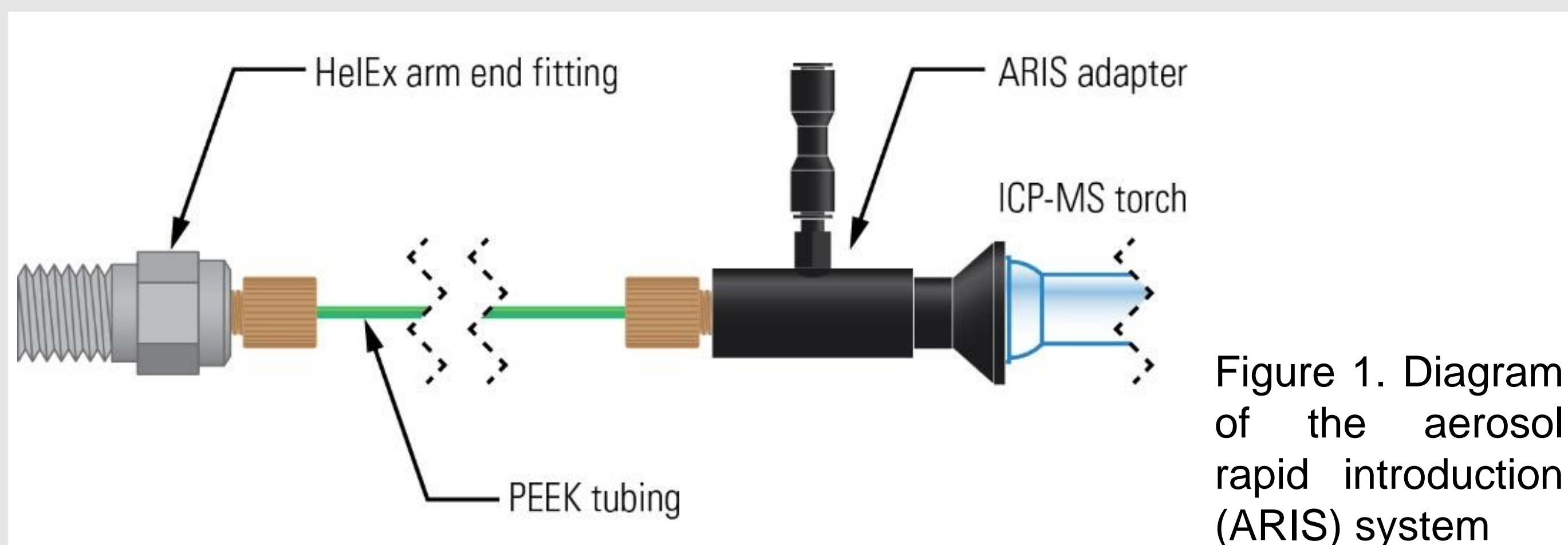


# HIGH-SPEED SUB-MICROMETER LASER ABLATION-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY IMAGING OF A DNA-BINDING $^{103}\text{Rh}$ -INTERCALATOR IN SINGLE CELLS USING THE ARIS

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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  $^{103}\text{Rh}$ -intercalator. The highest  $^{103}\text{Rh}$  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  $^{103}\text{Rh}$ -intercalator [2].



## 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).

- After 24 h, fixation in ethanol-series.
- Incubation at 20°C for 15 min with 500  $\mu\text{L}$  of 1:50 diluted 500  $\mu\text{M}$  DNA-binding  $^{103}\text{Rh}$ -intercalator (Fluidigm Corporation, San Francisco, CA, USA).
- Washed with 2 mL of MaxPar® Cell Staining Buffer and dried under  $\text{N}_2$  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  $\mu\text{m}$  square spot size, 50 Hz repetition rate, 2.98  $\text{J cm}^{-2}$  laser energy density and 500  $\mu\text{m s}^{-1}$  scan speed in line scanning mode.
- ICP-MS:  $^{238}\text{U}$  (1 ms dwell time).

Sub-micrometer LA-ICP-MS imaging of a 150 x 200  $\mu\text{m}^2$  area with multiple cells:

- LA: 1  $\mu\text{m}$  circular spot size, 100 Hz repetition rate, 2.98  $\text{J cm}^{-2}$  laser energy density, 10  $\mu\text{m s}^{-1}$  scan speed in horizontal line scanning mode with a 500 nm vertical interspacing (overlapping lines). The cells were ablated quantitatively.
- ICP-MS:  $^{31}\text{P}$  (17 ms dwell time) and  $^{103}\text{Rh}$  (17 ms dwell time) gives a total scan cycle time of 40 ms.
- Pixel acquisition rate of 25 pixels  $\text{s}^{-1}$  and pixel size of 0.4 x 0.5  $\mu\text{m}^2$

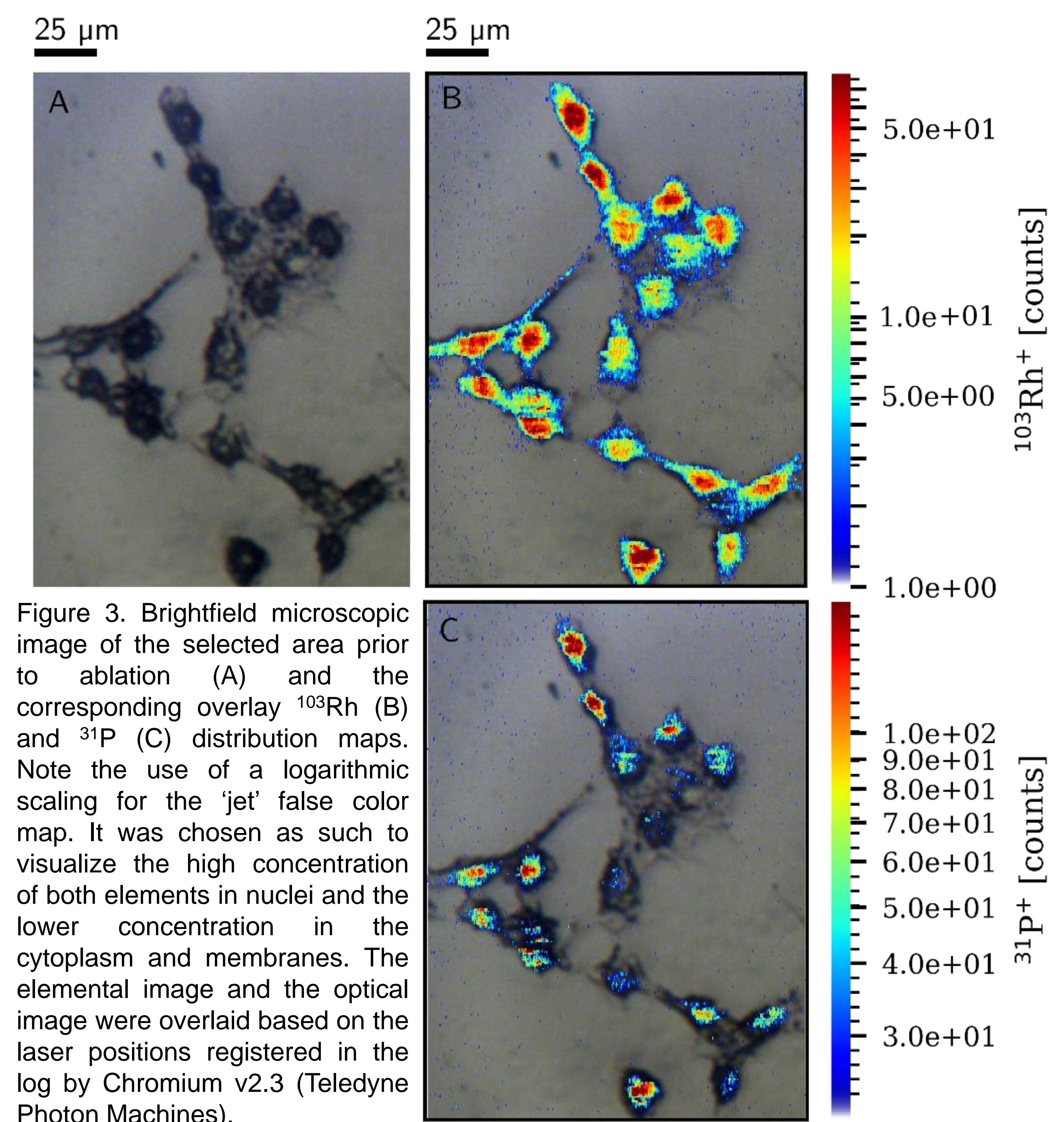
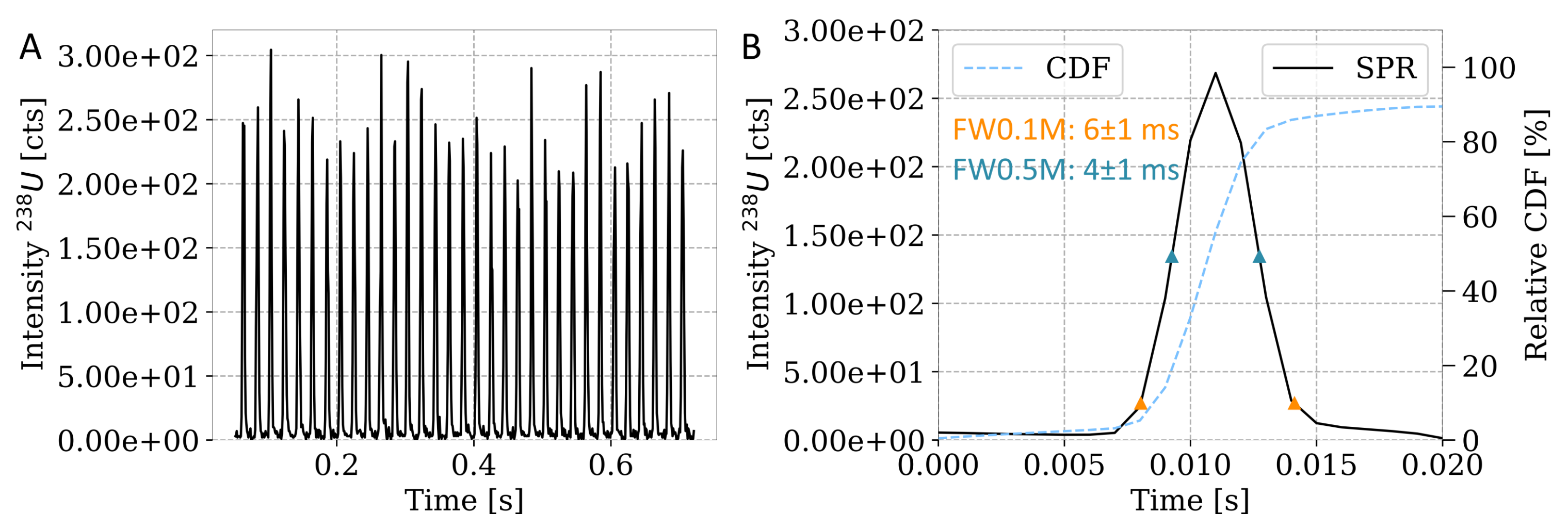
## Conclusion

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 noticeable 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- $\mu\text{m}$  scanning of layered materials, J. Anal. At. Spectrom. 30 (2015) 119–125. [2] Teledyne CETAC Technologies, Application note: High-speed sub-micrometer imaging of sub-cellular structures in single cells using ARIS, 10/02/2017

## Results and discussion

- Under optimal conditions, an average pulse response duration of  $6 \pm 1$  ms (FW0.1M,  $n=1,000$ ) was obtained.
- The highest  $^{103}\text{Rh}$  signal response was observed in the cells' nuclei and corresponds with the  $^{31}\text{P}$  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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