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From single cells to our planet—recent advances in using mass spectrometry for spatially resolved metabolomics

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Spatial information in the form of 3D digital content has been increasingly integrated into our daily lives. Metabolomic studies parallel this trend with spatial and time resolved information being acquired. Mass spectrometry imaging (MSI), which combines qualitative and quantitative molecular information with spatial information, plays a crucial role in mass spectrometry-based metabolomics. The lateral spatial resolution obtained by MSI continues to improve and allows mass spectrometers to be used as molecular microscopes—enabling the exploration of the cellular and subcellular metabolome. Towards the other end of the scale, MS is also being used to map (image) molecules on our skin, habitats, and entire ecosystems. In this article, we provide a perspective of imaging mass spectrometry for metabolomic studies from the subcellular to planetary scale.

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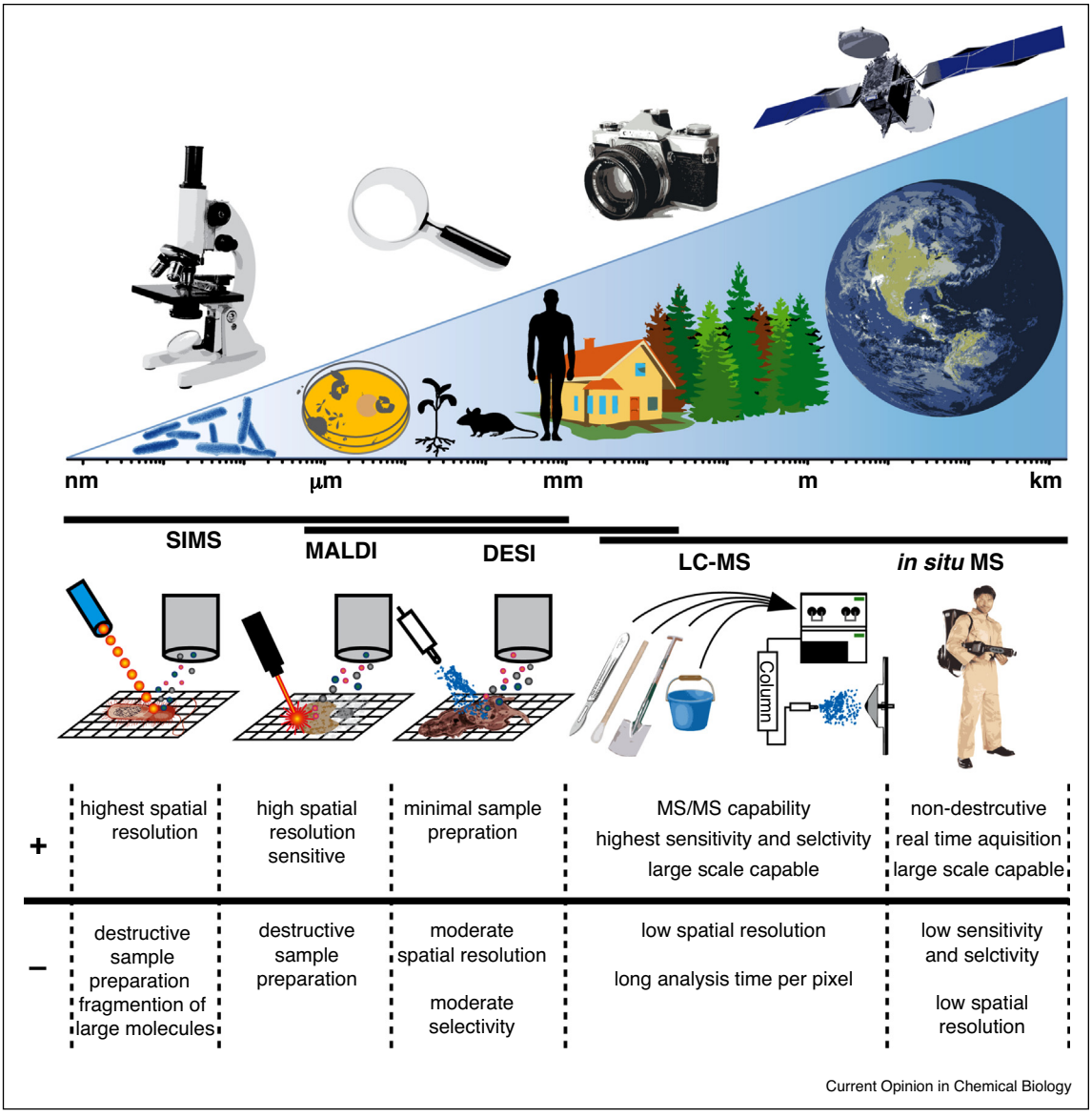
Introduction

Our world, especially the current and emerging scientific generation, grew up with 3D digital content. The introduction of 3D medical imaging such as X-ray computed tomography (CT) and magnetic resonance imaging (MRI) in the 1970s allowed us to better visualize our internal injuries and diagnose disease [1,2]. Decades later, a world without 3D digital information is unimaginable. We now interact daily with 3D digital content either in the form of movies, television, social media, and video games as well as virtual [3] and augmented reality [4]. Furthermore, the introduction of 3D printing technologies [5], 3D laser scanners [6] and structure from motion [7] has made it incredibly easy to transform digital objects into real objects and vice versa. 3D imaging and fabrication technology is becoming increasingly used in scientific studies (e.g., prototyping analytical equipment [8] and ion manipulation [9], production of custom medical equipment [10] and LASER scanning [11]). However, the molecular and spatial world in which we live has been left relatively unexplored. Spatial metabolomics aims to measure the metabolome, for example, the totality of molecules present in a sample, across spatial dimensions ranging from nanometers (subcellular) to kilometers (entire environments to planetary scale). The questions concerning our molecular environment (i.e., three spatial dimensions and time) are extremely interesting and vital to improving our understanding of physiology, health, and environment (locally and globally). Discussion of the current mass spectrometry-based spatial metabolomics is blended with our perspectives of each method's potential.

General remarks

Mass spectrometry is a powerful chemical measurement tool. It is sensitive and specifically detects molecules as charged ions. Metabolomic studies have been traditionally focused on answering the questions, "what metabolites and at what quantity are they present?" While the answers to such questions continue to drive the field, the study of complex systems is incomplete without spatial (and temporal) information. Mass spectrometry imaging (MSI) is a method by which molecular information is obtained over two or three spatial dimensions. Multiple ionization sources are available for MSI, offering different lateral spatial resolutions (nano- to kilometers) that can be used to answer spatial metabolomic questions across a wide range of scales as illustrated in Figure 1. The ionization source and mass analyzer define the analytical figures of merit, such as lateral spatial resolution, mass resolution, duty cycle, and MS/MS capability. However, there are a few instrument independent characteristics which are generally important for MSI experiments. The time required for data acquisition increases following an inverse squared relationship with lateral spatial resolution [12]. The sensitivity of mass analysis must increase with decreased lateral spatial resolution. Generally, quantitative analysis is poorer compared to traditional extraction and analysis by liquid chromatography–MS (with the caveat that such analysis destroys spatial information). MSI, regardless of the specific method, has been used

Figure 1



Scale of spatial metabolomics and corresponding mass spectrometry imaging methods. The spatial scale of samples range from nm (e.g., bacterial cells) to km (e.g., planetary). Optical imaging techniques, depicted above the spatial scale, is analogous to the MSI methods (below the spatial scale) in regards to the spatial resolution at which they acquire data. Sample scales and optical image generation is correlated to the matching MSI technique with typical spatial resolution. The highest resolution in nm range is obtained by SIMS followed by MALDI and DESI in the μm range. Upper limits are typically in cm range and either defined by the size of the sample and/or measuring time. For higher spatial scale imaging from mm to km range, collection and dissection of samples is combined with (micro) extraction and LC–MS analysis; alternatively, samples are analyzed *in situ* by MS. A brief comparison of the strengths and weaknesses of each technique is shown on the bottom of the figure; however, many are highly situational and study-dependent.

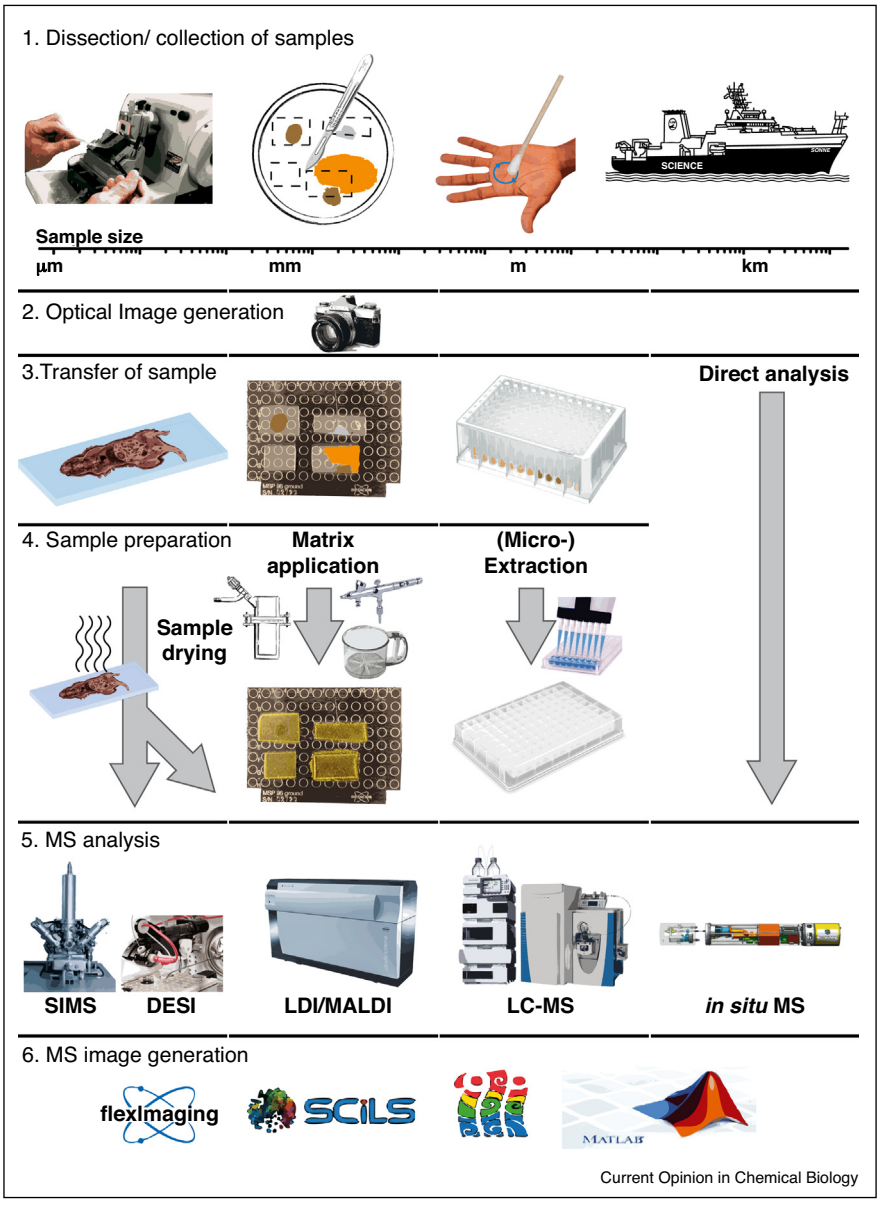
principally to explore chemo-spatial information at the microscopic scale, but also has vast potential in addressing the questions posed by spatial metabolomics on the macroscopic scale. The scale of the samples to be analyzed and the questions that are asked largely determines which MSI approach is most appropriate.

Secondary ion mass spectrometry (SIMS)

Historically, the first MSI experiments were performed using secondary ion mass spectrometry (SIMS) [13,14] which provides the highest lateral spatial resolution for cellular and subcellular exploration of the metabolome, currently. SIMS is performed by focusing a primary ion

beam at a sample surface inducing sputtering and secondary ion generation. The typical workflow for SIMS imaging is shown in Figure 2. The type of primary ion beam largely defines the lateral spatial resolution, sputter rate, depth resolution (i.e., static versus dynamic SIMS), and energy imparted to desorbed ions which can result in fragmentation [15]. Recent advancement of cluster ion beams is notable, allowing lateral spatial resolutions less

Figure 2



Experimental work flows applied for mass spectrometry imaging. The chart covers techniques applied for SIMS and nanoSIMS, DESI and nanoDESI, LDI, MALDI and LC–MS. Depending on the size of the sample or system which should be imaged, the experimental workflow typically starts with sectioning or dissection of the sample. For samples in the meter to kilometer range, a systematic sampling is performed in which spatial coordinates are recorded as sample metadata. After sampling and generation of initial optical images, the samples are transferred to target plates for SIMS, DESI, LDI and MALDI or in test tubes or microtiter plate for LC–MS. After transfer of the samples they can be either analyzed directly (SIMS, DESI and LDI) or further prepared, for example, MALDI imaging requires the matrix to be sprayed, sieved, or sublimated onto the samples. For LC–MS-based approaches, depending on the physical condition, samples are typically extracted using liquid solid extraction or solid phase extraction. After sample preparation, MS data is acquired by rastering the surface of the sample in which every mass spectrum recorded can be linked to its spatial coordinates (SIMS, DESI, LDI and MALDI), or by analyzing a set of samples of which each be traced back to their spatial origin of sampling (LC–MS approach). After MS acquisition, MS images are typically shown as heatmaps of single ion signals (e.g., extracted ion maps, EIMs). For the image generation, several vendor specific and vendor independent software packages are available. Besides this, MATLAB scripts are often used to visualize MSI data especially for experimental approaches and prototypes. Most recently, '*ili* a new software for LC–MS-based imaging, originating from former MATLAB toolboxes and is available through an open source license [55].

than 100 nm with moderate fluence. C_{60}^+ primary ion beams are commonly used for 3D imaging as the relatively large mass of the primary ion sputters material effectively while imparting relatively low energy thereby minimizing fragmentation [15]. Recent applications of 3D SIMS include the study of drug uptake in macrophages at the single cell level [16•] and the 3D analysis of the incorporation of peptides in MALDI matrix crystals [17]. The future remains promising for subcellular and cellular SIMS imaging with the coupling of new mass analyzers which provide improved mass resolution [18••], greater sensitivity, and the possibility of MS/MS [18••,19•].

Matrix-assisted laser desorption ionization (MALDI)

Matrix-assisted laser desorption ionization (MALDI) imaging was first reported by Caprioli *et al.* in 1997 [20] and is the most widely applied MSI method [21•]. MALDI imaging acquires mass spectrometric data from samples which are typically prepared as thin flat sections which are then coated with matrix, a chemical compound which absorbs light energy at the wavelength of the laser. An experiment without matrix would be laser desorption ionization (LDI) which has been used for MSI as well. The schematic workflow of both LDI and MALDI imaging is shown in Figure 2. The lateral spatial resolution typically ranges from a few μm to mm and is dependent on several factors, for example, matrix crystallization and laser diameter. The majority of MALDI imaging experiments are performed on linear MALDI-time-of-flight (TOF) platforms and have been used to detect small molecules, peptides [22,23] and proteins [24] from tissue sections, for example, *in vivo* studies. In addition to imaging of tissue sections, it is also possible to image microbial colonies directly from dried agar plates, Figure 3, visualizing the metabolomic content and interaction between co-cultured microorganisms [25,26]. Sequential analysis of several sections of a sample (agar or tissue section) and post analysis reconstruction produces three dimensional MS images [27–29]. Recent advances in microbial imaging include the application of an matrix spray device, which resulted in an higher homogeneity of the coated matrix and a higher spatial resolution [30•]. Other advances in instrumentation have increased spatial resolution to the sub μm range [31,32]. High mass resolution, a desirable feature in MSI, is typically achieved with MALDI systems coupled to quadrupole-TOF (qTOF) or Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers. Atmospheric pressure (AP) MALDI has been recently coupled to orbitrap mass spectrometers, yielding high mass resolution with high spatial resolution [33•]. Previously, one limiting factor was analysis time, but the recent introduction of MALDI-TOF systems with 10 kHz laser dramatically decreases analysis time. This particular improvement will have a significant impact on the feasibility of 3D images (which require the imaging of several tissue sections) as well as on high spatial resolution MALDI images of larger samples sizes [34••]. Besides new methods of matrix application and improvements of instrumentation, computational tools are being used to increase spatial resolution of the images generated—adapting a strategy used in super resolution microscopy and for satellite imagery, spatial resolution can be increased through image fusion [35••]. In this process, two or more images from different sources (e.g., optical image from a sample and corresponding MSI image) are mathematically fused into one image which represents the best characteristics of all information. Perhaps such strategies have the potential to push the spatial resolution of MALDI MSI field down to the scale of SIMS.

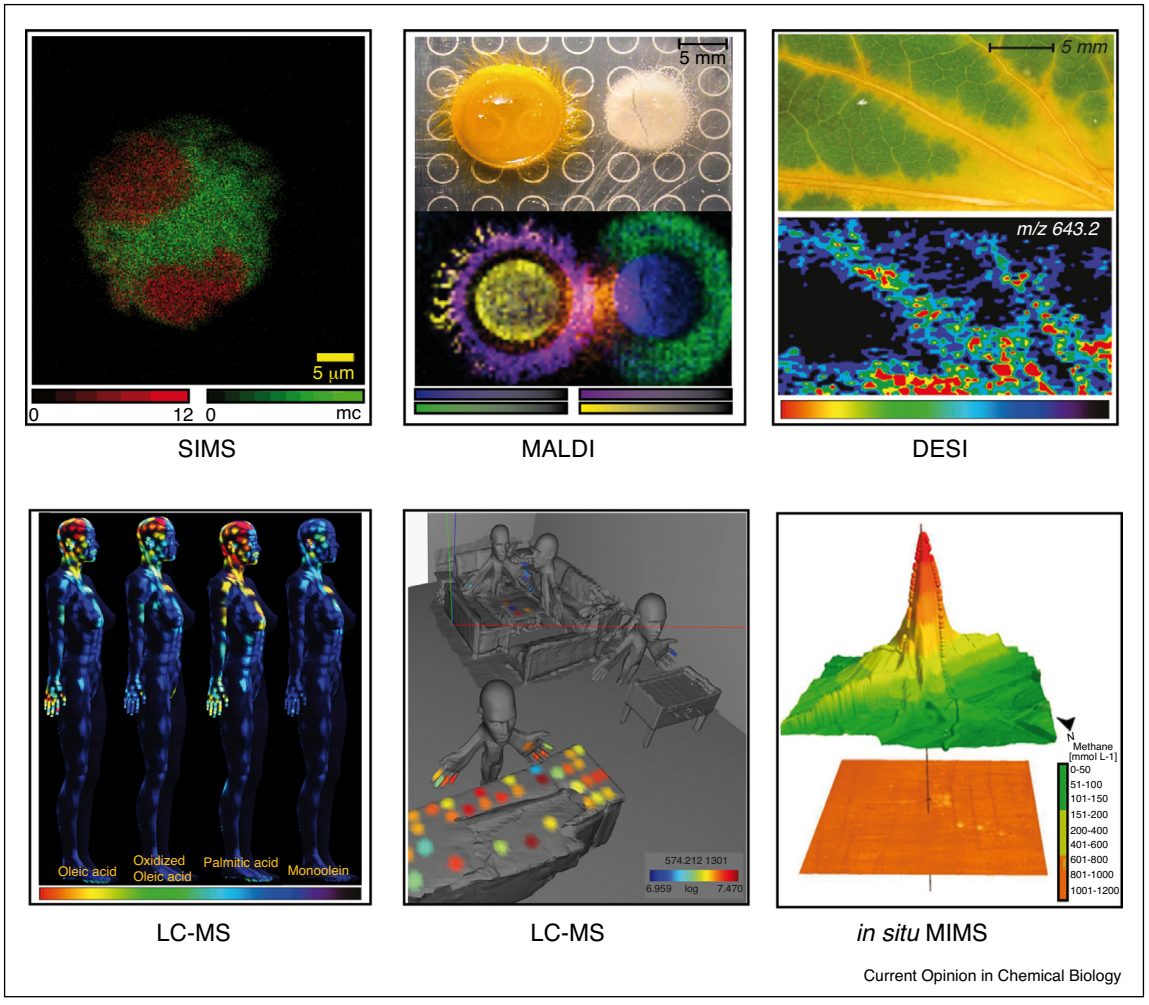
Desorption electrospray ionization (DESI)

The necessity of introducing a sample for imaging into the vacuum system of a mass spectrometer can be prohibitive, for example, SIMS imaging and most MALDI imaging systems. Ambient ionization methods, such as desorption electrospray ionization (DESI), are defined by the following characteristics: reduced or no sample pretreatment and the formation of ions at atmospheric conditions (pressure, humidity, temperature). Ambient ionization methods can also be used for MS imaging [36]. A typically DESI imaging workflow is shown in Figure 2. DESI imaging has been used to reveal the chemo-spatial relationships of plant metabolites in leaves [37] (Figure 3). Furthermore DESI has been applied for imaging of molecular differences associated with human brain cancer [38•], 2D distribution of fungal secondary metabolites [39] and 2D distribution of metabolites from bacterial co-cultures [40]. Further, 3D imaging of samples has been performed via the analysis of multiple sections and subsequent reconstruction, viz., 3D visualization of the chemical differences between grey and white matter in mouse brain [41]. Nano-DESI and laser ablation electrospray ionization (LAESI) offer some of the smallest lateral spatial resolutions and have also been widely applied [42,43]. As commercial ambient ionization source become available, the use of ambient ionization imaging is likely to increase, particularly on the 50–1000 μm lateral spatial resolution scale.

Liquid chromatography–mass spectrometry-based cartography (LC–MS)

The coupling of chromatographic separation, for example, capillary electrophoresis, gas chromatography, and liquid chromatography with mass spectrometry (LC–MS) has incontestably revolutionized chemical measurement and remains one of the most sensitive mass spectrometric techniques for the detection of many different organic molecules. Sample preparation before LC–MS typically destroys any spatial information associated with a sample; however, by measuring several samples, each of which with a defined spatial position (e.g., one pixel) LC–MS

Figure 3



Illustrative mass spectrometric images from different ionization techniques. The SIMS image shows the drug uptake of a macrophage. The molecular marker for the drug friodarone is I^- and shown in green. In the same image, the nucleus is represented in red through $HP_2O_6^-$ as molecular marker [16•]. The MALDI image shows two co-cultured myxobacteria colonies and their metabolic exchange [30•]. The DESI experiment was carried out on a tree leaf under ambient conditions [37]. Both LC–MS examples were acquired by swabbing skin or the habitats surface with ethanol soaked cotton swabs which were subsequently extracted, measured by LC–MS, followed by the generation of extracted ion maps [44•,45•]. The *in situ* MIMS image was acquired on a submergible mass spectrometer and shows the underwater methane concentration around a methane gas flare [46•].

represents a method of exploring the 3D chemical world in macro scale—referred to as 3D cartography. A typical workflow is shown in Figure 2. Through recent advances in spatial imaging tools, for example, 3D scanning or 3D reconstruction via photography, one is now more able to record scaffold models onto which MS information can be displayed. The chemical surface of human skin was mapped using this approach. The skin was swabbed with ethanol soaked cotton swabs in discrete areas followed by LC–MS analysis [44•]. The data were plotted upon a 3D rendering of a body, revealing distribution of human derived molecules, cosmetic and hygiene products as well as microbial compounds originating from the skin microbiome [44•]. An exemplary model of the human body is shown in Figure 3. In another study the molecular content of surfaces of human habitats and how the chemical inventory of our skin interacts with it was imaged [45•]. In Figure 3, an extracted ion map of a social gathering room after a happy hour event is shown. One can see how a particular molecular feature is spatially distributed, including upon the hands of people interacting with this environment. The depth of chemical information obtained by this method is unsurpassed by any other strategy, but can only be used to address questions that are compatible with poorer spatial resolution. Regardless, the information about the molecular nature of our body, homes, environment, and entire planet is revealing—generating more questions rather than

answers and will become an important aspect of imaging mass spectrometry.

In situ mass spectrometry

Exploring the chemical world on the largest scale is ideally performed outside of the laboratory, *in situ*. Mass spectrometry-based measurements have been taken from aircraft, submersible, and even on extra planetary craft (e.g., Mars rover Curiosity). These instruments are generally custom built and are coupled with electron ionization or gaseous discharge-based ionization sources. A popular methodology for continuous sampling and analysis is membrane inlet MS. A submersible instrument with a membrane inlet MS (MIMS) was used to analyze the concentration of methane in and around an underwater methane flare [46•]. An exemplary figure of an *in situ* 3D mapping experiment is shown in Figure 3. Curiosity, the Mars rover, is equipped with the Sample Analysis at Mars instrumentation package which includes a quadrupole mass spectrometer [47]. The resulting information has provided a better understanding of the chemical and isotopic composition of the Martian atmosphere—an improved chemical measure over the prior Viking missions [48]. With the miniaturization and development of ambient pressure ionization methods, this field will continue to expand in the future.

Data analysis and sharing

Facilitating the analysis and sharing of imaging data, several computational tools have been developed and are organized across multi-laboratory collaborations, such as the COST action on MS imaging (ms-imaging.org) or Metaspace2020 (metaspace2020.eu). Metaspace2020 has at least 100 high-resolution MS imaging data sets that are publicly accessible and can be analyzed through a web-browser. Current community efforts include the introduction of a common data format for imaging data such as imzML [49], compression of data [50] and data sharing [12,51•]. Several commercial software packages exist for the analysis of MSI data; however, several independent tools have been developed (or are under development) to facilitate data analysis and multi-platform data comparison. Examples are *OpenMSI* which uses an independent file format HDF5 [52], *SpectralAnalysis* a tool which covers the entire MSI data analysis [53], and msIQuant a viewer and analysis tool which uses the standardized imzML file format and includes several post processing options such as the above mentioned image fusion [54•]. For the visualization of LC–MS-based cartography one can use '*ili*, a software tool still under development but already accessible to the community [55], which generates extracted ion maps on 3D .stl files. '*ili* as well as all other software tools will improve data analysis, data sharing so that comparative analysis become possible, and ultimately such knowledge sharing needs to become a major facet in MSI—one facet that will parallel MSI development towards improved spatial and mass resolution.

Final remarks

Mass spectrometry imaging allows one to explore the metabolome, spatially. The adaptation of MSI is growing substantially as we transition from the 1D chemical analysis via spatial unaware extract analysis to exploring the 3D chemical world in which we live and the how that world changes over time. The different questions, and corresponding samples, will require MSI tools that cover a range of spatial resolutions. The power of MSI is furthered by capturing 3D spatial information onto which MS data can be plotted. People can process and interpret visual information better than any other information. Simply by adding the spatial location of sampling to common LC–MS analysis, brand new and arguably more insightful questions can be explored. The challenge of scale exists at both the small and large end of the spectrum, for example, subcellular and planetary, respectively, as does the challenge of increasing the sensitivity and specificity of chemical analysis. Mass spectrometry remains one of the most powerful techniques for chemical analysis; however, a large fraction of molecules that can be detected remain unannotated—potentially greater than 98% in LC–MS-based metabolomics [56•]. The only way to overcome these limitations is through building knowledge bases. Larger scale knowledge bases, including MSI data, should be connected so that we can also determine in what samples do the same molecules appear. This is important should we be able to ask molecular questions that cross all scales. Such knowledge base infrastructure for annotation is also being developed specifically for MSI. New developments in ionization techniques and mass analyzers will further improve the capabilities of MSI, especially when aided by new computational tools and community driven spectral databases such as GNPS [56•], open MSI [52], and Horizon2020.

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