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Laser ablation low-flow ICP-MS for elemental bioimaging

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Abstract

A laser ablation system (LA) was coupled to an in-house developed low-flow inductively coupled plasma ion source for mass spectrometry (ICP-MS). In this set-up, the low-flow torch showed best analytical performance at a total argon gas flow rate of only 1.27 L/min and a generator power of 900 W. The optimized system was applied to elemental bioimaging. Two different sections of one kidney sample were analyzed and images of the elements Al and Br from the staining agents hematoxylin and eosin, respectively, and I from contrast agent iodixanol were recorded selectively and with high sensitivity. For detection of the elements, the mass traces ^{27}Al, ^{79}Br, ^{81}Br, and ^{127}I were selected to demonstrate the system's analytical performance over a wide mass range. In total, the argon consumption could be reduced by > 90% compared to conventional ICP-MS systems.

Introduction

The analysis of the elemental distribution in biological samples has gained increasing research interests in recent years. Various biochemical species of elements have been identified as important components in organisms exhibiting beneficial or detrimental effects. Bioimaging of elemental distributions has therefore significantly gained importance and scientific recognition.[1-5] One of the most popular techniques in elemental bioimaging is the hyphenation of laser ablation with inductively coupled plasma mass spectrometry (LA-ICP-MS). This technique combines the high spatial resolution of laser ablation in the micrometer range with a highly sensitive, element selective detector system.[6-8] One of the major drawbacks of this technique is the high argon gas consumption of approximately 15-20 L/min. To generate well resolved images of organs and organ sections with LA-ICP-MS, small laser spot sizes and a fairly slow sample movement are necessary. Consequently, the measurement of an image takes several hours. This may result in a total argon consumption of several thousand liters of argon per bioimage.

Since the introduction of the ICP, several approaches were investigated to reduce the Ar consumption. To avoid the high cooling gas flows that constitute the largest portion of the ICP's gas consumption, alternative cooling strategies for the quartz torch were investigated. Most approaches dealt with water-cooling[9-13], air-cooling[13-15] and miniaturization[16, 17] of the ICP torch. Some work was focused on radiative cooling by means of ceramic materials, like Al_2O_3 and BN.[18, 19]

A torch for ICP coupled with optical emission spectrometry (ICP-OES) with only 1 L/min Ar consumption compared to Fassel-type torches was first presented by Klostermeier et al..[20] The authors called this new approach Static High sensitivity ICP (SHIP). As cooling medium, compressed air was used, thus allowing the complete removal of the conventional ICP's cooling argon gas flow. The torch geometry was changed significantly compared to Fassel-type torches: The main part of the quartz torch was bubble shaped and

contained a plasma that was formed like a sphere inside the load coil area of the ICP. It fit exactly into the load coil of a commercial ICP-OES spectrometer.

Scheffer et al. carried out first successful experiments to transfer this approach to an ICP-MS spectrometer. They still reported secondary discharges and polyatomic interferences which were caused by the interaction of plasma and the stream of cooling air.[21] In 2012, Pfeifer et al. presented an optimized version of this type of low-flow torch.[22] They developed a new sampling interface to minimize the interaction of plasma and cooling air stream. The removal of the secondary discharge was achieved by use of an electrostatic platinum shield. The limits of detection were found to be only slightly inferior compared to the conventional ICP-MS. However, the background of the low-flow torch was significantly lower than achieved with conventional torches. The authors could demonstrate the capability of the new low-flow ICP-MS in speciation analysis by its hyphenation with gas chromatography (GC) and high performance liquid chromatography (HPLC).

The torch presented here is based on these developments, but was further optimized with respect to the gas connectors and gas flow capillaries inside the fastening parts made of PEEK (polyether ether ketone), the electrical connections to improve plasma ignition, and positioning of the aluminum oxide sample injection capillary. The hyphenation of this new ICP-MS torch with a laser ablation system and the evaluation of its analytical potential in elemental bioimaging is described in this paper.

Instrumentation

Low-flow ICP-MS

An Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA, US) was used to detect the ablated material from the laser ablation system. The conventionally used ICP-Torch was replaced by a quartz torch and a PEEK fastening that were both in-house designed and manufactured to maintain a plasma with only little argon consumption (see Fig. 1). The fastening device was developed to position the torch

precisely within the ICP's load coil, but it also contains gas tubing, gas connectors, an electrical contact for the plasma ignition spark, as well as the electrical grounding contact for the grounded Pt shield for capacitive decoupling of the torch and the ICP. Cooling of the bubble-shaped quartz torch was achieved by use of compressed air. The sampling interface with an in-house manufactured sampler cone made of brass[22] and a commercially available nickel skimmer cone were used for ion sampling from the low-flow plasma. The sampler cone's orifice was 0.7 mm in diameter, while the skimmer cone's orifice had a diameter of 0.6 mm. Otherwise, the ICP-MS instrument was used as purchased. The pressure in the interface was slightly lower compared to conventional ICP-MS (1.69 x 10^2 Pa (low flow), 3.2 x 10^2 Pa (high flow)).

The quartz torch was moved as close as possible to the sampler tip. The latter was introduced into the orifice of the quartz torch to collect the analyte ions generated in the plasma. Only a small gap (ca. 0.2 mm) between the sampler base and the quartz torch was left. This gap ensured best sensitivity without allowing cooling air to enter the plasma zone, to avoid disturbing or even extinguishing of the plasma.[21]

The sample aerosol from the laser ablation system (see below) was introduced through an injector capillary (i.d. 1.0 mm) made of aluminum oxide. Positioning of the capillary and its precise direction to the central channel of the toroidal plasma was crucial to achieve best sensitivities. The position of this capillary had to be optimized prior to any series of analyses and could be fixed mechanically to keep reproducible conditions for all further measurements. The end of the injector capillary was positioned at the beginning of the torch bulb, which was completely filled by the plasma. The distance to the spherical plasma was kept as short as possible and was typically in the range between 1-2 mm.

Argon gas enters the torch through two concentrically oriented tubes: While the first is the injector capillary for the sample carrier gas flow, the second is the larger tube for the plasma gas flow around the injector tube (see Fig. 1). The plasma gas flow rate was set to the fixed value 0.42 L/min. Directly

controlled by the ICP-MS instrument, the sample carrier gas flow was guided through the ablation cell and was varied between 0.55 L/min and 1.00 L/min. Compromise conditions were set to the best match of high sensitivity and short washout times of the ablation cell. No further gases were used to maintain the plasma. Torch cooling was achieved by a stream of compressed air that was guided along the outer walls of the quartz torch. The pressure was set to 500 kPa (approximately 70 L/min) to prevent the torch walls from melting. The RF power was varied between 500 W and 1100 W. Extraction lenses and quadrupole parameters were tuned for best sensitivity.

For optimization of the ICP-MS for the analysis of gelatin standards, the mass trace m/z 127 (iodine) was detected in the transient measurement mode of the instrument with a dwell time set to 0.1 s. During imaging experiments of tissue sections, the dwell time was set to 0.5 s for iodine measurements at m/z 127. In case of hematoxylin and eosin (H&E) stained sections, dwell times of 0.4 s (^{27}Al), 0.3 s (^{79}Br) and 0.3 s (^{81}Br) were used, respectively. Data evaluation was performed using the data evaluation software ImageJ 1.48f (National Institutes of Health, Bethesda, MD, USA).

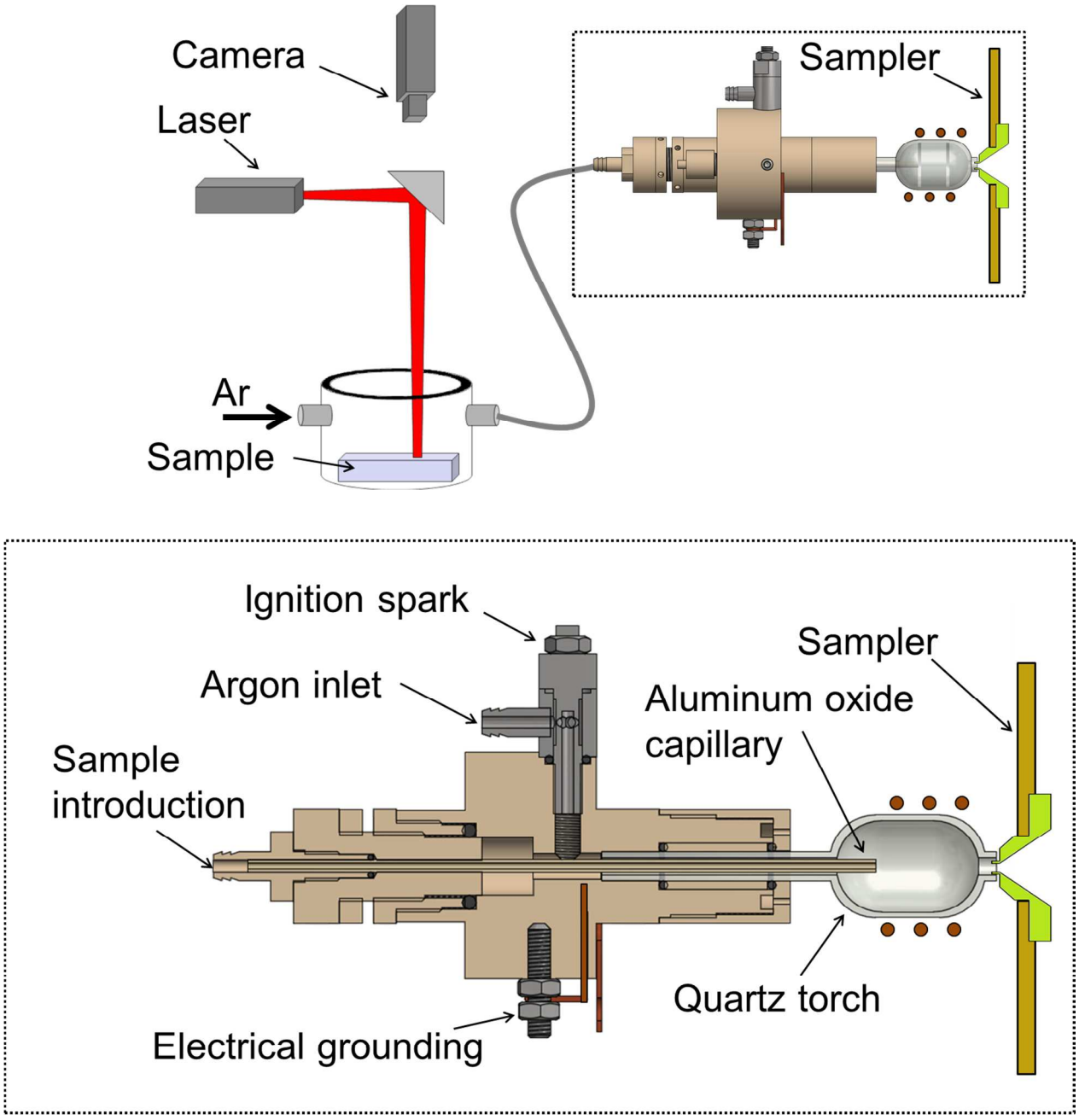

Fig. 1: Schematic setup of the LA-low-flow ICP-MS system (top) and detailed cross section of low-flow torch and fastening (bottom).

Laser ablation

A commercially available laser ablation system (LSX 213, CETAC Technologies, Omaha, NE, USA) with a frequency quintupled Nd:YAG laser at 213 nm was used. The LA system was equipped with a conventional cyclonic ablation cell with turbulent flow (50 mm (height) x 52 mm (i.d.)). The gelatin standards for optimization of the ICP-MS parameters were ablated in scanning mode with 100 µm spot size, 10 µm space between lines and 100 µm/s scan speed. The analyzed tissue sections were ablated in a line by line scan with 100 µm spot size. No space between the lines was left and a scan speed of 100 µm/s was applied. The laser parameters fluence and shot frequency were optimized to ensure a homogeneous ablation and sharp edged crater shapes. The laser shot frequency was varied between 10 and 20 Hz. Higher frequencies could not be applied with the given setup. The laser energy was varied between 4.9 J/cm^2 and 7.8 J/cm^2. The parameters were set to 7.2 J/cm^2, where an optimum signal to noise ratio is observed, and 20 Hz, respectively. To achieve fast wash out, the ablation cell was directly coupled to the low-flow ICP-MS by a transfer line with minimal distance. To achieve highest plasma stability, no further gases such as helium were used to transport the ablated material. Since some authors claimed that the exclusive use of argon shows advantageous wash-out behavior, the usage is well-known for biological samples.[23, 24]

Sample preparation

Gelatin sections

For optimization of gas flow and RF power, sections of gelatin standards containing a defined concentration of iodine (270 µg/mL) were prepared. Therefore, 100 mg of gelatin were added to 900 µL of an aqueous solution of 300 µg/mL iodine (Ultravist-300, Bayer Vital, Leverkusen, Germany). For homogenization, the solution was heated to 325 K (52 °C). Gelatin section samples of 12 µm thickness were prepared with a cryomicrotome at 253 K (-20 °C).

Kidney sections

Animal studies were carried out with approval of the state animal welfare committee (ZH1401) and in compliance with the German animal welfare legislation. Male Han-Wistar rats (Charles River, Sulzfeld, Germany) were intravenously dosed with the contrast agent iodixanol (Visipaque 320, GE Healthcare, Buchler GmbH & Co KG, Braunschweig, Germany) with a concentration of 4 g iodine/kg bodyweight. The animals were sacrificed after 24 hours, the left kidneys were removed and cryosections of 12 µm thickness were prepared. Tissue sections were stored at 255 K (-18 °C) until usage. Additionally, further kidney sections were stained by means of hematoxylin and eosin (H&E).

Results and discussion

Optimization of RF power and gas flow rates

Optimization was performed by ablating iodine containing gelatin standards (270 µg/mL) and averaging the obtained ICP-MS signals at m/z 127 over 100 dwell time periods. All relative standard deviation values were below 2%. The most important parameters for the low-flow ICP-MS were RF power and sample carrier gas flow rate.

The RF power was optimized in a range between 500 – 1100 W (see Fig. 2). It was found that higher forward powers resulted in higher intensities. However, above 900 W, ablation of quartz from the inner torch wall was observed. After short operation times at generator powers above 900 W, the sampler cone and its sampling orifice tend to be coated with redeposited quartz material. This results in signal depression and corresponding intensity drift of the ICP-MS signals. To a limited extent, this drift may be compensated applying an internal standard correction method.[25-28] This correction procedure is only sufficient for short term measurements and not suited for longer lasting imaging purposes.[22] Therefore, all measurements were performed at 900 W RF power. At this power value, no quartz deposition on the sampler was observed even after several hours of LA-ICP-MS imaging time.

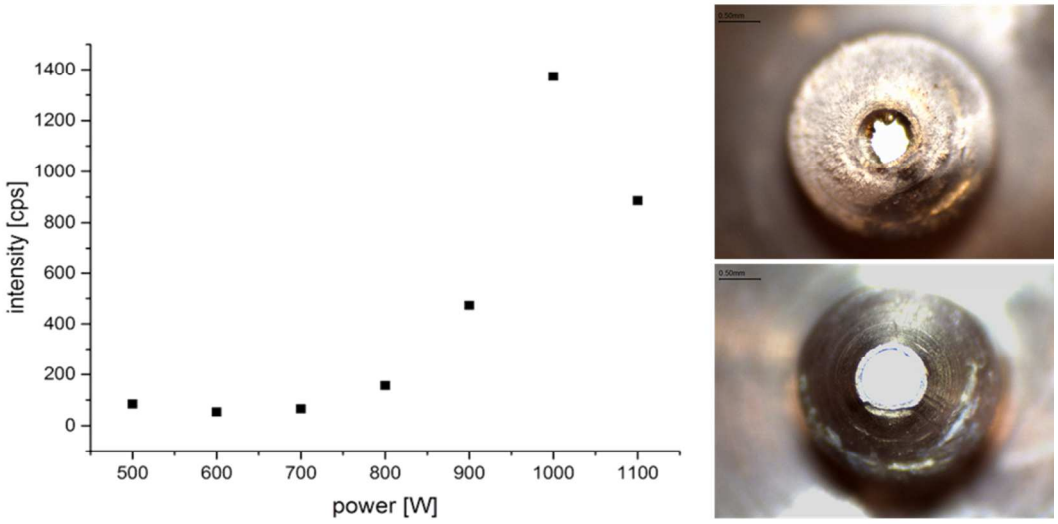

Fig. 2: Optimization of ICP forward power. Right top: Sampler tip after ca. 10 min with plasma RF power > 1000 W. Right bottom: Sampler tip after 270 min of continuous sample introduction at 900 W RF power.

The second optimized parameter was the sample carrier gas flow through the laser ablation cell. This gas stream transports the sample from the ablation cell into the plasma. It affects not only the plasma properties significantly, but also the transient peak form generated by the ablated material and introduced into the plasma. On the one hand, a lower gas flow deteriorates the wash out behavior from the laser ablation cell and causes broadening of the ICP-MS signals due to longer washout and transport times from the laser ablation cell into the ICP. Too long washout times reduce the spatial resolution for imaging purposes. On the other hand, the injector gas velocity directly depending on the sample carrier gas flow rate has an important influence on the location of highest analyte ion abundance within the plasma.[29, 30] Since the sampler tip position inside the orifice of the low-flow torch is almost fixed, the injector gas velocity has to be optimized carefully to precisely adjust the sample ion's location as close to

the sampler orifice as possible. Consequently, the best compromise for the sample carrier gas flow rate in view of sensitivity and spatial resolution of the imaging system had to be found.

Typical gas flow rates for the laser ablation cell in combination with a commercial ICP-MS system were 700 mL/min He and 400 mL/min Ar.[31] No helium was used in the measurements presented here. Accordingly, the sample carrier gas flow rate was varied between 550 mL/min and 1000 mL/min. The best sensitivity was achieved at 650 mL/min Ar gas flow, but at the cost of too long washout times. The highest gas flow rate at 1000 mL/min results in poor sensitivity of the ICP-MS. The best compromise between signal intensity and spatial resolution turned out to be a sample carrier gas flow rate of 850 mL/min. Summing up injector and plasma gas flows, the total Ar gas flow into the plasma was only 1.27 L/min. These gas flows were adjusted for all measurements. The generator power was set to 900 Watt for all imaging measurements.

Bioimaging of kidney sections

To visualize the distribution of the contrast agent iodixanol in rat kidney, cryosections were analyzed with a spot size of 100 µm. For this purpose, the isotope ^{127}I was detected using the optimized low-flow ICP-MS system (see Fig. 3). The elemental image shows an inhomogeneous distribution of iodine in the kidney. During the analyses, no signal drift was observed. As expected, highest concentrations of iodine with intensities of up to 160000 counts per second were detected in the cortex. These results correlate to previously published data by computer tomographical (CT) imaging.[32] Investigations with a conventional high flow LA-ICP-MS setup by Reifschneider showed good correlation with the results in the present study.[33] Lower concentrations of the contrast agent were detected in medulla and pelvis of the kidney.

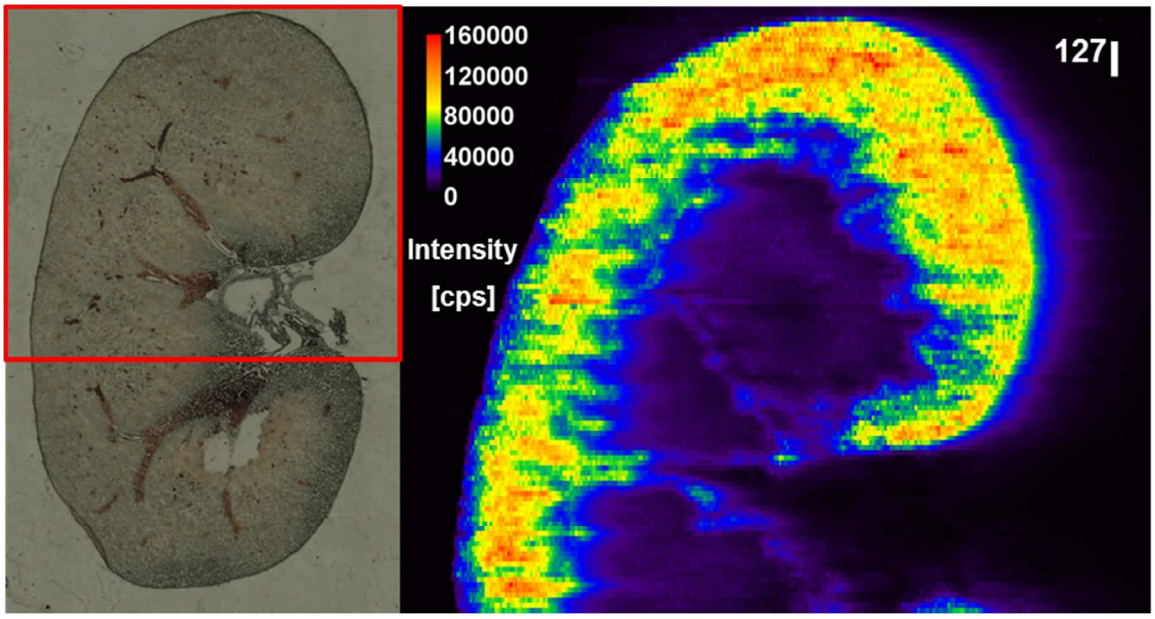

Fig. 3: Microscopic image of a rat kidney tissue slice after delivery of the contrast agent iodixanol (left image). The ablated area is marked with a red box (left). The iodine distribution is presented in the right image as detected with the LA-low-flow ICP-MS system at m/z 127.

Next to the investigation of the iodinated contrast agent iodixanol in the kidney cryosection, a parallel section of the same kidney was used for hematoxylin and eosin staining. These stains contain bromine (eosine) and aluminum (hematoxylin) and are therefore accessible to bioimaging by means of ICP-MS.[31] The kidney section was analyzed for the distribution of ^{27}Al, ^{79}Br and ^{81}Br (see Fig. 4 for the bromine isotopes). While aluminum turned out to be homogeneously distributed over the whole kidney (data not shown), the highest concentration of bromine was detected in the cortex of the kidney and lower concentrations in the medulla and the pelvis. This was confirmed by the analysis of both bromine isotopes, which expectedly show the same distribution.

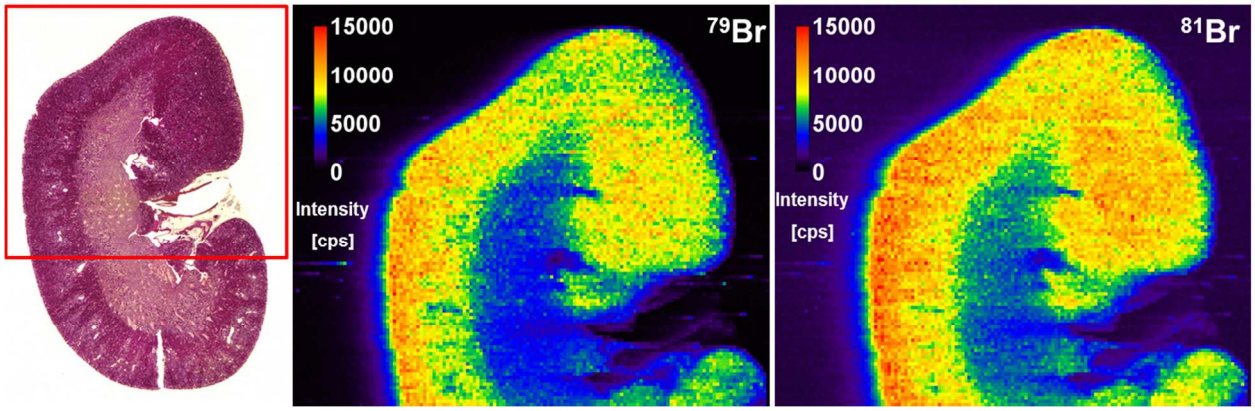

Fig. 4: Left: Microscopic image; the ablated area is marked with a red box (left). The bromine distribution image of a kidney cryosection was detected by LA-low-flow ICP-MS at m/z 79 (middle) and m/z 81 (right).

Conclusion

The first low-flow laser ablation ICP-MS system for elemental bioimaging was developed within this work. After initial optimization of RF power and sample carrier gas flow rates, elemental images of biological tissues containing iodine, aluminum and bromine as target elements were recorded to prove the performance and the robustness of the approach.

An RF power value of 900 W was found to be best suited for imaging purposes. The sample carrier gas flow rate was also optimized and the best compromise between signal intensity and spatial resolution was found to be 0.85 L/min. The second gas flow of the new low-flow ICP torch, namely the plasma gas flow, was 0.42 L/min and could not be changed. Hence, the total Ar consumption of the presented system could be reduced significantly to 1.27 L/min.

Future developments will be focused on the use of improved ablation cells. Laminar flow profiles in newly designed cells shall allow shorter wash-out times of the ablated sample aerosol. Consequently, the

sample carrier gas flow rate can be reduced without losing spatial resolution. In turn, the reduction of the sample carrier gas flow rate would increase the ion sampling efficiency and result in higher signal intensities of the ICP-MS system and thus lower limits of detection. Additionally, the lack of experience regarding the introduction of helium into the low-flow torch was a reason to start the present study first with argon as cell gas. Further experiments with He/Ar gas mixtures are planned for future work.

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