analysis.[135] In a different untargeted study, the capabilities of DART-MS to explore cold-tolerance in *Arabidopsis thaliana* accessions were compared with a UHPLC-MS-based method. PCA performed on DART mass spectral data was not able to provide sufficient information for sample discrimination, whereas the UHPLC–MS-based method did, possibly due to better metabolome coverage.[136]

One of the largest advancements that ambient-MS has brought to plant biology is its ability to image metabolites on the surface of plant tissue. DESI-MSI, for example, has allowed for mapping secondary metabolites, such as surface anti-fungal chemicals, in tropical seaweed. Lane et al. found that these chemicals were localized in specific patches of the seaweed.[137] Other studies have analyzed leaf imprints by DESI-MSI, gaining sensitivity and signal enhancement at the expense of losing *in vivo* or *in situ* capabilities. Imprinting can be done on porous surfaces to indirectly image tissues. For example, the fluctuations of glycoalkaloids present in sprouted potatoes infected by the phytopathogen *Pythiumultimum* were monitored by imprint-imaging DESI-MS in positive ion mode with minimal sample preparation.[138] The plant metabolic response to pathogen invasion was indicated by a decrease of the relative abundance of potato glycoalkaloids α-solanine at *m/z* 706 and α-chaconine at *m/z* 722, and the increase of the relative intensity of solanidine at *m/z* 398, solasodenone at *m/z* 412, solanaviol at *m/z* 430, solasodiene at *m/z* 396, solaspiralidine at *m/z* 428, γ-solanine/γ- chaconine at *m/z* 560, β-solanine *m/z* 706, and β-chaconine at *m/z* 722.[138] Direct DESI-MSI of leaves from the Katsura tree (*C. japonicum*) allowed for the identification and spatial distribution of non-fluorescent chlorophyll catabolites. Leaves imprinted with porous polytetrafluoroethylene provided increased signals for catabolites that were not detected in the more direct analysis.[139] Thunig et al. used a porous Teflon surface to reproducibly analyze the leaves of *Hypericum perforatum* and *Datura stramonium* with indirect DESI-MSI.[140]

Resulting from a combination of atmospheric pressure (AP) MALDI[141] and infrared (IR) MALDI,[142] AP IR-MALDI[143] has been used to analyze plant tissues and image metabolite distribution on strawberry skin. AP IR-MALDI is a soft ionization technique taking advantage of water present in biological samples. Water molecules absorb infrared radiation, leading to evaporation and metabolite expulsion from a plume of droplets formed with the sampled material without significant damage to the plant tissues.[143] A different experimental setup was designed by O'Brien et al. for spatially resolved ambient IR laser ablation-MS (AIRLAB-MS), using an infrared microscope with an infinity-corrected reflective objective and a continuous flow solvent probe.[144] The technique was able to determine the differences in nicotine concentrations between different parts of the leaves of wild-type and mutant tobacco plants.[144]

LAESI-MS has been extensively used in plat metabolomics studies.[145] IR LAESI-MSI was applied to analyze the depth and spatial distribution of metabolites in the Zebra plant (*Aphelandra squarrosa*) leaves. In the study, the authors identified 36 metabolites involved in pathways associated with the yellow variegated areas of the plant.[30] Three-dimensional (3D) images of plant tissues were obtained on various plant tissues to gain an additional spatial distribution dimension for understanding metabolite distribution.[30] LAESI-MS has also been used for cell by cell imaging of the purple onion (*Allium cepa*), which allowed finding differences in purple pigment concentrations between epidermal cells and has been used to distinguish gland cells from cells in the leaf away from the gland in *C. aurantium* (Figure 7).[146] The analysis of *A. cepa* provides an example of how LAESI-MSI can achieve relative quantitation in single cells.[147] This onion species was also used as a model to verify the feasibility of analyzing metabolites from a single cell using PESI-MS, allowing for direct sampling of live cells.[148] Differences between cell types and subcellular compartments of the same cell were observed based on the detected metabolites, which comprised fructans, lipids, and flavone derivatives.[148] In a PESI-inspired approach, Nakashima et al. developed a pressure PESI-MS with internal electrode capillary (IEC-PPESI-MS) which enabled high spatial-resolution cell sampling, precise post-sampling manipulation, and high detection sensitivity for *in situ* single-cell metabolite profiling.[149] The capability of the technique was evaluated with the analysis of amino acids, organic acids, carbohydrates, and flavonoid in stalk and glandular cells that form a trichome unit in tomato plants (*Solanum lycopersicum L.*), showing metabolic differentiation between two cell types as well as among different types of trichomes.[149] Etalo et al. performed a series of experiments on plant tissues to highlight the usefulness as well as the challenges of using LAESI-MSI for *in situ* metabolomic analysis.[150] The water content dependence was seen to affect the material ablation process and to be critical for efficient ionization. Thus, the authors suggested making careful comparisons between samples with different water content, either naturally or as a result of sample storage. The major downside of IR-based methods is the long analysis time (up to 3 min in some cases). Desorption spot sizes typically range between 50 and 250 μm, providing lower spatial resolution than other MSI techniques such as MALDI or SIMS.

Foodomics

Foodomics has only been relatively recently defined as a distinct field of study comprising food science and nutrition.[151, 152] In recent times, much attention has been given to preventive medicine, food quality and safety, and genetically-modified foods. The broad applicability of metabolomics to food science has been extensively reviewed.[153–155] The way in which food is metabolized has a major impact in human health; we are what we eat. By far the most popular ambient MS technique used in foodomics has been DART-MS. Applications in food quality and safety using DART-MS have been extensively reviewed.[154, 156] Examples include metabolomic fingerprinting for differentiating common carp muscles according to feeding history,[157] beer origin recognition,[158] authentication of milk and milk-based products,[159] differentiating vegetables from organic and conventional farming,[160] and studying ripening stages in fruit.[161]

Sample preparation protocols for DART-MS foodomic studies involve either metabolite extraction or grinding into powder. In most cases, DART-MS has been implemented within targeted metabolomics workflows. Based on previous knowledge about the classes of metabolites of interest, DART parameters that affect sensitivity, including gas temperature, position of the sample, and gas flow rate, require optimization before analysis. As mentioned in the introduction, sample preparation steps, if any, also have a drastic impact on the type of compounds that are detected. Single-drop liquid-liquid-liquid microextraction (SD-LLLME) coupled to DART-MS was developed to quantitatively target low levels of phytohormones in fruit juice.[162] Cajka et al. used polar and non-polar solvents for metabolite extraction from chicken meat and feed before analysis.[163] Multivariate statistical approaches using DART-MS data successfully differentiated chicken meat according to the animal diet. DART-MS has also been used for animal fat authentication based on triacylglycerol profiles used to build a linear discriminant model for classification,[164] as well as for olive oil authenticity assessment and adulteration screening.[165] TM-DART-MS has shown to be able to effectively ionize triacylglycerols, diacylglycerols, and free fatty acids in vegetable oils, during their thermally-induced oxidation.[165] DART mass spectra and PCA were used to assess compositional differences between heated and non-heated vegetable oil samples.[166] By means of TM-DART-MS, vegetable oils, phytosterols enriched margarines, butters and animal oil were satisfactorily differentiated with characteristic ions for each sample type, allowing sample classification based on phytosterol profiles.[167]

Additional metabolomics studies on food have been performed using REIMS, DESI-MS, and EASI-MS. REIMS allowed rapid lipidomic profiling of food grade meat products; a PCA-LDA model successfully classified animal tissue with different anatomical origin, breed, and species.[168] DESI-MS was proven to be a fast and simple technique for the detection and analysis of triglycerides in oil- or fat containing food samples.[169] EASI-MS allowed quality control of Amazonian vegetable oils based on triacylglycerol fingerprints,[170] and was used for quality control of vegetable and animal fats.[171]

Summary and Perspective

Ambient MS techniques have shown great capabilities and unique contributions in metabolomic studies in recent years. The ability to analyze samples in real time without the need for extensive sample preparation offers a unique opportunity to better understand the metabolism of a variety of species and subspecies where high throughput sampling is desirable. Ambient MS has allowed for rapid analysis of samples with little sample preparation further extending its applicability to a wide variety of sample types. In this review, we used selected publications to broadly illustrate and discuss applications of ambient MS in metabolomics.

Despite the growing popularity of ambient MS in metabolomics, key challenges remain. Matrix effects resulting in spectral overlap, differences in ionization efficiency, and ion suppression cannot be mitigated as with LC-MS methods, and remain a challenge for most ambient MS-based methods. Therefore, the comparison of metabolomic results from different sample types is limited with ambient MS methods since ion suppression is tissue-composition dependent. Untargeted analysis with imaging methods suffer from higher limits of detection than targeted approaches due to matrix effects. In addition, sampling reproducibility needs to be further improved. As with many other analytical methods, metabolite coverage, data analysis, and identification needs more development. Improvements to databases would further the metabolic coverage of these methods. Regardless of the current limitations, the scope of applications will only increase with improvements to existing ambient MS technologies and the advent of new ones.

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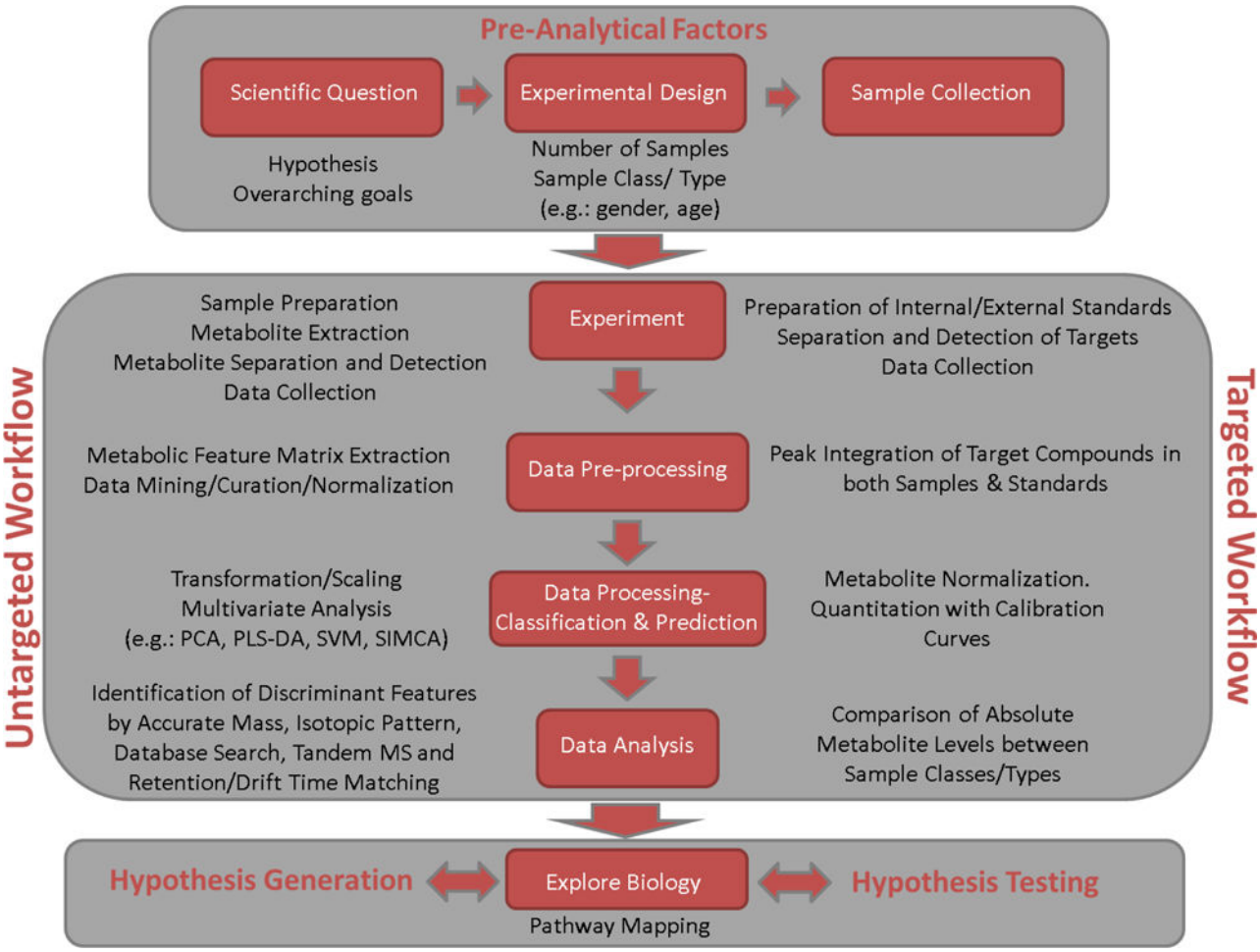

Figure 1.
MS-based untargeted and targeted metabolomics workflows.

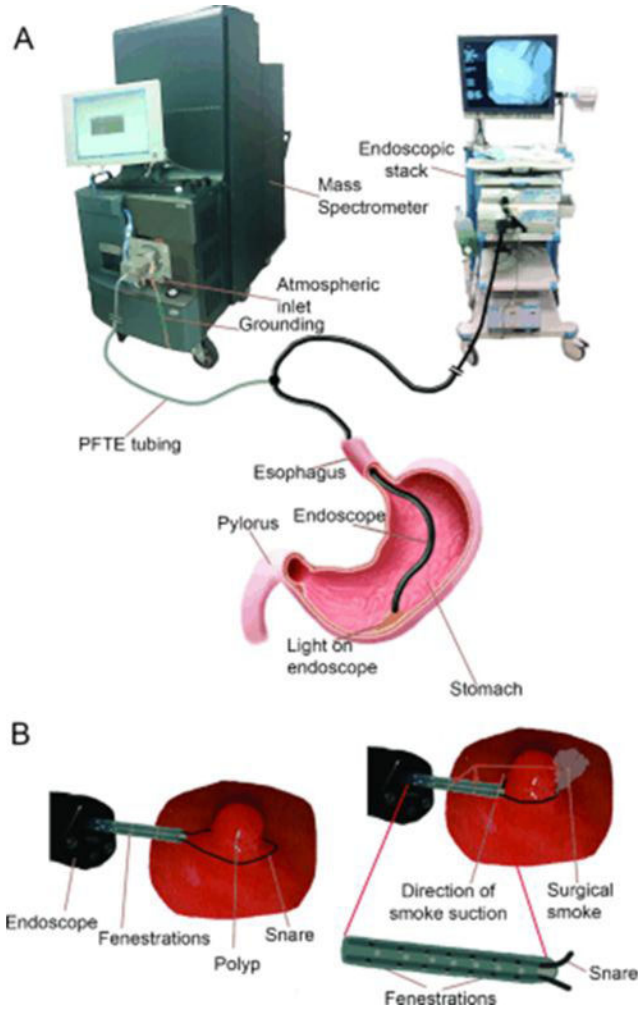

Figure 2.

Endoscopy experimental setup. A) The polypectomy snare was equipped with an additional T-piece in order to establish direct connection between the electrode tip and the mass spectrometer for the transfer of electrosurgical aerosol. B) Resection of GI polyps by using a commercial snare. The polyp is captured with the snare loop, which is tightly fastened around its base. Electrosurgical dissection is performed and the generated aerosol is aspirated through the fenestrations created on the plastic sheath of the snare.[78] Reproduced from Ref. 78 with permission from John Wiley and Sons ©2015.

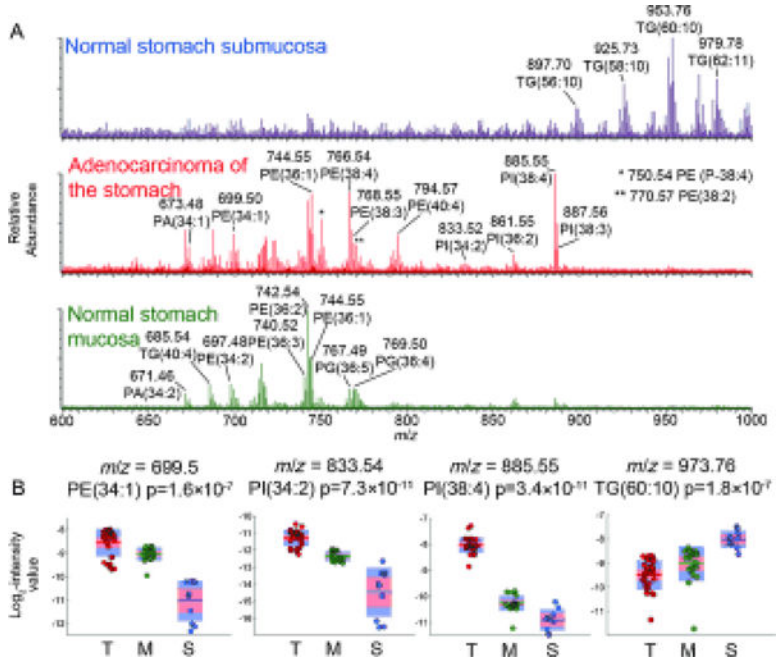

Figure 3.

A) Mass spectra from gastric mucosa, gastric submucosa, and adenocarcinoma tissue recorded ex vivo using a modified Xevo G2-S Q-ToF mass spectrometer. Cancerous and healthy mucosal tissues feature mainly phospholipids in the m/z 600–900 region, whilst submucosa features triglyceride and phosphatidylinositol species in the m/z 850–1000 region. B) Comparison of the abundance of selected peaks showing significant differences between cancerous and healthy tissues in the range m/z 600–1000 using Kruskal–Wallis ANOVA, $p<0.005$. T=tumor, M=mucosa, S=submucosa.[78] Reproduced from Ref. 78 with permission from John Wiley and Sons ©2015.

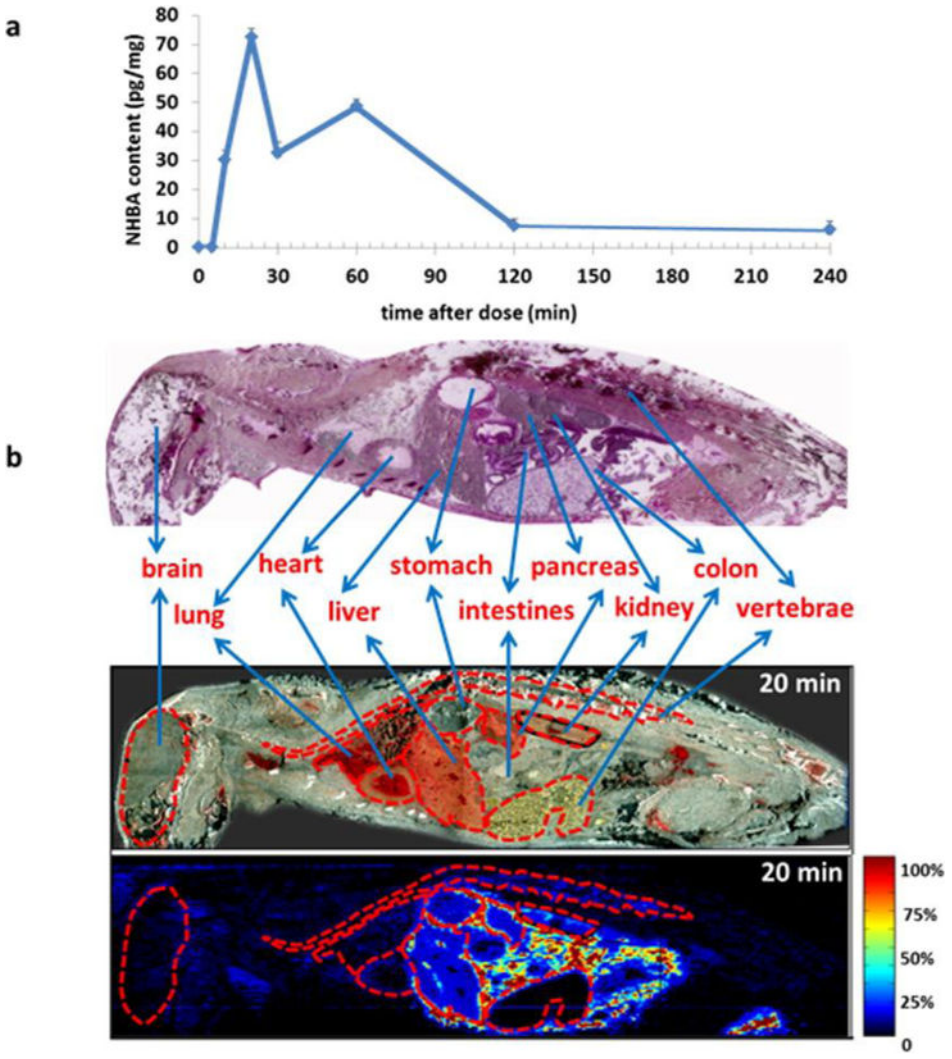

Figure 4.

NHBA content in the rat brainstem and whole-body distribution. (a) NHBA content in the brainstem of rats euthanized at various time points after dosing, as measured by quantitative LC-MS/MS in MRM mode. (b) Whole-body distribution of NHBA (40 mg/kg via intraperitoneal injection, followed by euthanasia 20 min later) acquired by AFADESI-MSI (MRM, m/z 374.2 → 242.0). Organ regions are outlined. Spatial resolution = 300 μm × 500 μm. ((Top panel) HE-stained, whole-body rat tissue section at 20 min after NHBA administration; (middle panel) optical image of rat tissue section; and (bottom panel) MSI image of rat tissue section.[97] Reproduced from Ref. 97 with permission from the American Chemical Society ©2015.

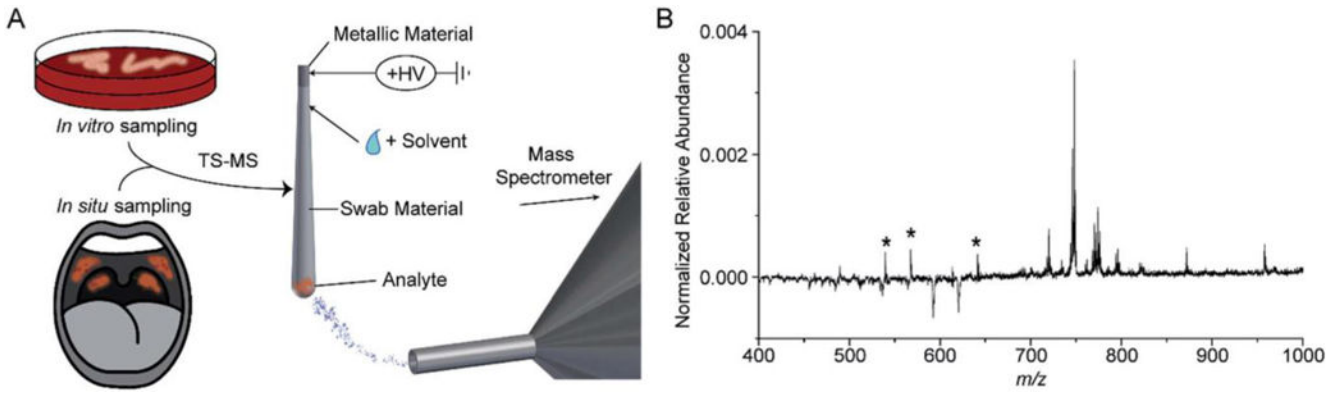

Figure 5.

(A) Schematic of sampling from culture or patient throat swab and subsequent analysis by TS-MS. (B) Negative ionization mode TS-MS spectra of a single colony of S. pyogenes sampled from culture. Peaks of negative relative abundance, after subtraction, and those annotated by asterisks are attributable to background. The other peaks with positive relative abundance are tentatively identified as bacterial phospholipids.[67] Reproduced from Ref. 67 with permission from the Royal Society of Chemistry ©2014.

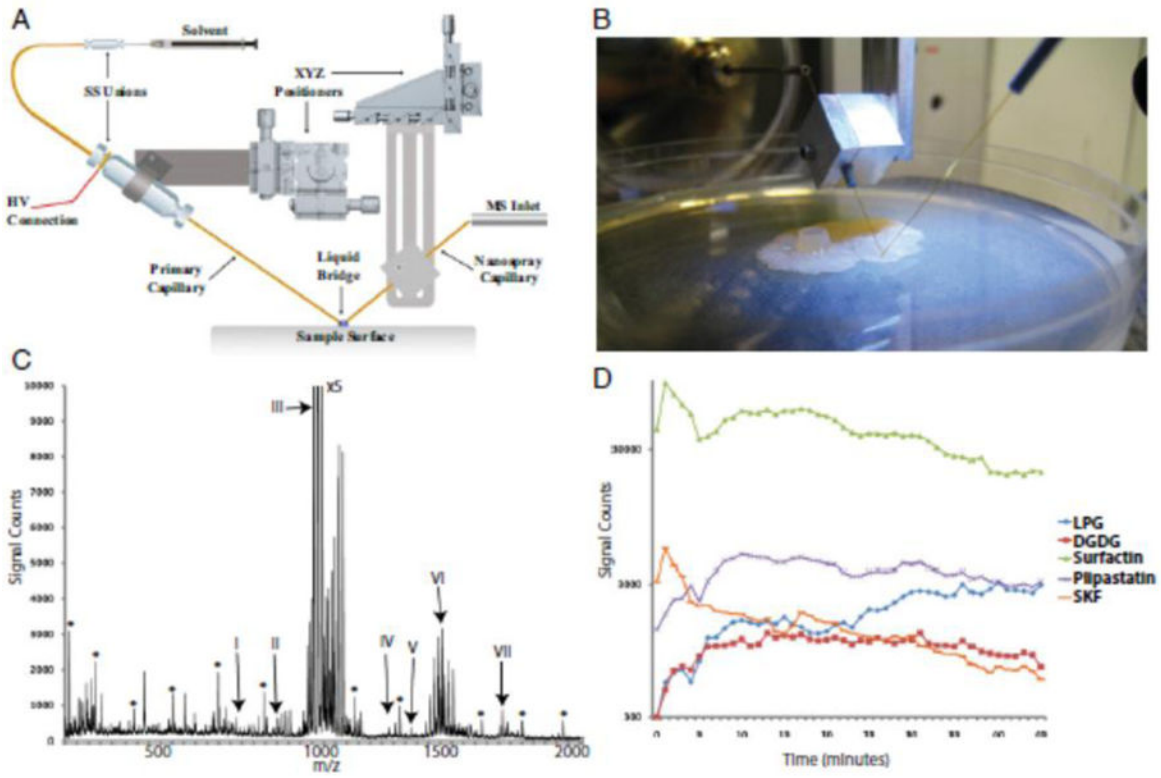

Figure 6.

Analysis of microbial colonies directly from petri dishes using nanoDESI.[122] Reproduced from Ref. 122 with permission from the National Academy of Sciences ©2012.

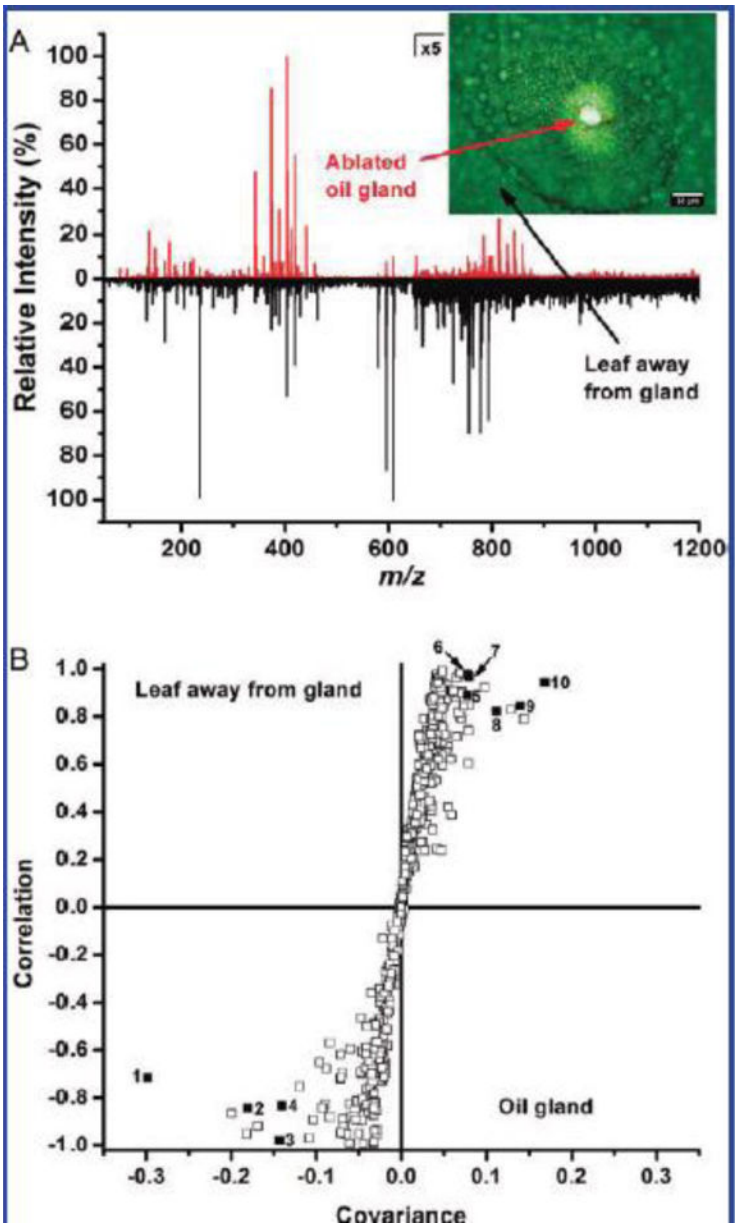

Figure 7.

(A) Positive-ion LAESI mass spectra from n = 6 to 8 oil gland cells (pooled from the ablation of the center of two glands) of a C. aurantium leaf (red trace on top) and n = 6 to 8 cells from the leaf away from the gland (pooled from two ablation spots) (black trace in the bottom). The inset shows a microscope image of an oil gland with the ablation mark (scale bar is 50 μm). The ablated spot is ~30 μm in diameter. (B) S-plot produced by OPLS-DA of the spectra showed that many metabolites strongly correlated with either the oil gland cells (n = ~25) or cells in the leaf away from the gland (n = ~25). The 10 metabolites with serial numbers (SN) (solid squares) indicated in the figure are identified in Table S4 of the Supporting Information.[146] Reproduced from Ref. 146 with permission from the American Chemical Society ©2011.

Table 1

Comparison of Ambient MS techniques

Technique	Acronym	Mechanism	Major Advantages	Major Disadvantages
Desorption Electrospray Ionization	DESI	Surface liquid extraction followed by electrospray-like ionization	• Broad metabolite coverage • Salt tolerant • Imaging of a wide range of surfaces possible • Commercially available	• Quantitation may be difficult
Easy Ambient Sonic-Spray Ionization	EASI	High velocity gas generates ions trough sonic spray mechanisms	• Voltage free • Positive and negative ions are simultaneously generated • Cleaner mass spectra • Well suited for permeable solid membranes	• Lower sensitivity • High gas flows required
Liquid Micro Junction Surface Sampling Probe	LMJ-SSP	Surface liquid extraction followed by ESI or APCI	• Can be coupled to different ionization techniques • Very sensitive • Low sample requirements • Can be coupled to LC for additional separation	• Delay between extraction and ionization
Direct Analysis In Real Time	DART	Thermal desorption followed by plasma ionization	• Rapid and simple analysis • *In vivo* analysis possible • Commercially available • Real time capabilities	• Requires analytes to be volatile or semi-volatile • Some plasma gases can be costly
Probe Electrospray Ionization	PESI	Sharp needle electrospray probe	• High salt tolerance • Very small sample size • *In vivo* analysis possible • Real time analysis • No needle clogging • Low ion suppression	• Reproducibility affected by needle sampling depth • Susceptible to cross-contamination
Laser Ablation Electrospray Ionization	LAESI	Laser ablation coupled to ESI	• Larger mass range	• Signal intensity correlated with

Technique	Acronym	Mechanism	Major Advantages	Major Disadvantages
			• Commercially available	water content making comparison of different sample types more difficult
Rapid Evaporative Ionization Mass Spectrometry	REIMS	RF electrosurgery coupled to remote sampling MS	• In situ tissue sampling • Commercially available	• Relatively energetic sampling/ ionization conditions, some degree of fragmentation.
Paper Spray Mass Spectrometry	PSMS	High voltage applied to wet porous media producing electrospray and in situ cleanup	• Good reproducibility with commercial cartridges of internal standards. • Inexpensive and simple to set up • Modifications can be made to accommodate a wider variety of sample types	• Ionization efficiency is affected by paper tip sharpness and type of paper used. • Sample deposition method has to be accurately controlled