

OVERVIEW

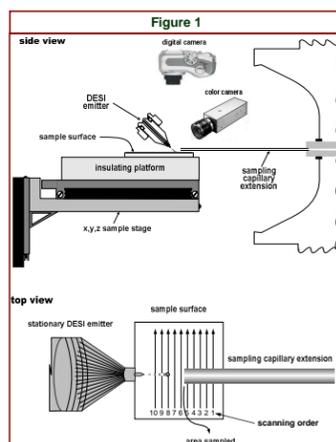
- Desorption electrospray ionization mass spectrometry (DESI-MS) is rapidly developing as a surface sampling/ionization source for the interrogation of a wide variety of analytes on a broad range of surfaces under ambient conditions, and can be used to obtain chemical images of the surface analyzed.
- Imaging was accomplished using uni- and bi-directional scanning with the former proving superior by providing more accurate signal-to-surface spatial location assignment.
- Upfront technical (e.g. scan lane spacing) and physical parameters (e.g. spray settings) inherently affect the best possible image quality theoretically achievable.
- Changing sampling capillary-to-surface distance during imaging may result in signal loss and incorrect spatial assignment of the area sampled on the surface.
 - Image analysis automation concept and associated software (HandsFree Surface Analysis[®]) were developed to control the sampling capillary-to-surface distance. This system enables "hands-free" reoptimization of the sampling capillary-to-surface distance during surface scans to achieve maximum DESI signal and to ensure correct spatial assignment of the area sampled to its true surface location.

INTRODUCTION

- This research is focused on understanding and controlling parameters affecting imaging quality using desorption electrospray ionization mass spectrometry [1-3].
- Recently, the technique was used to demonstrate imaging of analyte bands on TLC plates [4] and imaging endogenous compounds in rat brain tissue [5-6].
- To-date investigations focused on the effect of spray parameters on the VISIBLE spatial resolution/spot size [2,4-6].
- In this work we
 - map the high impact region of the spray plume and its effect on accurate signal-to-surface spatial location assignment
 - study the effect of scanning direction/mode (uni- or bi-directional scanning) on image quality
 - study the effect of the sampling capillary-to-surface distance, which may change during the course of imaging, on image quality
- Image analysis automation concept and associated software were developed to control the sampling capillary-to-surface distance during surface scans to achieve maximum DESI signal and to ensure accurate spatial assignment of the area sampled.
- Dosed tissue was analyzed and chemical images of drug and drug conjugate were constructed.

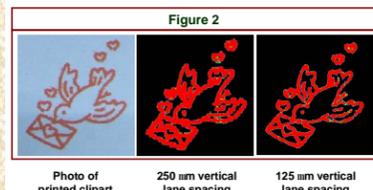
EXPERIMENTAL

- Chemicals. HPLC grade acetonitrile and methanol were purchased from Burdick & Jackson (Muskegon, MI).
- Lines on Glass Slides. Lines were drawn on 75x25 mm microscopic glass slides (Fisherfinest, Cat. No. 12-544-2, Fisher Scientific, Pittsburgh, PA) and fully frosted microscopic glass slides (Fisherfinest, Cat. No. 12-544-5CY, Fisher Scientific) using red permanent markers (Fine Sharpie (Series no. 30000) and UltraFine Sharpie (Series no. 37000), Sanford Corporation, Oak Brook, IL). The principal dye in the red ink was rhodamine B, determined by its parent ion at m/z 443.3 in the positive ion mode mass spectrum and by product ion spectrum (not shown).
- Imaging Printed Lines/Cliparts/Shapes on Copy Paper. Lines/clipart images/shapes were printed on copy paper (Hammermill Great White Copy, Item # 96700, International Paper Company, Memphis, TN) using an Epson Stylus Color 600 printer with its default S020089 color cartridge and the distribution of the most intense n -mer of a polymer additive in the ink at m/z 689.3 was monitored. Both the full mass spectrum of the ink and the product ion spectrum of m/z 689.3 were recorded in positive ion mode (not shown).
- DESI-MS. The manual- and computer-controlled x, y, z sample stage coupled to the LCQ DECA instrument is shown in Figure 1.

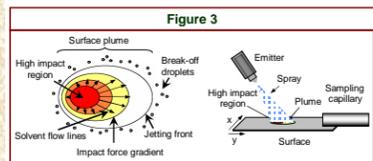


- The mass spectrometers used were (a) a 4000 QTrap hybrid triple quadrupole/linear ion trap mass spectrometer (MDS Sciex, Concord, Ontario, Canada) in the tissue imaging experiments and (b) ThermoFinnigan LCQ Deca ion trap (Thermo-Finnigan, San Jose, CA, USA) in all other experiments.
- In case of the 4000 QTrap, an extended, heated particle discriminator, while in the case of the LCQ DECA instrument the extended atmospheric sampling heated capillary allowed the instruments to be interfaced with automated MS2000 x, y, z robotic platforms (Applied Scientific Instrumentation Inc., Eugene, OR) for imaging purposes. For more details on instrumental setup see ref. 4.

- Effect of Scan Lane Spacing on Vertical Image Resolution.** An 11x10 mm clipart image was printed on copy paper and the distribution of the most intense n -mer of a polymer additive in the ink at m/z 689.3 was used to create the chemical image. Printed image was scanned horizontally in unidirectional mode.

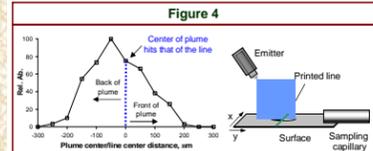


- Effect of Spray Geometry on Image Resolution.** Possible to obtain vertical resolution higher than that expected based on plume size, because the "sweet spot" (high impact area where desorption takes place) is smaller than plume diameter (see Figure 2). General plume diameter is 300-400 μm with our setup.



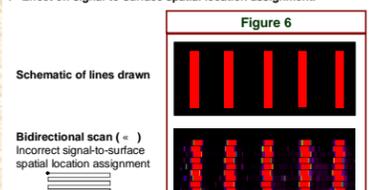
- Physical spray parameters that change the plume (location, size, pressure profile) possibly change "sweet spot", i.e. image resolution as well:
 - incident angle
 - spray-to-surface distance
 - compactness of the spray (divergence angle at the spray tip)

- Mapping the high impact region of the plume. A 100 μm wide line parallel to X axis was printed on paper. Surface moved continuously parallel to X axis and moved in 50 μm steps at every 0.5 min parallel to Y axis. Ion current of an ink component was recorded and averaged in these time intervals and plotted as the function of observed plume center/line center distance:

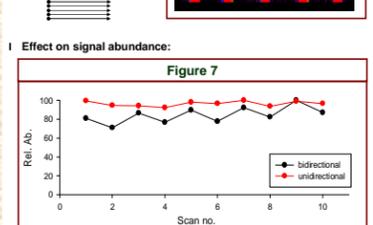


- Signal is asymmetric, back of the plume contains the "sweet spot" \rightarrow must be considered at signal-to-surface spatial location assignment

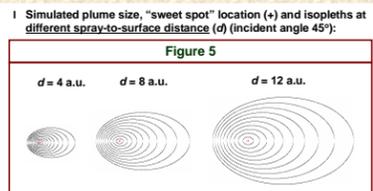
- Scanning Mode.** 5 vertical lines were drawn with red UltraFine Sharpie on a glass slide and scanned in 10 horizontal lanes with 500 μm spacing. The projected surface distribution of the principal dye (rhodamine B) was determined using bi- and unidirectional scanning modes.



- Effect on signal abundance:



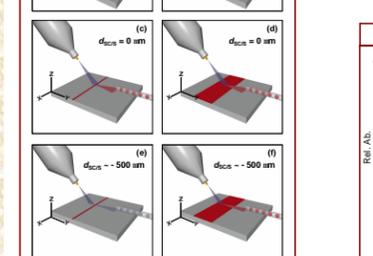
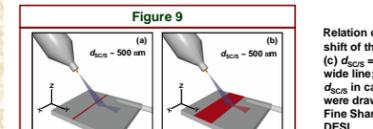
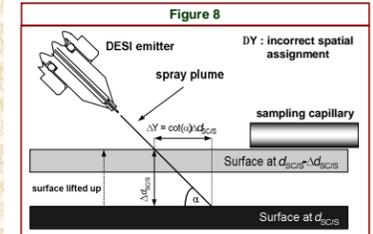
- Simulated plume size, "sweet spot" location (+) and isopleths at different spray-to-surface distance (d) (incident angle 45°):



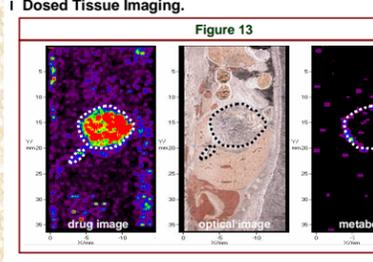
- Smaller distance: smaller, more compact sweet spot/higher resolution
- If uncontrolled, this distance can continuously change during scan and result in incorrect spatial assignment of the area sampled on the surface (see Figure 8 and related section)

RESULTS AND DISCUSSIONS

- Sampling capillary-to-surface distance (d_{SCS}).



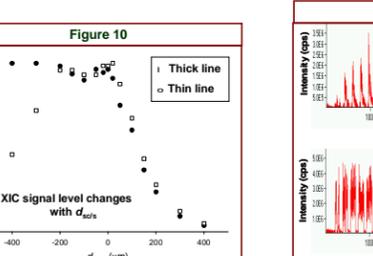
- Dosed Tissue Imaging.



- Non-optimal and/or changing d_{SCS} results in signal loss and incorrect spatial assignment of the area sampled on the surface during imaging experiments.
- Image analysis automation concept and associated software (HandsFree Surface Analysis[®]) were developed to control d_{SCS} .
- This automated system enables "hands-free" reoptimization of d_{SCS} during a surface scan to achieve maximum DESI signal and to ensure correct spatial assignment of the area sampled to its true surface location.

- $d_{SCS}=0$ μm: the sampling capillary just barely touched the surface. $d_{SCS}<0$ μm: gap with the specific distance between the sampling capillary and the surface. $d_{SCS}<0$ μm: the surface was lifted up from the $d_{SCS}=0$ μm state with the specific distance (while bending the flexible capillary).

- Relation of the sampling capillary and the surface showing the shift of the area sampled by the spray plume for (a) positive d_{SCS} , (c) $d_{SCS} = 0$ μm and (e) negative d_{SCS} , in case of sampling a 0.5 mm-wide line; and for (b) positive d_{SCS} , (d) $d_{SCS} = 0$ μm and (f) negative d_{SCS} in case of sampling a 2 mm-wide line parallel to the X-axis were drawn on frosted glass slides with red UltraFine Sharpie and Fine Sharpie pens, respectively, and the lines were sampled by DESI.

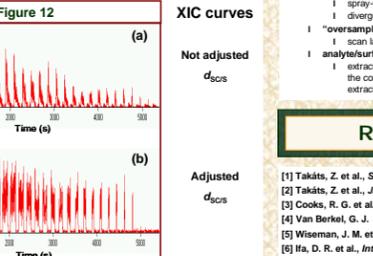


- Mouse dosed orally with sulforaphane at 90 mg/kg. Sacrificed and frozen 3 hours post dose and 40 μm sagittal section prepared.
- 5 μL/min, 80/20 (v/v) ACN/H₂O
- 14 x 40 mm; 81 lanes with 500 μm spacing
- 100 μm/s scan rate, ca. 3.5 hr total time
- SRM transitions:
 - SRM 178 → 114
 - SRM 485 → 178

- Vertical image resolution higher (smaller details) than expected based on the vertical plume size is possible because of the high impact region ("sweet spot") size. Imaging with 125 μm vertical scan lane spacing was achieved.

- Bidirectional scanning results in incorrect signal-to-surface spatial location assignment, while unidirectional scanning provides more accurate signal-to-surface spatial location assignment.
- Non-optimal and/or changing sampling capillary-to-surface distance results in signal loss and incorrect spatial assignment of the area sampled for test purposes.
 - Image analysis automation concept and associated software (HandsFree Surface Analysis[®]) were developed to control the sampling capillary-to-surface distance.
 - The automated system enables "hands-free" reoptimization of the sampling capillary-to-surface distance during surface scans to achieve maximum DESI signal and to ensure correct spatial assignment of the area sampled to its true surface location.

- Study the effect of
 - physical spray parameters on image quality
 - incident angle
 - spray-to-surface distance
 - divergence angle
 - "oversampling" on image quality
 - scan lane spacing lower than vertical size of "sweet spot"
 - analyte/surface interaction on tissue imaging
 - extraction efficiency, i.e. observed signal truly relates to the concentration of analyte or result of different extraction efficiency from different organs (pseudoinage)



- Results:
 - Parent drug was found in the stomach
 - well-detectable quantity
 - Drug conjugate was found in the stomach wall
 - low signal abundance
- Gas flow rate to obtain maximum signal level was relatively high (60 a.u., Analyst Software 1.4) and the analysis damaged the tissue

CONCLUSIONS

NEAR FUTURE

REFERENCES

[1] Takáts, Z. et al., *Science* 2004, 306, 471-473.
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 [6] Ha, D. R. et al., *Int. J. Mass Spectrom.* 2007, 259, 8-15.

ACKNOWLEDGMENTS

Dr. Julian Philips (Thermo Electron) is thanked for the loan of the LCQ DECA mass spectrometer.
 The microspray head used to fabricate the DESI emitter was provided through a CRADA with MDS Sciex (ORNL02-0662).
 Study of the fundamentals of DESI and DESI-MS was supported by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, US Department of Energy.
 ORNL TTED Royalty Funds provided support for the development and modification of the surface control and imaging software.
 ORNL is managed and operated by UT-Battelle, LLC, for the US Department of Energy under Contract DE-AC05-00OR22725.