Comparing C$_{60}^+$ and (H$_2$O)$_n^+$ clusters for mouse brain tissue analysis

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Time-of-flight SIMS is applied to the analysis of single cells and different types of biological tissue samples enabling the generation of images with high spatial resolution and chemical specificity. However, the low yield of secondary ions from this type of sample still remains a challenge. This low yield could potentially be increased by enhancing the protonation of ions with the presence of water. Here, we have explored the application of a prototype water cluster ion beam for the analysis of mouse brain tissue samples. A series of experiments acquired with 20 keV (H$_2$O)$_{3000}^+$ and 20 keV (H$_2$O)$_{5000}^+$ were compared with 20 keV C$_{60}^+$, showing ion yield enhancement when a (H$_2$O)$_n^+$ cluster ion is employed in the analysis. The results have demonstrated the potential benefits provided by the use of (H$_2$O)$_n^+$ clusters for the analysis of mouse brain tissue samples. © 2014 The Authors. Surface and Interface Analysis published by John Wiley & Sons Ltd.

Keywords: SIMS; tissue analysis; cluster comparison; H$_2$O; C$_{60}$; imaging

Experimental

A whole mouse brain was obtained using ethically approved procedures from the Faculty of Life Sciences, the University of Manchester. It was sectioned in a Cryo-Microtome (Wolfson Molecular Imaging Centre, The University of Manchester, UK) at

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Each section was 5 μm in thickness with sagittal orientation, and these were thaw-mounted on silicon wafers. The substrates were sonicated in methanol before mounting the tissue sections. The sections were stored at −80 °C until the day of analysis. They were desiccated for 1 h at room temperature prior to SIMS analysis.

Time-of-flight SIMS analysis was performed on a J105-3D Chemical Imager described previously by Fletcher et al. Data were collected using 20 keV C$_{60}^+$, 20 keV (H$_2$O)$_{3000}^+$ and 20 keV (H$_2$O)$_{4500}^+$ as primary ion beams (Ionoptika Ltd). An independent variable sample bias voltage was optimised for each experiment to compensate for charging during the experiments. Positive ion images were acquired using 650 × 650 μm field of view with 64 × 64 pixels. The pixel sizes were chosen to match the measured beam diameter. The structures studied are the caudate and putamen (striatum) of the brain, which are easy to identify because of their striated appearance.

Results and discussion
Comparing 20 keV C$_{60}^+$ and 20 keV (H$_2$O)$_n^+$ for mouse brain tissue analysis

Images of the striatum of the brain were acquired using 20 keV C$_{60}^+$ and 20 keV (H$_2$O)$_{3000}^+$. We present an image acquired with 20 keV C$_{60}^+$ and an accumulated ion dose of 2.5 × 10$^{12}$ ions cm$^{-2}$. The spectrum is mainly dominated by characteristic ions from the grey matter and white matter of the brain, i.e. phosphocholine head group m/z 184 and cholesterol m/z 369, respectively (Fig. 1A). Ion peaks from a higher mass range (500–1000 Da, where intact lipids species can be found) are also visible but are low in signal.

After an accumulated ion dose of 2.5 × 10$^{12}$ ions cm$^{-2}$ with C$_{60}^+$, analysis with 20 keV (H$_2$O)$_{3000}^+$ was carried out on the same area with a primary ion fluence of 5 × 10$^{11}$ ions cm$^{-2}$. The result from this experiment (Fig. 1B) shows a clear increase in total signal intensity even after the analysis with C$_{60}^+$. The total ion signal intensity is almost three times higher than the signal for the initial experiment with C$_{60}^+$. The signal is particularly enhanced for peaks in a higher mass range (500–1000 Da), where one can observe seven times more intensity compared with the initial analysis with C$_{60}^+$.

Two more images with 20 keV (H$_2$O)$_{3000}^+$ and 20 keV (H$_2$O)$_{4500}^+$ were acquired on two new areas of the striatum of the brain (Fig. 2). The ion dose for each experiment was 5 × 10$^{11}$ ions cm$^{-2}$. The spectra of these images show the same trend for the distribution of the peaks dominated by the phosphocholine head group peak. Cholesterol and glycerophospholipids are visible with both water cluster primary ions. Again, there is a clear enhancement of the signal intensity in comparison with C$_{60}^+$ (Fig. 1)

![Figure 1](https://example.com/figure1.png)

Figure 1. Analysis of the same area of the brain striatum with two different cluster ion beams. The area of analysis covered 650 × 650 μm in positive ion mode. (A) Total ion spectrum and total ion image from the 20 keV C$_{60}^+$ analysis with an accumulated ion dose of 2.5 × 10$^{12}$ ions cm$^{-2}$ and a beam diameter of 7 μm. The total ion image shows the striated anatomical features of this area of the brain. (B) Analysis of the same area of the striatum with 20 keV (H$_2$O)$_{3000}^+$ with an ion dose of 5 × 10$^{11}$ ions cm$^{-2}$ and a beam diameter of 9 μm. Single ion images showing the distribution of phosphocholine head group (m/z 184) and a cholesterol fragment (m/z 369) are also presented.
especially for ion species in a mass range 500–1000 Da. Figure 2 shows a particular enhancement (two times more intensity) of the secondary ion yield for peaks in this mass range when using \((\text{H}_2\text{O})_{4500}\) clusters compared with \((\text{H}_2\text{O})_{3000}\) although we have to consider a possible variation in the sample’s composition even within the same structure of the mouse brain.

**Simultaneous beam experiment: analysis with 20 keV \(\text{C}_{60}^+\) with a 5 keV \((\text{H}_2\text{O})_{3000}\) in DC mode**

A dual beam approach was carried out by analysing a new area of the striatum with 20 keV \(\text{C}_{60}^+\) (semi-continuous analysis beam) with a 5 keV \((\text{H}_2\text{O})_{3000}\) beam in continuous mode Direct Current (DC) simultaneously. The purpose of this experiment was to exploit the spatial resolution capabilities of the \(\text{C}_{60}^+\) beam while using a low energy \((\text{H}_2\text{O})_{3000}\) DC beam to increase the ionisation efficiency. After analysis with 20 keV \(\text{C}_{60}^+\) with an accumulated ion dose of \(5 \times 10^{12}\) ions cm\(^{-2}\) and a spot size of 7 \(\mu\)m, an additional DC 5 keV \((\text{H}_2\text{O})_{3000}\) defocussed beam was directed at the region of interest and further images acquired using \(\text{C}_{60}^+\) sputtering from the same area with both beams impacting the surface at the same time. The results from this experiment (Fig. 3) show a continuous loss of secondary ion yield while analysing with \(\text{C}_{60}^+\). However, there is a significant increase of secondary ion yield when the DC 5 keV \((\text{H}_2\text{O})_{3000}\) is activated.

The intensity of ions such as the cholesterol fragment \((m/z\ 369)\), phosphocholine head group \((m/z\ 184)\), and a glycerophosphocholine lipid \((m/z\ 798)\) show a decay in intensity after accumulation of dose from \(\text{C}_{60}^+\) analysis before the signal intensity is recovered by using the DC 5 keV \((\text{H}_2\text{O})_{3000}\) cluster beam. The recovery of the signal intensity can be found in all the peaks in the mass spectrum and therefore cannot be attributed to changes in the composition of the sample as a function of depth.

**Conclusions**

We have shown that \((\text{H}_2\text{O})_n^+\) clusters can be successfully applied for the analysis of mouse brain tissue samples. The results from experiments with \(\text{C}_{60}^+\) sputtering were compared with \((\text{H}_2\text{O})_n^+\) analyses, and an increase in secondary ion yields was observed when using 20 keV \((\text{H}_2\text{O})_{3000}\) clusters and 20 keV \((\text{H}_2\text{O})_{4500}\) clusters. We also investigated a dual beam approach using 20 keV \(\text{C}_{60}^+\) and 5 keV \((\text{H}_2\text{O})_{3000}\). The objective of this approach is to fully exploit the spatial resolution capabilities from the \(\text{C}_{60}^+\) as a primary ion beam with the presence of a low energy \((\text{H}_2\text{O})_n^+\) beam.

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**Figure 2.** Analysis of two different regions of mouse brain striatum using a water cluster ion source. In each case, the field of view is \(650 \times 650\ \mu\)m, and the analysis was in positive ion mode. (A) Analysis with 20 keV \((\text{H}_2\text{O})_{3000}\). (B) 20 keV \((\text{H}_2\text{O})_{4500}\). Both experiments were acquired with a beam diameter of \(\sim 10\ \mu\)m.

**Figure 3.** Comparison between 20 keV \(\text{C}_{60}^+\) (top) and the combined bombardment of 20 keV \(\text{C}_{60}^+\) with 5 keV \((\text{H}_2\text{O})_{3000}\) (DC) (bottom) spectra. Total ion images from both experiments are also displayed for each analysis.
operating in DC mode, obtaining an increase in the ionisation efficiency even after accumulating damage from the C60 + beam. There are clear benefits for the use of (H2O)n+ clusters for the analysis of mouse brain tissue, although further investigation is required to set the optimal conditions.

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