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Development of capabilities for imaging mass spectrometry under ambient conditions with desorption electrospray ionization (DESI)

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Dedicated to Don Hunt, in admiration and with affection.

Abstract

Aspects of the development of mass spectrometry over the past three decades are briefly reviewed and growth points in the subject are identified. Molecular imaging by mass spectrometry is one such growth area. The development of a capability for 2D chemical imaging of surfaces is described, based on the combination of a desorption electrospray ionization (DESI) ion source with an automated surface stage capable of *x*, *y* translational motion. The lateral resolution of this new system is found to be less than 200 microns, using a test ink pattern. Chemical imaging of surfaces is demonstrated using model examples of organic and biological systems: (i) imaging of a 2D pattern written in different colored inks on photographic paper and (ii) imaging of thin coronal sections of rat brain tissue fixed onto a glass microscope slide. In both cases, full mass spectra are recorded as a function of *x*,*y*-position on the surface. In the chemical imaging example, the distributions of the two different inks on the paper surface were mapped by tracking the abundance of the intact organic cation which characterizes each particular ink dye. In the tissue imaging example, distributions of specific lipids in coronal sections of rat brain tissue were followed from the abundance distributions in 2D space of the deprotonated lipid molecules recorded in the negative ion mass spectra. These latter distributions reveal distinct anatomical features of the rat brain. The results of these studies demonstrate the feasibility of performing surface imaging studies using DESI and show that at this stage of its development it has a lateral spatial resolution of a few hundred microns.

Keywords: Ambient ionization; Tissue imaging; SIMS; MALDI; History of mass spectrometry

1. Introduction

1.1. Donald F. Hunt and the development of mass spectrometry

An Honor Issue of a journal provides a special opportunity to examine the subject of the honoree and, by looking back in time, to seek a clearer understanding of the factors that have contributed to its present state. Simultaneously, the occasion provides the more risky opportunity to look ahead and attempt to identify embryonic features of the current science which may become prominent. This paper seizes both these opportunities.

One of us (RGC) has known Don Hunt for more than three decades. Acting as consultants to Finnigan Corporation, we met annually with company scientists at the close of the annual ASMS Conference during the 1980's to review the state of mass spectrometry. Inevitably this fiscally meritorious but vexing schedule put us into an introspective mood and for a number of years running we discussed at length our very different plans for our careers. Don had a clear plan for his future scientific journey, mapped out in terms of the decades to come. He was leaving the environmental/small molecule decade and about to enter the protein era, to be followed by the immunochemistry epoch and then, finally, the brain chemistry/consciousness decade. He has largely stuck to this remarkably ambitious plan (many of the topics he intended to study where not subjects then considered even remotely within the scope of mass spectrometry). My original criticism – that it can hardly be interesting if you can predict it – has withered away in the light of his spectacular achievements in all but the last of these areas.

The start of Don Hunt's independent career (1968) coincided with the rise of physical organic and mechanistic mass spectrom-

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etry. The established practitioners of mass spectrometry, many engaged in thermochemical and energetic measurements on elements or small inorganic compounds, were suddenly joined by a large group of newcomers, trained in organic chemistry, who set new directions for the subject. It was a time not unlike the present with the entry of biologists into mass spectrometry and the transformation being made by proteomics. The pioneer organic mass spectroscopists had innumerable fascinating chemical systems to study and it took some years before intellectual rigor was reimposed and the early naiveté of these mechanistic chemists gave way to more rigorous ion structure and mechanistic approaches. To his credit, Don Hunt was one of the more restrained members of the fraternity in this era when the science of mass spectrometry, mirrored the peccadilloes of the larger society.

The next new wave of activity to reach mass spectrometry was the analysis of complex environmental samples and the determination of their constituents. This endeavor was launched by new capabilities in instrumentation, particularly those associated with combinations of gas chromatography and mass spectrometry. With his training in organic chemistry, Hunt was able to make important contributions to this area rapidly. He recognized the environmental importance of molecules that form negative ions, and used the relatively new method of electron capture to efficiently and selectively generate ions from these compounds. He also developed a method of simultaneously analyzing and detecting positively- and negatively-charged ions using a quadrupole mass filter.

A combination of concepts (soft ionization and then product ion spectra for direct complex mixture analysis [1] and instrumental developments (e.g., reverse sector MIKES instruments [2],) prepared the ground for the next development in organic mass spectrometry, the rise of tandem mass spectrometry. Working fast, Hunt was able to build a prototype of the first commercial triple quadrupole [3] within months of learning of the Enke/Yost experiments [4]. Subsequently, he and Jeff Shabanowitz exhibited equal deftness in regard to injection of high mass ions into ion cyclotron resonance spectrometers [5,6] and in developing separation methods with the speed, resolution and performance needed to allow proteolytic peptide mixtures to be characterized and their protein precursors to be identified [7].

It is worth noting, especially for those who come to the work of Donald Hunt from the biological sciences, just how large a contribution he and his students have made to the instrumentation of mass spectrometry. Both top-down and bottom-up protein sequencing methods owe much to his work. He also contributed significantly to the ion trap revolution and his student George Stafford made the key invention – mass selective ion ejection [8] – in this area.

1.2. Current growth points in mass spectrometry

Any list of entries covering the sub-title topic is necessarily subjective. What cannot be in question is the fact that mass spectrometry has for many years been developing vigorously. In part, this is the result of its uniquely wide reach: implicitly, mass spectrometry is the science of ions (formerly, gas-phase ions but, increasingly, ions in air and even in solution). Using a very broad definition, atoms and molecules fall into two classes, charged and non-charged, giving mass spectrometry one of the two major classes of material! In this view, the subject embraces the fundamentals, practice and applications of a very broad swath of science and technology indeed. Such a definition, which will not please all, has the advantage of signposting new opportunities for the rapid growth of mass spectrometry and comes with the promise of continued re-invention of the subject.

We see three important growth points in mass spectrometry and have directed most of the efforts of our lab towards them. They are (i) *ambient mass spectrometry*, the attempt to move many of the essential steps in mass spectrometry out of the vacuum and into the ambient environment. These include ionization [9], in which samples are ionized in the ordinary environment (hence very rapidly), as well as ion activation and ion/molecule reactions [10]. (ii) *Preparative mass spectrometry*, the experiment in which ions are collected, stored and the resulting material used for subsequent experiments [11–13]. Although a difficult experiment, preparative mass spectrometry holds promise for separation of proteins and their collection in their biologically active forms, as well as applications in catalysis preparation and materials science nanofabrication [14]. (iii) *Imaging mass spectrometry*, the third growth area, has a long history in elemental imaging but molecular and especially biomolecular imaging is a newer and extremely exciting subject. It is worth noting that the subject of this paper combines two of these three growth nodes. . .imaging mass spectrometry and ambient ionization. . .to arrive at a new method of imaging ordinary materials in their native forms.

1.3. Imaging by mass spectrometry

Mass spectrometry of surfaces, essentially the interrogation of the spatial distributions of chemical constituents of a material or sample, has become an important technique in materials science and is an emerging area in the biological sciences. The desorption/ionization (DI) of atoms/molecules from surfaces under high vacuum utilizing laser pulses [15–17], fast atom bombardment [18], and high energy atomic or molecular ion beam impact including molecular clusters of keV translational energy [19] has been the subject of intense research and development over the last half century. Technological and phenomenological advances in DI techniques, such as the development of matrix-assisted laser desorption/ionization (MALDI) [20] and secondary ion mass spectrometry (SIMS) [19,21] has allowed the interrogation, including the imaging, of complex samples (e.g., intact biological tissue) after their introduction into the vacuum system.

More recent advances in mass spectrometry have moved some of the steps taken in the analysis of samples to outside of the vacuum environment; in these experiments the sample is ionized under ambient conditions [9]. Desorption electrospray ionization (DESI) [9,12] is the principal method in this new family of ionization methods and, in this work, methodology is investigated that allows chemical imaging of surfaces, including biological tissues.

DESI belongs simultaneously to two families of ionization methods, the spray methods which include electrospray ionization (ESI) [22,23], and the desorption ionization (DI) methods [20,21,24,25]. DESI uses an electrosprayed solvent to examine condensed phase samples at atmospheric pressure. It therefore allows direct and rapid analysis of surfaces with limited or no sample preparation while lifting the requirement that the sample be introduced into a vacuum system [12]. Ionization of molecules present on the sample surface occurs upon impact of the ESI-originated, charged droplets with the surface. The methodology has been applied to the analysis of trace levels of many classes of compounds including peptides, proteins, nucleotides [26], endogenous and drug metabolites [27,28] and nitro-aromatic compounds [29]. Semi-quantitative results have been obtained from profiling (linear imaging) of plant tissue, *in situ*, for alkaloid content [30] while a computer-controlled moving stage has been used for direct analysis of dyes and other compounds on chromatography plates [31]. In another example, DESI was combined with an ion mobility time-of-flight mass spectrometer to probe the conformations of proteins desorbed from an insulating surface [32]. The analysis of drug formulations has been demonstrated using DESI mass spectrometry both in tandem with ion mobility spectrometry [33] and alone [34].

Imaging mass spectrometry based on MALDI and SIMS is becoming a powerful technique for analyzing histological sections of biological tissues [35,36], in spite of the fact that to date these techniques have required that the sample be confined to the high vacuum region of the instrument, severely limiting any further chemical or physical manipulation of the sample. MALDI imaging, as developed principally by Caprioli et al. [35], yields rich information on the spatial distributions of proteins and peptides from intact biological tissue after matrix deposition. Although the method has primarily been applied to proteins, MALDI has seen some use in the investigation of distributions of lipids [37] and drug molecules [38] from tissue surfaces. SIMS imaging of biological materials, principally developed by Winograd and by Todd, yields chemical information on lower molecular weight species but at very much higher spatial resolution (<1 μm) [36,39–42].

The first MS imaging experiments on the spatial distribution of components in tissue samples under ambient conditions, reported in 2004 [12], used DESI to profile plant tissue for alkaloid distributions using a line scan. These experiments were followed by line profiles of thin sections of liver tissue [43]. In these latter and far more important studies, the distributions of several phospholipids were profiled in metastatic human liver adenocarcinoma tissues when 20 consecutive spots on the tissues were analyzed as the sample was moved in 1 mm increments. The important conclusion from this study was that the intensity distributions of particular sphingomyelin species could be used to distinguish the non-tumor and the tumor regions of the tissue.

In this previous work, a manual surface stage was used to map the distributions of molecules on the surface using DESI, although in this work, linear surveys rather than 2D maps were produced. In the present study we employ an automated DESI platform to record 2D MS images under ambient conditions at atmospheric pressure. We demonstrate this capability with two examples: first, by imaging the m-star (m^*) symbol written on photographic paper using two different inks, rhodamine 6G and Basic Blue 7; and second, by imaging thin, coronal sections of rat brain tissue. Selected ion images of the surfaces are created and show the distinctive distributions of the dyes in the inks on the surface, in the former case, and of lipids in the latter. In addition, we investigated the lateral spatial resolution by scanning the surface position.

2. Experimental

2.1. Materials and sample preparation

The m-star symbol (m^*), our laboratory logo, was drawn on glossy photographic paper (Inkjetphoto, International Paper, Stamford, CT) using blue and red permanent markers (ExtraFine Sharpie, Sanford Corporation, Oak Brook, IL). The principal dyes in the inks, rhodamine 6G, and Basic Blue 7, were readily recognized by the cations observed at *m/z* 443.5 and *m/z* 478.4, respectively. To measure the linear resolution of the experiment, parallel lines were drawn on the same paper using an ink-jet printer. The ink cartridge was doped with crystal violet (intact cation, *m/z* 372.3) and four lines of 0.1, 0.2, 0.5, and 0.8 mm in width were printed onto glossy photographic paper separated in each case by distances of 1.0 mm (center-to-center). Positive ion detection was used for the all the ink experiments and methanol was used as the spray solvent at a volumetric flow rate of 1.5 μL/min. The nebulizing gas pressure was 100 psi.

For brain tissue imaging experiments, frozen rat brain from a male Sprague–Dalley rat (Harlan Industries, IN) was cut into 4 μm sections using a Shandon Cryostat (Thermo Electron, San Jose, CA) at −19 °C. Serial sections were made and directly thaw mounted onto a general microscope glass slide (Erie Scientific, Portsmouth, NH). After sectioning, the sections were stored at −80 °C and dried in a vacuum dessicator for up to 2 h prior to analysis. For brain tissue imaging, the negative ion mode was used and methanol:water (1:1 v/v) was employed as the spray solvent at a volumetric flow rate of 1.25 μL/min. The nebulizing gas pressure was 100 psi.

2.2. Desorption electrospray ionization

The DESI ion source utilized in these studies was based on an early prototype design [26] from Prosolia Inc., (Indianapolis, IN). The DESI ion source consists of an inner capillary (fused silica, 50 μm i.d., 150 μm o.d.) (Polymicro Technologies, AZ), for delivering the spray solvent, and an annular outer capillary (250 μm i.d., 350 μm o.d.), for delivering the nebulizing gas. The surface moving stage includes two motorized slides (Princeton Research Instruments Inc., Princeton, NJ) capable of 3 in. × 2 in. travel, in *x* and *y*, respectively with a 3 in. × 3 in. polycarbonate block to which can be secured a 3 in. × 1 in. microscope glass slide. The DESI ion source is mounted onto an *x*, *y*, *z* manual translational moving stage coupled with a 360 degree

rotational stage in order to adjust the incident impact angle. A 0.5 mm i.d. stainless steel capillary was utilized for ion collection and transport into the mass spectrometer. The stainless steel capillary extended 3 in. from the mass spectrometer, allowing the surface stage to be moved in two-dimensions under the ion source.

2.3. Mass spectrometry and image acquisition

All DESI-MS spectra were acquired using a Thermo Finnigan LTQ (San Jose, CA) linear ion trap mass spectrometer equipped with the custom-built, automated DESI ion source described above. The mass spectra were acquired in profile mode and AGC (automatic gain control) was turned on for the ink experiments and off for the tissue experiments. For the "m*" experiment, the MS injection time was set at 500 ms and 2 microscans were summed for each pixel in the image. Imaging experiments were performed by continuously scanning the surface (area 9 mm × 6 mm) in the *x*-direction (see Fig. 1 for orientation) at a surface velocity of 133 μm/s while acquiring mass spectra every 1.07s in full scan mode over the range *m/z* 250–500. Each step in the *y*-direction was 330 μm. As a result, an array of 70 × 18 points (1260 mass spectra) was collected. The parallel lines were rastered at different velocities 50, 100 and 200 μm/s using tip sizes of either 10 or 50 μm. For the lateral resolution determination using crystal violet, the single ion monitoring (SIM) mode of the ion at *m/z* 372.3 ± 0.7 was employed. The MS injection time was set at 250 ms and 2 microscans were summed.

For the tissue imaging studies, the MS injection time was set at 250 ms and two consecutive scans were summed for each pixel in the image. Imaging experiments were performed by continuously scanning the surface (area 13.2 mm × 10.2 mm) at a surface velocity of 200 μm/s while acquiring mass spectra every 0.67 s. Each step in the *y*-direction was 300 μm resulting in the collection of 3366 mass spectra from an array of 99 × 34 points.

2.4. Software

BioMap (freeware, www.msi.maldi.org) was used to process the mass spectral data to generate two-dimensional coordinates of the surface versus intensity. BioMap is an image analysis software platform that can be used with a variety of imaging tools. An import filter for data conversion of Xcalibur™ raw data files acquired on the Thermo Finnigan LTQ into ANALYZE™ format was written in-house.

3. Results

3.1. Desorption electrospray ionization

In DESI, multiply-charged primary droplets impact the surface and are scattered as secondary droplets that carry the analyte and a fraction of the charge and mass of the primary droplet. Subsequently, ion formation from the scattered secondary droplets proceeds either by ion emission (ion evaporation model) or by evaporation of neutral solvent molecules (charged residue model) to produce gaseous ions, just as happens also in electrospray ionization. [44] Because of this similarity, the spectral characteristics (i.e., formation of multiply charged molecular ions of the form $[M + nH]^{n+}$ or $[M - nH]^{n-}$) are very similar to those of ESI even though the sample is in a different physical state (condensed phase vs. solution phase).

A DESI ion source with an automated surface stage is shown schematically in Fig. 1. In this configuration, charged droplets generated in the ion source are delivered to a surface that has been automated to provide two-dimensional translation under the spray tip. A custom-built stainless steel capillary inlet to the mass spectrometer is utilized to enable the surface to move underneath the inlet in the course of a surface imaging experiment. The ion transfer tube is heated to aid evaporation of solvent from the secondary droplets emitted from the surface. The position of the DESI source relative to the surface and inlet of the MS is adjustable allowing fine tuning of the signal intensity.

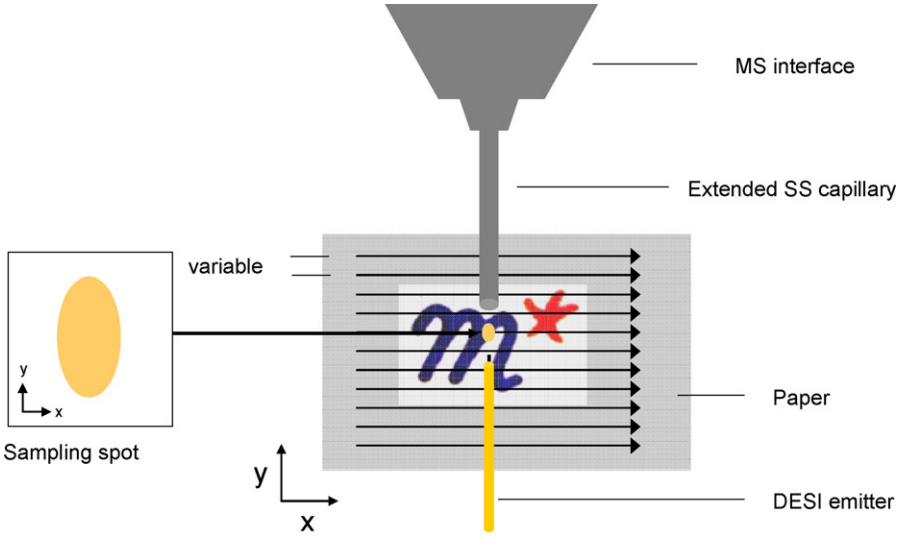

Fig. 1. Schematic illustration showing the automated surface stage DESI ion source.

3.2. Analysis of ink patterns to test spatial resolution

The analytical spot size of DESI depends on the capillary size and the volumetric flow rate of the spray solvent, among a number of other parameters [26]. The spray emanating from the capillary tip is conical and the spray pattern of the droplets impacting the surface has an ellipsoidal shape. As such, the spot width in the *x*-direction (see Fig. 1 for definitions of coordinates) is expected to be smaller than that in the *y*-direction.

In order to test the spatial resolution of the DESI ion source employed for these studies patterns of black ink containing crystal violet were printed onto glossy photographic paper (Fig. 2A). Each lane in Fig. 2A represents a particular set of consecutive lines of varying width (i.e., lane 1 = 100 μm; lane 2 = 200 μm; lane 3 = 500 μm; lane 4 = 800 μm). In these experiments, the surface was scanned over one set of lines (one lane) at a time at a rate of 50 μm/s in the direction indicated by the arrows in Fig. 2A. The tandem mass spectrum of the peak at *m/z* 372.3 detected from the black ink is shown in Fig. 2B. Each lane (1–4) was analyzed in the single ion monitoring mode (SIM *m/z* 372.3 ± 0.7) and the ion current was recorded as a function of time (or position, since the scan was linear with time).

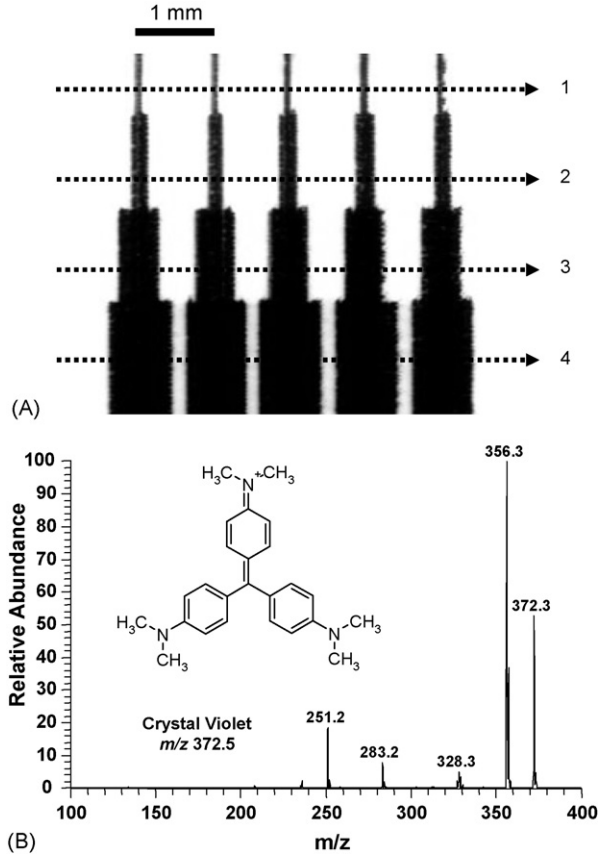

Fig. 2. (A) Ink pattern generated on glossy photographic paper to test lateral spatial resolution. Each lane (1–4) represents the line scan trajectory while operating in the single ion monitoring mode at *m/z* 372.3 ± 0.7 for crystal violet (B) product ion MS/MS spectrum of the peak *m/z* 372.3 (intact cation of the dye, Crystal Violet) on paper.

Fig. 3 shows the results of scanning the pattern in the *x*-direction, as indicated by the arrows shown in Fig. 2A. In lane 1, where the line spacing is 1 mm (with 900 μm dead space between lines), the results show baseline separation and no "carry-over" between lines. Lanes 2–4 also show baseline separation of the signals and no carry-over, but the peaks change from a roughly gaussian to a plateau shape. The plateau is observed when the line is wide enough such that the whole spot can fall onto it. The width of the ion beam corresponds to that line width where the signal just begins to plateau. This occurs between 100 and 200 micron width, indicating the ion beam has a diameter of approximately 200 microns in the *x*-direction, and this is also the resolution in this direction. This result is consistent with the demonstrated ability (lane 4) to resolve features on a surface that are separated by 200 μm. Note that the serrated tops of the peaks are ascribed to electronic and ion statistical noise. Note too, that when the scan speed across the surface is decreased there is an increase in the ion current (assuming no depletion in sample, which is supported by the fact that rescanning gives a similar spectrum) and this gives better signal to noise and an increase in effective resolution. Finally, it should be noted that scanning in the *y*-direction gives a resolution which is, within the error of this rough measurement, indistinguishable to that in the *x*-direction.

It was suggested earlier that reducing the capillary inner diameter would produce smaller spot sizes and so improve the spatial resolution [26]. Therefore, we constructed a DESI source using 10 μm inner diameter (i.d.) fused silica capillary and tested the lateral spatial resolution using the same pattern as in the previous experiments. Overall, there was no significant improvement in the lateral spatial resolution due to reducing the capillary i.d., although the expected reduction in signal intensity was noted. It is likely that the size of the outer capillary carrying the nebulizing gas has an important effect on spot size and hence resolution.

3.3. DESI imaging

For 2D DESI imaging, the sample is either placed onto a target (e.g., microscope glass slide) or is analyzed *in situ* and is moved under the fixed spray nozzle to expose an area of interest. Ion images of the sampled area showing the spatial (2D) distributions of the intensity of a selected ion (or set of ions) can be created from the data.

In order to test the feasibility of performing molecular imaging studies using DESI, the m* symbol was written onto photographic paper using two different inks, Basic Blue 7 (the letter m) and rhodamine 6G (the symbol *), as described in section 2.1. Fig. 4A shows a photograph of the m* symbol (9 mm × 6 mm) with arrows indicating the direction of each consecutive scan which subsequently allowed for reconstruction of the ion image. Fig. 4B and C show DESI mass spectra recorded from the surface of the photographic paper at the m and * positions, showing the intact cations of Basic Blue 7 at *m/z* 478.4 and rhodamine 6G at *m/z* 443.5, respectively. The corresponding ink structures are shown in the insets. The area analyzed was approximately 5.4×10^7 μm^2; it was scanned in both the *x* and *y* directions to produce an array of 70 × 18 (1260 pixels). The resulting spatial

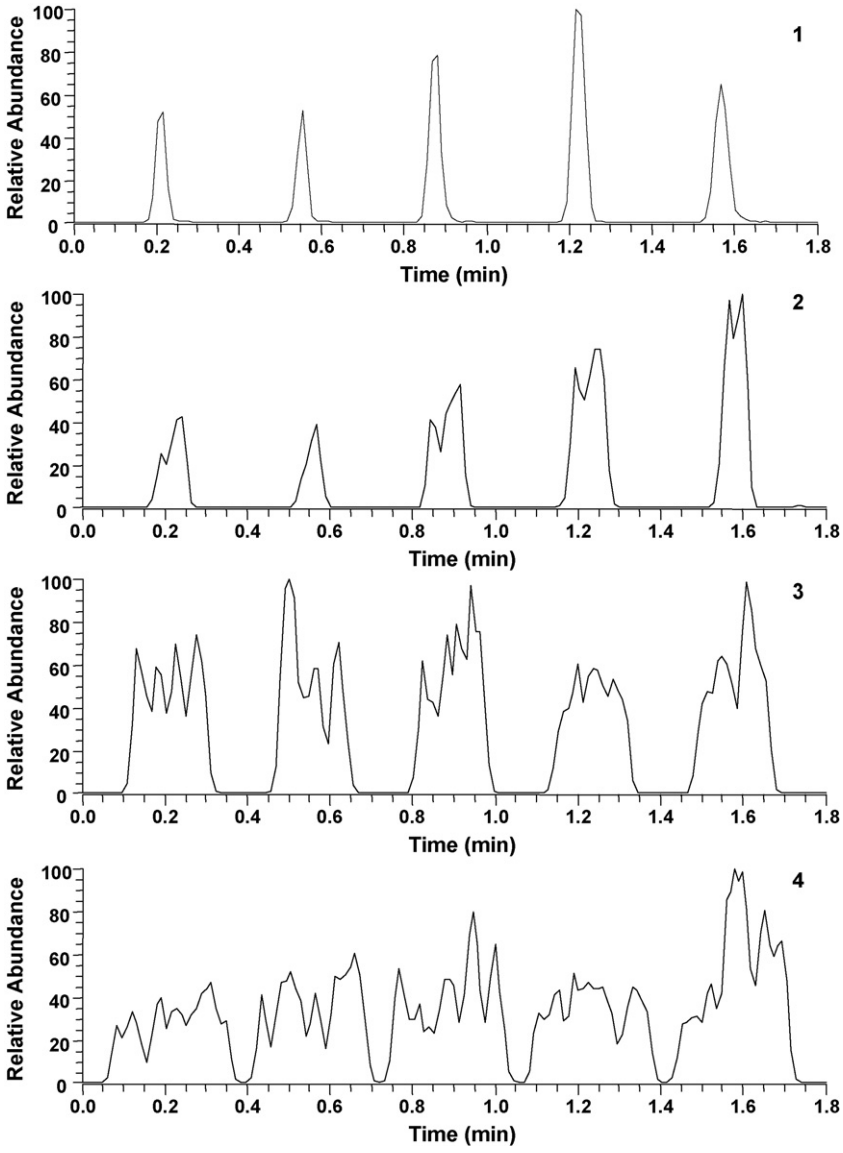

Fig. 3. Total ion current recorded in single ion monitoring mode for the peak at *m/z* 372.3 for each successive lane (1–4) shown in Fig. 2.

distributions of the two inks on the surface plotted in *x* and *y* versus intensity are shown in Fig. 4D. The molecular ion image of the m* symbol distinctly reveals the features of the original image as shown in Fig. 4A. The curvatures in the lines of the m and * are resolved to the extent that they can be correlated to positions on the original image. There are no contributions in the mass spectra due to cross-contamination of the dyes or to carry-over effects.

The ability to record spatial and molecular information simultaneously on surfaces is a particularly powerful approach, especially when limited preparation of the surface is required and the sample is under ambient conditions. Previous work has demonstrated that rich chemical information can be obtained from line scans across intact biological tissues after cryosectioning and mounting onto a glass slide [43]. More recently, 2D molecular ion images of particular lipids in rat brain were recorded using an automated DESI ion source [45]. In parallel work, Van Berkel and Kertesz reported on the use of an automated DESI ion source for the imaging of analytes separated on TLC plates [46]. In Fig. 5 the spatial distributions of two lipids in a coronal section of rat brain are shown. Chemical assignments were confirmed by MS/MS and by comparison to ESI mass spectra of authentic samples [47–49]. Fig. 5A shows the selected ion image of the peak at *m/z* 810, which corresponds primarily to the deprotonated form of phosphatidyl serine (38:4) It is found that the signal is most intense in the region corresponding to the white matter in the brain, specifically the corpus callosum. In addition, other structural features are evident in the ion image e.g., the lateral ventricles and the anterior commissure. The ventricles in the rat brain are clearly resolved in the corresponding ion images, indicating that a spatial resolution of better than 400 μm is achieved. Fig. 5B shows the spatial distribution of the peak at *m/z* 225, the deprotonated form of myristoleic acid (14:1). The spatial distribution of this ion shows that myristoleic acid is uniformly distributed in the tissue section.

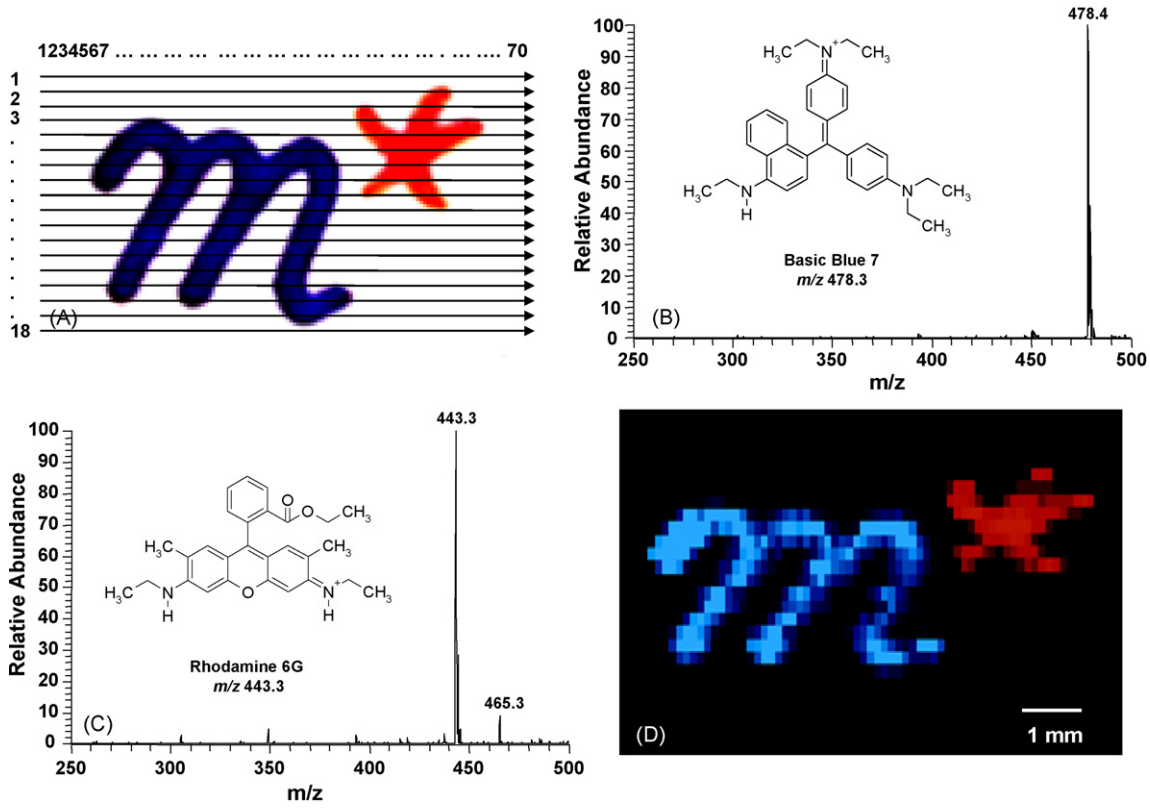

Fig. 4. (A) Digitally scanned optical image of the m* symbol written on glossy photographic paper. Arrows indicate the direction of each scan. (B) Positive ion DESI mass spectrum of Basic Blue 7 on the paper using 100% methanol as the spray solvent. (C) Positive ion DESI mass spectrum of rhodamine 6G on the paper using 100% methanol as the spray solvent. (D) Molecular ion image of the m* symbol recorded by DESI.

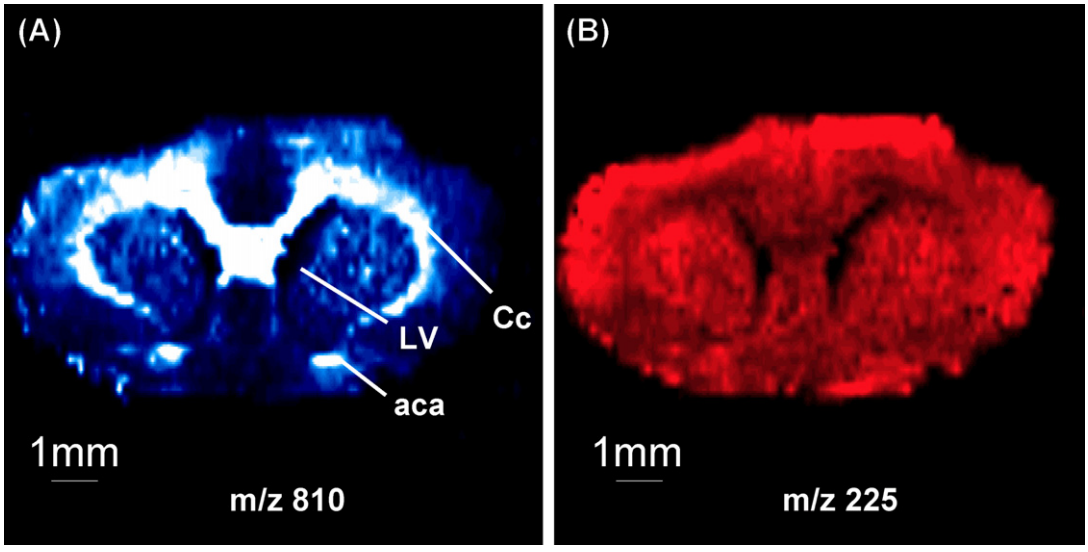

Fig. 5. (A) Selected molecular ion image (*m/z* 810) as recorded in full scan, negative ion mode from a 4 μm coronal section of rat brain tissue. (B) Selected molecular ion image of the peak at *m/z* 225 recorded in full scan, negative ion mode from the same tissue section.

4. Conclusions

The application of DESI-MS to automated surface imaging is demonstrated, first from an ordinary surface (i.e., ink on paper) and second from histological sections of rat brain tissue. The present design allows an achievable lateral resolution of better than 400 μm in the rat brain tissue and considerably better than this in the ink experiments (ca. 200 μm) using 50 μm spray tips. The samples are held in the ambient environment and can be manipulated or processed by other means too—before, after or during the image acquisition. The differences in resolution observed between the biological and non-biological samples are likely to be due to secondary effects associated with weaker ion signals and higher scan rates in the tissue sample

rather than more fundamental effects but this remains to be demonstrated.

The DESI imaging experiment is complementary to MALDI imaging in a number of important ways, including the fact that MALDI is most readily applied to proteins and peptides while DESI works most readily for lipids. In addition, MALDI gives a higher resolution but requires more exacting sample preparation. More experiments are necessary to increase the resolution of DESI, especially if sub-cellular resolution is intended. (There are many problems where this is not needed, as illustrated by the example discussed above.) One approach, for instance, can be the use of nano-sprays to produce spots of 50 μm or less in size, however the correlation between the area sampled and the signal intensity is expected to decrease the sensitivity of such experiments. As mentioned before, reducing the capillary i.d. from 50 to 10 μm resulted in reduction in signal intensity without increasing the resolution. Other experiments can be performed to increase the intensity of small spots, for instance, using a larger orifice of entrance to the mass spectrometry or an atmospheric pressure ion-funnel to concentrate the reflected droplets/ions. Also, in the future, we expect to see more attention given to the use of chemically selected reagents which are added to the spray solution in order to selectively or more efficiently ionize particular classes of compounds by DESI. We also expect further improvements in data processing and in the use of MS/MS scans, high resolution and supplementary ion mobility measurements to improve the quality of the information obtained.

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