Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the Analysis of the Spatial Distribution of Trace Elements in Biological Systems

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ABSTRACT

Purpose: To demonstrate the capability for elemental imaging using laser ablation (LA) hyphenated with inductively coupled plasma mass spectrometry (ICP-MS).

Methods: Different biological samples, such as plants or rat kidney were cut in thin sections and analyzed. To compare results, samples were additionally analyzed using fluorescence and brightfield microscopy[1].

Results: Images showing the distribution of different trace elements were obtained.

INTRODUCTION

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is widely recognized as a powerful technique for the determination of trace elements in a variety of sample types. It is able to directly and quantitatively assess the amount of an element (e.g. toxic elements such as As or Hg, but also essential elements such as Cu, Zn, or Se). In some cases, not only the concentration, but also the lateral distribution can provide important information when investigating biological systems. Laser ablation imaging is becoming a well-established tool to visualize both naturally occurring and artificially introduced trace elements; however, there are challenges to overcome in order to achieve high lateral resolution and image contrast.

MATERIALS AND METHODS

Sample Preparation

Tobacco stems and petioles were embedded in hydroxyethyl cellulose (an embedding medium with low Ca content), frozen and cryosectioned into 30 µm thin sections. They were then transferred onto quartz glass sample mounts (also with lower Ca background than usual borosilicate glass sample mounts). Rat kidneys were embedded into Technovit® and sectioned into 5 µm thin sections. Subsequently, they were transferred onto glass sample mounts. Rat livers were frozen, cryosectioned into 5 µm thin sections and transferred onto glass sample mounts.

Test Method

For the analysis, a Teledyne CETAC Technologies LSX-213 G2+ laser ablation system was coupled to a Thermo Scientific™ iCAP™ TQ ICP-MS. The iCAP TQ ICP-MS was configured with a high sensitivity interface (Table 1) to ensure the detection of analytes even in low concentrations and small amounts of ablated sample. Prior to the measurements, all plasma and interface related settings were tuned automatically and were fully tailored to the LA-based sample introduction by using the autotune procedures provided in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS) Software.

Table 1. Instrument Settings

iCAP TQ ICP-MS	
Injector	2.5 mm i.d., quartz
Interface	High Sensitivity (2.8 mm) Skimmer insert; Ni Cones
RF Power	1550 W
CRC Flow	SQ-O_2 and TQ-O_2 modes: 0.4 mL·min^{-1}
Teledyne CETAC Analyte G2+ Excimer LA System	
Ablation Cell Gas Flow	0.8 L·min^{-1} He
Spot Size	25 µm
Scan Speed	75 µm · s^{-1}
Laser Energy	~4.5 J·cm^{-2}
Repetition Rate	10 Hz

Data Analysis

Both devices (laser ablation system mass spectrometer) were controlled using the Qtegra ISDS Software. Through the use of dedicated plug-ins, full synchronization of data acquisition, allowing unattended routine operation for overnight runs, was possible. Data acquisition was accomplished using the tQuant virtual evaluation of the Qtegra ISDS Software. Data reduction and image construction were accomplished using self-developed software.

What is Elemental Imaging?

Elemental images or maps refer to an LA-ICP-MS analysis that provides information on the elemental distribution across a two-dimensional area of a sample, for example across the surface of a biological tissue section. As the laser is fired at the sample surface, the sample is moved at a defined and constant rate. This means that the time profile of a line scan can be translated into a distance profile. Gathering multiple profiles across the sample generates a 2D image of the elemental distribution in the sample (3D after moving the laser sampling point in the vertical axis), where signal intensity is directly proportional to concentration.

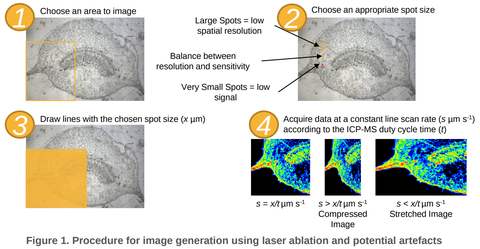

Figure 1. Procedure for image generation using laser ablation and potential artefacts

RESULTS

For the analysis, three different measurement modes were applied:

- SQ-O_2 – single quadrupole mode with collision/reaction cell (CRC) pressurized with oxygen as reaction gas.
- TQ-O_2 – triple quadrupole mode with CRC pressurized with oxygen as a reaction gas, first quadrupole (Q1) set to analyte mass (M^+) and third quadrupole (Q3) set to product ion mass (MO^+).

Q3 set to product ion mass, transmitting **^{96}[SeO]$^+$**, removing **^{80}Ar$^+$**

Q2 filled with reactive gas (O_2): **^{80}Se$^+$** → **^{96}[SeO]$^+$**

Q1 set to analyte mass, transmitting **^{80}Se$^+$**, eliminating **^{96}Mo$^+$**

For plant samples such as tobacco *(Nicotiana tabacum)*, nutritional elements are analytes of high interest. One of these elements is Ca, which contributes to plant wound sealing and defence and is crucial for signal transduction in eukaryotic cells in general. Changes in the calcium distribution across growth stages or differences between wild types and genetically modified plants can give valuable information about all these mechanisms. Even though LA-ICP-MS is a powerful tool for bio-imaging, access to calcium by SQ-ICP-MS is complicated by both background gas interferences (e.g. $^{40}Ar^+$ on $^{40}Ca^+$, $^{40}Ar^4H e^+$ on $^{44}Ca^+$) as well as those generated by the sample matrix (e.g. $^{39}K^1H^+$ on $^{40}Ca^+$).

Phosphorous and sulphur are used for visualization in many biological samples as they are present in all living cells and provide clear structural information. They are, however, difficult to access via traditional SQ-ICP-MS due to the presence of intense interferences from background (gas) species (e.g. $^{16}O^{16}O^+$ on $^{32}S^+$).
For comparison of single quadrupole and triple quadrupole performance, a tobacco petiole thin section (Figure 1) was analyzed in SQ-O_2 mode (right half of the petiole, Figure 2) and TQ-O_2 mode (left half of the same petiole, Figure 3). To avoid interferences in both modes, a mass shift reaction with oxygen has been applied to all three analytes ($^{31}P^+ \rightarrow {}^{31}P^{16}O^+$, $^{32}S^+ \rightarrow {}^{32}S^{16}O^+$, $^{44}Ca \rightarrow {}^{44}Ca^{16}O^+$).

Comparison of SQ and TQ modes for the analysis of tobacco petioles

As can be seen in Figure 3 and Figure 4, the mass shift reaction with oxygen successfully removes most interferences for P (Figure 3 and Figure 4, left) and S (Figure 3 and Figure 4, middle) in SQ- and TQ mode. Consequently, the general structure of the petiole cross section can be differentiated from the background very clearly. Noticeably, the embedding medium, which surrounds the sample, is contaminated with high amounts of P, which can be seen in both modes.
For Ca (Figure 3 and Figure 4, right), the mass shift reaction with oxygen itself is not sufficient for effective interference removal. This leads to a blurry image in SQ mode where only the Ca hotspots are clearly visible. Those hotspots correspond to idioblasts, specialized cells that accumulate calcium oxalate as a defense against herbivory. In contrast, when TQ mode is applied, the Ca distribution in the whole section can be visualized, including the finer trichome structures, as the background concentration caused by the interferences is decreased to zero.

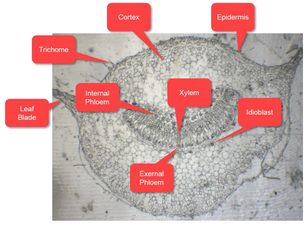

Figure 2. Visible light image of a thin section from a tobacco petiole, indicating major structures.

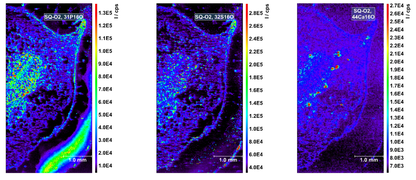

Figure 3. Elemental distribution maps for the SQ-O_2 analysis of $^{31}P^{16}O^+$ (left), $^{32}S^{16}O^+$ (middle) and $^{44}Ca^{16}O^+$ (right). All intensity values are shown in cps.

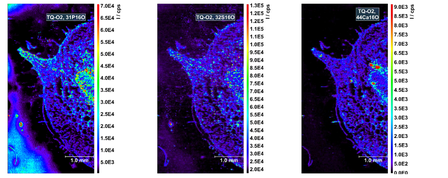

Figure 4. Elemental distribution maps for the TQ-O_2 analysis of $^{31}P^{16}O^+$ (left), $^{32}S^{16}O^+$ (middle) and $^{44}Ca^{16}O^+$ (right). All intensity values are shown in cps.

Comparison of SQ and TQ modes for the analysis of rat kidneys

For thin section samples derived from animals, e.g. in clinical research studies, it is common that the distribution of more than just one element is of high interest. Therefore, the mass imaging mode has to be a good compromise between the number of analytes and obtainable sensitivity and spatial resolution. Similar to plant samples, many of the elements of interest are suffering from interferences on their major isotopes, such as iron (e.g., $^{40}Ar^{16}O^+$ on $^{56}Fe^+$) or selenium (e.g., $^{40}Ar^{40}Ar^+$ on $^{80}Se^+$).

For comparison of single quadrupole and triple quadrupole performance, two parallel thin sections of a rat kidney have been analyzed in SQ-O_2 (Figure 5 & 6) and TQ-O_2 (Figure 7 & 8) modes. Oxygen has been chosen as a cell gas here to allow mass shift reactions of Se and avoid the strong Ar interferences on mass. As can be seen in Figure 6, neither the Fe nor the Se distributions in the rat kidney thin section are clearly visible in SQ-O_2 mode.
For $^{57}Fe^+$ (Figure 6, left), the background intensities are still too high due to the formation of $^{40}Ar^{16}O^1H$ and $^{40}Ar^{17}O^+$ in the CRC, so differentiation of background and sample is very difficult to achieve. For $^{77}Se^{16}O^+$ (Figure 6, right), the background intensities are close to zero, but the intensities of the minor Se isotope at m/z 77 are too low for visualization.

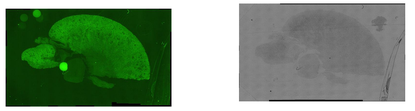

Figure 5. Fluorescence (left) and bright field microscopic image (right) of the rat kidney thin section to be analyzed in SQ-O_2 mode.

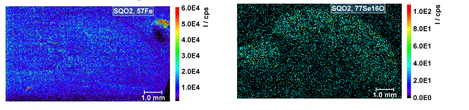

Figure 6. Elemental distribution maps for the SQ-O_2 analysis of $^{57}Fe^+$ (left) and $^{77}Se^{16}O^+$ (right). All intensity values are shown in cps.

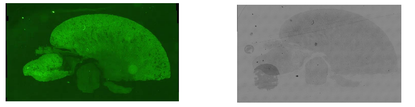

Figure 7. Fluorescence (left) and bright field microscopic image (right) of the rat kidney thin section to be analyzed in TQ-O_2 mode.

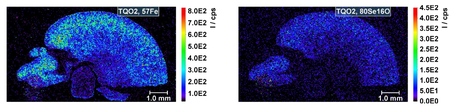

Figure 8. Elemental distribution maps for the TQ-O_2 analysis of $^{57}Fe^+$ (left) and $^{80}Se^{16}O^+$ (right). All intensity values are shown in cps.

In contrast to the SQ-O_2 results, the TQ-O_2 mode enables visualization of both Fe and Se distributions (Figure 8). For ^{57}Fe (Figure 8, left), the application of the first quadrupole as a mass filter removes the $^{40}Ar^+$ precursor ions so they cannot react with oxygen in the cell, and therefore lowers the background intensities successfully to reveal the detailed distribution within the sample.
For $^{80}Se^{16}O^+$ (Figure 8, right), the intensities are still relatively low due to the minimal amount of Se present in the sample. As the TQ-O_2 mode removes argon based precursor ions, and furthermore the $^{80}Ar_2$ dimer does not oxidize, effectively all interferences at this m/z have been removed. The major isotope of Se can be used in this case (m/z 80 with mass shift to m/z 96), allowing the Se distribution within the sample to be visualized even at low concentrations.

CONCLUSIONS

- The LA-ICP-MS system described has been shown to be ideally suited for the high spatial resolution bioimaging analysis of various elements in thin sections in both single and triple quadrupole analysis modes.
- The use of triple quadrupole technology in the iCAP TQ ICP-MS system clearly improves the images produced for analytes such as Ca (through the analysis of $^{44}Ca^{16}O^+$ at m/z 60), iron (through the analysis of $^{56}Fe^{16}O^+$ at m/z 72) or Se (through the analysis of $^{80}Se^{16}O^+$ at m/z 96).
- This also enables a wider dynamic range and cleaner backgrounds to reveal additional structural information not detectable by traditional single quadrupole ICP-MS.

REFERENCES

1. Application Note 43358, Thermo Fisher Scientific

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TRADEMARKS/LICENSING

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