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Cryogenic (H₂O)ⁿ-GCIB-SIMS imaging

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We use this protocol and it's working

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Abstract

The protocol describes the imaging of frozen-hydrated biological sample using high resolution mass spectrometry imaging, water gas cluster ion beam secondary ion mass spectrometry (H₂O)_n-GCIB-SIMS).

Materials

HPLC plus water, Sigma

Liver tissue section (Cryomicrotomed at the thickness of 10 microns)

Liquid nitrogen

Instrumentation: a buncher-ToF SIMS instrument, J105 3D Chemical Imager (Ionoptika, Southampton, UK. Abbv. J105)

- 1 Both $(\text{H}_2\text{O})_n$ -GCIB and C_{60} -SIMS were performed on a buncher-ToF instrument, J105 3D Chemical Imager (Ionoptika, Southampton, UK. Abbv. J105). The water cluster ion beam is pulsed through a pulser in the gun column, where the distance to the sample surface is 0.533 m. Beam tuning was assisted with an oscilloscope (Tektronix TDS 2024, USA) with detection by a secondary electron detector (SED). The singly-charged $(\text{H}_2\text{O})_n$ cluster size at beam energy of 70 kV with a time of flight (ToF) of 103 μs was calculated using the ToF equation as $n = 30,900$ (Figure S15). The SED offset was 8 μs . Beam focus was measured by scanning a 1000 mesh grid (Agar Scientific, Essex, UK). The average beam spot sizes were calculated using 20/80 percent of maximum intensities and were $1.60 \pm 0.01 \mu\text{m}$ and $1.16 \pm 0.45 \mu\text{m}$ for 70 keV $(\text{H}_2\text{O})_{30k}^+$ and 40 kV C_{60}^+ , respectively (Figure S14). The beam dither was adapted to match the image pixel size. The mass resolution $m/\Delta m$ was 6875 around m/z 100, and 10,000~12,000 up to m/z 2000. The live readout of mass resolution was from the software, Ionoptika SIMS Mainframe during the data acquisition.

The gold coated Si wafer with the frozen-hydrated mouse/human liver tissue section was plunged into liquid nitrogen and inserted to the pre-chilled cold sample stage in J105 instrument and kept at 100 K during GCIB-SIMS imaging. This cryogenic sample handling preserved the frozen-hydrated state thus maintaining the chemical gradients in the tissue section.

Guided by the anatomical features on the semi-serial H&E stained section, an area of interest was selected for SIMS imaging in negative ion mode using a 70 keV $(\text{H}_2\text{O})_{30k}^+$ beam. The acquisition was in negative ion mode with 256×256 pixels using a 2×2 tiled image mode for mouse liver tissue sections, or 768×768 pixels using a 3×3 tiled image mode for human liver tissue sections. Each tile covers $400 \times 400 \mu\text{m}^2$ (3.1 μm per pixel) for each section. With 1 pA of beam current and 296 shots per pixel, the ion doses were 3.01×10^{12} ions/ cm^2 each tile.