Microscopic Imaging of Glass Surfaces under the Effects of Desorption Electrospray Ionization

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Microscopic imaging techniques were used to study sample removal from a glass surface by desorption electrospray ionization (DESI). Changes in fluorescence or absorption images of organic dyes were used to track the impact of the DESI spray on a smooth surface. Time-resolved images revealed rapid removal of the dye from an elliptical region impacted directly by the spray, followed by slower redistribution of the sample across the surface by flowing solvent and/or spray gases. The persistence of a mass spectrometric signal long after the dye was removed from the central impact region indicated that ions were being produced using processes other than impact of the spray on the surface. Placement of a barrier between the DESI spray tip and the mass spectrometer inlet provided additional evidence that ions were being produced at the ends of rivulets streaming away from the central impact region.

Many of the most important developments in mass spectrometry (MS) have been advances in ionization techniques. The creation and development of certain ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), have opened up vast new areas of research that have permanently shaped the field of mass spectrometry. Recent research in ionization sources has expanded the field of MS to include the analysis of surfaces using ambient ionization techniques.

A wide array of novel ambient surface ionization techniques has been described recently. The more heavily researched of these ionization sources include desorption electrospray ionization (DESI), direct analysis in real time (DART),[1] atmospheric solids analysis probe (ASAP),[2] and plasma-assisted desorption/ionization (PADI).[3] A common characteristic of these ambient sources is the ability to generate an ionization medium such as a gas, plasma, or spray. This ionization medium is then directed at the analyte to cause localized ionization from a surface. The capability of localized, real-time, ambient sampling from surfaces fills a crucial niche in mass spectrometry and has already been demonstrated for spatial sampling and chemical mapping of complex systems.[4]

Of the ambient surface ionization sources, DESI has been the most heavily investigated and has been shown to work for a large number of applications.[5−17] We have limited the scope of this paper to the DESI source, which is well-documented, solvent-based, and fairly simple in construction. The first DESI paper authored by the Cooks group described the DESI source but did not discuss possible ionization mechanisms.[18] Subsequent papers by the Cooks group and other groups have put forth a variety of mechanistic studies to address suggested theories regarding the method of analyte transport into the mass spectrometer during the desorption process.[19−21] The research has typically involved comparison of MS data obtained through operation of the DESI source using different operating parameters. Three mechanisms have been proposed by the Cooks group: charge transfer, droplet pickup, and neutral volitization.[22] Of the mechanisms that have been proposed, the droplet pickup mechanism requires solvent

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to make contact with the surface. The Cooks group later demonstrated significant solvent contact with the surface by showing droplet reflection from the surface, providing evidence for the droplet pickup mechanism.[23] Cooks' work was further supported by simulations that showed the formation of thin films of solvent on the surface, from which droplets of analyte were be ejected into the air.[24,25] These droplets were then assumed to undergo ESI-like processes to produce gas-phase ions.

DESI has been proven to be effective with a variety of sample types and surfaces. On one extreme, trace amounts of analyte are coated on a smooth, well-characterized surface. On another extreme, the analyte is dispersed in a bulk solid. In cases where the sample is deposited on a substrate, the substrate can be smooth or rough, hydrophobic or hydrophilic. It is unlikely that a single simple model can account for all ion production by DESI, and the parameter space associated with the technique is so large that it is impractical to attempt to cover all possible types of spray-surface interactions in a single study. There have been multiple reports of the use of microscopy to study the interaction of a DESI source with analytes on a surface. The Van Berkel group has observed spray patterns on variety of surfaces.[26−28] They have identified three regions formed by the DESI spray.[26] A majority of the spray plume impinges on a central region at high velocity, creating an elliptical spot. The second region is formed from the DESI spray when jets of solvent extend from the central region. The third region consists of slow-moving droplets of solvent that land on the surface outside of the first two regions. Van Berkel et al. also showed that the most efficient region for sample transport into the mass spectrometer is the central elliptical region.[26] Bereman and Muddiman used fluorescence spectroscopy to quantify analyte removal during DESI analysis,[29] but they did not obtain high-resolution images of the spray location. More recently, Greene et al. published a method for reproducibly creating surfaces for DESI analysis using glass-coated Rhodamine B slides.[30] Using conventional and confocal microscopy, they imaged individual spots that had been exposed to the DESI spray for a controlled amount of time, with particular attention being paid to the distribution of dye from the "crater". Their study emphasized the effects of operating parameters on the removal of dye from the surface. Because of the thickness of the dye layer that was coated on their slides, the work of Greene et al. falls somewhere between the extremes of sampling a trace material from a smooth substrate and sampling a bulk solid. Despite the appeal that microscopy has for studying the interaction of a DESI spray with a surface, the approach does have some important limitations. Upright microscopes are difficult to use with a DESI source, because the source interferes with microscope objectives. Samples can be removed from the source for offline analysis, but real-time imaging of the interaction of the spray with the surface is impractical.

Herein, we describe our system for the direct analysis of surfaces undergoing ambient ionization using fluorescence and absorption microscopy. The hardware approach used in our study avoids a conflict between the ion source and the optics, using an inverted microscope for surface imaging of the active spray location. Like other uses of microscopy to study DESI, our approach suffers from some limitations. The surface under study must be optically transparent. However, using thin coatings on a glass surface, we can study subtle interactions between the spray and the surface in real time. Imaging of the active region exploits two advantages inherent in surface-ambient ionization techniques. One of these advantages is the ability to detect analyte applied to a surface without extensive sample preparation. This advantage can be exploited for imaging by applying sample to a surface suitable for microscopy. In addition, it has been shown that ambient ionization sources can be adapted to operate at a significant distance from a mass spectrometer, with the sample transported to the mass spectrometer through an extension of the sampling interface.[5,6] Therefore, it is possible to extend the mass spectrometer interface over the stage of an inverted microscope and optically monitor the surface of a sample while recording mass spectra from the same sample.

This research demonstrates the feasibility of sampling from a microscope stage without a significant loss of signal. We provide the first set of real-time images of the DESI footprint region, which provides insight into the different regions of the DESI spray outlined by the Van Berkel group. Visual observation of the spray has led us to design additional experiments to understand how the solvent interacts with the analyte on the surface.

EXPERIMENTAL SETUP

DESI Source. The general layout of the DESI source and mass spectrometer inlet is illustrated in Figure 1. The DESI source was constructed in-house and is similar to other home-built DESI emitters.[23,31] Two fused-silica capillaries were used to produce the spray tip and mounted using a stainless steel Swagelok T-union. The inner capillary had an inner diameter of 75 μm and an outer diameter of 150 μm. The outer capillary had an inner diameter of 250 μm and an outer diameter of 350 μm. The inner capillary was mounted to protrude ~0.2 mm from the tip of the outer capillary. The inner capillary was run through the back of the T-union to a syringe that was set to deliver a solvent that was composed of a 50/50 (%) solution of spectroscopic-grade methanol and 18 MΩ water at a rate of 2.0 μL/min. A high-voltage power supply was connected to the syringe and run at +4900 V for all experiments. Nitrogen was used as the nebulizing gas and was introduced into the base of the T-union at 140 psi. The conditions were chosen to optimize the signal into the mass spectrometer. Variation of the spray angle within a range of 50°−60° and variation of the nitrogen pressure over a range of 80−200 psi caused small changes in the spray footprint that were comparable to those introduced by simply disassembling and reassembling the DESI emitter.

To operate the DESI source over the microscope, a special mount was constructed. The mount was designed to allow

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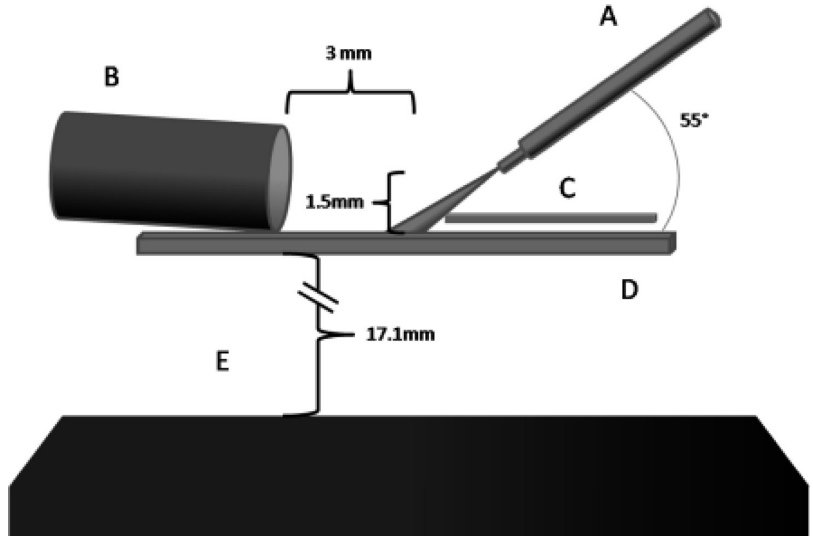

Figure 1. Schematic showing the basic experimental setup for imaging DESI surfaces over the microscope. Legend: (A) DESI spray capillary, (B) transfer line to mass spectrometer, (C) electronic shutter, (D) microscope slide, and (E) microscope objective. (Image not to scale.)

reproducible mounting of the DESI source over the microscope, which was secured on an optical breadboard. The mount consisted of three aluminum posts, a three-dimensional (3-D) stage, an aluminum arm, a rotational stage, and a plastic holder for the T-union. The aluminum posts were fastened to the breadboard to elevate the system relative to the microscope. The 3-D stage was then bolted to the aluminum posts, and an aluminum arm was attached to the stage to extend over the microscope. The stainless steel T-union was then placed into the plastic mount, which was attached to the rotational stage and connected to the aluminum arm. The spraying angle was set to 55° for all experiments, and the needle was positioned to be 1.5 mm above the surface of the slide at all times.

Mass Spectrometer. All mass spectrometry (MS) data were acquired using an LCQ Classic mass spectrometer and Xcalibur software package. The mass spectrometer was mounted on a massive translational stage that allowed positioning of the mass spectrometer relative to the microscope with submillimeter precision.

Previous research has shown that it is possible to use transfer lines to acquire nonproximate MS data.[5,6] Similar to other previously reported nonproximate detection experiments, a stainless steel transfer line was extended from the mass spectrometer to the microscope. The transfer line extended 41 cm from the LCQ heated capillary and had an inner diameter of 1.8 mm and an outer diameter of 2.5 mm. The transfer line was positioned over the microscope stage using steel rods and a micropositioning system. For most experiments, the transfer line was positioned to be in direct contact with the slide. For the simultaneous imaging/sampling experiments, the transfer line was positioned just above the microscope slide (<1 mm) to reduce the amount of background signal acquired during the experiment.

For DESI experiments that did not require simultaneous optical and mass spectrometric data acquisition, glass slides were positioned using steel rods to be directly in front of the heated capillary with no modifications to the mass spectrometer. The DESI source was positioned ~5 cm from the tip of the LCQ heated capillary.

Imaging. Images were taken using a Nikon TE-2000U inverted microscope set at a magnification of 4× and an Andor Luca-S CCD driven by Andor Solis imaging software attached to the camera port. An argon-ion laser set to a wavelength of 488 nm was used as the excitation source for fluorescence imaging. A rotating beam diffuser was used in conjunction with a lens to eliminate laser speckle and provide uniform illumination of the optical field.[32] Fluorescence images were taken with 20-s exposures, unless otherwise noted, and the gain on the CCD was set at 50. For high-speed imaging, either ambient light or the incandescent lamp of the microscope was used, depending on the exposure time necessary for the image.

Shutter. An electric shutter was designed in house to control the access of the spray plume to slide surfaces. Design constraints on the shutter required that it not come in contact with the surface of the slide while being low enough to easily pass under the DESI tip. The shutter consisted of two metal bars separated by a plastic plate the width of a microscope slide. The shutter was mounted to two solenoids, which were used to open and close the shutter, thereby opening or closing the path between the DESI emitter and the slide surface. The shutter was controlled using a PCI-6034E card with a BNC-2110 control box (National Instruments, Austin, TX), using a custom, in-house LabVIEW program.

Slide Coating. Commercially acquired precleaned microscope slides were cleaned with Alconox detergent, followed by nitric acid and methanol, and then were rinsed with deionized water and left to air-dry. Solutions of Rhodamine B Base (RBB) (Sigma Aldrich, St. Louis, MO) and Methyl Violet 10G (Matheson Coleman & Bell, Gardena, CA), also known as Crystal Violet (CV), were prepared in pure methanol at varying concentrations. Cleaned sides were dipped in solution and retracted at a rate of 0.5 cm/s, using a motorized stage controlled via LabVIEW, following a procedure characterized in detail by Hanley and Harris.[32] Slides were left to dry before being removed from the stage. One side of the slide was designated as the top and the underside was wiped clean of any dye, using methanol. All slides

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used in the experiment were used the same day that they were coated.

For the most concentrated Rhodamine B Base solution (8×10^{-6} M), the coating procedure produced a density of 3.5×10^{12} molecules/cm^2 on the surface. We estimate, based on the dimensions of the molecule, that a full monolayer would have a surface density of $10^{13}-10^{14}$ molecules/cm^2, so the coatings produced by this procedure were substantially less than a monolayer.

Slides coated with thicker coatings of CV were prepared by adding Crystal Violet solution dropwise to the slide. Dropwise addition of the dye solution continued until it was observed, upon allowing the solvent to evaporate, that an almost-opaque continuous layer of dye formed in a region large enough for imaging on the microscope.

METHODS

Fluorescence Intensity Calibration. To ensure that the fluorescence intensities recorded in the microscopic images were proportional to dye concentrations on the surface, we coated a series of slides with RBB by dipping them in solutions with concentrations ranging from 1×10^{-6} M to 1×10^{-5} M. The slides were then imaged with the CCD, and the average intensities plotted as a function of the original solution concentration. The fluorescence signal was linear up to a dye concentration of 8×10^{-6} M.

Time-Resolved Measurements. The interactions of a DESI spray with a surface occur on a range of time scales. Capture of as much of that range as possible with equipment available to us required several different experimental approaches. We recorded the fastest signals by imaging a subarray of the CCD at a frame rate of 144.5 Hz, corresponding to exposure times of 7 ms. The fluorescence signals from RBB were not adequate at such short exposure times, so we backlit a thick coating of CV with the built-in incandescent illuminator on the microscope and recorded absorption images.

The electronic shutter provided intermediate time resolution. The shutter was capable of opening and closing within a few milliseconds, making it possible to expose the surface of the slide to the DESI spray for tens of milliseconds, followed by static recording, using fluorescence imaging, of changes to the surface that occurred while the shutter was open. The static experiments were performed with slides that had been dipped in an 8×10^{-6} M RBB solution. The shutter also allowed us to precisely time the beginning and end of the experiments.

The slowest experiments were again recorded in the absorption mode with CV as the test analyte. A relatively thick coating of CV was prepared by evaporating multiple aliquots of 4×10^{-5} M CV on a slide. The slide was placed on the microscope, the mass spectrometer was activated, and full-frame video was recorded at a frame rate of 2 Hz. Once a baseline had been established, the shutter was opened, and both mass spectra and video were recorded for 3 min before the shutter was closed. Because the signal from the mass spectrometer was noisy, the experiment was repeated seven times and selected ion signals were averaged to give a clear picture of the time evolution of the mass spectral signal.

Rivulet Experiment. A glass coverslip was mounted to a translational stage and positioned over the microscope, so that the coverslip was orthogonally oriented to the microscope stage and to the DESI tip, creating a barrier between the spray and the transfer line of the mass spectrometer (see Figure 2). The coverslip was positioned to be 2 mm from the DESI tip and 3 mm from the transfer line. The lower edge of the coverslip was positioned using the translational stage to be ~0.3 mm above a slide. This positioning allowed for the spray to impinge directly on the microscope slide and the rivulets formed during the process to pass under the coverslip. A slide partially coated in 9.2×10^{-4} M CV was placed on the microscope stage, so that coated portion of the slide was on the side of the barrier opposite the DESI source, several mm away from the coverslip. The uncoated side was then sprayed for 30 s while being imaged with the CCD. After 30 s, the microscope stage was repositioned to bring the coated portion of the slide to the edge of the coverslip, but with the analyte still on the side opposite the DESI emitter. The only contact between the DESI spray and the sample was via the flow of the rivulets underneath the barrier. The time-dependent signal from CV was recorded on the mass spectrometer during the entire experiment.

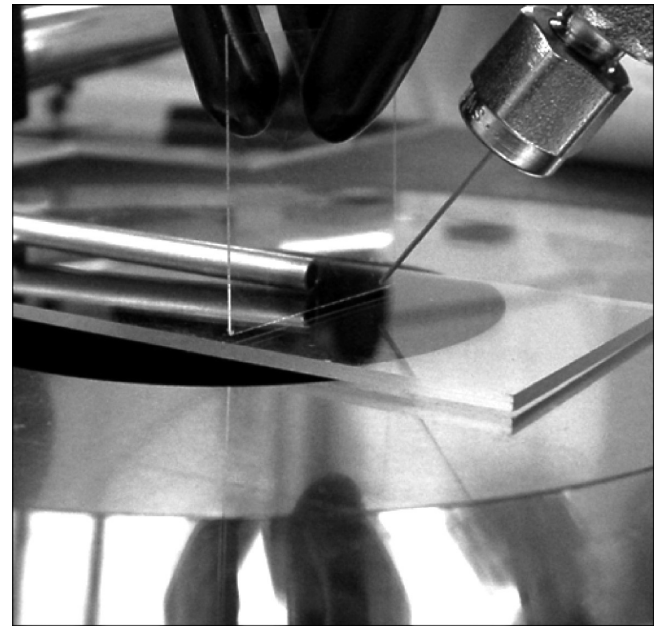

Figure 2. Photograph of the layout used in the rivulet experiment.

Safety Considerations. *The high voltage applied to the DESI capillary is potentially hazardous.* Care should be taken to use a high-voltage supply with limited current capacity, to insulate the high-voltage cable and connection to the DESI source, and to ground the working surfaces. The DESI source produced small quantities of methanol vapor, so the source should be used in a well-ventilated environment.

RESULTS AND DISCUSSION

Relation to Previous Work. Upon viewing the DESI spray through a microscope, one immediately notes the presence of droplets localized in a central region with additional random droplets falling outside this central region. Figure 3 shows a typical spray profile, pieced together from multiple images of the footprint region generated by spraying a slide coated with RBB for 30 s. The needle of the DESI source was positioned to spray solvent

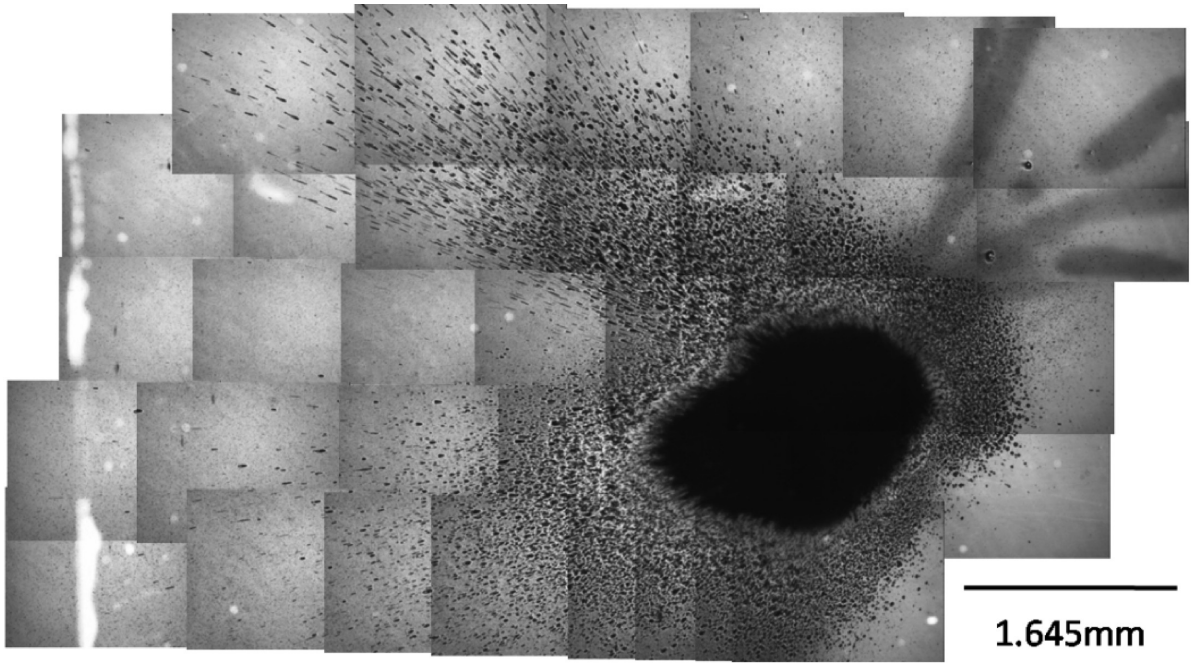

Figure 3. The spray footprint generated by spraying a slide coated with a homogeneous layer of Rhodamine B Base (RBB). The spray plume was in contact with the surface for 30 s. Individual fluorescence images were taken over the microscope using laser excitation and 20-s CCD exposures. In false color, brighter regions indicate the presence of more dye.

from right to left, with respect to the image. The dimensions of an individual image under 4× magnification are 1.645 mm × 1.240 mm. The effect of the rivulets can be seen at the edges of the spray footprint, whereas complete removal of dye is observed in the central high-flow-rate region. The regions outside of the spray footprint are disturbed, indicating the presence of slower-moving droplets. Some of the droplets at the top of the image have elongated profiles, indicating much-faster-moving droplets that were likely generated from bouncing off the glass slide in the central region of the spray.

Nature of the Spray on a Glass Surface (Fluorescence). A fluorescent dye coating is useful for imaging, as long as the coating is fairly homogeneous and is thin enough to avoid self-absorption. Our coating procedure yields a linear fluorescence signal with dye concentrations on slides of up to 8.0×10^{-6} M RBB. A dipped slide imaged on the microscope is shown in Figure 4A. The same spot was exposed to the DESI spray by opening the electronic shutter for 30 ms; the resulting image is shown in Figure 4B. It is apparent, even at an exposure of only 30 ms, that the dye is already being disturbed on the surface of the slide. The presence of the slower-moving droplets in the DESI spray is evidenced by the small areas of dye removal that can be seen outside the central region of the entire spray footprint. Although there is no evidence of rivulet formation after only 30 ms of exposing the slide to the spray, the initial solvent has displaced enough dye to yield an increase in fluorescence signal downstream from the spray.

Figure 4C shows a 200-ms exposure to an unsprayed location on the slide. The shape of the spray footprint is better defined by 200 ms, and the footprint itself has some indication of the direction of solvent flow, based on the dye remaining in the central region. The possible formation of rivulets is seen extending around the impact region. Another 200-ms exposure to the same location (see Figure 4D) shows that the dye is further displaced from the central region, with much of it collecting downstream from the spray. Residual dye in the center spot of Figure 4C has been removed from Figure 4D, and the borders of the footprint region are more defined. After several additional 200-ms exposures (see Figure S-1 in the Supporting Information), the footprint region has expanded with an almost-complete removal of the dye from the central location.

Based on variations in solvent flow rate, and the height of the DESI needle, the appearance of the spray can vary considerably. These variations include the shape, the amount of solvent present on the surface, the formation of rivulets, and the length of these rivulets. It is important to note that the shape of the spray footprint is influenced by the construction of the tip of the DESI source. As has been observed previously, no spray footprint is ever perfectly symmetrical.[26] This is likely due to small variations in the position of the inner capillary relative to the center of the outer capillary. With the microscope, it is possible to focus on the emitter tip while spraying. In almost all cases, the inner capillary is off-center, leading to asymmetry in the spray footprint. In addition, as a general practice, we constructed the DESI source so that the inner capillary would extend 0.1−0.3 mm beyond the end of the outer capillary. Longer lengths would occasionally cause the inner capillary to visibly oscillate.

Figure 5 shows variations of dye displacement using 200-ms shutter exposures of 1, 2, and 3 μL/min flow rates, respectively. The increase in solvent flow causes a notable increase in dye displacement over time. Figure 5A is similar in appearance to Figure 4B. Figure 4B was taken with only 30 ms exposure but double the flow rate. Neither case shows much dye displacement. Figure 5B is taken under the same conditions as those described in Figure 4C and is useful in showing the variation in the footprint introduced by disassembly and reassembly of the DESI source.

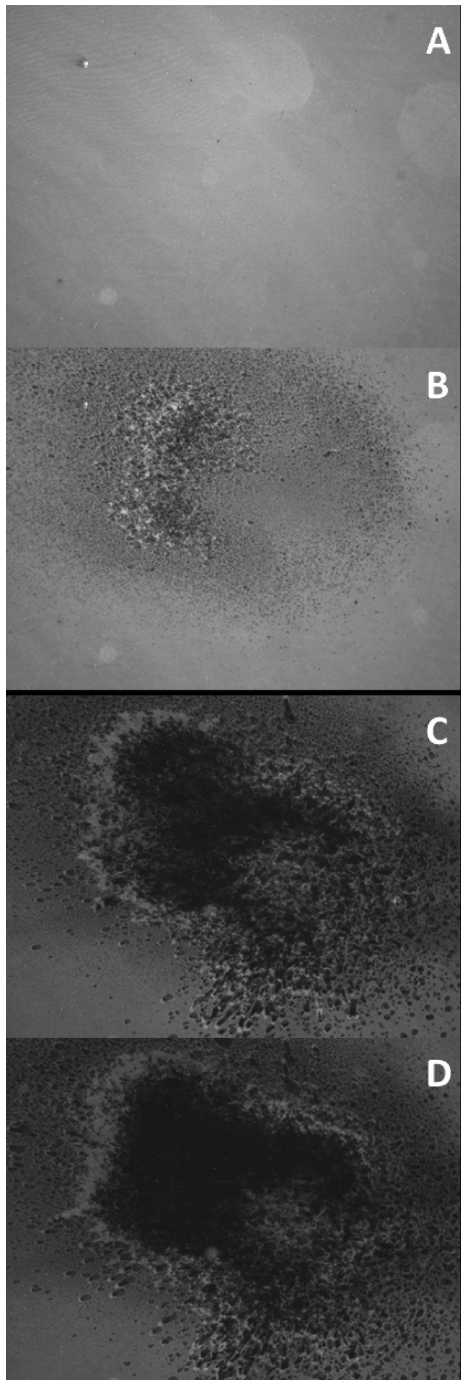

Figure 4. Fluorescence images taken (A) before and (B) after a slide coated in RBB was exposed to the DESI spray for 30 ms. Fluorescence images taken after a spray exposure of (C) 200 ms and (D) another 200 ms exposure at the same location.

Figure 5C shows an expanded footprint region which has far less residual dye remaining within the footprint than was the case at 2 μL/min. The increase in solvent flow has created more rivulets that have extended further in all directions.

If any signal generated more than a second into the spraying process of a thin coating is to be explained, it likely is not generated from dye being displaced from the central region, but instead from the interaction of the rivulets with the dye on the edges of the footprint. Thus, we propose that ions are generated when the solvent evaporates at the end of the rivulets, transporting dissolved analyte into the gas phase. This process leaves a majority

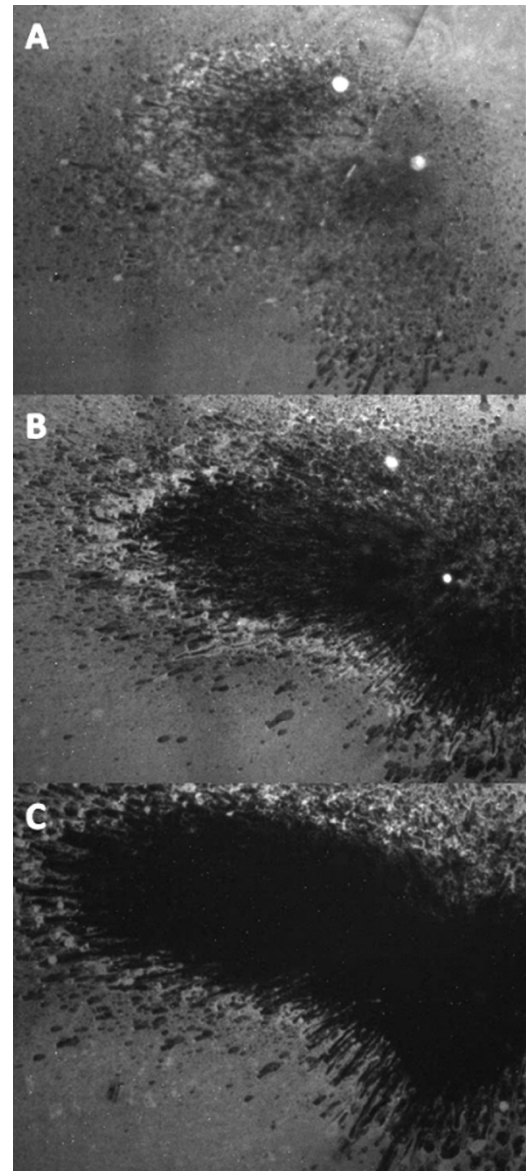

Figure 5. Fluorescence images taken of a slides coated in RBB and exposed to the DESI source at a rate of (A) 1 μL/min, (B) 2 μL/min, and (C) 3 μL/min. Images taken with exposure times of 10 s.

of the analyte on the surface for continued interaction with the rivulets. As far as we are aware, analyte transport into the gas phase from the ends of charged droplets on a surface has not been previously suggested. Studies in which analyte spots were rastered by approaching with the spray from different directions provide evidence for this theory.[26] Approaching a spot with the rivulets of the spray region still generates some signal, even when the central region is not in contact with the analyte. This indicates that ionization can occur at the end of the rivulets.

We have considered the possibility of Taylor cones forming on the rivulets, but have yet been unable to find any evidence of such using the microscope. Further research is needed to identify the mechanism of analyte transport when signal persists after all the analyte is removed from the central elliptical region.

Van Berkel et al. noted that operating on smooth surfaces creates a "washing" effect, which they conclude would be detrimental to a DESI analysis in certain circumstances.[26] They further state that "it is likely that a certain amount of jetting occurs

on all surfaces". Although a majority of the sample seems to be pushed away by the spray, instead of being ionized, this washing effect by the spray is an unavoidable effect of the DESI technique on glass surfaces. The images in Figure 4 indicate that the washing effect may extend the sampling time with prolonged exposure of analyte to the solvent at the edges of the spray footprint.

Simultaneous Imaging. Despite the washing effect, we have noted that signal in the mass spectrometer persists far longer than the time necessary to remove dye from the central region of the spray footprint. To identify the source of the prolonged signal, an experiment was designed to monitor the ion signal in the mass spectrometer while imaging the analyte removal from the surface over the microscope. Based on the rate of sample removal seen in the previous experiments, a 3-min run was chosen to give ample time for the sample to be thoroughly displaced from the spray footprint.

The selected-ion monitoring (SIM) of the $[M\text{-}Cl^-]^+$ peak at 372.2 ± 0.5 amu was averaged across seven runs and is shown in Figure 6A. Immediately prior to closing the shutter 3 min into the run, the signal remains at 30% of its maximum intensity. These experiments were performed with the DESI spray plume impinging on an area where much of the dye had formed small crystals in a fairly homogeneous layer, as seen in Figure 6B. From the images, it is evident that the dye quickly accumulates along the edges of the footprint region. Further investigation shows that the slow-moving droplets cause the dye to migrate from regions outside the spray footprint inward, toward the central region of the spray footprint. Figure 6B shows the edges of the spray at frames 20 and 170, sampled at 2 Hz. The region surrounding the footprint is disturbed by the slow-moving droplets, and over the course of the run, the dye crystals surrounding the entire spray footprint can be seen migrating toward the spray. In the regions where the migration is the fastest, the border between the spray footprint and the outer region breaks down, presumably because of analyte transfer into the gas phase. If the dye were to continue to migrate in this fashion, a much-larger region could effectively be sampled. A video of this dye migration phenomenon can be seen in Video S-1 in the Supporting Information.

DESI samples are often prepared by spotting low-concentration solutions on a small region of a surface for analysis. In such a case, the slow-moving droplets would provide little if any benefit for increasing the longevity of signal. In cases where the amount of sample in an area far exceeds the spray footprint size, the slow-moving droplets may play a significant role in the ionization of the system. However, migration of analyte outside the spray footprint may not be possible on surfaces that are not smooth; thus, analyte migration may be unique to DESI analysis of glass slides with sample areas that are large, compared to the size of the spray footprint.

High-Speed Imaging. In an effort to identify what occurs in the first few seconds of the spray plume coming in contact with the surface, we recorded high-speed video of the rivulets as they crossed an undisturbed surface. Figure 7 shows a series of successive images taken at high speeds. The first set of images (Figure 7A) show the first milliseconds of the spray on a coated CV slide and are enlarged to enhance detail. Image 7A-i records the 7−14 ms after the shutter was opened. As the dye is first

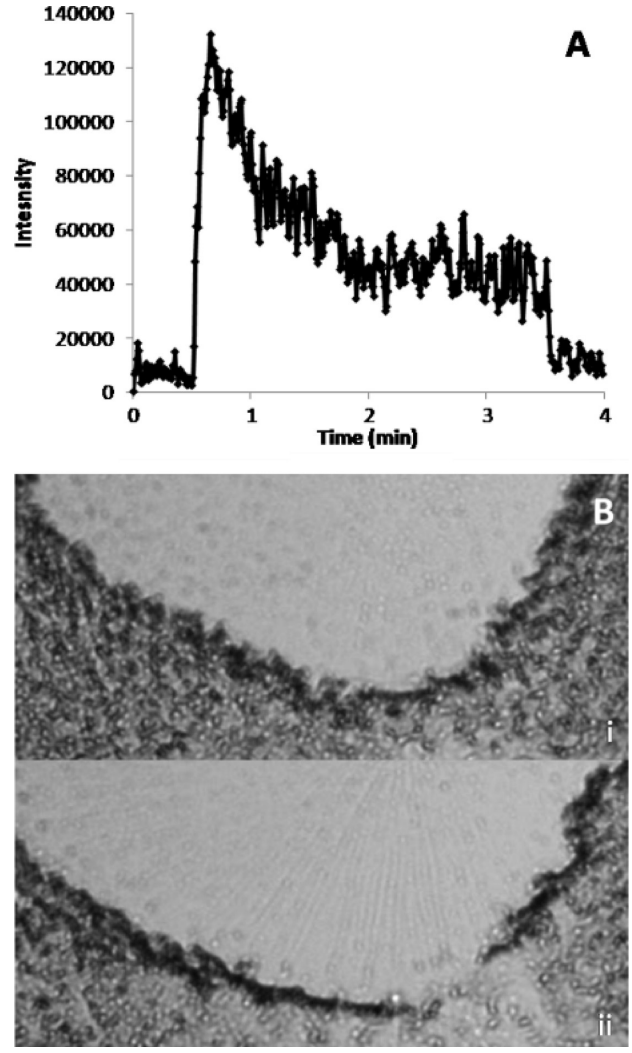

Figure 6. (A) Plot showing the time-dependent ion signal during prolonged exposures of single spots to the DESI spray. The curve is the average of seven 3-min runs, each of which was started on a fresh dye surface. The shutter was triggered 30 s into each run. (B) Still images of the spray footprint (i) 10 s into a run and (ii) 85 s into a run.

disturbed, it is dissolved unevenly, mostly because of the lack of symmetry of the spray plume. The next frame, taken ~7 ms later, reveals three things: (1) the washing effect occurs almost immediately, (2) the inner region is already mostly cleared of the dye, and (3) the solvent is rapidly being pushed toward the outer edge of the footprint. The slower-moving droplets are also plentiful, because they produce a very marked spotted pattern to the right of the spray footprint. As noted previously, the dye is rapidly spreading. In the third frame, the dye seems to be almost completely removed from the center while the solvent is pooling against the footprint boundary.

Figure 7B shows selected frames for the next few seconds and demonstrates the spread of the rivulets across the slide. It can be concluded that much of the dye from the center of the footprint region is being channeled along the rivulets outside the spray footprint, as they become visibly darker. The rivulets seem to form at the edge of a layer or a film of solvent in the central region. In addition, aggregation of the dye along the sides of the channels that lead to the rivulets is observed. The formation of the rivulets and the rate at which they cross the slide varies with each frame.

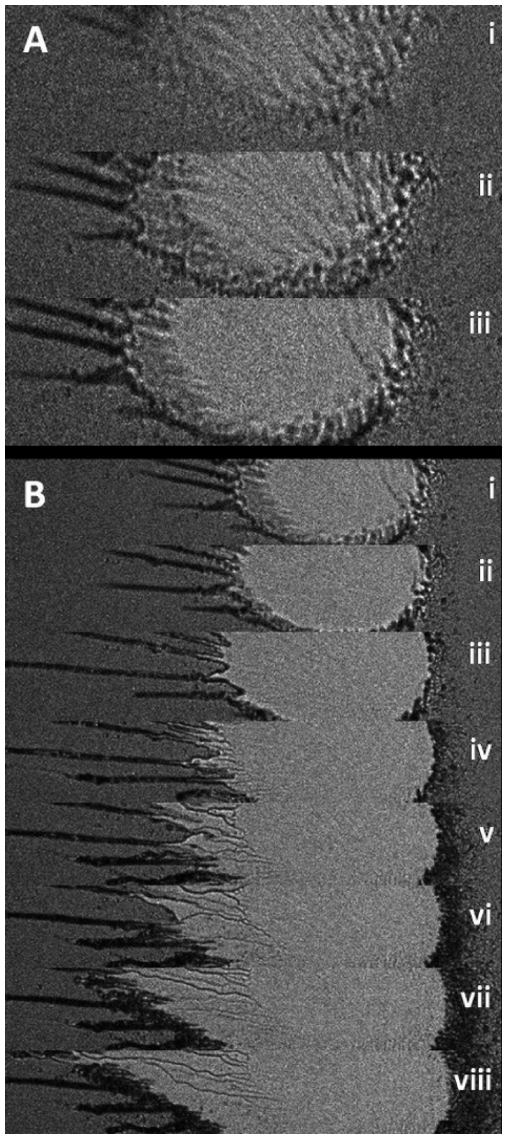

Figure 7. High-speed images of a slide coated in Crystal Violet (CV) as it is exposed to the DESI spray. Each image is a 7-ms exposure. The first three frames (shown in panel A) are successive images enlarged to show detail, showing the initial contact of the spray with the surface and the rapid spreading of solvent across the surface as it dissolves the dye and carries it to the edges of the footprint, taken at times of (i) 14−21 ms, (ii) 21−28 ms, and (iii) 28−35 ms. The next set of images (B) are nonsuccessive and show the spreading of the dye footprint and the accumulation of the dye on the edges taken at starting times of (i) 28 ms, (ii) 42 ms, (iii) 76 ms, (iv) 180 ms, (v) 491 ms, (vi) 907 ms, (vii) 2048 ms, and (viii) 3536 ms.

The position of an individual rivulet can be measured to get a rough estimate of velocity across the slide. Here, the first rivulet to extend beyond the edge of the image was traveling at a rate of 22 mm/s between two frames and had slowed to a rate of 17 mm/s by the next frame (data not shown). Small droplets seen in individual frames are moving faster than can be recorded by the CCD, which suggests additional processes may be involved, such as that shown by Venter et al. in finding droplets above the surface that move at high speeds after colliding with the surface.[23]

Additional frames in Figure 7B show how the spray footprint continues to expand during the spraying process. The rivulets travel in random paths, colliding with the dye and continuously dissolving it and depositing it further downstream. The slow-moving droplets continue to spread out from the spray footprint to the right of the stream. The entire process shown here occurs within ~3.5 s, which is the time at which the DESI signal is strongest in the mass spectrometer. A video of the high-speed imaging is available in Video S-2 in the Supporting Information. The video includes 729 frames recorded at a rate of 144.5 frames per second (144.5 Hz).

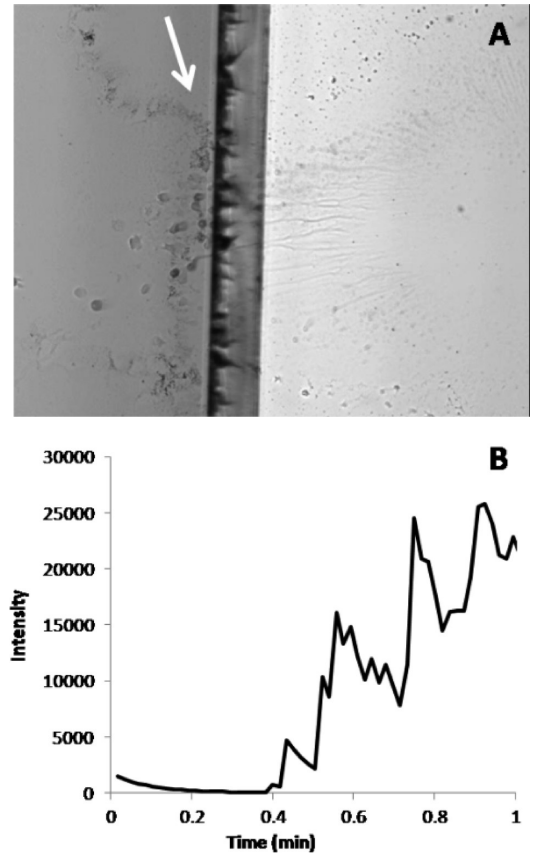

Figure 8. (A) Image of a DESI spray on a glass slide in which the rivulets are passing underneath a glass coverslip and coming into contact with a light coating of CV, as indicated by the white arrow. (B) Time-resolved MS signal at *m*/*z* 372.34. The slide was moved into the position illustrated in panel A 30 s into the run.

Rivulet Experiment. This experiment used a simple physical barrier in the form of a coverslip to ensure that none of the spray made direct contact with the analyte (see Figure 2). By preventing the central region of the spray footprint from interacting with the analyte, we were able to conclusively demonstrate that analyte removal and ionization can occur solely via the interaction of the solvent and analyte at the ends of the rivulets. Figure 8A shows the rivulets passing underneath the coverslip into the dye on the left of the slide. Figure 8B shows the CV selected-ion monitoring (SIM) profile mass peak at 372.34 $[M\text{-}Cl^-]^+$ over the course of the run. Initially, the SIM profile showed only background levels at 372, but after the slide was moved so that the rivulets were coming in contact with the dye, the intensity of the SIM profile increased for the remainder of the run. Although the intensity of the analyte peak was much weaker when compared to a direct spraying of the analyte, the increase in signal definitively showed that ionization was occurring at the ends of the rivulets. A video of the entire run is provided in Video

S-3 in the Supporting Information. This experiment does not eliminate the possible contributions of small ejected droplets, as described by Costa and Cooks, to the ionization signal.[24,25] However, it does prove that droplets that splashed from the surface are not the sole source of the ion signal.

CONCLUSION

The desorption electrospray ionization (DESI) source has been successfully coupled with an inverted microscope to characterize the spray/analyte interaction on the surface through fluorescence and high-speed imaging. The images reveal water jets (which we refer to as rivulets) picking up the bulk of the analyte and carrying it to the end of the spray footprint, where it is then deposited. When thin coatings of analyte are sprayed, dye from the central elliptical region of the spray footprint is removed quickly and is likely ionized outside of the central region. Our experiments indicate that operation of the DESI source using ideal operating parameters create rivulets on glass surfaces and that these rivulets contribute to the overall process of analyte transfer into the mass spectrometer. The images indicate that the slow-moving droplets that land outside the spray footprint may contribute to prolonged analyte signal by causing the analyte to migrate toward the spray footprint. Further work remains for analyzing the source and nature of these droplets' contribution to ionization, as well as the spray interaction on nonglass surfaces.

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SUPPORTING INFORMATION AVAILABLE

Figure S-1 shows a DESI footprint from multiple 200-ms shutter exposures. Video S-1 is a video of DESI spray during simultaneous experiment using a thick coating of Crystal Violet (CV) on a glass slide. Video S-2 is a high-speed video of CV removal at 144.5 Hz. Video S-3 is a video of DESI spray on the surface during the coverslip experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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