Introduction

During the last 50 years, natural product research has benefited from the rapid advancement of MS (mass spectrometry) instrumentation, which enabled highly sensitive analysis of biological samples [1]. New MS ionization sources such as ESI [2] and MALDI [3] have revolutionized analytical chemistry, leading their inventors to receive the Nobel Prize in Chemistry in 2002. These “soft” ionization techniques enable the direct observation of natural compounds as intact ions and forever transformed MS from a niche tool of analytical chemistry to a ubiquitous technique utilized in life sciences research. Coupled to GC (gas chromatography) and LC (liquid chromatography), hyphenated MS instruments are now established worldwide as indispensable tools for high-throughput workflows. Being the most sensitive and the most powerful method, LC-MS nowadays represents the backbone of targeted and untargeted metabolomics approaches to detect and elucidate extremely low-abundance metabolites occurring in any type of biomass used in natural product research [4]. However, MS does not provide any information concerning the spatial and temporal distribution of metabolites in a biological sample. IMS complements traditional metabolomics and chemical
analyses by combining the qualitative and quantitative molecular information with spatiotemporal information, providing the capability to map specific molecules to 2D or 3D coordinates of the original sample [5].

IMS started developing more than two decades ago as the high-resolution frontier of material science and biology [3, 6, 7], and later gained large popularity in peptide and protein analyses, particularly in medicine and cancer diagnostics [8–11]. As of today, IMS has established itself as a highly efficient and reliable tool in capturing the image of both large and small molecules in various life science disciplines. IMS techniques combine the specificity, selectivity and sensitivity of MS with spatially resolved chemical information [3]. The ionization source or sampling probe is rastered across the sample surface to induce desorption of organic compounds into the gas phase, where they get ionized and differentiated through their m/z [12]. This process is repeated sequentially over the whole sample until the mass spectra associated with every position on the sample are acquired. The ions of interest are then displayed in different colors and superimposed on the sample’s picture, correlating with the natural distribution and the abundance of each m/z ion throughout the sample. The higher the abundance of the molecular ion at a certain raster position, the greater the intensity of color that is displayed [13]. IMS does not require traditional sample extraction, which not only saves time and a tedious procedure but also eliminates problems (e.g., the production of artifacts). Moreover, depending on the type of IMS application, analysis of volatile and nonvolatile compounds may be achieved at the same time [14]. An IMS dataset contains a picture for each detected analyte [15]. IMS provides an untargeted in situ analysis of molecular species from complex samples to single cells, and from biological macromolecules to small metabolites, where metabolic signatures can be correlated with the histological and morphological features [16]. It provides scientists “molecular eyes” or “molecular microscopes” for analyzing the surface, tissue, and even cellular and subcellular metabolomes [5] as well as studying the dynamics and biological functions of metabolites of interests in a given organism. By providing a simplified analytical workflow and a chemical specificity at the morphological level that was never possible before, IMS is now emerging as a revolutionary field at the interface between chemistry and (micro)biology. Not surprisingly, the IMS approach has been employed in over 100 microbiological studies since its first use in this field in the early 2000s [12]. Currently, it is being successfully applied to several areas including biomarker identification, tissue specific biosynthetic processes, microbe-microbe and host-microbe interactions, functional ecology, drug discovery, and chemical ecology.

Early IMS techniques, such as SIMS and MALDI, require the sample to be ionized at high vacuum. SIMS was first reported by Castaing and Soldzian in 1962 [17] and is the oldest IMS technique. Under high vacuum, a beam of high energy primary ions is focused onto the sample, physically perturbing the surface by a process known as “sputtering” [18], generating secondary ions, which are analyzed by the mass spectrometer [19]. One type of SIMS experiment called dynamic SIMS uses a continuous beam of primary ions to ablate the surface of the sample, allowing 3D as well as 2D imaging experiments [20, 21]. The primary advantage of SIMS is spatial resolution, as the ion beam can be focused down to 50 nm, enabling imaging on a subcellular level [22]. IMS-MALDI has been applied to the subcellular localization of biomarkers and metallo-drugs in cancer cells [23, 24], as well as antibiotics in individual bacteria [25]. SIMS imaging has also been used to study nitrogen fixation in microbial communities [26] and marine invertebrates [27], as well as carbon flux in terrestrial worms [28]. As a result of the high-energy beam of ions, IMS typically exhibits hard ionization, producing many fragments instead of molecular ions, potentially leading to the loss of information.

The well-established technique of MALDI-MS has a characteristic soft ionization, which produces a high ratio of molecular-to-fragment ions. MALDI imaging was first reported by Caprioli et al. [8], who mapped certain peptides and proteins in various coated tissue sections or blotted imprint of the sections [8]. In a typical MALDI-IMS experiment, the sample is coated or co-crystallized with a light-absorbent matrix and irradiated with a pulse from a UV or IR laser. The matrix absorbs the radiation, transferring energy to the sample and aiding in ionization [29]. MALDI has several disadvantages, including high chemical noise in the low mass range (<300 m/z) originating from the matrix components that may suppress crucial small molecule ions and the requirement for the sample to be mounted on a conductive surface. The laser desorption/ionization process also destroys the sample during MALDI-IMS. However, in comparison to several other IMS techniques discussed below, MALDI-IMS has superior spatial resolution (from >5 µm up to >100 µm) [12]. Other advantages of MALDI-IMS include the wide mass range (from 300 m/z up to >5000 m/z) with which it can operate, good tolerance of variations in sample geometry, and a wide variety of established protocols for imaging, especially of microbial colonies [30]. With selection of a proper matrix, many types and sizes of compounds, ranging from proteins to lipids, peptides or secondary metabolites, can be visualized [12]. MALDI-IMS is the most established and most frequently used IMS technique in natural product research. Mainly led by the Dorrestein group at UC San Diego (USA), MALDI-IMS has been used in many excellent studies, for example, in identification of the biosynthetic origin of natural products.
from a bacterium-sponge symbiont [31], mechanisms of coral-associated bacterium to protect its host from pathogenic fungi [32], and in examination of complex molecular interplays including suppression, enhanced production, biotransformation, and other metabolic exchanges in polymicrobial co-cultures [33]. MALDI imaging has been widely applied to natural product research, examining the spatial distribution of compounds in plants [34–39], bacteria [40,41], cyanobacteria [31,42], and various marine invertebrates [31,42–46].

In contrast to SIMS and MALDI, which operate under vacuum, recent IMS applications are embracing techniques that can be performed at ambient conditions with as little sample preparation as possible, such as LAESI-IMS and DESI-IMS. LAESI-IMS, invented by Nemes and Vertes and first described in 2007 [47], combines a mid-IR laser with an electrospray source. The sample is ablated by the laser, and the resulting cloud of particles is ionized as it passes through the electrospray plume [48]. The primary advantages of LAESI-IMS are that non-flat samples may be analyzed and that very little sample preparation is required [49]. Since the laser ablation removes a layer of the sample with each pulse, LAESI-IMS may be used for 3D as well as 2D sample analysis [50]. One key limitation of LAESI-IMS is that the sample needs to be rich in water in order to absorb energy sufficient for ablation from the laser pulse [47]. As LAESI-IMS physically removes layers of the sample during ablation, it is not suitable for analyses in which the sample needs to be analyzed repeatedly (e.g., for a time course study). Applications of LAESI in natural product research include the investigation of bacterial biofilms [51,52] and in the imaging of living plant tissues [50,53,54]. Combining LAESI-IMS analysis with bioassay has been shown to be a powerful tool for drug discovery [55].

Compared to other IMS techniques, DESI-IMS presents several advantages that render it well suited for natural products chemistry. Because SIMS-IMS and MALDI-IMS are both performed under vacuum, the samples must usually be freeze-dried before the analysis, making these techniques incompatible with living tissue. DESI differs from SIMS, MALDI, and LAESI in that it is considered a minimally destructive ionization method, allowing samples to be repeatedly analyzed in a time course experiment. The relatively limited sample preparation required for DESI-IMS analysis combined with minimally destructive sampling of living tissue enable DESI-IMS to fill a niche inaccessible by other IMS methodologies. These properties make DESI-IMS a potent tool for the investigation of microbe-microbe and host-microbe interactions. In this review, we focus on DESI-IMS technique, discussing the principles of the ionization mechanism, instrument optimization, sample preparation, and current applications in natural products chemistry, including terrestrial or marine microorganisms, plants, and animals, and finally its wide use in medical biochemistry and clinical research.

**DESI-IMS**

In 2004, DESI was introduced as a novel ESI technique by Cooks’ group at Purdue University (USA) [56]. DESI relies on a soft ionization technique similar to ESI in LC-MS, which delivers mass spectra with very low fragmentation in either positive or negative ionization mode. The simple workflow and the ease with which a DESI source can be connected to existing mass spectrometers contribute to rising popularity of DESI in different fields of analytical chemistry. One of the main advantages in comparison to other IMS techniques is that DESI does not require extensive sample preparation such as matrix fixation in MALDI-IMS, resulting in a simplified analytical procedure for a rapid spatial and temporal identification of chemicals in intact biological samples, all under ambient conditions [56,57]. DESI-IMS can be applied to investigate highly complex sample surfaces, from polymeric materials to biological tissues and even fluids [58], allowing for the detection and semi-quantification of a variety of polar or non-polar small molecules [56,59–61].

**Chemical and physical aspects of the ionization mechanism**

In DESI, high-velocity ionized solvent droplets desorb the analytes of interest directly from the sample surface, where compounds are solvated in a thin film. To achieve the chemical desorption, solvents are electro-sprayed under high voltage through an emitter capillary producing charged “primary” droplets, which are nebulized and then directed toward the sample. Metabolites located on the sample surface are in this way desorbed into gaseous “secondary” droplets delivering molecular ions entering the MS inlet where m/z values are measured [62,63] (Fig. 1). Depending on the polarity of the metabolite of interest, different spray solvents from the DESI source can be used for imaging. For the analysis of both polar and non-polar compounds, standard solvents such as MeOH or ACN are frequently used, usually with the addition of 2–5% H2O, but different solvent compositions have been employed according to the specific needs and application type [64,65]. Overall, DESI-IMS has been shown to have a high degree of tolerance toward salt adducts formation and ionization suppression [66]. Interestingly, while the electrodynamics of droplets formation from the liquid ESI cone-jet have been thoroughly studied [67], the physical mechanism responsible for progeny droplet formation and impact at the sample surface during the DESI event is still elusive and not completely understood. Due to the high-pressure environment and the high kinetic energy of the molecules produced in DESI [58], the desorption process does not seem to depend on the physical particle sputtering mechanisms that govern gas ion collisions techniques such as SIMS [18,58,68,69]. Instead, several studies on ion-surface scattering processes in vacuum suggest that, during DESI, the molecules from surfaces may be ionized and released following a mechanisms known as “chemical sputtering” [70,71]. Fluid dynamics studies that simulated the electrochemical desorption mechanisms of DESI hypothesized that stochastic momentum-transfer events are the major forces leading to the formation of analyte-containing droplets [63,72]. These results also highlight the strong dependence on the contact angle and surface wettability of the materials as some of the major variables that govern the DESI process and that eventually affect the way metabolites are picked up from the surfaces and analyzed [72]. A modified version of DESI is nano-DESI, in which the microscale solvent extraction and desorption are achieved using two separate capillaries surrounding the sample in a “liquid bridge” [73,74]. Notably, nano-DESI is most suitable for direct sampling and IMS chemical profiling of small-scale wet
surfaces that would suffer ablation from the strong nebulizing gas of DESI such as living microbial colonies in agar media and associated biofilms [74–76].

**DESI instrumental parameters and geometry**

Due to the sensitive intrinsic properties, optimization of different parameters prior to DESI analysis is routinely required in order to obtain the best desorption and ionization of metabolites in the sample and the highest reproducibility between experiments. The correct adjustment of these parameters will strongly influence the outcome of the analysis and the final imaging resolution. Among the most critical parameters are the electrospray solvent flow rate (e.g., 0.1–5 µL/min) and voltage (1–8 kV), and the overall geometry of the DESI system, particularly the distance of sample surface to the DESI spray nozzle tip (d1) and to the MS-inlet (d2), as well as their respective incident and collection degree angles (α and β) [58,77,78] (Fig. 1). Common application guidelines of the instrument recommend utilizing a geometry configuration with larger tip-to-surface distances (d1 = 5–8 mm), smaller incident angles (α = 20–50°), and higher voltages (3–8 kV) for small molecules such as lipids or aromatic hydrocarbons, and opposite range of settings (d1 = 1–2 mm; α = 60–90°, 1–4 kV) for larger molecules, such as proteins or nucleic acids [58]. Solvent flow rate and sprayer distance from the sample surface influence the quality of the analysis as they affect droplet sizes and their speed. At 2 µL/min solvent flow rate and 1130 kPa nebulizing gas pressure, droplets have been measured to have an average diameter of 2–4 µm and an impact velocity of 120 m/s [63]. High nebulizing flow rates are generally preferred as they produce smaller and faster droplets, which increase the desolvation efficiency. However, the upper limit is reached when droplets generated are too small and may evaporate before reaching the surface resulting in a lack of signal. Thus, given each geometry setup, the relationship between solvent and gas flow rates will strongly determine the final imaging resolution [77]. A typical spatial resolution can range between 50 and 200 µm, with an average resolution of 40–60 µm [79] and the highest resolution reported at 35 µm during phospholipid analysis in mouse brain tissue in 2012 [79–81]. Increased resolution can be achieved by nano-DESI thanks to nano-spray ability to deliver droplets at lower velocity (4 m/s), allowing a gentle deposition of charged reagents with minimal splashing and a spatial imaging to <12 µm [73,82].

**Sample preparation**

DESI-IMS has been successfully applied in several areas ranging from clinical medical research to pharmacology, and from natural product chemistry to chemical ecology. According to the application and the nature of the biological tissue, sample preparation may differ. DESI analysis is usually restricted to flat and preferably hard surfaces. Samples with smooth and regular surfaces can be usually analyzed directly with no sample preparation. In case of non-flat surfaces (for instance animal organs and tissues), cryosections can be prepared following commonly established IMS protocols [83]. In case of plants, preparation of DESI samples can be achieved in different ways. Flowers and leaves are often problematic as they present soft, irregular, and very absorbent surfaces, which may result in low or instable signal during imaging.

In addition, most land plants contain wax cuticles on their leaf surface that are highly hydrophobic and can be difficult to penetrate by spraying solvents. As an alternative to direct analysis on plant surfaces, indirect sample analysis can be performed via “imprinting” of metabolites for instance on glass slides. However, ablation of metabolites deposited on the glass surface may occur rapidly, resulting in a loss of signal intensity over repeated measurements. Sorbent materials such as porous PTFE or TLC (thin-layer chromatography) silica plates appear more suitable for indirect tissue analysis by sample imprinting. Both PTFE and TLC plates have good absorbing properties that retain metabolites from the plants until desorption with DESI and have been applied for the imprinting of various plant organs and tissues [70–72,83]. TLC is a cheaper solution with similar performance to PTFE, but overall PTFE is currently reported as the best solution for DESI-IMS analysis of plant materials in terms of both reproducibility and quantitative ability [81,85–87].

**Data analysis**

The datasets produced by DESI spatial mass scanning typically contain a large set of information and thus requires intensive
post-acquisition analysis for data extraction, visualization, and interpretation. In this respect, developments in bioinformatics tools such as machine learning algorithms allows for advanced high-throughput data analysis [88]. For instance, deep learning and unsupervised neural network based methods have been successfully applied for the analysis of complex dataset from 3D-DESI-IMS of tumor tissue, allowing for high dimensionality reduction and data clustering normally not achievable by classical linear methods [89]. Recently, a collaborative initiative sponsored by EMBL (European Molecular Biology Laboratory) in the framework of the European Research Program Horizon 2020 tackled the need for high-throughput analysis of high dimensionality data from MALDI and DESI-IMS and developed an open-source method that is available to test in its beta uploading using the MetaSpace cloud-server (http://metaspace2020.eu/). A similar project has been undertaken by the community of OpenMSI (https://openms.nersc.gov), which provides a high-performance web-based platform for the storage and management of IMS data, including tools for visualization and statistical analysis [90].

Applications of DESI-IMS in Natural Product Research

Application of DESI-IMS on microorganisms

Since 2008, DESI-IMS has been successfully applied for chemical profiling of metabolites located on surfaces and/or internal tissues, studying single or mixed microbial cultures and chemical interactions between them [Table 1]. However, the use of DESI-IMS on soft and irregular surfaces (such as agar media) is still a challenge today because the strong nebulizing gas of DESI can provoke an aperture in some cultures. Indeed, direct IMS of living colonies has mostly been accomplished by nano-DESI-IMS, more suitable method to imaging wet surface, on living communities such as *Shewanella oneidensis*, *Bacillus subtilis*, *Streptomyces coelicolor*, *Mycobacterium smegmatis*, and *Pseudomonas aeruginosa* for investigation of various chemical families (e.g., lipopeptides, rhamnolipids, quinolones, phenazines, glycopeptidolipids) [74]. However, analysis of *B. subtilis* by DESI-IMS or by nano-DESI-IMS has revealed, in both cases, the presence of surfactin-type lipopeptides.

One of the first applications of DESI-IMS directly on bacterial cultures aimed at identification of bacterial species based on their chemistry and particularly on the basis of their lipid constituents [91, 92]. For example, the cyclic lipopeptide surfactin C15, a well-known metabolite produced by *Bacillus* sp., was observed on agar plates, permitting the taxonomical identification of *B. subtilis* directly on Petri dish [91]. DESI-IMS was also employed to analyze lipid composition on 16 bacterial samples to distinguish bacterial species and even subspecies using bacterial samples suspended in 70% EtOH. Chemotaxonomical identification of the bacterial species was based on the distribution of several major classes of lipids, including phosphotydylethanolamines, phosphotydylglycerols, and lysophospholipids, in positive and negative ionization modes. This lipidomic-type approach allowed the taxonomical characterization of several bacterial species including *Staphylococcus au-
reus*, *Escherichia coli*, *B. subtilis*, and *Salmonella* sp. [92]. The lipidomic analysis of bacterial strains by DESI-IMS enabled the differentiation of Gram-positive and Gram-negative bacteria [92], providing new opportunities for microbiological research.

DESI-IMS has also been used for analyzing natural products (primary and secondary metabolites) directly from bacterial and/or fungal cultures. For instance, metal scavenging siderophores were imaged directly from agar culture of *Streptomyces wadaya-mensis* [93], whereas several new polyhydroxyanthraquinones were identified as quorum sensing inhibitors in the guttate-forming *Penicillium restrictum* [94], an endophytic fungus isolated from the stems of a milk thistle, *Silybum marianum* (L.) Gaertn. (Asteraceae). The DESI-IMS study showed that the polyhydroxyanthraquinones were produced by fungal mycelia and were expressed differentially over time. Also, the potent quorum sensing inhibitory polyhydroxyanthraquinones were observed to be concentrated at the fungal surface, whereas less potent compounds were diffused through the culture medium [94], illustrating the power of DESI-IMS to determine the spatiotemporal distribution as well as the specific production of natural products. Further investigation of bacterial central carbon metabolism performed by Jackson et al. [95] on *E. coli* allowed the detection of 13 out of 17 selected central carbon metabolites (i.e., metabolites involved in the integration of pathways of transport and oxidation of main carbon source inside the cell). Similarly, DESI-IMS was shown to be an effective method to understand chemical interactions/exchanges (e.g., antagonistic fungal interactions [96, 97], fungal/bacterial interactions [98], or metabolic exchange between bacterial species [99] directly). The interaction of micro- and macroorganisms with other organisms often relies on the production of secondary metabolites. Specific metabolite exchanges such as two lactones derivative (T39 butenolide and harzianolide), one hexaketide metabolite (sorbicillinol), and an unknown metabolite (m/z [M + H]+ 319.1) were revealed during the co-culturing of the commercially relevant phytopathogenic fungus *Moniliophthora roreri* (causing fungal cacao pest) and the fungal biocontrol agent *Trichoderma harzianum* [96]. Likewise, the production of phomopsinone A (α-pyrone derivatives) and T-2 toxin (mycotoxin) was shown during the co-culture of fungus *Fusarium sp.* and fungus *Clothesomyces aquaticus* [97]. A proteobacterium, *Burkholderia seminalis* isolated from *Saccharum officinarum* L. (Poaceae) roots, was shown to inhibit the growth of multiple cacao pathogens by the production of rhamnolipids, and other unidentified metabolites [98]. DESI-IMS imaging of *B. subtilis* and *S. coelicolor* co-culture has highlighted the ability of *B. subtilis* to silence antibiotic production (in particular the production of benzo isochromane quinone polyketide actinorhodin) in *S. coelicolor*, giving *B. subtilis* a fitness advantage over *S. coelicolor* [99]. Such studies employing DESI-IMS do not only provide a better understanding for microbial interactions, but also offer new ecological perspectives—for example, the ability *T. harzianum* or *B. seminalis* to act as biocontrol agent against *M. roreri* and other cacao pathogens, respectively. Chemical imaging of microbial co-cultures has potential to be used as a tool for the discovery and the localization of new bioactive metabolites generated by the co-microorganisms. Those co-microorganisms modulate the activation or the suppression of specific metabolite production.
### Table 1 Application of DESI-IMS in microbial natural products.

| Organism | Max no. of microorganisms in mixtures | Applications | Sample preparation | ionization mode | Known chemical family/compounds visualized | References |
|----------|--------------------------------------|--------------|--------------------|-----------------|------------------------------------------|------------|
| **Fungi** |                                      |              |                    |                 |                                          |            |
| T. harzianum; M. roteri | 1–2 | Antagonistic interaction of fungi | Imprint | Positive | Dehydrohistyltryptophanidyldiketopiperazine; histidylnortryptophanidyldopiperazine; roquefortine C; roquefortine D; meleagrin-C13O; glandicoline A; glandicoline B; meleagrine; epineoxaline; oxaline; peptai-bols (harzianins and trichotoxins); T39 butenolide; harzianolide; sorbicillin | [96] |
| P. restrictum | 1 | Spatial and temporal distribution of quorum sensing inhibitors metabolites from the guttate-forming fungus | Cross section (thickness: 15 µm) and imprint | Negative | 2-Hydroxyemodic acid; 1′-hydroxyisorhodoptilometrin; 1′-hydroxy-2′-ketoisorhodoptilometrin; (−)-2′-isorhodoptilometrin; desmethylermoquinone; 2-chloroemodic acid; emodin | [94] |
| C. aquaticus; Fusarium sp. | 2 | Interaction of an antifungal compound producing fungus and a mycotoxin producing fungus | Direct analysis using cardboard inserts and cross section | Positive | Phomopsinone A; T-2 toxin | [97] |
| **Fungi/bacteria** |                                    |              |                    |                 |                                          |            |
| B. seminalis; M. perniciosa; P. capsici; P. palmivora; P. citrophthora | 1–2 | Antagonistic interaction between bacteria and fungi and oomycetes | Direct analysis on dehydrated agar plate | Positive and Negative | Glycero-phosphoethanolamine and glycero-phocholine classes; phospholipids (glycerophosphatidic acid and glycerophosphoglycerol); rhamnolipids (Rha-Rha-C14-C14 and Rha-Rha-C15-C14); pyrrolnitrin; glycrophosphoserine; glycero-phosphoinositol; pyochelin | [98] |
| **Bacteria** |                                    |              |                    |                 |                                          |            |
| B. subtilis; S. coelicolor | 2 | Metabolic exchange between microbial species | Millipore HA filter imprint | Negative | Surfactin; plipastatin; actinorhodin | [99] |
| 16 bacterial strains | 1 | Characterization of different bacterial species | Bacterial suspensions | Positive and Negative | Lipids | [92] |
| B. subtilis | 1 | Characterization of bacterial species | Intact biofilm on membrane (filter paper) | Positive and Negative | Lipopeptide (surfactin) | [91] |
| E. coli | 1 | Temporal detection of central carbon analysis | Bacterial extracts on nonporous Teflon substrate | Negative | Central carbon metabolites (13 metabolites) | [95] |
| S. wadayamensis | 1 | Protocol allowing direct IMS of agar cultures: application on metal scavengers siderophores | Direct analysis on dehydrated agar plate and imprint | Positive | Siderrophore (protonated desferrioxamine; ferrioxamine B; Al-desferrioxamine B complex); antimiycin isoforms | [93] |
| **Pathogens/plants** |                                    |              |                    |                 |                                          |            |
| P. ultimum; S. tuberosum | – | Plant metabolic response to pathogen invasion | Imprint | Positive | Glycoalkaloids (α-solanine and α-chaconine; solanidine; solasonine; solanavioi; solasonine; solaspiralidine; γ-solanine/γ-chaconine; β-solanine and β-chaconine) | [100] |
Table 2  Application of DESI-IMS on macroorganism-derived natural products.

| Organism | Applications                                                                 | Sample preparation               | Ionization mode | Known chemical family/compounds visualized                                                                 | References |
|----------|------------------------------------------------------------------------------|----------------------------------|-----------------|----------------------------------------------------------------------------------------------------------------|------------|
| Plants   |                                                                              |                                  |                 | Serpentine; vindoline; catharanthine; 19-S-vindoline; like-catharanthine; perivine; alstonine; tabersonine isomers; dihydrotabersonine; ajmalicine; hydroxytabersonine; decaetoxyvindoline; 5-adenosylmethionine; akuammicine; methoxytabersonine; lochnerinine; echitovenine; deacetoxyvindoline; vindoline; strictosidine; anhydrovinblastine; vinblastine | [84]       |
| C. roseus| Identifying varietal differences, toxic metabolites production, changes in metabolites during growth, pest/pathogen attack, and natural stresses | TLC imprints                     | Positive        | Hydroxynitrile glucosides (hydroxynitrile glucosides; osmaronin) | [101]      |
| H. vulgare| Chemical profiling                                                           | Direct and imprints on porous Teflon substrate | Positive and Negative | Hyperforin; hypericin; adhyperforin; pseudohypericin; protopseudohypericin; protohypericin; atropine; scopolamine; hexose sugars; sucrose; papaverine; morphine | [81]       |
| H. perforatum; D. stramonium; P. somniferum | Spatial distribution on leaves, petals and/or seeds of three plants species | Imprint on porous Teflon substrate | Positive and Negative | Tetracosanic acid; hexacosanoic acid; octacosanoic acid; melissic acid; quercetin; quercitrin; isobaric mixture of isoquercitrin; hyperoside; hypericin; protohypericin; pseudohypericin; protopseudohypericin; C26, C28 and C30 fatty acids; hyperforin; rutin; hyperforin; adhyperforin | [102]      |
| H. perforatum | Methods development for the direct imaging of soft plant tissues | Direct analysis                  | Negative         | Cj-NCC-1 and 2; Hv-NCC-1; So-NCC-2; Ox-NCC-1 (chlorophyll catabolite, polyfunctionalized linear tetrapyrrole); phenylboronic acid with diol substituent of the chlorophyll catabolite; | [85]       |
| D. binectariferum | Spatial and temporal distribution of rohitukine and related compounds during various stages of seed development | TLC imprints                      | Positive         | Rohitukine; acetylated and glycosylated rohitukine | [104]      |
| C. japonicum; L. styraciflua; O. virginiana | Rapid identification and spatially resolved relative quantification of chlorophyll degradation products in complex senescent plant tissue matrixes | Imprint using porous PTFE substrate | Positive and Negative | Cj-NCC-1 and 2; Hv-NCC-1; So-NCC-2; Ox-NCC-1 (chlorophyll catabolite, polyfunctionalized linear tetrapyrrole); phenylboronic acid with diol substituent of the chlorophyll catabolite; | [85]       |
| Algae    |                                                                              |                                  |                 | Bromophycolide A and B; callophycoic acid | [105]      |
| C. serratus| Natural products chemistry and chemical ecology to provide ecological roles of metabolites | Algal sample preserved with 10% formalin in seawater and then affixed to PTFE substrates | Negative         | Bryophycolide A and B; callophycoic acid | [105]      |
| P. neurymenioides | Natural products chemistry and chemical ecology to provide ecological roles of metabolites | Direct analysis by glue fixation on glass slides | Positive         | Neurymenolide A | [106]      |
| Animals  |                                                                              |                                  |                 | Phospholipids; AMMOENG 130                                      | [57]       |
| D. rerio | Characterization, distribution and metabolism of a toxic ionic liquid         | Cryosection using CMC (carboxymethyl cellulose), 50-µm tissue sections | Positive         | Phospholipids; AMMOENG 130                                      | [57]       |
Plant-pathogen interactions have also been analyzed by DESI-IMS, linking plant responses to microbial pathogens as reported by Tata et al. [100]. Plants represent an important source of nutrients but very few studies on plant pathosystems (i.e., ecosystems defined by parasitism) are available. DESI-IMS was used to study the fluctuations of glycoalkaloids in sprouted potato tubers infected by the fungal phytopathogen *Pythium ultimum*. After eight days of infection, it was observed that metabolic pathways were affected by the phytopathogen to increase or decrease the levels of in total 12 glycoalkaloids [100]. Therein, the DESI-IMS study permitted to determine the plant metabolic changes on the production of glycoalkaloid after infection by the fungal phytopathogen and demonstrated its useful application in plant pathosystems.

In summary, DESI-IMS has so far been used on microorganisms for (i) taxonomical identification of species, (ii) production and spatiotemporal distribution of (surface) metabolites, (iii) identifying metabolic exchanges between bacterial species, (iv) antagonistic fungal/fungal and fungal/bacterial interactions, and (v) elucidation of metabolic responses of plants to pathogenic infections (Table 1). These studies open up new perspectives in (micro)biology, and in the understanding of biological systems from a chemical angle.

**Application of DESI-IMS on macroorganisms**

Several examples of DESI-IMS analyses have been reported on macroorganisms, including analyses on plants, algae, and fish (Table 2).

With regard to plants, DESI-IMS has principally been applied on the imprints of leaves [81, 84, 85, 101, 102], flowers and petals [84, 101, 102], fruits [103], and seeds [104] (Table 2). The cuticle of plants is dominated by metabolites such as fatty acids with aliphatic tails longer than 22 carbons. These VLCFAs represent a physical morphological barrier in leaves and petals, presenting an obstacle for DESI-IMS analysis. To overcome this difficulty, a ternary solvent system, CHCl₃-ACN-H₂O (1:1:0.04), was developed and used to analyze the cuticle itself as well as the subcuticular metabolites directly by DESI-IMS [102]. The leaves and petals of *Hypericum perforatum* L. (Clusiaceae) were analyzed in several studies [64, 70, 81, 82, 85], in which the detection and localization of several metabolites were reported. The phloroglucinol derivative hyperforin was shown to be localized in the translucent glands, while hypericin (an anthraquinone derivative) was present in the dark glands. A number of other phloroglucinol, flavonoid, and anthraquinone derivatives known from *Hypericum* sp. (adhyperforin, pseudohypericin, protopseudohypericin, protohypericin, rutin, quercetin, phloroglucinol), as well as VLCFAs, were detected by indirect analysis (imprints) [81] or direct analysis using a ternary solvent system and/or after surface extraction by CHCl₃ [102]. Similarly, the distribution of leaf metabolites of *Datura stramonium* L. (Solanaceae) was analyzed, revealing the presence of the major tropane alkaloids atropine and scopolamine. Both compounds were located principally in the leaf ribs and veins, suggesting their transport within the plant [81].

Similar approaches were also performed on other plants, such as *Papaver somniferum* L. (Papaveraceae) [81], *Catharanthus roseus* (L.) G.Don (Apocynaceae) [84], *Hordeum vulgare* L. (Poaceae) [101], *Ceridiphyllum japonicum* Siebold & Zucc (Ceridiphyllaceae), *Liquidambar styraciflua* L. (Hamamelidaceae), *Ostrea virginiana* K.Koch (Corylaceae) [85], *Lotus japonicus* (Regel) K.Larsen (Leguminosae), *Manihot esculenta* Crantz (Euphorbiaceae) [87], *Ginkgo biloba* L. (Ginkgoaceae) [104], and strawberry (*Fragaria x ananassa* Duch. [Rosaceae]) [103], indicating the capability of DESI-IMS to map various classes of metabolites (e.g., terpenoid indole alkaloids, hydroxynitrilic glucosides) on various plant organs (Table 2). The spatial and temporal distribution of rohitukine, a chromone alkaloid with anti-inflammatory, anticancer, and immunomodulatory activities, was imaged during the seed development of *Dysoxylum binecateniferum* Hook.f. (Meliaceae). Rohitukine accumulation was shown to be more important during seed development and its increase was specifically located in the embryo, cotyledons, and to a lesser extent in the seed coat [104]. DESI-IMS studies on leaves, flowers, or seeds were performed to determine metabolite distribution at a specific time or during various plant developmental stages.

DESI-IMS has also been used to study molecular distribution of bioactive natural products in marine organisms, such as seaweeds (Table 2). The DESI-IMS analysis of the tropical red seaweed *Callophybus serratus* revealed the presence of the macrocyclic halogenated-benzoxates, bromophycolide A and B on the surface of the alga [105]. Both compounds inhibit the growth of *Lindra thalassiae*, a marine fungus that infects diverse hosts ranging from brown algae to seagrasses. The natural concentrations of these two metabolites were shown to be more than sufficient to display an antifungal activity in the algal surface patches, suggesting the importance of secondary metabolites for chemically mediated biological processes [105]. The compounds were observed also within internal algal tissue by DESI-IMS. The fact that bromophycolides are not homogenously distributed on the algal surface but associated with distinct patches could be due to the presence of tissue damage, indicating the chemical defense of *C. serratus* to fungal infection. Seaweeds and corals often occur in close proximity in the same environment. As coral reefs are declining globally, seaweeds are commonly replacing corals. However, the mechanism of the increasing coral-algal interactions is poorly understood. Andras et al. [106] employed DESI-IMS to visualize...
### Table 3  Application DESI-IMS in medicine and clinical research.

| Tissues/samples | Applications                                                                 | Sample preparation                                      | Ionization mode | Known chemical family/compounds visualized                                                                 | References |
|-----------------|------------------------------------------------------------------------------|----------------------------------------------------------|-----------------|------------------------------------------------------------------------------------------------------------|------------|
| Medicine        | Tissues smears                                                               | Tumor sectioned (cryosection) on glass slides with PTFE spots | Negative        | Lipids (fatty acids and phospholipids)                                                                   | [111]      |
| Human brain tumors | Molecular diagnosis of human brain tumors                                  | n.a.                                                     | Negative        | Lipids                                                                                                     | [107]      |
| Squamous cell carcinomas | Rapid determination of tumor stroma ratio                                  | Tissue slices using frozen tumor samples (cryosection)    | Negative        | Lipids                                                                                                     | [113]      |
| Tumor and normal breast tissues | Lipidomic comparison of normal rat breast tissues and neoplastic tissues from animals belonging to the DMBA chemical carcinogenesis rat model | Tissues (cryosection)                                    | Negative        | Lipids                                                                                                     | [114]      |
| Human tissue specimens including normal ovarian tissues, BOT and HGSC samples | Identification of potential predictive markers of disease aggressiveness: application on serious ovarian tumors | Tissues (cryosection)                                    | Positive and Negative | Saturated and unsaturated fatty acids; sphingolipids; ceramides; cardiolipins; glycerophosphoethanolamines; glycophosphoglycerol; glycerophosphoserines; glycerophosphoinositol; glycerophosphocholines; triacylglycerols; sphingomyelins | [115]      |
| Cerebrum tumor tissue | Correlation between lipidomic data and glioma diagnostic information | Tissues (cryosection)                                    | Positive and Negative | Lipids                                                                                                     | [112]      |
| Human cancer tissues and human fluids (blood, urine) | Cancer diagnostic and paper spray ionization for therapeutic drug monitoring | Tissues (cryosection) and direct fluids on paper spray | Positive        | Glycerophosphocholines                                                                                     | [116]      |
| Urine samples | Metabolites and drugs analysis                                               | Extracted urine samples on Teflon slides                  | Positive        | Temazepam; oxazepam; desmethyldiazepam; para-hydroxymazepam; alprazolam; codeine; morphine; oxyphenethanol; amphetamine; 11-Nor-9-carboxy-delta9-tetrahydrodihydrocannabinol; delta9-tetrahydrocannabinol | [117]      |
| Histological sections of brain, lung, kidney and testis | Spatial distribution of drugs directly from histological sections | Histological sections                                    | Positive        | Clozapine; N-desmethylclozapine; clozapine-N-oxide                                                      | [60]       |
| Human tissue samples | Detection of metastatic breast and thyroid cancer in lymph nodes | Tissue samples (cryosection)                             | Negative        | Lipids                                                                                                     | [118]      |
| Prostate tissue | Diagnosis of prostate cancer                                                 | Tissue sections (cryosection)                            | Negative        | Krebs cycle metabolites; carbohydrates; lipids                                                          | [108]      |
| Tumor tissues | 3D-DESI imaging to study metabolic heterogeneity of cancer                  | Tissue sections                                           | Negative        | n. a.                                                                                                     | [89]       |
| Mouse brain tissue | Lipidomic profiling in individual mouse preimplantation embryos and oocytes | Fresh dry samples                                         | Negative        | Lipids; phospholipids                                                                                     | [119]      |
| Oocytes and preimplantation embryos of bovine species | Lipidomic profiling                                                        | Brain tissue sections                                     | Positive        | Lipid metabolism (free fatty acids; phospholipids, cholesterol-related molecules; triacylglycerols)      | [120]      |

n. a.: information not available
and quantify neurymenolide A, an unusual cyclophane derivative isolated from the red alga *Phaeolcarpus neurymenoides*. Neurymenolide A is an allelopathic agent occurring on the surface of the live alga that damages the coral *Porites rus* by direct contact with the alga. This study elegantly shows the potential of DESI-IMS in chemical ecology studies. In our laboratory, we have been applying DESI-IMS to Baltic Sea plants and seaweeds to determine their surface metabolome, in combination with classical metabolomics approaches (Fig. 2).

There is only one study that has reported the use of DESI-IMS on the whole body zebrafish (*Danio rerio*) by cryosectioning [43]. This study aimed at localizing the accumulation of a mildly toxic ionic liquid AMMOENG 130 in zebrafish tissues, after exposure of the zebrafish to varying concentrations of this liquid. The ionic liquids are found in large quantities in detergents and softeners, and are potentially toxic. In particular, AMMOENG 130 was found to be very toxic against *D. rerio* (i.e., more toxic than 13 out of 15 common ionic liquids). DESI-IMS experiment on *D. rerio* revealed the accumulation of this toxin in the respiratory and nervous system of the fish, suggesting that it is potentially a neurotoxin. Consequently, DESI-IMS analysis of macroorganisms opens new avenues in other research fields such as ecotoxicology for biomonitoring of toxic compounds.

### Application of DESI-IMS in medicine and clinical research

Similar to MALDI-IMS, the dominant application area of DESI-IMS is medical research, where it enables a rapid and accurate method to visualize biomarkers in biological tissues. In particular, DESI-IMS has been demonstrated to be very efficient in lipid composition analysis. Lipids play key roles in various cytological processes and in cell signaling; hence, the analysis of lipid composition in biological tissues is crucial to support morphological investigations, diagnosis, and characterization of pathophysiological processes such as neurodegenerative and cardiovascular diseases, as well as neoplastic processes. Tissue profiling of lymphoma, gliomas, gastric, liver, prostate, and breast cancer have all showed how drastic change in lipid composition can be used as biomarkers. DESI-IMS analysis of macroorganisms opens new avenues in other research fields such as ecotoxicology for biomonitoring of toxic compounds.

Eberlin et al. [107] used lipidomic data as biomarkers to rapidly classify 36 human gliomas including oligodendroglioma, astrocytoma, and oligoastrocytoma at different histological grades and varied tumor cell concentrations. The combination of DESI-IMS, multivariate analysis, and machine learning was applied to recognize glioma subtypes and grade on the basis of the World Health Organization tumor classification system. The analysis resulted in a recognition capability of greater than 99% and a cross-validation of more than 97% based on 128 peaks for the subtype, 123 peaks for the grade, and 136 peaks for the concentration. Furthermore, small molecule analysis (Krebs cycle intermediates metabolites and carbohydrates) combined with lipid composition was used to distinguish prostate cancer from normal tissues and to improve the accuracy of prostate cancer identification [108]. These models permitted the differentiation of prostate cancer from benign specimens with nearly 90% accuracy per patient. Glucose/citrate ratio, in parallel to lipids composition, could also be used to accurately monitor the occurrence of prostate cancer [108]. In the majority of cancer diagnoses by DESI-IMS, lipid composition was of key importance to distinguish tumor tissues from normal tissues (Table 3) [108]. DESI-IMS was used to record the spatial intensity distribution of a drug, clozapine, directly from histological sections of brain, lung, kidney, and testis [60], in addition to cancer diagnosis applications. In another study, Miyamoto et al. [109] combined microscopy, MALDI-IMS and nano-DESI to reveal changes in glucose metabolism and the accumulation of sphingomyelin metabolites in the glomeruli of the diabetic mice fed with high-fat diet. This shows the great potential of the technique in various areas of medical research, including metabolic disorders.

On the whole, DESI-IMS has been successfully applied in various research fields including microbiology, ecotoxicology, ecology, forensics, embryology, chemistry, and medicine, presenting a fast, easy, and reliable direct/indirect analysis of biological samples. DESI-IMS has found applications in other research fields—for instance, in astrophysics to detect and identify the spatial distribution of organic compounds on meteorites’ surface [110] indicating its potential in so many other disciplines.

### Conclusions and Future Perspectives

IMS techniques have already opened new avenues in several areas of life sciences, including medicine, microbiology, and natural product chemistry. The IMS surpasses all existing molecular analysis methods (i.e., genomics, transcriptomics and metabolomics) by giving crucial and simultaneous spatial information on molecules. Therefore, various IMS techniques with different ionization modes and workflows are being adopted by scientists to address questions presented by spatial metabolomics on various biological systems. However, all IMS techniques have drawbacks and limitations in instrumentation, sample preparation, metabolites annotation, resolution, and data analysis. Considering their ever-increasing popularity, it is foreseeable that the IMS techniques will rapidly improve to move forward and find even more applications in life and medical sciences, and potentially in industry. Specifically, IMS techniques will bring metabolomics research to a paradigm shift and completely change the way we interrogate biological systems and provide new approaches in drug discovery and medicine. With future developments, IMS has the power to transform our ability to interrogate natural processes for example, better understanding host-microbe and microbe-microbe interaction, biosynthesis of natural products, chemical ecology, virulence factors, and disease states. Continuous improvement will be crucial for the advancement of IMS and its contribution in our understanding of life and metabolic processes.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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