Molecular depth profiling directly on a rat brain tissue section using fullerene and bismuth cluster ion beams

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18th Sanibel Conference on Mass Spectrometry

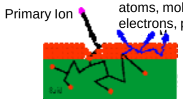
Abstract

The capabilities of time of flight secondary ion mass spectrometry (TOF-SIMS) have been recently greatly improved with the arrival in this field of polyatomic ion sources [1,2]. This technique is now able to map at the micron scale intact organic molecules in a range of a thousand Daltons or more, at the surface of tissue samples, and many biomedical applications are expected. Nevertheless, this remains a surface analysis technique, and three-dimensional information on the molecular composition of the sample could not be obtained due to the damage undergone by the organic molecules during their irradiation. The situation changed slightly with the low damage and low penetration depth of the C_{60} fullerene ion beams. Several recent studies have shown the possibility of organic molecular depth profiling using this kind of beams onto model samples [3]. This possibility has been tried out directly onto a rat brain tissue section, which is the most commonly used biological tissue model in TOF-SIMS imaging method developments. The tissue surface has been sputtered with a 10 keV energy fullerene ion beam, and surface analyses were performed with a 25 keV Bi_3^+ ion beam at regular time intervals. The total depth which was analyzed was more than two microns, with total primary ion doses of more than 10^{16} ions.cm^{-2}. Although not in contradiction with results previously published but with much lower doses, it is found that the molecular damage remains too large, thus making molecular imaging very difficult at such a scale. In addition, most of the lipids, which are usually the main observable molecules in TOF-SIMS, are found to be concentrated close to the sample surface in the first hundreds of nanometers [4].

Definitions:

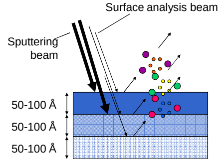
In SIMS studies, projectiles (primary ions) penetrate into the bulk for only several hundreds of Ångströms. The solid is damaged along this depth, because projectiles are slowed down by successive elastic collisions with atoms of the sample. Those atoms are displaced and molecules are destroyed.

Secondary ions originate only from the first 50-100 Å [5].



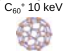
The experiment:

Dual beam depth profiling:
1. C_{60} beam to sputter with a limited damage
2. Bi_3^+ beam to analyse with a good efficiency

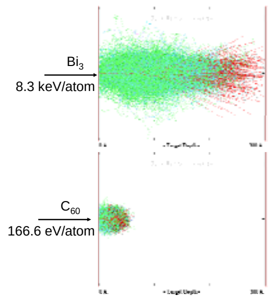


Surface analysis beam: Bi_3^+ 25 keV

Sputtering beam: C_{60}^+ 10 keV



Simulations:



Simulations performed with SRIM software (www.srim.org) of the penetration of a bismuth ion, element of a Bi_3 cluster, and of a carbon ion, element of a C_{60} cluster, into adipose tissue. A fullerene ion, which is very efficient in sputtering, has a much lower penetration depth than a bismuth ion. Thus the damage is reduced below the surface.

	C_{60}	Bi_3
Energy/atom (eV)	166.6	8333
Energy (keV)	10	25
(dE/dx) (eV/Å)	485	229
Projected range (Å)	22	212

Materials and methods:

Experiments performed with a TOF-SIMS IV (Ion-Tof GmbH, Münster, Allemagne) time-of-flight mass spectrometer, located in Tascon GmbH company (Münster, Germany) and equipped with the two following primary ion sources:

- A bismuth liquid metal ion source: Bi_3^+ ions are selected.
- A fullerene ion source, from which C_{60}^+ ions are selected.

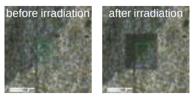
The experiments are performed as follows:

- 1 sec irradiations with C_{60}^+ at 10 keV (2nA) over 300x300 μm^2; 2.8x10^{13} ions.cm^{-2} each irradiation,
- Followed by surface analysis with Bi_3^+ 25 keV (0.1 pA @ 200 μsec) over 110x110 μm^2, 1.5x10^{10} ions.cm^{-2} at each analysis step.

The total C_{60}^+ fluence is several 10^{16} ions.cm^{-2}.

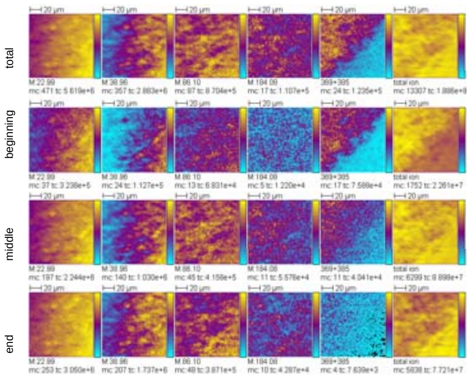
Irradiations located at the edge of the *corpus callosum* at the surface of a rat brain tisssue section (15µm) deposited onto a stainless steel plate.

The total sputtered depth is measured after the irradiation with a KLA Tencor profilometer (Surface Profilometer - Alpha Step 500, précision ~1.5 %).



Video images of the 300x300 µm² sputtered area at the edge of the corpus callosum; the green square corresponds to the 100x100 µm² area analyzed by Bi_3^+

Experimental results: images



Ion images, total (sum along the irradiation) and recorded at the beginning, in the middle and at the end of the irradiation

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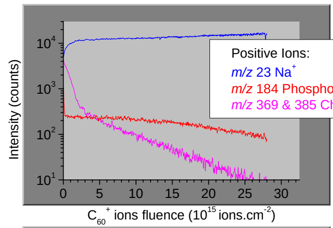
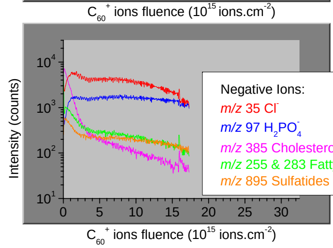
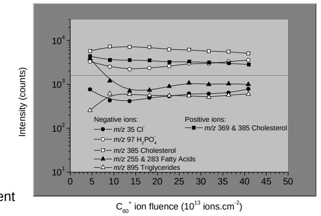
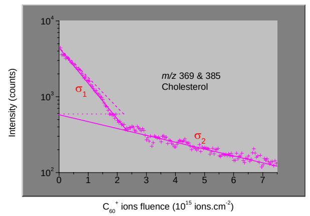
Experimental results: damage cross sections

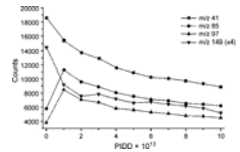




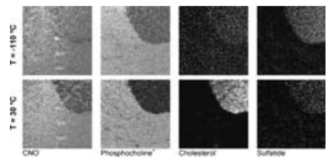
Same data with different enlargments of the *x*-axis scale







Comparison with results obtained by Fletcher *et al* with C_{60} onto cellulose ([M+H]$^+$ *m/z* 149) (Fletcher *et al* [6]).



Lipids are known to concentrate at the surface during reheating of freeze dried tissue sections (Sjövall *et al* [7]).

Conclusion:

- Although the damage cross section is lower with C_{60} ions than with cluster of heavy ions such as bismuth clusters, and although the penetration depth is much smaller with C_{60} than with Bismuth (22 Å instead of 212 Å), the damage remains important and the secondary ion yields undergo an important decrease during the sputtering process.
- Two different regimes are revealed by the irradiations: at the beginning, and up to a fluence of 2.8x10^{15} ions.cm^{-2}, the secondary ion yields decrease ~10 times faster than above this value.
- Nevertheless the damage measured during the early beginning of the irradiation is not in contradiction with results obtained onto model layers in the literature.
- It seems that most of the lipids might have been concentrated at the surface, in a depth estimated to ~200-300 nm. This can be in agreement with the results obtained by Sjövall *et al*, who concluded that such a lipid migration could occur during the reheating of the freezed sections.
- Below the lipid-concentrated layers, the yields are too low to provide images with enough count rates.

Although C_{60} ion beams provide reduced damage and high sputtering rates, depth profiling over several microns in a tissue section remains a challenge.

References:

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