# ToF-SIMS cluster ion imaging of hippocampal CA1 pyramidal rat neurons

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## ARTICLE INFO

## ABSTRACT

Recent studies have demonstrated the power of time-of-flight secondary ion mass spectrometry (ToF-SIMS) cluster ion imaging to characterize biological structures, such as that of the rat central nervous system. A large number of the studies to date have been carried out on the "structural scale" imaging several mm$^2$ using mounted thin sections. In this work, we present our ToF-SIMS cluster ion imaging results on hippocampal rat brain neurons, at the cellular and sub-cellular levels. As a part of an ongoing investigation to examine gut linked metabolic factors in autism spectrum disorders using a novel rat model, we have observed a possible variation in hippocampal *Cornu ammonis* 1 (CA1) pyramidal neuron geometry in thin, paraformaldehyde fixed brain sections. However, the fixation process alters the tissue matrix such that much biochemical information appears to be lost. In an effort to preserve as much as possible this original information, we have established a protocol using unfixed thin brain sections, along with low dose, 500 eV Cs$^+$ pre-sputtering that allows imaging down to the sub-cellular scale with minimal sample preparation.

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## 1. Introduction

Over the past few years, there has been an almost exponential increase in the number of studies that showcase the inimitable capacity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) in the analysis and characterization of biological materials. Such specimens include sectioned rat and mouse brains and single cells [1–6]. Most recently, 3D images have been reconstructed from shallow image depth profiles on individual cells [7].

Prior to ToF-SIMS, it was all but impossible to efficiently obtain "fingerprintable", molecular mass spectral information (meaning polyatomic fragments with more than two atoms) as a function of spatial distribution, especially at the submicron level. Techniques such as electrospray with MS$^n$ (where $n = 2$ indicates tandem MS via triple quadrupole detection or $n > 2$ with an ion trap) require sample extraction and/or homogenization, which eliminates the possibility of extracting any spatial information from the chemical constituents. MALDI-ToF provides only limited spatial distribution on a coarser scale than ToF-SIMS. Indeed, even more mature surface analytical techniques with imaging capability that provide at least some chemical information such as FTIR, Raman and to a lesser extent high resolution XPS are limited in the analysis and

characterization of biological samples by coarser spatial resolution, lower sensitivity, and possible severe sample damage. Arguably the most influential factor in facilitating the highly successful incursion of ToF-SIMS into the medical and biological fields relates directly to recent important technological advances in the instrumentation; in particular, the cluster primary ion sources (Au$_x^+$, Bi$_x^{y+}$, C$_{60}^+$). These sources combine perhaps the two most desirable and indispensable capabilities in this regard: high spatial resolution (submicron level is not uncommon nowadays) imaging and "soft" ionization resulting in unprecedented secondary ion yields of increasingly larger mass, molecular species. Much ground-breaking work has been done with cluster ion sources by Arlinghaus, Hagenhoff, Winograd, Vickerman and others. For excellent recent reviews see for example, Johansson [8] and Brunelle [9].

Of course, with such technological advances comes the desire to clearly image smaller and smaller features such as cells (or indeed *parts* of cells) whilst still extracting the most (i.e. highest mass) chemical information possible. To this end, sample preparation and experimental design become paramount in achieving these goals. Many of the ToF-SIMS studies cited above were carried out on thin tissue sections, typically 10's of microns thick, prepared using well established techniques (or customized variants thereof). These have been employed for decades by researchers in the biological/medical field. The goal is to have a UHV compatible sample with minimal topography, that also reflects the initial

biochemical conditions in the living tissue or cell *as closely as possible*; a veritable "snapshot" of life in cross-section as it were.

Much previous work involving individual cell imaging used cultured cells, and oftentimes the selection of cells was based upon a favourably large size ($>50\ \mu m$). In this work, we used $Bi_3^+$ primary ions, following a short 500 eV $Cs^+$ pre-sputter treatment ($\sim 5e + 14\ ions/cm^{2)}$), to image sub-cellular features in rat *Cornu ammonis* 1 (CA1) hippocampus pyramidal neurons, which are typically only $\sim 20\ \mu m$ in size, in the surrounding neuropil. The increase in overall negative secondary ion yield and improved image quality appeared to effectively outweigh the effects of a low energy $Cs^+$ ion dose. These samples were part of an ongoing study using rats as a model investigating propionic acid, a dietary and enteric bacterial short chain fatty acid, as a possible environmental trigger for autism spectrum disorders. ToF-SIMS was thought to be an ideal tool to examine propionic acid's widespread effects on gene expression, lipid metabolism, cytoskeletal regulation and immune function [10,11]. The additional effects of paraformaldehyde tissue fixation as part of the initial sample preparation on the ability to spatially resolve sub-cellular features in neurons are explored. The fixation process arrests autolysis and putrefaction as well as coagulates soluble and structural proteins by aldehyde crosslinking [12]. This results in reduced topography for subsequent microscopic investigation. Xia and Castner [13] have used fixation processes to preserve the structure of adsorbed protein films for ToF-SIMS analysis. Finally, the extent to which chemical information is lost due to the particular fixation process is addressed.

## 2. Materials and methods

From continuing studies [10,11], adult Long Evans rats were intraventricularly infused with propionic acid (0.4 μl of a 0.26 M solution) or 0.1 M phosphate buffered saline vehicle (pH 7.5), twice daily for 7 days through chronically implanted indwelling canullae. Rats were sacrificed by decapitation, and brains were removed and immediately frozen at $-70\ ^\circ C$ with OCT-embedding. Thin coronal dorsal hippocampal sections were cut on a microtome (Leica RM2125) from the OCT-embedded brains in a cryostat operated at $-20\ ^\circ C$. The thin sectioned brain films with a thickness of $\sim 30\ \mu m$ were placed on positively charged glass slides. Unfixed samples had no further treatment and were introduced into the instrument in the frozen state. The fixed samples were prepared using standard methods by transcardiac perfusion/postfixation with ice cold 4% paraformaldehyde in 0.1 M phosphate buffered saline under deep sodium pentobarbital anaethesia [10]. All ToF-SIMS analyses were performed at ambient temperature. All animal procedures were in accordance with guidelines of the Canadian Council on Animal Care and approved by the University of Western Ontario Animal Use Committee.

An ION-ToF (Gmbh) ToF-SIMS IV equipped with a Bi liquid metal ion source was employed in this study. The primary analysis beam was 25 keV $Bi_3^+$ operated in the Burst Alignment mode [14] with a 10 kHz repetition rate and a pulse width of 100 ns. The target current was $<0.2$ pA and the spot size was estimated to be $\sim 300$ nm. Mass analysis was performed via a single stage reflectron ToF analyzer, at 2 V with 10 keV post acceleration. A pulsed, low energy (18 eV) electron flood was employed to neutralize sample charging; the current was maintained below $\sim 20\ \mu A$ maximum to avoid sample damage. A second 500 eV $Cs^+$ beam, with 1 nA primary current and a raster size of $500\ \mu m \times 500\ \mu m$ was used to pre-sputter areas of interest prior to $Bi_3^+$ imaging.

## 3. Results and discussion

Fig. 1 depicts selected negative secondary ion images acquired from the pyramidal layer of the CA1 hippocampus on an unfixed tissue sample. A 30 s, $Cs^+$ pre-sputter was applied prior to $Bi_3^+$ imaging. The $C_xH_y^-$ image is a sum of several images, with $x = 1-3$ and $y = 0, 1$. The image denoted as $m/z$ 185 is tentatively assigned to $C_{12}H_{25}O^-$.

The structure imaged appears to be a pyramidal neuron in cross-section. More specifically, the size of the roughly round structure delineated especially in the CN- image is consistent with a neuronal soma structure ($\sim 20-30\ \mu m$). The prominent, void-

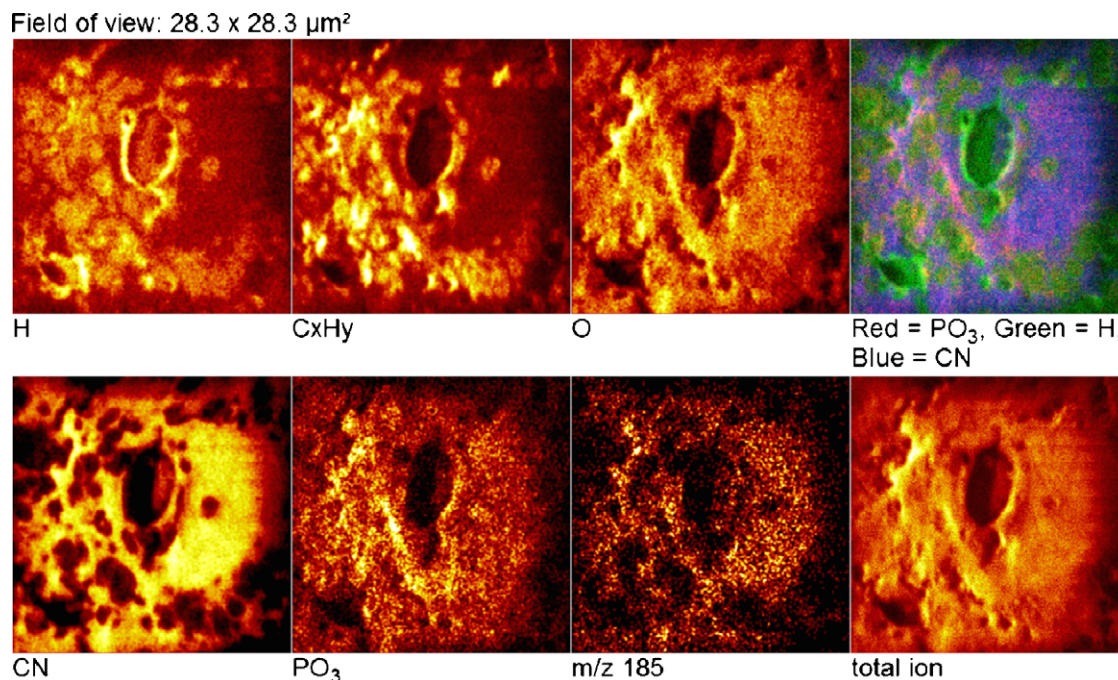Field of view: 28.3 x 28.3 μm²



**Fig. 1.** Negative secondary ions images of CA1 hippocampal neuron in unfixed tissue, following 30 s, 500 eV $Cs^+$ pre-sputter.