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Technical Note

High-speed sub-micrometer imaging of sub-cellular structures in single cells using ARIS

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INTRODUCTION

The focus of many bioimaging techniques, such as fluorescence microscopy and imaging mass spectrometry, has been slowly evolving from imaging the distribution of species on the macroscale level to the cellular and sub-cellular level. LA-ICP-MS has followed this trend, as it has been realized that the elemental distribution on a cellular and sub-cellular level provides insights into the mechanistic processes driving the elemental distribution observed at the macroscale level. While LA-ICP-MS imaging at the sub-cellular level is not yet mainstream, the number of papers devoted to this topic is rapidly increasing. The ARIS is a recently introduced robust low-dispersion add-on to the HelEx II cell, greatly enhancing the scanning speeds (< 40 ms washout) and sensitivity of the system, which can be used to achieve a spatial resolution higher than that of conventional setups. The performance of this setup is not limited by the washout of the LA system any longer, but rather limited to the sensitivity of the mass spectrometer. In this proof-of-concept study, we have demonstrated the use of an Analyte G2 193 nm ArF*excimer-based laser ablation system (Teledyne Photon Machines, Bozeman, MT, USA) equipped with the ARIS device coupled to an Agilent 7900 ICP-mass spectrometer (Agilent Technologies Inc., Tokyo, Japan) to image single cells at a scale of 500 nm.

EXPERIMENTAL SECTION

MDAMB231 X4 breast cancer cells of human origin, overexpressing the chemokine receptor 4 (CXCR4) were seeded onto 2.5 mm diameter coverslips in Dulbecco's minimum essential medium (DMEM), containing 10% fetal bovine serum and penicillin/streptomycin (Life Technologies Inc., Carlsbad, CA, USA). After 24 hours, the cells were fixed using an ethanol-series. The cells were then incubated at room temperature for 15 min with 500 µL of 1:50 diluted 500 µM DNA-binding ^{103}Rh-intercalator solution (Fluidigm Corporation, South San Francisco, CA, USA) and subsequently washed with 2 mL of MaxPar® Cell Staining Buffer (Fluidigm Corporation, South San Francisco, CA, USA). The cell plates were air-dried and placed in 1 inch brackets within the standard 9-hole sample holder. A small region of 150 x 200 µm^2 containing multiple cells was scanned using a laser spot size of 1 µm, with (overlapping) horizontal scan lines at a vertical interspacing of 500 nm. By overlapping ablation positions, an effective sampling area (and lateral resolution) equal to a quarter of the spot size area is achieved. The repetition rate was set to 100 Hz, the scan speed to 10 µm s^{-1}, and the laser energy density was determined to be 2.98 J cm^{-2}. The laser was operated in fixed dosage mode (stage-priority triggering) to achieve better positioning accuracy during ablation. In this approach, 25 pixels were acquired every second (dwell times: 17 ms for ^{31}P and for ^{103}Rh, total scan cycle time: 40 ms). The total analysis time for this area was approximately 1 h 40 min.

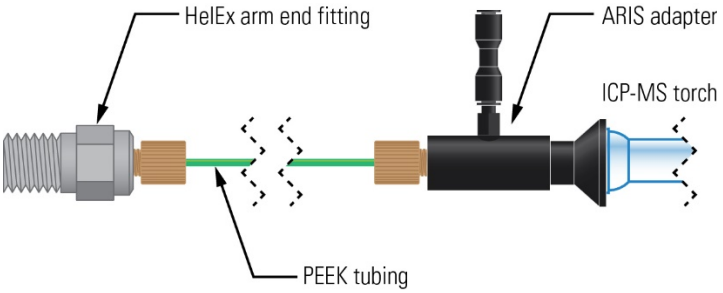

Figure 1: Diagram of the Aerosol Rapid Introduction System (ARIS).

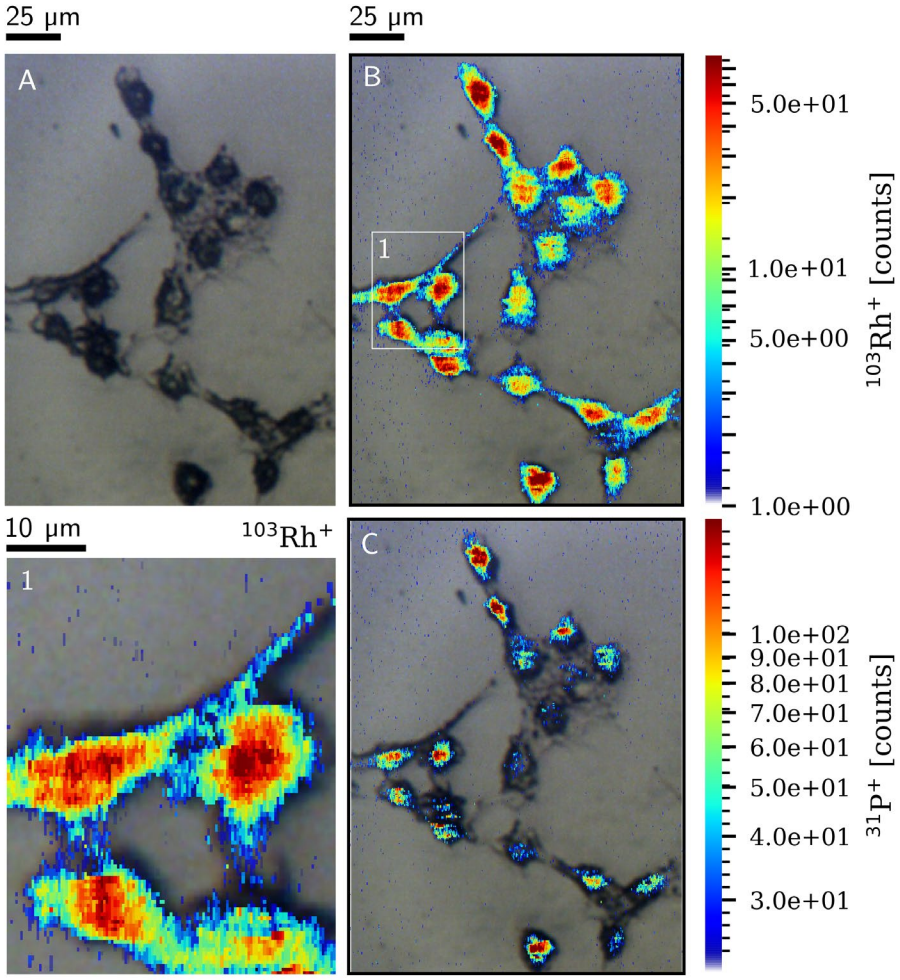

Figure 2: Images of the ^{103}Rh and ^{31}P signal overlaid on a brightfield microscopy image of the ablated zone prior to ablation. A) Microscopic image acquired *a priori*. B) Overlay with the 'jet' colormap of the ^{103}Rh signal with a figure inset (1). Note that a logarithmic scaling was used. C) Overlay with the 'jet' colormap of the ^{31}P signal. Note that a logarithmic scaling was used.

RESULTS AND CONCLUSION

The images thus acquired are displayed in Figure 2; these images are generated using an inverse logarithmic scaling of the transparency channel and a logarithmic scaling of the 'jet' colormap. As can be observed in these images, the contrast ratio of this image is remarkably high, considering the extreme lateral resolution that was achieved. All fine details of the cells' outline can be discerned. As expected, the Rh-intercalator is highly concentrated in the cells' nuclei, whilst trace amounts are also present in other cell compartments. The phosphorus channel also reflects the position of the nucleus, due to the high density of phosphate groups in the DNA. In conclusion, a LA-ICP-MS setup equipped with the ARIS device is well-suited to image the intracellular metal distribution down to a scale of 500 nm.

REFERENCES

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