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Dynamic SIMS analysis of cryo-prepared biological and geological specimens

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Abstract

The modification of a dynamic magnetic sector secondary ion mass spectrometry (SIMS) instrument to permit the analysis of frozen biological and geological samples is described. The SIMS instrument used for this study combines SIMS analysis with the generation of ion-induced secondary electron images, allowing direct superposition of the SIMS image onto the image of cellular structures. Secondary ion maps have been acquired with sub-micron resolution, permitting the characterisation of sub-cellular elemental distributions in plant cells and human fibroblast cells, as well as the distribution of chemical impurities at grain boundaries in polar ice samples. This cryo-preparation technique clearly extends the applicability of SIMS analysis to a wide range of samples.

Keywords: Cryo; SIMS; Biological; *Pteris vittata*; Fibroblast cells; Polar ice

1. Introduction

Dynamic secondary ion mass spectrometry (SIMS) is a well established surface analytical technique that has been routinely applied to a variety of geological, metallurgical and electronic materials. SIMS has several characteristics that are advantageous for the analysis of biological specimens, primarily the ability to detect all elements and isotopes, higher sensitivity than many other analytical techniques and good spatial resolution. However, the greatest factor limiting research in these areas is the sample preparation necessary to chemically and structurally stabilise the cells [1,2]. Although analysis of frozen-hydrated samples using SEM–EDX is popular in the biological sciences, the cryo-preparation method is not a very well established technique within the SIMS community. The literature documents only a few system modifications to permit cryo-analysis, usually of static TOF–SIMS instruments better suited to the analysis of thin films, organic polymers and other delicate specimens [3–6].

The magnetic sector SIMS system used in this work has previously been used for the analysis of tumour cells [7], wheat root [8] and wheat grain [9], but all analyses were subject to the artefacts inherent with chemical fixation methods. The modifications to this system permitted analyses previously not possible and provided complementary analysis to SEM methods already available. Three case studies of the work performed to date are presented in this publication.

2. Instrumentation

An Oxford Instruments' Electron Microscope CryoTrans 1500 Cryo-Preparation System was adapted for connection to an existing magnetic sector SIMS instrument, previously described elsewhere [9]. Adapter flanges were made to mount the cryo-preparation chamber to a front port and to connect the liquid nitrogen dewar and gas feeds to the stage onto a side port. The typical operating pressure of the main chamber was 2×10^{-8} mbar in cryo-mode. A schematic diagram of the modified SIMS instrumentation is shown in Fig. 1.

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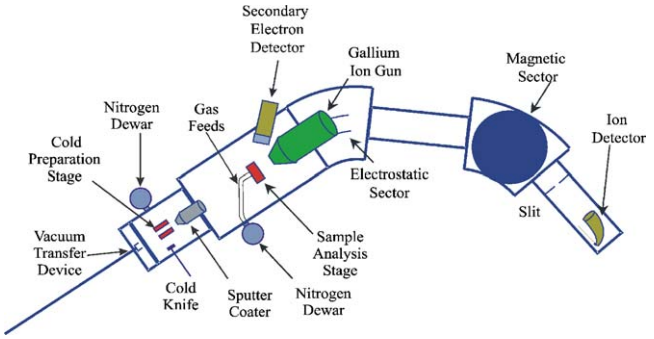

Fig. 1. A schematic diagram of the modified magnetic sector SIMS instrument at the University of Bristol.

3. Experimental

It took approximately 15 min for the preparation and analysis stages to reach temperatures of −160 °C (or below) and −170 °C (or below), respectively. The preparation stage was directly cooled from the liquid nitrogen dewar, whilst the analysis stage was indirectly cooled by the continuous flow of nitrogen gas (pre-cooled by passing through liquid nitrogen). Samples were prepared as outlined in the relevant sections, plunge frozen into nitrogen slush, gold coated in the preparation chamber and transferred to the analysis stage. Secondary electron images were obtained and if frosting of the surface had occurred during sample preparation then the stage temperature was raised to −90 °C for up to 10 min to sublime the excess ice. Following sublimation, the stage heater was switched off and the temperature of the analysis stage was decreased between −170 and −192 °C, where it was maintained during analysis. The gas flow through the stage was reduced during the acquisition of images at high magnification to prevent vibration.

Secondary electron images, spectra and ion maps were collected using a primary ion beam of 1 nA and a diameter of 100–200 nm, corresponding to a current density of 5 A cm^{-2}. Images were obtained in 640 × 480 pixel format, each taking 90 s to acquire. Contrast and brightness were automatically adjusted. Spectra were obtained by scanning through the mass range of interest, usually 0–140 Da in 0.05 Da steps, each with a duration of 100 ms (giving an overall time of 280 s). A magnification of between 200 and 1000× was used, which corresponds to a square region of analysis with sides of between 650 and 130 μm. Images were coloured and combined using the in-house software ''Combine2. exe'' [10].

4. Results and discussion

4.1. P. vittata frond tissues

Several species of plants have evolved which are capable of taking up extraordinary quantities of metals/metalloids and concentrating them in their above-ground biomass. These plants are known as hyperaccumulators and are a promising method of cheap, environmentally sound remediation of contaminated soils. The Chinese brake fern, *Pteris vittata*, a known hyperaccumulator of arsenic (As), was analysed in the cryo-SIMS, to assess the suitability of the technique for the sub-cellular localisation of the hyperaccumulated As. Young *P. vittata* plants were treated with 500 μM sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) in hydroponic solution for 5 days. Leaf samples were mounted with Tissue-Tek®, plunge frozen and then cut with a razor blade to expose clean sections through cells. The negative ions at 91 and 107 Da, indicative of AsO^- and AsO_2^-, respectively, were used to identify As within cells. Initial analyses of mature *P. vittata* pinnae suggested that As was more concentrated in epidermal tissues than in mesophyll regions and high As-signals originated from regions corresponding to the vacuoles of cells. The detection of As was complicated by low count rates and the emission of polyatomic clusters from the biological matrix, but using this technique As could be mapped in regions of higher As-concentration, as shown in Fig. 2, in which the AsO_2^- signal is overlaid onto a secondary electron image of the frozen hydrated sample.

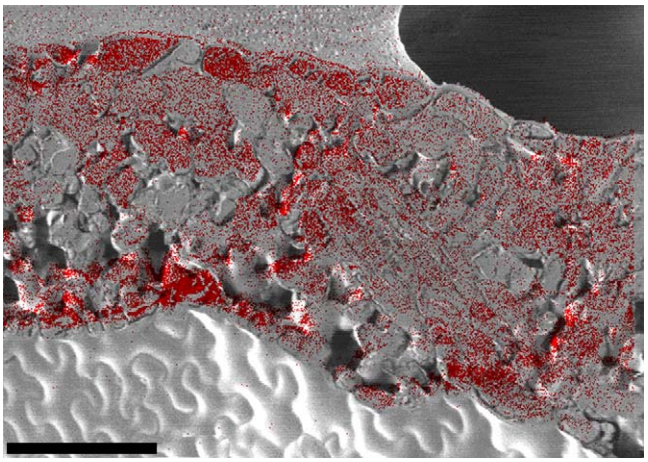

Fig. 2. Secondary electron image of *P. vittata* leaf section (upper epidermis shown uppermost), with AsO_2^- map overlaid in red. Scale bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

4.2. Human fibroblast cells

Following the replacement of a diseased joint with an artificial prosthesis, the wear processes induced by constant activity release billions of tiny debris particles from the artificial joint. Cells in the body ingest many of the particles, inducing a chain of events that leads to osteolysis, where bone around the implant detaches, loosening the implant. This necessitates a difficult operation to replace the dysfunctional implant [11]. Using the cryo-SIMS, Cr was located in fibroblast cells after exposure to Cr^{3+} for 7 h. Maps were acquired of different ions, including, Ca^+, Cr^+, CN^-, and PO_2^-, as shown in Fig. 3. The cellular morphology was visible in all the maps except that of Cr^+, which occurs as particles. The particles were also rich in Ca (pink/purple colour in Fig. 3(a)) and phosphate (blue dots in Fig. 3(b)). The oval shape is intended to help the viewer locate common points on all the maps and arrows indicate the positions of three cell nuclei.

It is clear from these maps that dosing fibroblast cells with dissolved Cr^{3+} results in particulate matter in the cell culture