HHS Public Access

Author manuscript

Acc Chem Res. Author manuscript; available in PMC 2023 December 11.

Published in final edited form as:

Acc Chem Res. 2023 September 19; 56(18): 2526–2536. doi:10.1021/acs.accounts.3c00382.

Desorption Electrospray Ionization Mass Spectrometry: 20 Years

Nicolás M. Morato,

Department of Chemistry and Bindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, United States

R. Graham Cooks

Department of Chemistry and Bindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, United State

CONSPECTUS:

Mass spectrometry (MS) is one of the most widely used technologies in the chemical sciences. With applications spanning the monitoring of reaction products, the identification of disease biomarkers, and the measurement of thermodynamic parameters and aspects of structural biology, MS is well established as a universal analytical tool applicable to small compounds as well as large molecular complexes. Regardless of the application, the generation of gas-phase ions from neutral compounds is a key step in any MS experiment. However, this ionization step was for many years limited to high-energy approaches that required gas-phase analytes and thus it was restricted to volatile samples. Over the last few decades, new methodologies have been developed to address this limitation and facilitate ionization of biological molecules. Electrospray ionization (ESI) is the most broadly used of these methods, as it facilitates the ionization of intact polar compounds from solution.

Twenty years ago, our group reported a new ionization method that uses a charged solvent spray to impact a surface, generating ions from *objects* rather than just *solutions* and doing so directly in the ambient environment with no vacuum requirements and little to no sample preparation. This method was termed desorption electrospray ionization (DESI), and it initiated a new field that would come to be known as ambient mass spectrometry. The simplicity and wide applicability of the DESI technology—and the tens of ambient ionization methods developed subsequently—revolutionized the MS analysis of complex materials for their organic components, especially for *in situ* applications.

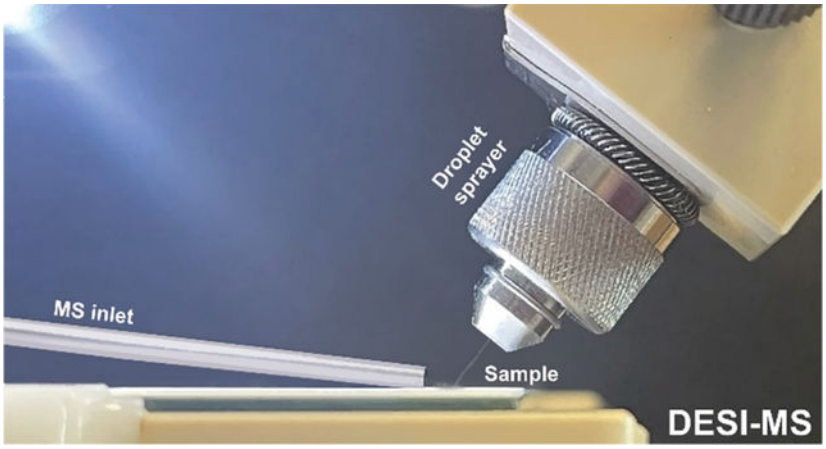
This Account describes the history of DESI, starting with the development of the technique from early electrosonic spray ionization (ESSI) experimental observations as well as the studies leading to the understanding of its mechanism as a “droplet pick-up” phenomenon involving sequential events (*i.e.*, thin film formation, solid–liquid extraction, secondary droplet generation, and ESI-like ionization from these droplets). We also overview the developments and applications of the technology that have been demonstrated by our group during the last two decades. In particular, we describe (i) the use of DESI for tissue imaging, one of its more significant applications to

Corresponding Author: **R. Graham Cooks** – Department of Chemistry and Bindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, United States; cooks@purdue.edu.

The authors declare no competing financial interest.

date, and its extension to intraoperative clinical diagnosis; (ii) the integration of the technology with portable instrumentation for *in situ* analysis, especially when coupled with tandem mass spectrometry (MS/MS); (iii) the use of DESI microdroplets as microvessels to accelerate organic reactions by orders of magnitude compared to those in bulk solution; and (iv) the combination of all these capabilities for automated high-throughput experiments aimed at accelerating drug discovery.

Graphical Abstract



INTRODUCTION: HISTORY OF MOLECULAR IONIZATION

Mass spectrometry (MS) is the science and technology of ions. Not surprisingly, the generation of ions from *neutral* samples, *i.e.*, ionization, is a key step in any MS experiment. In fact, for many years this step limited MS analysis to volatile organic compounds. In the early decades of organic MS, ionization was performed by electron ionization (EI), which produces highly reproducible ionization/fragmentation events through electron bombardment that deposits a broad distribution of internal energies into the nascent molecular ions. Collisions with 70 eV electrons typically provide both molecular weight and structural information, and the reproducibility of the spectra makes the method well suited to the creation of databases.[5] However, because EI requires volatile analytes, and most organic molecules are involatile, extensive derivatization strategies were commonplace. This disadvantage is shared by chemical ionization (CI), which uses EI on a gas (*e.g.*, methane) to form reagent ions that subsequently ionize minor gas-phase analytes through ion–molecule reactions.[6] This method allows control of the type of molecular ion generated (*e.g.*, protonated molecules) and also (based on the thermochemistry of the ion–molecule reaction) the energy deposition and hence the extent of fragmentation.

The MS analysis of nonvolatile organic compounds started to take form around 1970 with the implementation of the new ionization methods of field and plasma desorption (FD and PD, respectively). FD utilizes a combination of heat and a high electric field to ionize a solid sample deposited on a dendritic emitter,[7] whereas PD uses fission fragments (typically of ^{252}Cf) to generate a short-lived nanosized plasma that rapidly heats and ionizes compounds deposited on a thin foil.[8] These approaches were followed by the application

of secondary ion mass spectrometry (SIMS) to organic samples. SIMS is an atomic ion generation technique based on the bombardment of surfaces with primary ions of keV energies that sputter atoms and secondary ions from materials. In its static mode, achieved using a low-dose primary ion beam, analysis is limited to less than a monolayer to avoid resampling of the damaged surface. Static SIMS was successfully applied to the formation of organic ions from deposited samples.[9] A key advance was the use of a matrix in a large excess relative to the analyte to yield *soft ionization* (*i.e.*, low internal energy and hence little or no fragmentation) and to increase the ionization efficiency. This key observation regarding the effects of matrices on ionization was made in our group in 1981.[10] Significant dilution (10–10^3×) of fragile samples in an ammonium chloride matrix enhanced the SIMS signal of intact ions and reduced their fragmentation, allowing the analysis of biological molecules (*e.g.*, sugars and amino acids) as their metal adducts or protonated species.[10] The counterintuitive role of the added matrix in the dissipation of excess internal energy was key also to the implementation of liquid SIMS,[11] the closely related liquid-matrix technique of fast atom bombardment (FAB),[12] and matrix-assisted laser desorption ionization (MALDI),[13] which is now a widespread technique for the analysis of large molecules, including proteins.

Electrospray ionization (ESI)[14] was introduced in the 1980s as a method that provided intact molecular ions from analytes *in solution*. ESI provided an avenue for the analysis of nonvolatile biological compounds, small and large, the latter being facilitated by the formation of multiply charged species and consequently lying within the accessible *m*/*z* (mass-to-charge ratio) range of existing mass spectrometers. Nanospray (nESI) later emerged as a higher-efficiency approach for the analysis of aqueous samples;[15] the efficiency increase followed directly from the smaller size of the droplets generated compared to traditional forced-flow, pneumatically assisted ESI.

DEVELOPMENT OF DESORPTION ELECTROSPRAY IONIZATION (DESI)

Following reports on the efficiency of nESI, some our lab's work in the early 2000s focused on the development of a modified ESI technique termed electrosonic spray (ESSI), which used a variable-potential microelectrospray together with a supersonic gas jet for nebulization, therefore providing access to ultrafine initial droplets at low temperatures.[16] Experiments showed that this method can generate fully desolvated protein ions at atmospheric pressure, providing narrow charge-state distributions and excellent survival of complexes.[16] An important observation concerned the ESSI beam divergence as a function of the spray distance (Figure 1); increased distance decreases the sampling efficiency, although signal-to-noise ratios better than 30 are obtained even at large spray distances (>3 m).[16]

These distance experiments led to the observation by Zoltan Takats that neutral material deposited adventitiously on the MS inlet from a previous *previous* experiment could be ionized by a spray of pure solvent. This accidental discovery was appreciated as being important, as it represented a new ionization technique that shared features of the two main classes of methods (then, as now): spray methods (*e.g.*, ESI) and desorption methods (*e.g.*, MALDI and SIMS). The original name for the new experiment, used in the first patent

filing, was spray ambient desorption ionization (SADI). This name explicitly recognizes the characteristic of ionizing condensed phase samples using spray ionization. The later name desorption electrospray ionization (DESI) does this too, although it emphasizes the relationship to ESI over the hybrid character of the method (Figure 2).

DESI EXPERIMENT AND MECHANISM

DESI was initially described in 2004.[1] Its main feature is the ability to generate ions from *objects* rather than just from *solutions* and to do so directly in an ambient environment. The DESI experiment utilizes a charged solvent spray generated with a constant (μL/min) solvent flow rate and a high-speed nebulizing gas jet (typically nitrogen), a characteristic shared with ESI and other spray ionization methodologies. The charged microdroplets in this spray can be directed at virtually any surface, somewhat like the projectiles used in desorption ionization methods (*e.g.*, keV energy ions in SIMS). The exact ionization mechanism that follows this interaction of the spray and the surface, however, remained elusive for several years, something not uncommon during the development of MS ionization methods.

Several ionization mechanisms were initially considered for DESI, including chemical sputtering, evaporation/gas-phase ionization, shockwave phenomena, and—primarily—a process described as “droplet pick-up”. Chemical sputtering is the simultaneous desorption and ionization of molecules at surfaces *under vacuum* due to charge and momentum transfer from arriving projectiles. However, momentum transfer was quickly proven unlikely in DESI due to the low velocities (ca. 100 m/s) and small energies (0.6 meV per impacting water molecule) in arriving droplets of ca. 3 μm diameter.[17] Such data also challenged any shockwave mechanism, which would require projectile velocities greater than the speed of sound.[17] Additional experiments to determine the internal energy of DESI-generated ions provided distributions similar in shape and mean value to those obtained using ESI or ESSI, suggesting the occurrence of a similar phenomenon, *i.e.*, the generation of gas-phase ions from droplets.[18] Computational fluid dynamics modeling of DESI,[19] together with empirical evidence on the large effects of the nebulizing gas pressure and the surface-to-sprayer distance on droplet velocities and MS signal intensities,[17] suggested hydrodynamically driven transport of secondary droplets.

Consolidation of these studies confirmed a droplet pick-up mechanism (Figure 3) involving (i) surface wetting followed by (ii) solid–liquid microextraction into the generated thin film and (iii) stochastic momentum-transfer events between impacting droplets and the solvent film that produce secondary droplets from the DESI surface. Small progeny droplets fly toward the mass spectrometer at low angles carrying dissolved analytes, being pneumatically driven by the nebulizing gas jet and by the drag from the vacuum interface at the instrument inlet.[19] Ionization occurs from these droplets through well-known ESI mechanisms (*i.e.*, ion evaporation and charge residue models).[20] Note that the key microextraction event acts in essence as an *in situ* and online sample purification step which, in combination with the inherent contactless analysis (*i.e.*, the sample is never in contact with the spray emitter nor does it pass through any capillary), provides unique advantages in terms of the complexity of the samples that DESI can handle. A particular example is the exceedingly high tolerance

of DESI toward high-concentration nonvolatile salt solutions, a difficult challenge for ESI-based methodologies.

Although the high momentum of the primary droplets makes them nonsusceptible to Coulombic effects, electrostatic effects are observed in DESI, with the surface itself being identified as an electrochemically active element. In fact, the DESI experiment can be characterized as a surface-dependent DC capacitor, which provides the highest currents using superhydrophobic materials with low surface energies and extremely high contact angles.[21] Porous PTFE therefore serves as an excellent DESI substrate and has seen much use over the years. Note that other surface properties, including roughness, analyte affinity, and conductivity, also play a role in DESI by altering the charge distribution or affecting the desorption and microextraction efficiency.

The dependence of DESI on the spray hydrodynamics enables the tuning of source parameters for particular analytes, providing an additional degree of specificity to the analysis. As expected considering the DESI mechanism, the solvent selection (and the use of any additives, *e.g.*, surfactants, acids, salts) is important as it affects not only the extraction efficiency but also the sampled area and the secondary droplet size.[22]

AMBIENT MS(/MS) ANALYSIS

Early on, the ability to obtain MS information from ordinary samples in their native environment without any sample simplification (preparation or separation) by easily generating ions outside of the mass spectrometer was highlighted as revolutionary.[23] DESI, closely followed by DART (direct analysis in real time),[24] opened the door for a new field that became known as *ambient mass spectrometry*.[20] Over the years, many other ambient ionization methods have emerged, creating a sort of alphabet soup, with more than 30 methods by 2010 and with the number still increasing. Examples from our group include DAPCI (desorption atmospheric pressure chemical ionization),[25] LTP (low-temperature plasma),[26] and PSI (paper spray ionization).[27]

The unique benefits of ambient ionization, in conjunction with the signature advantages of MS (*i.e.*, speed, sensitivity, and chemical specificity),[20] have resulted in numerous ambient mass spectrometry applications. DESI in particular allows qualitative and quantitative applications to the sensitive detection of explosives, propellants, and chemical warfare agents;[1,28] drugs, metabolites and pharmaceuticals;[20,29] proteins;[30] microorganisms;[31] and individual embryos, oocytes, and stem cells;[32] as well as natural products directly from their biological matrices (*e.g.*, alkaloids in plant leaves[1]).Moreover, the combination of tandem mass spectrometry (MS/MS) and DESI (or ambient ionization in general) constitutes a unique approach for the rapid and direct analysis of complex samples. The application of MS/MS to complex mixture analysis was pioneered at Purdue in the late 1970s by combining MS/MS, in the form of MIKES (mass-analyzed ion kinetic energy spectrometry), with soft ionization by CI.[33,34] The advantages of DESI over CI, together with the advances in MS/MS, have created a general approach to high-speed *in situ* analysis when used with portable mass spectrometers.

DESI IMAGING

DESI inherently provides spatial control over the sampling event, meaning that the impacting spray can be directed easily to a particular spot on a sample surface (typically achieved by moving the surface rather than the sprayer). This feature quickly established DESI as an imaging technique with lower spatial resolution than MALDI but advantages in the lack of sample preparation and ambient ionization. DESI found its main and ongoing application in the direct analysis of tissue sections, particularly for diagnostics based on lipids and metabolites.[35] Many types of human and animal tissues have been imaged using DESI, a few examples from our group being brain,[35,36] kidney,[37] prostate,[38] spinal cord,[39] crystalline lens,[40] and arterial plaques.[41] Note that these measurements are not limited to endogenous compounds but can also be utilized to study the spatial distributions of xenobiotics and their metabolites.[42] Extension to other types of sample systems, for instance, plant material (directly or imprinted onto a flat substrate),[43] ink in documents,[44] or chemical traces in latent fingerprints[45] (an application that was featured in an episode of the popular TV series CSI), further demonstrated the wide applicability of DESI and the advantages of ambient MS imaging.

Over the years, technological and data processing advances have improved DESI imaging capabilities. For instance, the initial spatial resolution of 1 mm achieved using manual sample control was rapidly improved to typical values better than 200 μm—with access even to 35 μm[46]—through optimization and automated continuous-velocity 2D rastering that are still in use. Additionally, postacquisition analysis of feature-rich two-dimensional ion images using multivariate methods and other computational tools proved useful for the study of biological samples, particularly to classify regions of tissue sections as diseased/healthy,[37] and to generate 3D DESI images after the analysis of multiple sequential sample sections.[47]

DESI-BASED CLINICAL DIAGNOSTICS

The depth of molecular information obtained by DESI tissue imaging makes this approach valuable as a molecular pathology technique for clinical diagnostics. In fact, during multiple imaging studies, highly accurate classifications of human disease state using lipid and metabolite profiles have been reported for renal carcinoma,[37] prostate cancer,[38] and brain cancer,[36] as well as arterial plaque progression[41] and spinal cord injuries,[39] among other examples. Additionally, the use of nondestructive histologically compatible solvent systems allows subsequent analyses to be performed on sections already screened by DESI in order to augment the molecular and biological information and correlate such information across the different methods.[48] For instance, DESI can be followed by MALDI for the correlation of lipid and protein information and, significantly, by standard histopathological analysis (*e.g.*, H&E) for the correlation of molecular features with morphological features.[48]

Despite the success of this approach, traditional DESI imaging workflows based on tissue sections are not compatible with point-of-care applications (whether *in vitro* or *in vivo*), and this is where the molecular information provided by DESI could have the largest impact in clinical decision making. This fact led to a methodological change in which tissue smears rather than sections are used and the information on spatial distribution of analytes was

traded for speed and simplicity in the analysis. Smears can be generated rapidly *in situ* from minimal amounts of tissue without any specialized instrumentation (or the regulatory burden of *in vivo* studies) to provide relevant molecular profiles of the sample[3,49,50] (Figure 4). Most importantly, as no spatial distribution is sought, smear analysis can be carried out in just a minute or two using a simple rastering pattern to average for inhomogeneities.

Using this modified DESI methodology, we have focused for several years on the molecular diagnosis of brain cancer, in particular the assessment of the isocitrate dehydrogenase mutation (IDH) status. This determination would provide highly valuable and actionable information to surgeons if the results were available during surgery. Currently this information (from genomics) is only available postoperatively. Together with clinician teams, our group has installed mass spectrometers in surgical rooms and evaluated the performance of real-time intraoperative (*in vitro*, not *in vivo*) DESI-MS analysis.[3,49] In multiple studies (several dozen patients each), good correlations were observed between lipid and metabolite profiles and tissue class (*e.g.*, parenchyma vs glioma) or tumor cell percentage,[3,50,51] while virtually perfect classifications have been obtained for IDH genotyping using a targeted approach for the detection of the oncometabolite 2-hydroxyglutaric acid.[3,49,51] In some cases, its signal is normalized to endogenous glutamate in a simultaneous MS/MS experiment[51] (Figure 4).

PORTABLE DESI AND NONPROXIMAL SAMPLING

Point-of-care DESI applications extend beyond the clinical field, for instance, to the detection of explosives and toxic chemicals. In this context, we coupled DESI with a nonproximal sampling approach using a stainless-steel flexible ion transport tube with a control arm, which physically extended the inlet of the mass spectrometer, separated the DESI source from the instrument, and provided spatial control over sampling.[52] Distances up to 3 m between the mass spectrometer and the sample are accessible with this setup,[52] which was subsequently extended to 10 m and optimized using three DESI sprayers and an ion transfer funnel to allow larger surface area sampling.[25] Surprisingly, whether with one or multiple sprayers, it was observed that the chemical background significantly decreases with distance, providing higher signal-to-noise ratios despite an overall reduction in absolute intensities.[25,52] Notwithstanding the long transfer distances, low nanogram levels of multiple chemical warfare agents, explosives, active ingredients, peptides, and illicit drugs were successfully detected from a variety of surfaces, including laptops, pharmaceutical tablets, and directly from the fingers of volunteers (Figure 5).[25,52]

In situ DESI analysis is best carried out by coupling it to a portable (“mini”) mass spectrometer. Specific examples include transportable[53] (Mini 8; cylindrical ion trap; 0.087 m^3; 38 kg) and hand-held[54] (Mini 10; rectilinear ion trap; 0.014 m^3; 14 kg) instruments, both with atmospheric pressure interfaces that use a small inlet diameter (254 and 127 μm in the Mini 8 and 10, respectively).[53,54] In addition, we have developed and coupled DESI to a lighter-weight battery-powered instrument (Mini 10.5, 3–4 h of operation on batteries, rectilinear ion trap, 0.014 m^3; 10 kg)[55] equipped with a discontinuous atmospheric pressure interface (DAPI) that allows standard nebulization gas pressures to be used for DESI. Nanogram limits of detection were demonstrated with these portable systems for

some pesticides, chemical warfare agents, illicit drugs, and small biomolecules, showcasing the potential of portable DESI-MS platforms in on-site forensic, environmental, and security applications or in planetary exploration missions.[53–55]

Despite these examples, it still seems to the authors that insufficient attention in MS goes to *in situ* complex mixture analysis using ambient ionization in combination with small, portable, and MS/MS-capable instrumentation. In most such applications, the use of high mass resolution—perhaps one of the biggest driving forces in current MS instrumentation development—is not needed, and this type of instrument is not fieldable anyway.

CHEMICAL REACTIONS IN DESI MICRODROPLETS

The DESI spray, typically a pure solvent, can be easily altered to include a variety of additives. These additives can been utilized to perform reactions simultaneously with the desorption ionization process, an approach termed reactive DESI.[28] Initially explored additives (acids, *e.g.*, trifluoroacetic and hydrochloric acids and salts) were used to form complexes of the additive cation/anion and the neutral analyte, especially where sites for facile protonation/deprotonation were absent in the analyte molecule. For instance, applications of this methodology to the analysis of explosives (*e.g.*, RDX, HMX, and TATP)[28] and olefins were demonstrated.[56] Olefins in particular were analyzed using silver cationization which, in combination with multistage tandem mass spectrometry (MS^n), allows for online isomer differentiation.[56]

Reactive DESI can also be utilized for the formation of new *covalent* bonds, primarily for online derivatization of compounds with low ionization efficiencies or to enhance the specificity of the analysis. For instance, addition of tetramethyl urea to the DESI spray leads to the generation of an acylium ion that undergoes an Eberlin reaction with any cyclic acetals present in the sample.[57] Not-easily ionizable steroids can be analyzed by reactive DESI using Girard T or betaine aldehyde as spray additives, as they introduce a charged quaternary nitrogen (*i.e.*, charge labeling) in the reaction product.[2,58] Dinitrophenylhydrazine has also been used for the detection of carbonyls as their hydrazone derivatives, in particular for low molecular weight aldehydes.[39] These strategies, either those based on complexation or those involving covalent bond formation, have been successfully implemented in the direct analysis of such complex samples as tissue sections in imaging experiments.[39,41,56]

Redox chemistry, as perhaps is evident, is also accessible through DESI experiments. Both oxidation and reduction reactions were observed soon after the first description of DESI and exploited for the sensitive detection of aromatic species after reduction to odd-electron anions,[28] or for the oxidation of saturated hydrocarbons followed by online betaine aldehyde derivatization of the resulting alcohols to give positively charged hemiacetals, a useful approach for the ambient analysis of petroleum distillates.[59] Importantly, these redox phenomena were found to be chemically rather than electrochemically controlled and easily tunable (*i.e.*, favored vs avoided) by experimental conditions, with their main driving force being an *in situ* reaction with gas-phase radicals generated through the discharge induced by high spray voltages.

The key feature of reactive DESI is that reactions occur *in situ* within the short time scale of the analysis. This factor was explicitly recognized in 2011 when we first reported the acceleration of chemical reactions occurring in secondary DESI microdroplets[2] (Figure 6). The rates of acid and base-catalyzed reactions were observed to increase in these small (≤1 μm) droplets compared to bulk solutions, a phenomenon that was quickly recognized as being associated with the droplet surface (which is enhanced by a $1/r$ factor) and initially explained through an increased reagent concentration and colocalization, together with a dramatically different pH environment in the small droplets.[2] Over the years, a great many studies on microdroplet accelerated reactions have confirmed the key role of the solvent–air interface at the droplet surface.[61,62] The mechanistic explanation involves the combined effects of partial solvation (*i.e.,* an intermediate state between solution and gas phase where energy barriers are reduced relative to those for bulk solution) and chemical effects of the high surface field as a result of generating highly reactive species, primarily water radical cations/anions.[60–64] These two factors are thought to be the main causes of the large reaction rate increases (up to 10^6) observed in microdroplets and, to a smaller extent, in their 2D analogs: thin films.[62] It is worth recalling that solvation can change the rate of a reaction by as much as 12 orders of magnitude, as comparisons of bulk solution and ion/molecule reactions show.[65] Accelerated gas/solution reactions—first observed with DESI—have implications for the basic understanding of chemical phenomena, such as the prebiotic origin of peptides and homochirality,[66] and a vast range of practical applications ranging from material synthesis to drug discovery.[67] As such, this set of phenomena provides mass spectrometry with a synthesis component, a feature that may even become of comparable importance to its analytical capabilities.

Note that both thin films and microdroplets are generated during the DESI process, providing straightforward access to reaction acceleration and facilitating, for instance, the rapid screening of reaction mixtures. In practice, access to these accelerating conditions is favored by longer droplet flight times; therefore, short spray distances can be chosen to create “analytical” conditions and long distances for “synthetic” conditions. Also note that DESI droplets containing the products of accelerated reactions can be sampled by a mass spectrometer, or alternatively they can simply be intercepted in order to collect nanograms of reaction products for facile product characterization by other means or for assessment of their biological activities.[67]

HIGH-THROUGHPUT DESI

The simplicity and speed of DESI make it attractive for high-throughput applications. Approaches that included direct analysis of tablets on a moving band (akin to an industrial process line) or the sampling from standard 96-well plates using a geometry-independent DESI probe were early demonstrations of high-throughput analysis at rates of a few seconds per sample combined with excellent qualitative and quantitative performance.[29,68] However, in today’s era of automation and big data, subsecond analysis times are required for the efficient exploration of either chemical (*i.e.,* synthesis of compounds) or biological (*e.g.,* assessment of bioactivities) space. In this context, and through the DARPA *Make It* program, we exploited all the advantages of the DESI technology for the development of an automated

platform capable of complex sample analysis at ≥1 Hz rates in arrays of up to 6144 samples (Figure 7).[69]

The initial version of this system[70] was based on an almost direct translation of the DESI *imaging* capabilities to the *high-throughput* application using dense arrays of samples (initially organic reaction mixtures) deposited on a flat inert surface. The imaging of such an array can provide ion intensity maps (analogous to those obtained from a tissue section) that allow for the interrogation of MS features for each individual sample.[70] Combining this concept with automated fluid handling and plate transporting robotics, as well as automated control and data analysis software, resulted in an integrated platform that facilitated the screening of organic reactions at a rate of ca. 3 s per sample.[70] This approach was successful for the efficient optimization of several reactions under various conditions (*e.g.*, solvent, stoichiometry, and catalyst),[70,71] providing results that were subsequently used to guide scaled-up flow synthesis.[71]

Given this success, improvements were made to this imaging-based methodology, in particular to address the time lost moving through the empty space between samples, the redundant information acquired in the several scan lines over a single sample, and the nonproportionality between the analysis time and the plate density, all effects of the constant-velocity *line-by-line* rastering motion that characterizes tissue imaging experiments. For this reason, and taking advantage of the positional precision of the DESI stage and the fluid handling robotics, a new scan method based on the preanalysis calibration of the sample array position on the plate and a *spot-to-spot* jumping motion was implemented.[69,72] This approach overcame the limitations of the initial platform and allowed density-dependent throughputs better than one sample per second while providing results comparable to those of standard screening platforms (*e.g.*, LC-MS) in a fraction of the time and with significantly less solvent consumption.

To date, more than 20 reaction types (*e.g.*, *N*-alkylation, *N*-acylation and *N*-sulfonation, aldol addition, imine formation and reductive animation, nucleophilic aromatic substitution, nitrosation, Suzuki cross-coupling, thiol oxidation, aza-Michael, alkenylation, and sulfur fluoride exchange) have been studied using this system.[67,69–73] The platform provides online reaction acceleration (in secondary DESI microdroplets) allowing reaction screening without any bulk preincubation steps, significantly increasing the overall process throughput. Through these studies, the scaled-up synthesis of anticancer drugs has been optimized,[71] and recently the late-stage functionalization of complex bioactive compounds such as opioids has been demonstrated.[67,73] Note that, after the initial screening, samples can be re-examined, in particular by MS/MS, for online structural confirmation of reaction products.[69,72,73]

The tolerance of DESI to typical bioassay matrix components (*i.e.*, high concentrations of buffers, nonvolatile salts such as phosphates, and detergents), which are commonly inimical to MS analysis, allowed for the expansion of the system capabilities into biological space. Using DESI, the inherent advantages of MS (*i.e.*, speed, chemical specificity, and sensitivity) can then be fully exploited for high-throughput label-free bioactivity assessments that overcome limitations of traditional bioassay methodologies based on plate readers

(*e.g.*, reduced versatility, use of non-native substrates, introduction of interferences from coupled reactions, and safety concerns in the case of radiometric methods).[4] Note that such applications require *excellent quantitative performance*, which has been consistently demonstrated in high-throughput DESI despite of the short analysis times, the matrix complexity, and the remarkably small amounts of material deposited (typically in the high picogram to low nanogram level), using internal standards or product-to-substrate ion ratios.[4,69,74] The wide applicability of this approach has been demonstrated with multiple enzymatic systems,[4,69,74] and it is currently being extended to receptor- and cell-based assays. Moreover, high-throughput analysis of biological samples has also been achieved using high-density tissue arrays, an application that can efficiently support intraoperative MS diagnostics through the generation of classification models or biomarker discovery based on large sample cohorts.[51] Further integration of this approach with novel MS/MS scan methodologies such as 2D MS/MS (all fragments from all precursors) provides additional information that can be utilized in the generation of spectral databases, a recent example being the analysis of microorganisms.[75]

CONCLUDING REMARKS

Twenty years after its inception, DESI has found numerous applications across a wide variety of fields, a trend that should continue as the technology evolves. Of particular interest is its integration into standard of practice workflows for intraoperative diagnosis, particularly for real-time IDH genotyping of brain tumors, as well as its implementation in automated drug discovery. This latter, somewhat ambitious, goal relies on a combination of all the key features of DESI that were overviewed in this Account. The contactless high-performance analysis of complex condensed-phase samples without any workup, the precise and rapid control over the sampling event, and the reaction acceleration occurring in the secondary DESI microdroplets all converge into a DESI-based workflow for (i) reaction screening aimed at new drugs or analogs, followed by (ii) nanoscale synthesis through collection of successful accelerated reaction products and (iii) online label-free biological activity assessment of drug candidates.[67]

“It’s the greatest thing since night baseball!”, said John Fenn—inventor of ESI and Nobel laureate—about DESI ca. 2004.[23] Twenty years in, it seems the technology has justified that sentiment.

ACKNOWLEDGMENTS

The authors acknowledge all members, past and present, of the Aston Laboratories for Mass Spectrometry at Purdue University for their invaluable contributions to the development of DESI and the field of ambient mass spectrometry. The names of many of these people are included in the cited references. This work was supported by the National Center for Advancing Translational Sciences (Grant UG3TR004139).

Biographies

Nicolás M. Morato is a postdoctoral research associate at Purdue University in Prof. R. Graham Cooks group, where he also earned his Ph.D. in 2023. He received B.Sc. degrees in chemistry (2017) and industrial engineering (2018) from Universidad de los

Andes, Colombia. His research has focused on the development of ambient ionization methodologies for the rapid and simple analysis of complex samples.

R. Graham Cooks is the Henry Bohn Hass Distinguished Professor of Chemistry at Purdue University. He received Ph.D. degrees from the University of Natal (1965) and Cambridge University (1967). He is a pioneer in the field of mass spectrometry, and his research combines fundamental phenomena, instrumentation, and analytical applications. He is an elected member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the National Academy of Inventors.

REFERENCES

(1). Takáts Z; Wiseman JM; Gologan B; Cooks RG Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. Science 2004, 306, 471–473. [PubMed: 15486296]

(2). Girod M; Moyano E; Campbell DI; Cooks RG Accelerated Bimolecular Reactions in Microdroplets Studied by Desorption Electrospray Ionization Mass Spectrometry. Chem. Sci. 2011, 2, 501–510.

(3). Pirro V; Alfaro CM; Jarmusch AK; Hattab EM; Cohen-Gadol AA; Cooks RG Intraoperative Assessment of Tumor Margins during Glioma Resection by Desorption Electrospray Ionization-Mass Spectrometry. Proc. Natl. Acad. Sci. U. S. A. 2017, 114, 6700–6705. [PubMed: 28607048]

(4). Morato NM; Holden DT; Cooks RG High-Throughput Label-Free Enzymatic Assays Using Desorption Electrospray-Ionization Mass Spectrometry. Angew. Chem., Int. Ed. 2020, 59, 20459–20464.

(5). McLafferty FW A Century of Progress in Molecular Mass Spectrometry. Annu. Rev. Anal. Chem. 2011, 4, 1–22.

(6). Field FH Chemical Ionization Mass Spectrometry. Acc. Chem. Res. 1968, 1, 42–49.

(7). Beckey HD; Schulten H-R Field Desorption Mass Spectrometry. Angew. Chem., Int. Ed. 1975, 14, 403–415.

(8). Macfarlane RD; Torgerson DF Californium-252 Plasma Desorption Mass Spectroscopy: Nuclear Particles Are Used to Probe Biomolecules. Science 1976, 191, 920–925. [PubMed: 1251202]

(9). Benninghoven A; Sichtermann W Secondary Ion Mass Spectrometry: A New Analytical Technique for Biologically Important Compounds. Org. Mass Spectrom. 1977, 12, 595–597.

(10). Liu LK; Busch KL; Cooks RG Matrix-Assisted Secondary Ion Mass Spectra of Biological Compounds. Anal. Chem. 1981, 53, 109–113.

(11). Aberth W; Straub KM; Burlingame AL Secondary Ion Mass Spectrometry with Cesium Ion Primary Beam and Liquid Target Matrix for Analysis of Bioorganic Compounds. Anal. Chem. 1982, 54 (12), 2029–2034.

(12). Barber M; Bordoli RS; Elliott GJ; Sedgwick RD; Tyler AN Fast Atom Bombardment Mass Spectrometry. Anal. Chem. 1982, 54, 645–657.

(13). Hillenkamp F; Karas M; Beavis RC; Chait BT Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Biopolymers. Anal. Chem. 1991, 63, 1193A–1203A. [PubMed: 1897719]

(14). Fenn JB; Mann M; Meng CK; Wong SF; Whitehouse CM Electrospray Ionization for Mass Spectrometry of Large Biomolecules. Science 1989, 246, 64–71. [PubMed: 2675315]

(15). Wilm M; Mann M Analytical Properties of the Nano-electrospray Ion Source. Anal. Chem. 1996, 68, 1–8. [PubMed: 8779426]

(16). Takáts Z; Wiseman JM; Gologan B; Cooks RG Electrosonic Spray Ionization. A Gentle Technique for Generating Folded Proteins and Protein Complexes in the Gas Phase and for Studying Ion–Molecule Reactions at Atmospheric Pressure. Anal. Chem. 2004, 76, 4050–4058. [PubMed: 15253642]

(17). Venter A; Sojka PE; Cooks RG Droplet Dynamics and Ionization Mechanisms in Desorption Electrospray Ionization Mass Spectrometry. Anal. Chem. 2006, 78, 8549–8555. [PubMed: 17165852]

(18). Nefliu M; Smith JN; Venter A; Cooks RG Internal Energy Distributions in Desorption Electrospray Ionization (DESI). J. Am. Soc. Mass Spectrom. 2008, 19, 420–427. [PubMed: 18187338]

(19). Costa AB; Cooks RG Simulated Splashes: Elucidating the Mechanism of Desorption Electrospray Ionization Mass Spectrometry. Chem. Phys. Lett. 2008, 464, 1–8.

(20). Cooks RG; Ouyang Z; Takats Z; Wiseman JM Ambient Mass Spectrometry. Science 2006, 311, 1566–1570. [PubMed: 16543450]

(21). Volny,´ M; Venter A; Smith SA; Pazzi M; Cooks RG Surface Effects and Electrochemical Cell Capacitance in Desorption Electrospray Ionization. Analyst 2008, 133, 525. [PubMed: 18365123]

(22). Badu-Tawiah AK; Eberlin LS; Ouyang Z; Cooks RG Chemical Aspects of the Extractive Methods of Ambient Ionization Mass Spectrometry. Annu. Rev. Phys. Chem. 2013, 64, 481–505. [PubMed: 23331308]

(23). Cho A Mass Spectrometrists Salivate Over Recipe for Ions Alfresco. Science 2004, 306, 388–388.

(24). Cody RB; Laramée JA; Durst HD Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. Anal. Chem. 2005, 77, 2297–2302. [PubMed: 15828760]

(25). Cotte-Rodríguez I; Mulligan CC; Cooks RG Non-Proximate Detection of Small and Large Molecules by Desorption Electrospray Ionization and Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry: Instrumentation and Applications in Forensics, Chemistry, and Biology. Anal. Chem 2007, 79, 7069–7077. [PubMed: 17696318]

(26). Harper JD; Charipar NA; Mulligan CC; Zhang X; Cooks RG; Ouyang Z Low-Temperature Plasma Probe for Ambient Desorption Ionization. Anal. Chem. 2008, 80, 9097–9104. [PubMed: 19551980]

(27). Liu J; Wang H; Manicke NE; Lin J-M; Cooks RG; Ouyang Z Development, Characterization, and Application of Paper Spray Ionization. Anal. Chem. 2010, 82, 2463–2471. [PubMed: 20158226]

(28). Cotte-Rodríguez I; Takáts Z; Talaty N; Chen H; Cooks RG Desorption Electrospray Ionization of Explosives on Surfaces: Sensitivity and Selectivity Enhancement by Reactive Desorption Electrospray Ionization. Anal. Chem. 2005, 77, 6755–6764. [PubMed: 16255571]

(29). Chen H; Talaty NN; Takáts Z; Cooks RG Desorption Electrospray Ionization Mass Spectrometry for High-Throughput Analysis of Pharmaceutical Samples in the Ambient Environment. Anal. Chem. 2005, 77, 6915–6927. [PubMed: 16255590]

(30). Myung S; Wiseman JM; Valentine SJ; Takáts Z; Cooks RG; Clemmer DE Coupling Desorption Electrospray Ionization with Ion Mobility/Mass Spectrometry for Analysis of Protein Structure: Evidence for Desorption of Folded and Denatured States. J. Phys. Chem. B 2006, 110, 5045–5051. [PubMed: 16526747]

(31). Song Y; Talaty N; Datsenko K; Wanner BL; Cooks RG In Vivo Recognition of Bacillus Subtilis by Desorption Electrospray Ionization Mass Spectrometry (DESI-MS). Analyst 2009, 134, 838–841. [PubMed: 19381372]

(32). Ferreira CR; Pirro V; Eberlin LS; Hallett JE; Cooks RG Developmental Phases of Individual Mouse Preimplantation Embryos Characterized by Lipid Signatures Using Desorption Electrospray Ionization Mass Spectrometry. Anal. Bioanal. Chem. 2012, 404, 2915–2926. [PubMed: 23052870]

(33). Kondrat RW; Cooks RG; McLaughlin JL Alkaloids in Whole Plant Material: Direct Analysis by Kinetic Energy Spectrometry. Science 1978, 199, 978–980. [PubMed: 17752371]

(34). Kondrat RW; Cooks RG Direct Analysis of Mixtures by Mass Spectrometry. Anal. Chem. 1978, 50, 81A–92A.

(35). Wiseman JM; Ifa DR; Song Q; Cooks RG Tissue Imaging at Atmospheric Pressure Using Desorption Electrospray Ionization (DESI) Mass Spectrometry. Angew. Chem., Int. Ed. 2006, 45, 7188–7192.

(36). Eberlin LS; Norton I; Orringer D; Dunn IF; Liu X; Ide JL; Jarmusch AK; Ligon KL; Jolesz FA; Golby AJ; Santagata S; Agar NYR; Cooks RG Ambient Mass Spectrometry for the Intraoperative Molecular Diagnosis of Human Brain Tumors. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, 1611–1616. [PubMed: 23300285]

(37). Dill AL; Eberlin LS; Zheng C; Costa AB; Ifa DR; Cheng L; Masterson TA; Koch MO; Vitek O; Cooks RG Multivariate Statistical Differentiation of Renal Cell Carcinomas Based on Lipidomic Analysis by Ambient Ionization Imaging Mass Spectrometry. Anal. Bioanal. Chem. 2010, 398, 2969–2978. [PubMed: 20953777]

(38). Eberlin LS; Dill AL; Costa AB; Ifa DR; Cheng L; Masterson T; Koch M; Ratliff TL; Cooks RG Cholesterol Sulfate Imaging in Human Prostate Cancer Tissue by Desorption Electrospray Ionization Mass Spectrometry. Anal. Chem. 2010, 82, 3430–3434. [PubMed: 20373810]

(39). Girod M; Shi Y; Cheng J-X; Cooks RG Mapping Lipid Alterations in Traumatically Injured Rat Spinal Cord by Desorption Electrospray Ionization Imaging Mass Spectrometry. Anal. Chem. 2011, 83, 207–215. [PubMed: 21142140]

(40). Ellis SR; Wu C; Deeley JM; Zhu X; Truscott RJW; Panhuis MIH; Cooks RG; Mitchell TW; Blanksbya SJ Imaging of Human Lens Lipids by Desorption Electrospray Ionization Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2010, 21, 2095–2104. [PubMed: 20947369]

(41). Manicke NE; Nefliu M; Wu C; Woods JW; Reiser V; Hendrickson RC; Cooks RG Imaging of Lipids in Atheroma by Desorption Electrospray Ionization Mass Spectrometry. Anal. Chem. 2009, 81, 8702–8707. [PubMed: 19803494]

(42). Wiseman JM; Ifa DR; Zhu Y; Kissinger CB; Manicke NE; Kissinger PT; Cooks RG Desorption Electrospray Ionization Mass Spectrometry: Imaging Drugs and Metabolites in Tissues. Proc. Natl. Acad. Sci. U. S. A. 2008, 105, 18120–18125. [PubMed: 18697929]

(43). Ifa DR; Srimany A; Eberlin LS; Naik HR; Bhat V; Cooks RG; Pradeep T Tissue Imprint Imaging by Desorption Electrospray Ionization Mass Spectrometry. Anal. Methods 2011, 3, 1910.

(44). Ifa DR; Gumaelius LM; Eberlin LS; Manicke NE; Cooks RG Forensic Analysis of Inks by Imaging Desorption Electrospray Ionization (DESI) Mass Spectrometry. Analyst 2007, 132, 461. [PubMed: 17471393]

(45). Ifa DR; Manicke NE; Dill AL; Cooks RG Latent Fingerprint Chemical Imaging by Mass Spectrometry. Science 2008, 321, 805. [PubMed: 18687956]

(46). Campbell DI; Ferreira CR; Eberlin LS; Cooks RG Improved Spatial Resolution in the Imaging of Biological Tissue Using Desorption Electrospray Ionization. Anal. Bioanal. Chem. 2012, 404, 389–398. [PubMed: 22706326]

(47). Eberlin LS; Ifa DR; Wu C; Cooks RG Three-Dimensional Vizualization of Mouse Brain by Lipid Analysis Using Ambient Ionization Mass Spectrometry. Angew. Chem., Int. Ed. 2010, 49, 873–876.

(48). Eberlin LS; Liu X; Ferreira CR; Santagata S; Agar NYR; Cooks RG Desorption Electrospray Ionization Then MALDI Mass Spectrometry Imaging of Lipid and Protein Distributions in Single Tissue Sections. Anal. Chem. 2011, 83, 8366–8371. [PubMed: 21975048]

(49). Santagata S; Eberlin LS; Norton I; Calligaris D; Feldman DR; Ide JL; Liu X; Wiley JS; Vestal ML; Ramkissoon SH; Orringer DA; Gill KK; Dunn IF; Dias-Santagata D; Ligon KL; Jolesz FA; Golby AJ; Cooks RG; Agar NYR Intraoperative Mass Spectrometry Mapping of an Onco-Metabolite to Guide Brain Tumor Surgery. Proc. Natl. Acad. Sci. U. S. A. 2014, 111, 11121–11126. [PubMed: 24982150]

(50). Jarmusch AK; Pirro V; Baird Z; Hattab EM; Cohen-Gadol AA; Cooks RG Lipid and Metabolite Profiles of Human Brain Tumors by Desorption Electrospray Ionization-MS. Proc. Natl. Acad. Sci. U. S. A. 2016, 113, 1486–1491. [PubMed: 26787885]

(51). Morato NM; Brown HM; Garcia D; Middlebrooks EH; Jentoft M; Chaichana K; Quiñones-Hinojosa A; Cooks RG High-Throughput Analysis of Tissue Microarrays Using Automated Desorption Electrospray Ionization Mass Spectrometry. Sci. Rep. 2022, 12, 18851. [PubMed: 36344609]

(52). Cotte-Rodríguez I; Cooks RG Non-Proximate Detection of Explosives and Chemical Warfare Agent Simulants by Desorption Electrospray Ionization Mass Spectrometry. Chem. Commun. 2006, 2968–2970.

(53). Mulligan CC; Talaty N; Cooks RG Desorption Electrospray Ionization with a Portable Mass Spectrometer: In Situ Analysis of Ambient Surfaces. Chem. Commun. 2006, 1709–1711.

(54). Keil A; Talaty N; Janfelt C; Noll RJ; Gao L; Ouyang Z; Cooks RG Ambient Mass Spectrometry with a Handheld Mass Spectrometer at High Pressure. Anal. Chem. 2007, 79, 7734–7739. [PubMed: 17867653]

(55). Sokol E; Noll RJ; Cooks RG; Beegle LW; Kim HI; Kanik I Miniature Mass Spectrometer Equipped with Electrospray and Desorption Electrospray Ionization for Direct Analysis of Organics from Solids and Solutions. Int. J. Mass Spectrom. 2011, 306, 187–195.

(56). Jackson AU; Shum T; Sokol E; Dill A; Cooks RG Enhanced Detection of Olefins Using Ambient Ionization Mass Spectrometry: Ag+ Adducts of Biologically Relevant Alkenes. Anal. Bioanal. Chem. 2011, 399, 367–376. [PubMed: 21069301]

(57). Sparrapan R; Eberlin LS; Haddad R; Cooks RG; Eberlin MN; Augusti R Ambient Eberlin Reactions via Desorption Electrospray Ionization Mass Spectrometry. J. Mass Spectrom. 2006, 41, 1242–1246. [PubMed: 16888754]

(58). Wu C; Ifa DR; Manicke NE; Cooks RG Rapid, Direct Analysis of Cholesterol by Charge Labeling in Reactive Desorption Electrospray Ionization. Anal. Chem. 2009, 81, 7618–7624. [PubMed: 19746995]

(59). Wu C; Qian K; Nefliu M; Cooks RG Ambient Analysis of Saturated Hydrocarbons Using Discharge-Induced Oxidation in Desorption Electrospray Ionization. J. Am. Soc. Mass Spectrom. 2010, 21, 261–267. [PubMed: 19914089]

(60). Basuri P; Gonzalez LE; Morato NM; Pradeep T; Cooks RG Accelerated Microdroplet Synthesis of Benzimidazoles by Nucleophilic Addition to Protonated Carboxylic Acids. Chem. Sci. 2020, 11, 12686–12694. [PubMed: 34094463]

(61). Cooks RG; Yan X Mass Spectrometry for Synthesis and Analysis. Ann. Rev. Anal. Chem. 2018, 11, 1–28.

(62). Wei Z; Li Y; Cooks RG; Yan X Accelerated Reaction Kinetics in Microdroplets: Overview and Recent Developments. Annu. Rev. Phys. Chem. 2020, 71, 31–51. [PubMed: 32312193]

(63). Qiu L; Cooks RG Simultaneous and Spontaneous Oxidation and Reduction in Microdroplets by the Water Radical Cation/Anion Pair. Angew. Chem., Int. Ed. 2022, 61, No. e202210765, DOI: 10.1002/anie.202210765.

(64). Lee JK; Walker KL; Han HS; Kang J; Prinz FB; Waymouth RM; Nam HG; Zare RN Spontaneous Generation of Hydrogen Peroxide from Aqueous Microdroplets. Proc. Natl. Acad. Sci. U. S. A. 2019, 116, 19294–19298. [PubMed: 31451646]

(65). Garver JM; Fang Y; Eyet N; Villano SM; Bierbaum VM; Westaway KC A Direct Comparison of Reactivity and Mechanism in the Gas Phase and in Solution. J. Am. Chem. Soc. 2010, 132, 3808–3814. [PubMed: 20187638]

(66). Holden DT; Morato NM; Cooks RG Aqueous Microdroplets Enable Abiotic Synthesis and Chain Extension of Unique Peptide Isomers from Free Amino Acids. Proc. Natl. Acad. Sci. U. S. A. 2022, 119, No. e2212642119.

(67). Cooks RG; Feng Y; Huang K; Morato NM; Qiu L Re-Imagining Drug Discovery Using Mass Spectrometry. Isr. J. Chem. 2023, 63, No. e202300034.

(68). Manicke NE; Kistler T; Ifa DR; Cooks RG; Ouyang Z High-Throughput Quantitative Analysis by Desorption Electrospray Ionization Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2009, 20, 321–325. [PubMed: 19013081]

(69). Morato NM; Le MT; Holden DT; Graham Cooks R Automated High-Throughput System Combining Small-Scale Synthesis with Bioassays and Reaction Screening. SLAS Technol. 2021, 26, 555–571. [PubMed: 34697962]

(70). Wleklinski M; Loren BP; Ferreira CR; Jaman Z; Avramova L; Sobreira TJP; Thompson DH; Cooks RG High Throughput Reaction Screening Using Desorption Electrospray Ionization Mass Spectrometry. Chem. Sci. 2018, 9, 1647–1653. [PubMed: 29675211]

(71). Jaman Z; Sobreira TJP; Mufti A; Ferreira CR; Cooks RG; Thompson DH Rapid On-Demand Synthesis of Lomustine under Continuous Flow Conditions. Org. Process Res. Dev. 2019, 23, 334–341.

(72). Sobreira TJP; Avramova L; Szilagyi B; Logsdon DL; Loren BP; Jaman Z; Hilger RT; Hosler RS; Ferreira CR; Koswara A; Thompson DH; Cooks RG; Nagy ZK High-Throughput Screening of Organic Reactions in Microdroplets Using Desorption Electrospray Ionization Mass Spectrometry (DESI-MS): Hardware and Software Implementation. Anal. Methods 2020, 12, 3654–3669. [PubMed: 32701099]

(73). Huang K; Morato NM; Feng Y; Cooks RG High-Throughput Diversification of Complex Bioactive Molecules by Accelerated Synthesis in Microdroplets. Angew. Chem., Int. Ed. 2023, 62, No. e202300956.

(74). Kulathunga SC; Morato NM; Zhou Q; Cooks RG; Mesecar AD Desorption Electrospray Ionization Mass Spectrometry Assay for Label-Free Characterization of SULT2B1b Enzyme Kinetics. ChemMedChem. 2022, 17, No. e202200043.

(75). Szalwinski LJ; Gonzalez LE; Morato NM; Marsh BM; Cooks RG Bacterial Growth Monitored by Two-Dimensional Tandem Mass Spectrometry. Analyst 2022, 147, 940–946. [PubMed: 35166732]

KEY REFERENCES

- Takáts Z.; Wiseman J. M.; Gologan B.; Cooks R. G. Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science* 2004, 306, 471–473.[1] *Initial description detailing the development of DESI-MS*.
- Girod M.; Moyano E.; Campbell D. I.; Cooks R. G. Accelerated bimolecular reactions in microdroplets studied by desorption electrospray ionization mass spectrometry. *Chem. Sci*. 2011, 2, 501–510.[2] *First report of accelerated microdroplet reactions, which were initially observed in DESI*.
- Pirro V.; Alfaro C. M.; Jarmusch A. K.; Hattab E. M.; Cohen-Gadol A. A.; Cooks R. G. Intraoperative Assessment of Tumor Margins during Glioma Resection by Desorption Electrospray Ionization Mass Spectrometry. *Proc. Natl. Acad. Sci*. 2017, 114, 6700–6705.[3] *Intraoperative DESI-MS for brain cancer diagnosis*.
- Morato N. M.; Holden D. T.; Cooks R. G. High-throughput Label-Free Enzymatic Assays using Desorption Electrospray Ionization Mass Spectrometry. *Angew. Chem. Int. Ed*. 2020, 59, 20459–20464.[4] *High-throughput label-free biological assays toward DESI-based drug discovery*.

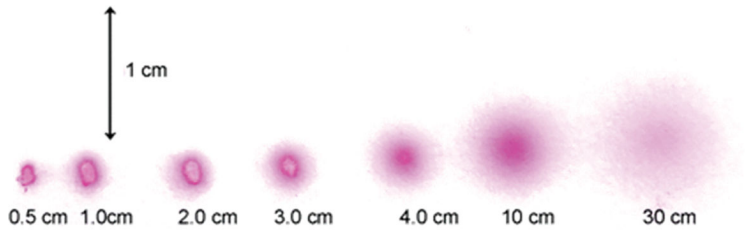


Figure 1.

Cross-section of ESSI spray recorded as a function of the distance from the spray tip. Reproduced with permission from ref 16. Copyright 2004 American Chemical Society.

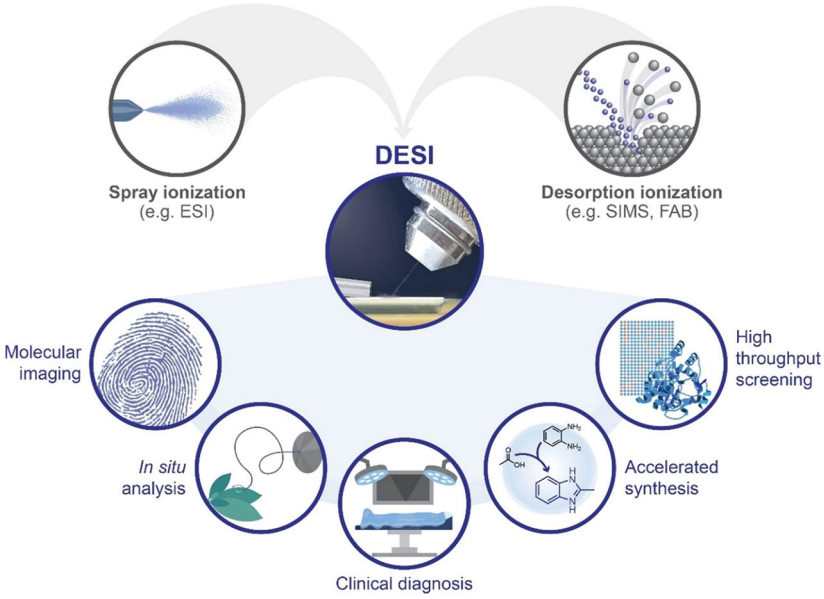


Figure 2.

Overview of DESI showcasing its relationship to both spray and desorption ionization methods, as well as some of its extensively demonstrated capabilities.

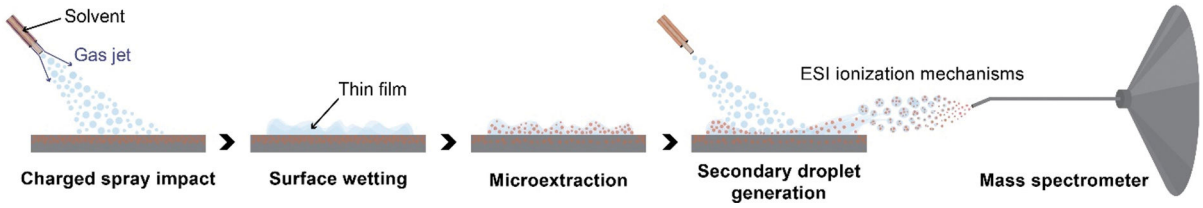


Figure 3.

DESI droplet pick-up mechanism. A charged spray impacts a sample surface, generating a thin film in which a microextraction event takes place. Secondary microdroplets that contain dissolved analytes are then ejected from this thin film. Finally, these droplets yield dry ions through well-known ESI mechanisms.

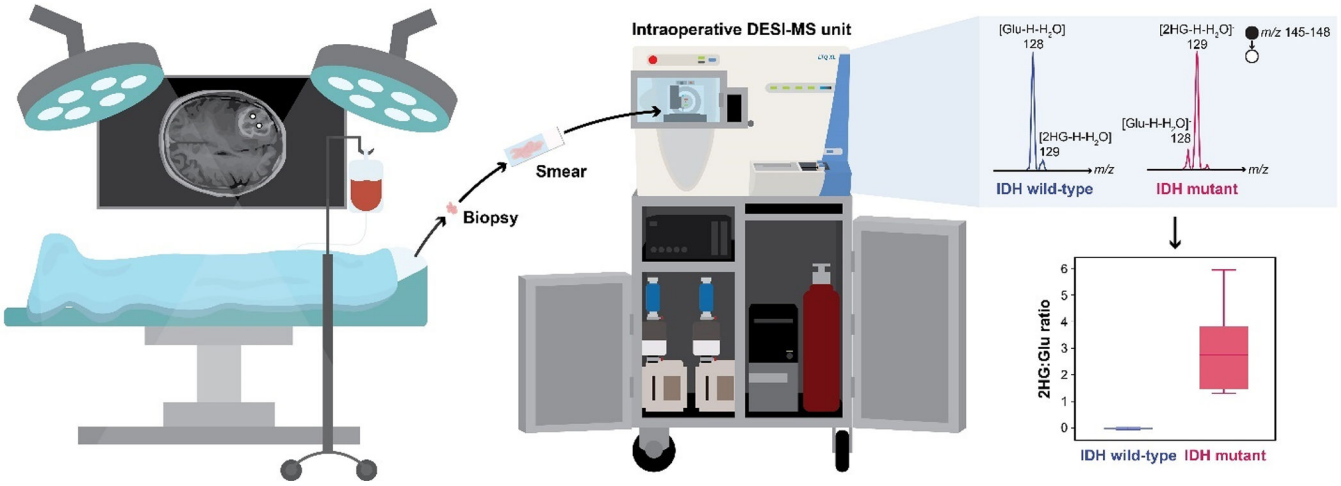


Figure 4.

DESI-MS intraoperative diagnostics. *In situ* analysis is carried out *in vitro* using small resected biopsies that are smeared on standard microscope glass slides and directly analyzed using DESI-MS. This approach accurately identifies the IDH mutation status of gliomas during craniotomy. Adapted with permission from ref 51. Copyright 2022 the Authors. Published by Springer Nature under a Creative Commons Attribution 4.0 International License.

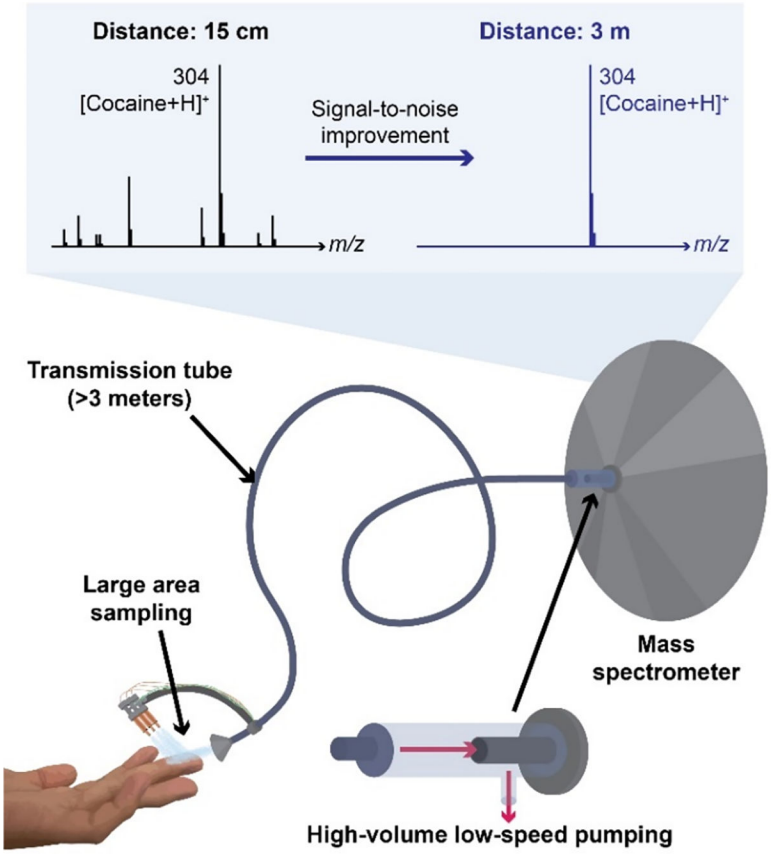


Figure 5.

Nonproximal DESI-MS using a flexible transmission tube (>3 m) coupled to the mass spectrometer inlet using high-volume low-speed vacuum pumping. A large heterogeneous area can be sampled using multiple sprayers simultaneously. Signal-to-noise ratios consistently improve with distance. Adapted with permission from ref 25. Copyright 2007 American Chemical Society.

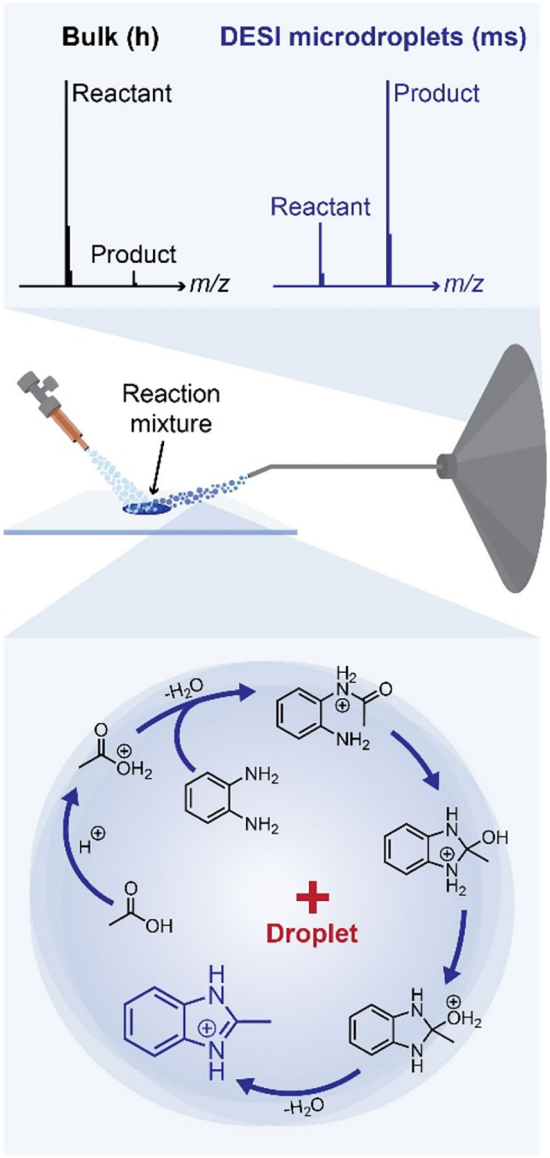


Figure 6.

DESI microdroplets facilitate accelerated chemical reactions, allowing for simultaneous synthesis and analysis. Many reactions, for instance, the condensation of phenyl diamines with carboxylic acids to yield benzimidazoles, are accelerated due to partial solvation of reagents, extremes of pH, and the high-electric field at the droplet–gas interface. Data from ref 60.

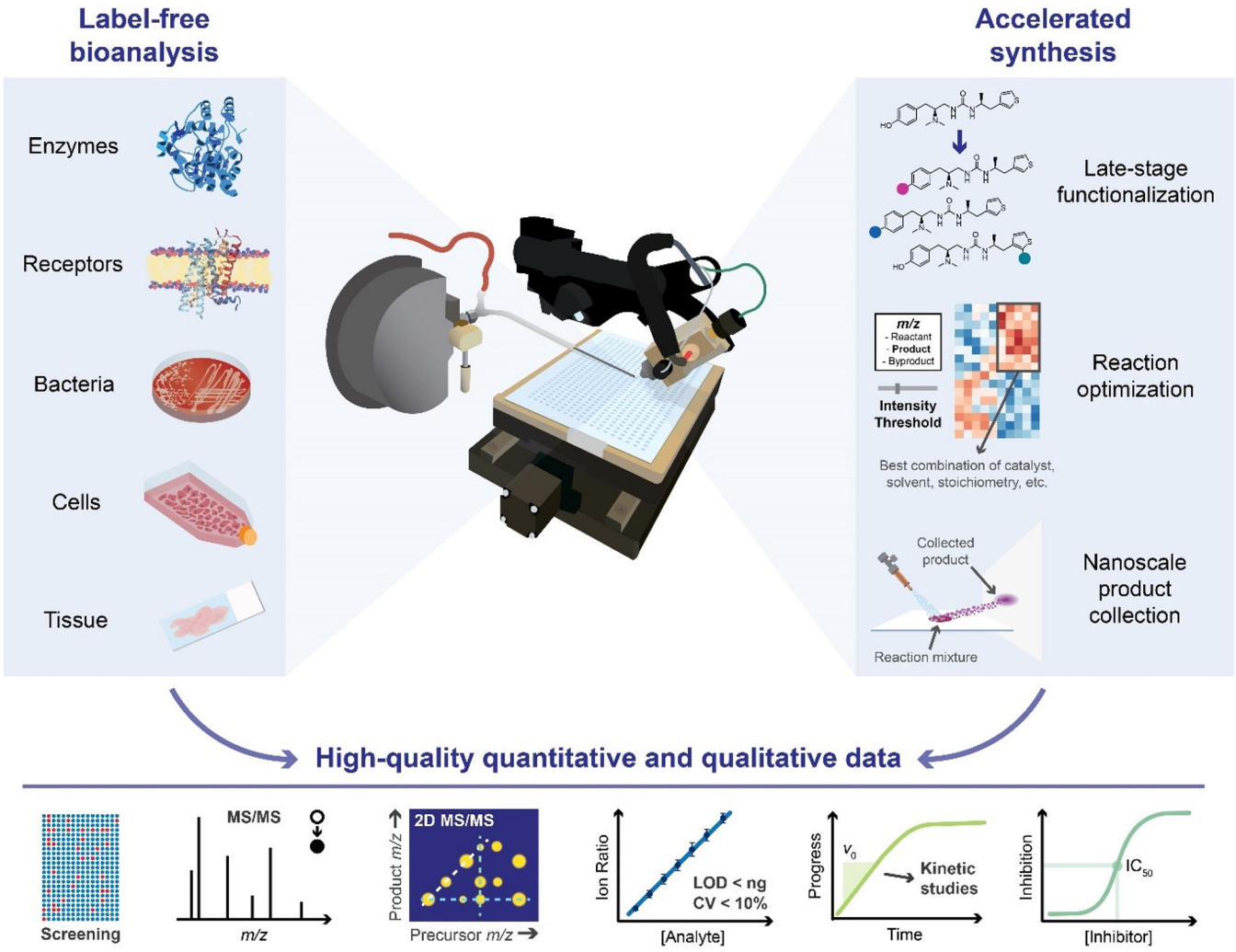


Figure 7.

Overview of some of the capabilities of high-throughput DESI-MS for biological analysis and accelerated organic synthesis. Note that both qualitative and quantitative experiments are accessible using this technology, with demonstrated excellent performance.