



# Cluster SIMS with a hybrid quadrupole time-of-flight mass spectrometer

A. Carado<sup>a,\*</sup>, J. Kozole<sup>a</sup>, M. Passarelli<sup>a</sup>, N. Winograd<sup>a</sup>, A. Loboda<sup>b</sup>, J. Wingate<sup>b</sup>

<sup>a</sup> The Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802, United States

<sup>b</sup> Applied Biosystems/MDS Sciex, 71 Four Valley Drive, Concord, Ontario, CA, United States

## ARTICLE INFO

### Article history:

Available online 14 May 2008

### Keywords:

ToF-SIMS  
Cluster SIMS  
 $C_{60}^+$   
Orthogonal  
QStar

## ABSTRACT

The new physics associated with cluster SIMS, i.e. reduced chemical damage enabling 3D dynamic imaging, and increased ion yields from organics samples, suggests that cluster sources may be suitable for use on commercial MALDI/electrospray (ESI) instruments. In efforts to investigate this approach to secondary ion analysis, a 20 keV  $C_{60}^+$  primary ion source by Ionoptika Ltd. was fitted to a commercial LC/MS/MS instrument; the QSTAR<sup>®</sup> XL system by Applied Biosystems/MDS Sciex. This instrument is capable of MS/MS, ion trapping, chemical imaging, and utilizes an orthogonal ToF, enabling use of a DC primary ion beam for imaging and data collection. The system employs high nitrogen pressure, typically several millitorr, in the sample region, as opposed to large extraction voltages, to facilitate the transmission of the secondary ions to the ToF region. In these initial experiments, it was demonstrated that ion signal generated by  $C_{60}^+$  bombardment can be enhanced by trapping in the collision cell and that secondary ions can fragment via collision induced dissociation (CID) to yield MS/MS information. In ToF-MS mode, efficiencies are comparable with pulsed primary beam ToF-SIMS instruments. Mass resolution of over 12,000 is routinely observed with mass accuracy in the 2 ppm range, which has important implications in accurate ion mapping in imaging mode.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

The emergence of cluster ion sources has significantly improved the prospects for organic sample analysis with SIMS due to their ability to produce enhanced secondary ion yields compared to liquid metal ion sources [1]. Despite this, low counts in imaging mode for traditional ToF-SIMS instruments still present a significant hurdle for scientists analyzing biological samples. One way to improve this situation is to capitalize on the ability of cluster sources to cause little or no damage to the surface that is being analyzed, allowing static limit restrictions to be reconsidered. In so doing, many more ions become available for analysis. Unfortunately, traditional ToF-SIMS instruments are not designed to take full advantage of this opportunity due to their requirement for small, well defined packets of ions to be ejected from the sample to allow flight time calculations.

Tandem mass spectrometry instruments designed for MALDI and electrospray ionization have often employed a time-of-flight region situated orthogonally to the quadrupole region in order to deal with ion beams generated in these types of experiments. By decoupling the ToF region from the secondary ion ejection process,

long pulses or continuous beams of secondary ions can be pulsed into the ToF. Furthermore, these instruments operate with negligible extraction voltages in the source region and at high nitrogen gas pressures in RF-quadrupole ion guides which when combined with orthogonal ToF injection enables mass resolution often exceeding that of traditional pulsed ToF-SIMS instruments. These attributes combined with the ability to perform tandem mass spectrometry and ion trapping make this type of instrument design well suited for cluster SIMS.

## 2. Experimental

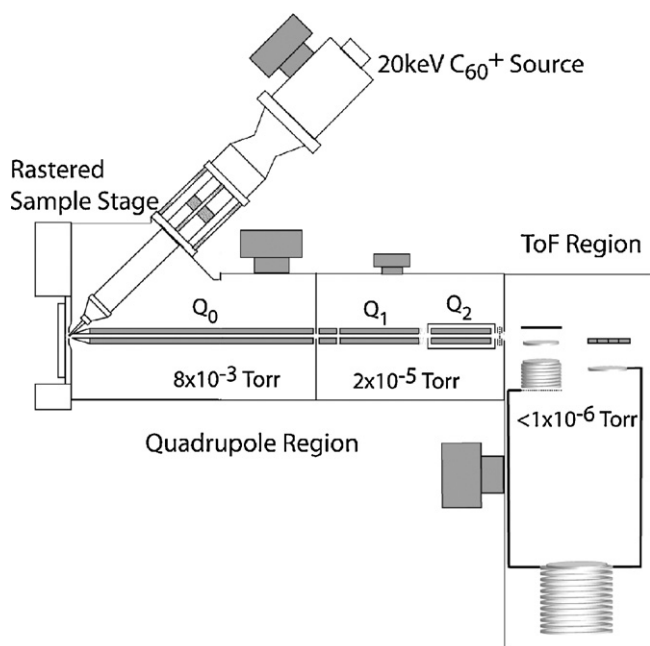
### 2.1. Instrumentation

The front end of a QSTAR<sup>®</sup> XL system, a hybrid LC/MS/MS instrument originally designed for MALDI and electrospray ionization mass spectrometry (Applied Biosystems/MDS Sciex) was modified to fit a 20 keV  $C_{60}^+$  source by Ionoptika Ltd. as shown in Fig. 1. The instrument consists of a sample region maintained at relatively high pressure, up to 1 Torr, a quadrupole region operated with a pressure gradient from  $5 \times 10^{-3}$  to  $2 \times 10^{-5}$  Torr, and an orthogonally situated time-of-flight region at less than  $1 \times 10^{-6}$  Torr.

The high nitrogen pressure in the sample and quadrupole regions serve two purposes. When low energy secondary ions

\* Corresponding author.

E-mail address: [ajc161@psu.edu](mailto:ajc161@psu.edu) (A. Carado).



**Fig. 1.** Illustration of the QSTAR<sup>®</sup> XL system by Applied Biosystems/MDS Sciex altered to operate with a 20 keV  $C_{60}^+$  ion source by Ionoptika Ltd.

collide with nitrogen, the transverse motion of the secondary ions is reduced, focusing the secondary ion beam through the center of the quadrupoles [2] which is critical to efficiency and mass resolution. Additionally, it has been shown that ions generated by MALDI and electrospray are “cooled” through collisions with high nitrogen pressure preventing fragmentation [3].

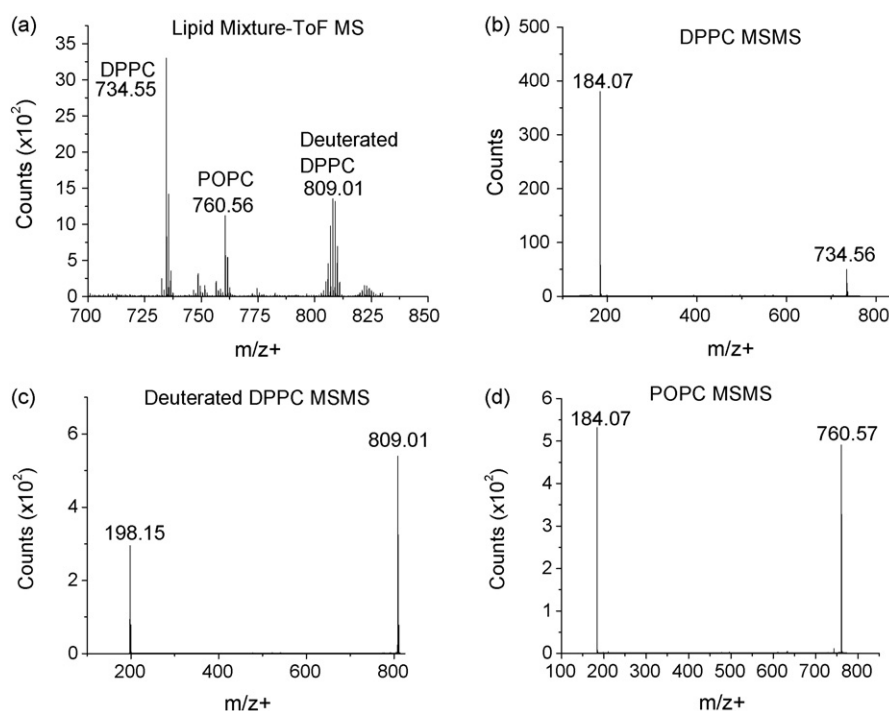
A detailed description of the operation of an orthogonal ToF can be found elsewhere [4], but briefly, the first quadrupole, designated  $Q_0$ , acts as an ion guide to funnel the secondary ions

into the quad region.  $Q_1$  is used for mass selection in MS/MS experiments or in low pass filter RF only mode for ToF-MS experiments and  $Q_2$  can act as an ion guide, an ion trap, and/or collision cell. Secondary ions are ejected from the sample plate at very low voltages, typically 10–20 eV, and swept into the quad region by nitrogen gas flow. After exiting  $Q_2$ , they enter the flight tube and are pulsed in a direction orthogonal to their direction of travel through the quadrupoles, thereby decoupling the secondary ion injection from the flight time separation. Data analysis is handled by Analyst<sup>®</sup> QS 2.0 software and sample stage motion is controlled by oMALDI<sup>™</sup> Server 5.0 software, both by Applied Biosystems/MDS Sciex.

The 20 keV  $C_{60}^+$  ion source, manufactured by Ionoptika Ltd., was operated with 6–15 picoamp (pA) current on the sample in DC mode. Details of this source can be found elsewhere [5]. In order to minimize  $C_{60}^+$  collisions with nitrogen, the beam region of the source was fitted with a sleeve that allowed it to be differentially pumped to below  $5 \times 10^{-6}$  Torr. It has been shown previously that  $C_{60}^+$  remains largely intact at this pressure [6]. In addition, an extended nose cone with a 100  $\mu\text{m}$  final aperture was designed to reduce the distance between the ion source and the sample to approximately 4 mm and to aid in maintaining relatively low pressures in the beam region. This configuration allows a maximum of several hundred pA of primary beam current to be delivered to the target with a spot size that is adjustable from 40–200  $\mu\text{m}$ .

## 2.2. Materials and sample preparation

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-d62-*sn*-glycero-3-phosphocholine 1,1,2,2-d4-*N,N,N*-trimethyl-d9 (deuterated DPPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were obtained from Avanti Polar-Lipids Inc. and were dissolved in a 90:10 solution of chloroform: methanol to a concentration of about 5 mg/ml. After making a mixture of the three lipids, a small aliquot was mixed



**Fig. 2.** MS/MS data from  $C_{60}^+$  generated secondary ions. Clockwise from upper left. (a) ToF-MS analysis of lipid mixture of DPPC ( $m/z^+$  734), POPC ( $m/z^+$  760), and deuterated DPPC ( $m/z^+$  809). (b) MSMS spectrum of DPPC. (c) MSMS spectrum of deuterated DPPC. (d) MSMS spectrum of POPC.

with a saturated solution of sinapic acid (Fluka) in methanol in a ratio of approximately 1:100 lipid: sinapic acid, and a sample was drop-dried directly on the stainless steel MALDI plate. Cholesterol was obtained from Sigma–Aldrich and 50 mg was dissolved in 5 ml chloroform. A small aliquot was then drop dried onto a stainless steel MALDI plate. A polydimethylsiloxane (PDMS) solution was made by soaking a 1 cm × 1 cm × 0.76 mm Sil-Tec silicone sheet manufactured by Technical Products Inc., in 5 ml hexane. This solution was then drop-dried onto a stainless steel substrate.

### 3. Results and discussion

#### 3.1. MS/MS of lipid mixture

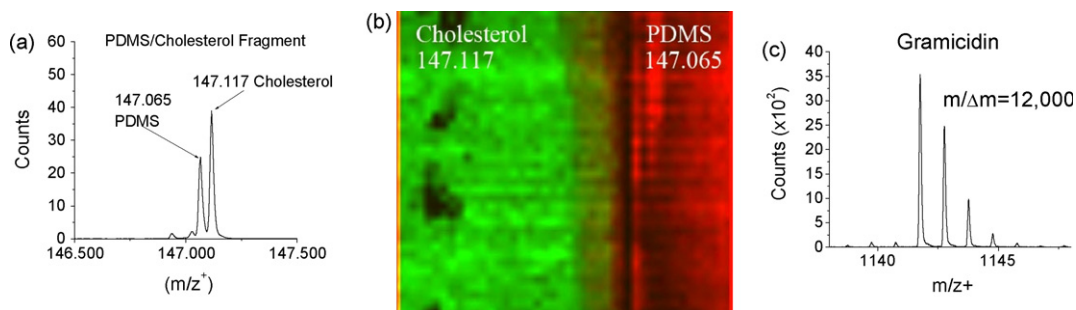
As a test of the ability of this instrument to perform MS/MS on biologically relevant molecules generated by SIMS bombardment, a lipid mixture of DPPC, POPC, and deuterated DPPC was analyzed as a thin film. Results of the lipid mixture analysis are shown in Fig. 2.

Sinapic acid was mixed with these lipids to compare with MALDI, although both ToF-MS and MS/MS experiments were performed successfully without the acid addition. (data not shown). A ToF-MS spectrum of the mixture as well as the corresponding MS/MS data from each protonated lipid molecular ion was obtained. MS/MS analysis of this mixture gives the familiar intense phosphatidylcholine head group fragment at  $m/z^+$  184 or the corresponding deuterated head group fragment at  $m/z^+$  198. This fundamental experiment illustrates the capabilities of this instrument to obtain molecular ion information of an important classification of biological molecules with sufficient intensity for MS/MS analysis.

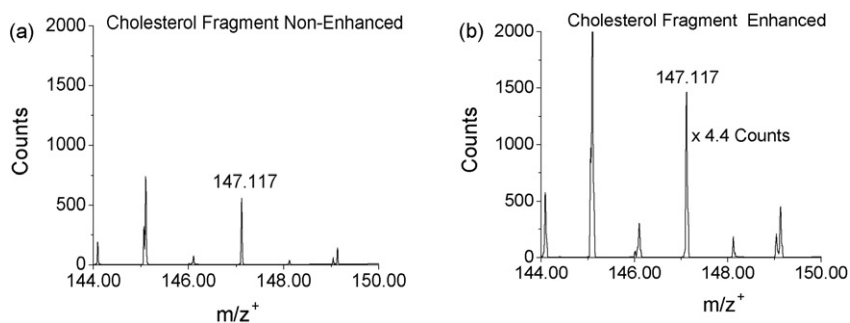
#### 3.2. High mass resolution chemical mapping

Orthogonal ToF pulsing combined with collisional focusing of the secondary ion beam through the quadrupole rods results in mass resolution of up to 12,000. This has the potential to reveal previously undetectable peaks that may be of importance to the SIMS scientist. As an example, MS/MS analysis of cholesterol revealed that the most intense peak in the fragmentation spectrum was mass 147.117 amu, similar to MS/MS fragmentation found by others [7]. The nominal mass of 147 has long been known to be indicative of PDMS, a prolific contaminant in SIMS experiments [8], and was therefore ignored. In order to investigate this further, after thoroughly cleaning two stainless steel sample substrates with hexane followed by methanol, one was coated with PDMS, and one with cholesterol. These two substrates were then placed adjacent to each other on a MALDI sample plate and imaged with a 40  $\mu\text{m}$  step size and a 2.5 pA DC primary beam current and a field of view of 1.5  $\text{mm}^2$ . Data was accumulated for 1 s per pixel. Results are shown in Fig. 3. Mass resolution with the current ToF setting is mass dependent with better results at higher masses. The peaks shown below in Fig. 3a. are at 8000 resolution, but results of  $m/\Delta m = 12,000$  have been obtained at mass 1141 amu. as shown in Fig. 3c.

These results illustrate the importance of mass resolution in chemical imaging. Even with the relatively low resolution of 8000, at least four peaks can be seen between masses 146.85, and 147.117 amu. We are currently investigating the identities of the other peaks at nominal mass 147 amu. Initial investigations into previous biological experiments done in this lab indicate that the 147.117 cholesterol fragment peak is often more intense than the common  $(M - \text{OH})^+$  369 amu and therefore may be a better choice as a cholesterol indicator.



**Fig. 3.** Orthogonal ToF-SIMS imaging of the junction of two sample plates; one coated with cholesterol, and one coated with PDMS. (a) The spectrum which contains both the PDMS and cholesterol fragment peaks separated by 0.052 amu. (b) Image of the junction between the two films. Field of view is 1.5  $\text{mm}^2$ . (c) Resolution of 12,000 for a gramicidin film.



**Fig. 4.** ToF-SIMS analysis of a cholesterol film (a) before and (b) after trapping the cholesterol fragment  $m/z^+$  147.117. Trapping in  $\text{Q}_2$  resulted in an increase in counts of 4.4 $\times$ , as well as enhancing the surrounding peaks.

### 3.3. Enhancement of cholesterol fragment by Q2 trapping

Reduced efficiency due to duty cycle issues inherent to orthogonal ToF instruments can be overcome or mitigated by trapping ions of interest in Q<sub>2</sub> and timing their release to coincide with the ToF pulse. Details of this mode of operation and further information regarding duty cycle can be found elsewhere [9]. Fig. 4 shows the results of a ToF-MS analysis of a thin film of cholesterol. On the left is the non-enhanced spectrum and on the right is after enhancement of  $m/z^+$  147.117 by trapping in Q<sub>2</sub>. Counts were increased in this case by a factor of 4.4. Ions surrounding the enhanced ion also experience an increase while ion intensity outside this window decreases. Unlike MS/MS experiments on cholesterol, this fragment is not a particularly intense peak in ToF-MS experiments on thin films or in biological samples when compared to the surrounding peaks, so a moderate increase in counts is significant.

### 4. Conclusions

The sputtering mechanism of C<sub>60</sub><sup>+</sup> allows for the possibility of entirely new types of instrumentation for ToF-SIMS analysis. An instrument that is capable of handling long or continuous beams of secondary ions enables the full advantages of cluster SIMS to be exploited. Increased secondary ions, the ability to collect information throughout the entirety of a depth-profiling experiment, ion trapping for increased sensitivity, and MS/MS information are all important advantages associated with this type of

secondary ion analysis. Initial results reported here indicate that commercial MALDI/ESI instruments are well suited for use with cluster SIMS ion sources. Successful MS/MS and ion trapping experiments were performed on secondary ions generated from a 20 keV C<sub>60</sub><sup>+</sup> primary ion source with mass resolution that is significantly improved over traditional ToF-SIMS instruments and at efficiencies that are comparable.

### Acknowledgements

The authors acknowledge Applied Biosystems/MDS Sciex for use of the QSTAR<sup>®</sup> XL Orthogonal ToF instrument, The National Institute of Health under grant #EB002016-13, and the National Science Foundation under grant #CHE-555314.

### References

- [1] N. Winograd, *Anal. Chem.* 77 (2005) 142a.
- [2] D.J. Douglas, J.B. French, *J. Am. Soc. Mass Spectrosc.* 3 (1992) 398.
- [3] A.N. Krutchinsky, I.V. Chernushevich, V.L. Spicer, W. Ens, K.G. Standing, *J. Am. Soc. Mass Spectrosc.* 9 (1998) 569.
- [4] A.V. Loboda, A.N. Krutchinsky, M. Bromirski, W. Ens, K.G. Standing, *Rapid Commun. Mass Spectrosc.* 14 (2000) 1047.
- [5] D. Weibel, S. Wong, N. Lockyer, P. Blenkinsopp, R. Hill, J.C. Vickerman, *Anal. Chem.* 75 (2003) 1754.
- [6] R.J. Cotter, B.D. Gardner, S. Iltchenko, R.D. English, *Anal. Chem.* 76 (2004) 1976.
- [7] B. Rossmann, K. Thurner, W. Luf, *Monatsh. Chem.* 138 (2007) 436.
- [8] J.C. Vickerman, A. Brown, N.M. Reed, in: J.C. Vickerman, A. Brown, N.M. Reed (Eds.), *Secondary Ion Mass Spectrometry, Principles and Applications*, Oxford University Press, 1989, pp. 113–115.
- [9] I.V. Chernushevich, *Eur. J. Mass Spectrom.* 6 (2000) 471.