Cryogenic (H2O)n-GCIB-SIMS imaging

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Liver Ln-Abs staining protocol

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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 $(H_2O)_n$-GCIB-SIMS).

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MATERIALS TEXT

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 $(H_2O)_n$-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 $(H_2O)_n$ 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±0.01 µm and 1.16±0.45 µm for 70 keV $(H_2O)_{30k}^+$ and 40 kV C_{60}^+, respectively (Figure S14). The beam dither was adapted to match the image pixel size. The mass resolution m/Δm 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 $(H_2O)_{30k}^+$ 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 × 400 μm^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.

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