

ATOMIC AND MASS SPECTROMETRY RESEARCH UNIT

HIGH-SPEED SUB-MICROMETER LASER ABLATION-INDUCTIVELY COUPLED PLASMA-

MASS SPECTROMETRY IMAGING OF A DNA-BINDING ¹⁰³Rh-INTERCALATOR IN SINGLE

CELLS USING THE ARIS

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Introduction

In most of the laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) setups, substantial aerosol particle dispersion is induced within the ablation cell and/or the aerosol transfer tubing, resulting in pulse response durations of 1-2 s (full peak width at 10% maximum, FW0.1M) [1]. The aerosol rapid introduction system (ARIS, Figure 1), a powerful low-dispersion add-on of the HelEx II ablation cell, was developed and launched recently; this system minimizes the aerosol particle dispersion generated within the aerosol transfer tubing and pulse response durations of < 40 ms (FW0.1M) can be achieved. The ARIS greatly enhances the scanning speed and sensitivity of the LA-ICP-MS setup without compromising on lateral resolution, which permits images of much higher lateral resolution to be acquired, compared to conventional setups. As bioimaging at the micro-scale and/or (sub-)cellular level is gaining importance in fluorescence microscopy and imaging mass spectrometry, and since the number of publications on this topic is still limited, this proof-of-concept study focuses on imaging single breast cancer cells of human origin (MDAMB231 X4), stained with a DNA-binding ¹⁰³Rh-intercalator. The highest ¹⁰³Rh signal response was observed in the nuclei of the cells and equipping the LA-ICP-MS setup with the ARIS device enabled intracellular imaging of the DNA-binding ¹⁰³Rh-intercalator [2].

Results and discussion

- Under optimal conditions, an average pulse response duration of 6 ± 1 ms (FW0.1M, n=1,000) was obtained.
- The highest ¹⁰³Rh signal response was observed in the cells' nuclei and corresponds with the ³¹P distribution due to the high density of phosphate groups in DNA. All fine details of the cell's outline can be discerned because of the high lateral resolution.

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Sample preparation

MDAMB231 X4 human breast cancer cells were seeded onto 2.5 cm diameter glass coverslips in Dulbecco's minimum essential medium with 10% fetal bovine serum and penicillin/streptomycin (Life Technologies Inc., Carlsbad, CA, USA).



Figure 2. (A) Fast-transient ²³⁸U signal response, expressed in counts, showing a number of single pulse responses (SPR) of in total 1,000 laser shots on NIST SRM612 (50 Hz). (B) In this graph, the average SPR profile, computed from the series of pulses is shown. A relative cumulative distribution function (CDF) was calculated, which displays how much of the aerosol created in a single shot is arriving into the plasma. The SPR shape is almost symmetric, which indicates that the system is in a laminar/transitional flow state with a relatively limited contribution of diffusion-induced and Taylor dispersion to the total dispersion of the aerosol packet.





B





³Rh

- After 24 h, fixation in ethanol-series.
- Incubation at 20°C for 15 min with 500 µL of 1:50 diluted 500 µM DNA-binding ¹⁰³Rh-intercalator (Fluidigm Corporation, San Francisco, CA, USA).
- Washed with 2 mL of MaxPar[®] Cell Staining Buffer and dried under N_2 flow. Instrumentation

LA-ICP-MS: Analyte G2 193 nm ArF* excimer-based LA unit (Teledyne Photon Machines, Bozeman, MT, USA) equipped with the HelEx II ablation cell and coupled to an Agilent 7900 ICP-MS instrument (Agilent Technologies, Tokyo, Japan) *via* the ARIS device.

Instrument settings and data acquisition conditions

Pulse response duration experiment on NIST SRM612 glass certified reference material (National Institute for Standards and Technology, Gaithersburg, MD, USA):

- LA: 10 µm square spot size, 50 Hz repetition rate, 2.98 J cm⁻² laser energy density and 500 µm s⁻¹ scan speed in line scanning mode.
- ICP-MS: ²³⁸U (1 ms dwell time).

Sub-micrometer LA-ICP-MS imaging of a 150 x 200 µm² area with multiple cells:

- LA: 1 µm circular spot size, 100 Hz repetition rate, 2.98 J cm⁻² laser energy density, 10 µm s⁻¹ scan speed in horizontal line scanning mode with a 500 nm vertical interspacing (overlapping lines). The cells were ablated quantitatively.
- ICP-MS: ³¹P (17 ms dwell time) and ¹⁰³Rh (17 ms dwell time) gives a total scan cycle time of 40 ms.



Figure 3. Brightfield microscopic image of the selected area prior ablation and the (A) corresponding overlay ¹⁰³Rh (B) and ³¹P (C) distribution maps. Note the use of a logarithmic scaling for the 'jet' false color map. It was chosen as such to visualize the high concentration of both elements in nuclei and the concentration in lower the cytoplasm and membranes. The elemental image and the optical image were overlaid based on the laser positions registered in the log by Chromium v2.3 (Teledyne Photon Machines).



Conclusion

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The robust low-dispersion add-on ARIS lowers the aerosol particle dispersion induced within the aerosol transfer tubing and a LA-ICP-MS system equipped with the ARIS is well-suited to image the intracellular metal distribution down to a scale of 500 nm. Due to the higher mass flux, the sensitivity of the instrument is drastically improved as compared to the standard Helex II setup. For biological material, a pixel acquisition rate of 25 – 30 pixels can be achieved on biological material without inducing any noticible image blurring effects. For glass material and most geological materials, pixel acquisition rates of 50-100 pixels/s are feasible.

References: [1] S.J.M. Van Malderen, J.T. van Elteren, F. Vanhaecke, Development of a fast laser ablation-inductively coupled plasma-mass spectrometry cell for sub-µm scanning of layered materials, J. Anal. At. Spectrom. 30 (2015) 119–125. [2] Teledyne CETAC Technologies, Application note: High-speed sub-micrometer imaging of subcellular structures in single cells using ARIS, 10/02/2017

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