Effect of low energy Cesium deposition on the secondary ion yield for biological samples

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ABSTRACT:
Multi-isotope Imaging Mass Spectrometry (MIMS) is a quantitative imaging methodology which combines SIMS analysis (using the NanoSIMS50 instrument) and the use of stable-isotope-tagged tracer molecules to study metabolism in vitro (cell culture) or in vivo (animal and human). By measuring $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, MIMS has unraveled fundamental biological questions concerning stem cell biology and metabolism [1-3] and is effectively used in microbiology [4, 5]. In this study, we investigated the effect of cesium deposition, prior to SIMS analysis, on the secondary ion (SI) yield for the analysis of the first atomic layers of biological samples. The effect of Cs coverage on organic samples is achieved by transfer of the NanoSIMS to thin polymer films and macro-molecules [6, 7]. In the present study, we show results for whole cell mounts and embedded sections. The Cesium deposition was performed directly in the instrument by rastering the area of interest with a 50 eV Cesium beam. The area covered can range from 20 x 20 µm to the millimeter range.

IN SITU CESIUM DEPOSITION

Figure 1: Schematic of the NanoSIMS. A 1.6 km Cesium ion beam of 50-330 nm diameter is focused on a sample and cesium ions and cesium ions are extracted. The voltage applied to the target is ~6 V and the cesium ions at the exit of the source have an 6 keV energy. The parameters for the immersion lens are: E0W = -9000 V, E0P = -8000 V and E0S = -7300 V. For cesium deposition the voltage on the sample (E0S) is set to 7700 V such slowing the cesium ions down to 30 eV of energy. The parameters for the immersion lens are: E0S = -6500 V, E0P = -520 V, E0D = -550 V.

QUANTIFICATION OF THE EFFECT OF CESIUM COVERAGE

Figure 2: Cs beam size in deposition mode. a) 3D Ion Current (IIC) image of a silicon chip. Field 120 µm, D0.5. Secondary ion signals enhanced in areas where cesium is in excess providing an indirect means of tuning the cesium beam size. The two squares correspond to the cesium beam size for E0D = 9000 V and 6000 V after 2 min of deposition with a D0.2 (1 µA) fixed probe. The probe size depends on the E0P value and the distance between the sample and the lens. b) ICC image 120 µm field with D0.5. The probe size is measured at the exit of the source. The Cs beam size is 1.5 µm for deposition with a 1 µA Cs beam current (E0S = 2000 V). As can be seen from the graph profile, the probe size is approximately 1/3 µm and homogeneous over 1 µm. The optimal probe size for deposition with a 1 µA Cs current is 1 µm (not shown here).

Figure 3: Cs deposition on biological samples: a) CN signal from mouse nucleated cell, 60 µm field, 4 min per plane. Cesium deposition conditions were 30 pA Cs beam current, 17 pA for 5 µm Cs ions from ROIs on Neurites from areas covered with cesium prior to analysis (red) and not covered (black). Data are the mean value of counts derived from several ROIs. The relative gain was estimated from the first 10 planes.

CONCLUSION:
In situ deposition offers three advantages: 1- a direct comparison of the effect of Cesium on a contiguous area, covered and uncovered with Cesium, under identical tuning conditions, 2- no associated damage to the sample, which remains under vacuum during the entire process (deposition and analysis), and 3- optimization of the deposition conditions (thickness of Cs) to achieve the maximum yield, which may vary with the nature of the sample (e.g. porosity, topography). The results show that yields for C', D' and CN' from the first 10 atomic layers are improved by a factor of 5 to 6 when cesium is deposited prior to MIMS analysis of both embedded sections and whole cell mounts.

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