TOFWERK
Time-of-Flight Mass Spectrometry

TELEDYNE
CETAC TECHNOLOGIES
Everywhereyoulook™

14306 Industrial Road
Omaha, NE 68144
USA
+1.402.733.2829
cetac.com

# Application Note

## High-Speed, High-Resolution, Multi-Element Imaging of Plant Root Cross-Sections to Highlight Nutrient Transport Pathways

*Yannick Bussweiler[1], Olga Borovinskaya[1], Søren Husted[2], Daniel Olof Persson[2], Thomas Hesselhøj Hansen[2], Ciprian Cosmin Stremtan[3]*
*[1] TOFWERK AG, Thun, Switzerland | [2] University of Copenhagen, Denmark | [3] Teledyne CETAC Technologies, Omaha, USA*

Knowledge of plant root functions in the uptake and transport of nutrients is of great importance, e.g., for the agricultural industry. However, the exact transport pathways and behavior of some of the key nutrients are still poorly understood. Modern microanalytical methods, such as bioimaging by LA-ICP-MS, are increasingly used to elucidate these plant root functions (e.g., Persson et al., 2016).

The combination of inductively-coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) and fast-washout laser ablation systems allows for multi-element imaging at much greater speed and higher spatial resolution than conventional LA-ICP-MS (e.g., Gundlach-Graham et al., 2015, Burger et al., 2015), and thus make it a promising tool to study plant roots. In this study, the technique was tested on root cross-sections (~20 µm thickness) of the plant *A. thaliana* and of wild barley. The samples were prepared at the University of Copenhagen following a protocol which involves encapsulating the roots in paraffin, cryo-sectioning, and freeze-drying them (Persson et al., 2016).

An Analyte G2 excimer laser (193 nm) equipped with a HelEx II cell and an ARIS device (Teledyne CETAC Technologies, USA) was used for fast washout of the ablated material (< 20 ms). The laser spot size was 3 µm, the repetition rate was 20 Hz, and the laser fluence was 3 and 1 J/cm$^2$ for the A. thaliana and barley roots, respectively. The TOFWERK icpTOF was run in normal mode, collecting ~ 33,000 mass spectra per second that were further integrated into one mass spectrum per pixel. Imaging was performed in spot-resolved mode, implying that each pixel in the image represents the signal from a single laser shot, with minimal overlap of neighboring pixels (see Bussweiler et al., 2017). Image acquisition was performed with TOFWERK's TOFpilot software, allowing real-time display of the images.

A total of 13 elements (with atomic masses of 23 and higher) were detected in the root samples, including Na, Mg, P, K, Ca, Mn, Fe, Cu, Zn, Br, Sr, Mo, and Ba. The total analysis time for the A. thaliana root sample (250 µm by 250 µm) was ~6 minutes, and for the barley root sample (525 µm by 534 µm) ~50 minutes.

**Analyte G2 Excimer Laser Ablation System shown equipped with HelEx II Tunable 2-Volume Cell.**

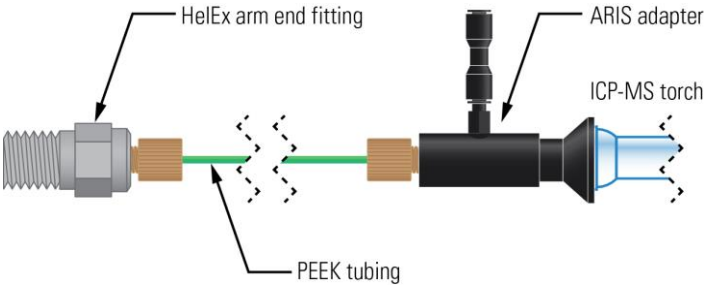


**Diagram of the Aerosol Rapid Introduction System (ARIS) .**

Since the icpTOF collects entire mass spectra with every measurement, images for multiple isotopes are available for some elements. The signals of different isotopes can be combined in order to obtain clearer elemental images (see Figures 1 and 2). In general, the detected cations are located in the cell walls and concentrated mostly around the stele of the

root (i.e., the central part). In some cases, the cations are concentrated around the epidermis of the root, e.g. Mg and Br in the barley root (Figure 2).

Based on this preliminary study, the combination of icpTOF and fast-washout laser ablation is well-suited for high-speed, high-resolution, multi-element imaging of plant samples, making it an important tool in future studies on root functions.

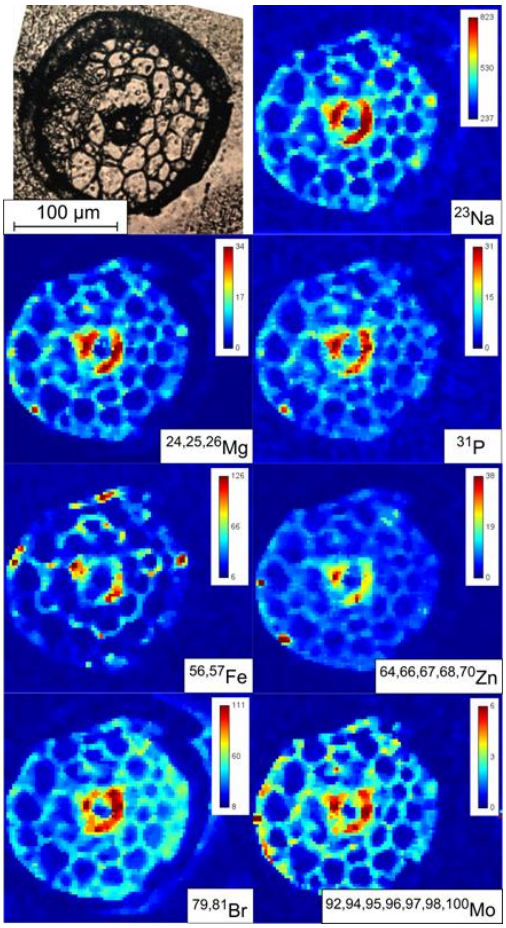


**Figure 1: Multi-elemental intensity images for a root cross-section of the plant A. thaliana recorded by LA-ICP-TOFMS. Where multiple isotopes were available, the images were combined to create clearer elemental images. The units are in ions per pixel. The total analysis time was ~6 minutes.**

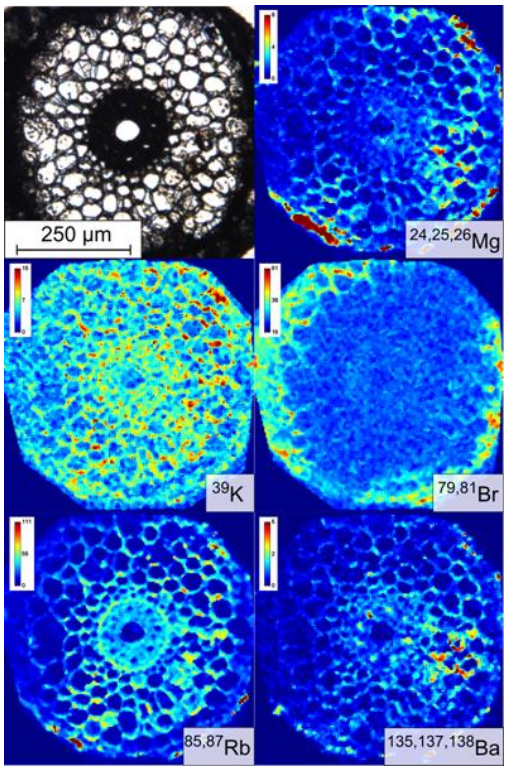


**Figure 2: Multi-elemental intensity images for a root cross-section of wild barley recorded by LA-ICP-TOFMS. Where multiple isotopes were available, the images were combined to create clearer elemental images. The units are in ions per pixel. The total analysis time was ~50 minutes.**

## REFERENCES

Burger, M., Gundlach-Graham, A., Allner, S., Schwarz, G., Wang, H.A., Gyr, L., Burgener, S., Hattendorf, B., Grolimund, D. and Günther, D., 2015. High-speed, high-resolution, multielemental LA-ICP-TOFMS imaging: Part II. Critical evaluation of quantitative three-dimensional imaging of major, minor, and trace elements in geological samples. Analytical chemistry, 87(16), pp.8259-8267.

Bussweiler, Y., Borovinskaya, O., Tanner, M., 2017. Laser Ablation and Inductively Coupled Plasma–Time-of-Flight Mass Spectrometry—A Powerful Combination for High-Speed Multielemental Imaging on the Micrometer Scale. Spectroscopy 32(5), pp.14-20.

Gundlach-Graham, A.W., Burger, M., Allner, S., Schwarz, G., Wang, H.A.O. and Gyr, L., 2015. High-speed, high-resolution, multi-elemental LA–ICP–TOFMS imaging: Part I. instrumentation and two-dimensional imaging of geological samples. Anal Chem, 87(16), pp.8250-8.

Persson, D.P., Chen, A., Aarts, M.G., Salt, D.E., Schjoerring, J.K. and Husted, S., 2016. Multi-element bioimaging of Arabidopsis thaliana roots. Plant Physiology, pp.pp-00770.