

Analysis of Trace Elemental Distribution in Plant Specimens

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ABSTRACT | In crop sciences, the genetic modification of crops to improve the uptake of trace elements such as Cu, Fe, and Zn is a major focus of research because it has direct implications for increasing micronutrient content and crop yield [1]. This work is critical for addressing problems in regions with poor soil nutrition. To determine the transportation mechanisms and signaling pathways that communicate demand for trace element uptake, it is necessary to localize these metals at the cellular level and across various growth stages of plants. The AttoMap micro x-ray fluorescence (microXRF) system provides the femtogram-level sensitivity and subcellular resolution needed.

INTRODUCTION

Understanding the spatial distribution of inorganic content in plant specimens is critical important to several agricultural and environmental disciplines, including:

- New **phytoremediation techniques**, in which plants are designed to remove toxic contaminants and reclaim polluted land;
- **Phytomining**, in which “hyperaccumulating” plants harvest precious minerals in an economic and environmentally-friendly way; and
- **Agricultural studies**, in which plant uptake of metals is modified to enhance crop growth, reduce the absorption of toxic elements, and increase the micronutrient value of crops

Despite the importance of trace metals in plants, analysis is challenging and typically requires the use of a synchrotron. These multi-hundred-million-dollar particle accelerator facilities produce brilliant beams of x-rays, enabling the high-resolution and high-sensitivity microXRF analysis required. However, synchrotron beamlines are oversubscribed, and access generally requires a peer-reviewed application process and, if granted, significant travel time and expenses.

Sigray AttoMap, a newly developed laboratory microXRF for agricultural research, was used in this study to analyze the uptake and partitioning of iron (Fe). This represents one of the most challenging use cases for microXRFs due to extremely low Fe concentrations (10^{-12} picogram-scale), which require parts-per-million (ppm) sensitivity for accurate measurement.

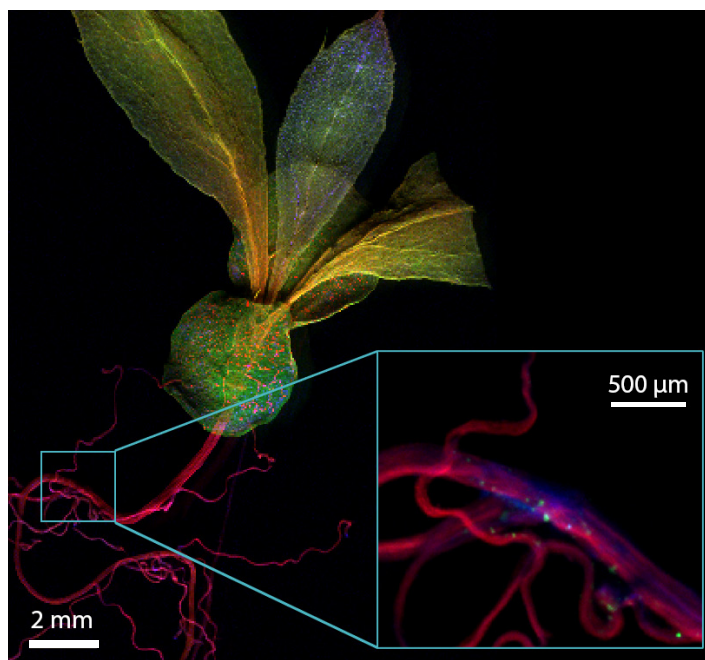


Figure 1. AttoMap Micro-XRF mapping of a hyperaccumulating seedling. Larger view is of a tricolor composite of K (red), Ni (blue), and Cl (green). Zoom-in of roots shows trace uptake of Mn (green). Courtesy of Dr. Antony van der Ent and Dr. Peter Erskine, University of Queensland, Australia.

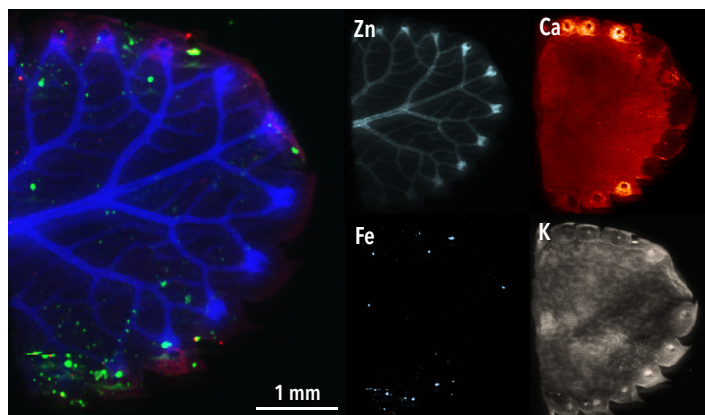


Figure 2. AttoMap Micro-XRF simultaneously provides elemental imaging for multiple elements. Left: tri-color composite of Zn (blue), Fe (green), and Ce (red). Right: individual channels for elements of interest. Courtesy of Cerege, CNRS, Aix-Marseille University.

Iron is critical for plant growth and plays a major role in respiration and photosynthesis. Approximately, 30% of the world's arable land is considered iron-limited for plant growth [2]. The study results indicate that the transporter protein, OPT3 (Oligopeptide Transporter 3), mediates Fe-loading into developing leaves, suggesting that OPT3 proteins regulate Fe demand signaling from shoots to roots.

METHOD

This study analyzed the leaves of a genetically modified *Arabidopsis* knockout (OPT3-3), courtesy of Prof. Olena Vatamaniuk (Associate Professor of Soil and Crop Sciences, Cornell University), alongside a control (wild type) sample to determine the potential role of the OPT3 protein. Leaves were sampled at different growth stages: one leaf was removed from the same plant at 16 days of growth and another at 19 days. All elements were simultaneously analyzed using Attomap microXRF to characterize the distribution of key plant growth-related minerals Ca, Zn, Mg, Fe, and K.

For the 16-day leaf, an area of 3.5 mm x 3.8 mm was mapped at a 10 μ m spot size and a 10 μ m step size. The x-ray source settings were configured to use a tungsten (W) target from the multi-target source, operated at 35 kV. Although tungsten (W) was selected due to interest in a broad range of elements, a copper (Cu) target is optimal when Fe (6.4 keV) is the sole interest. Follow-up studies aimed for even greater Fe sensitivity can be uniquely achieved using AttoMap's patented multi-target x-ray source.

The 19-day leaf (Figure 4, next page) was scanned over a 4.0 mm x 8.3 mm area at a spot size of 10 μ m and a step size of 15 μ m with source settings unchanged from the 16-day leaf.

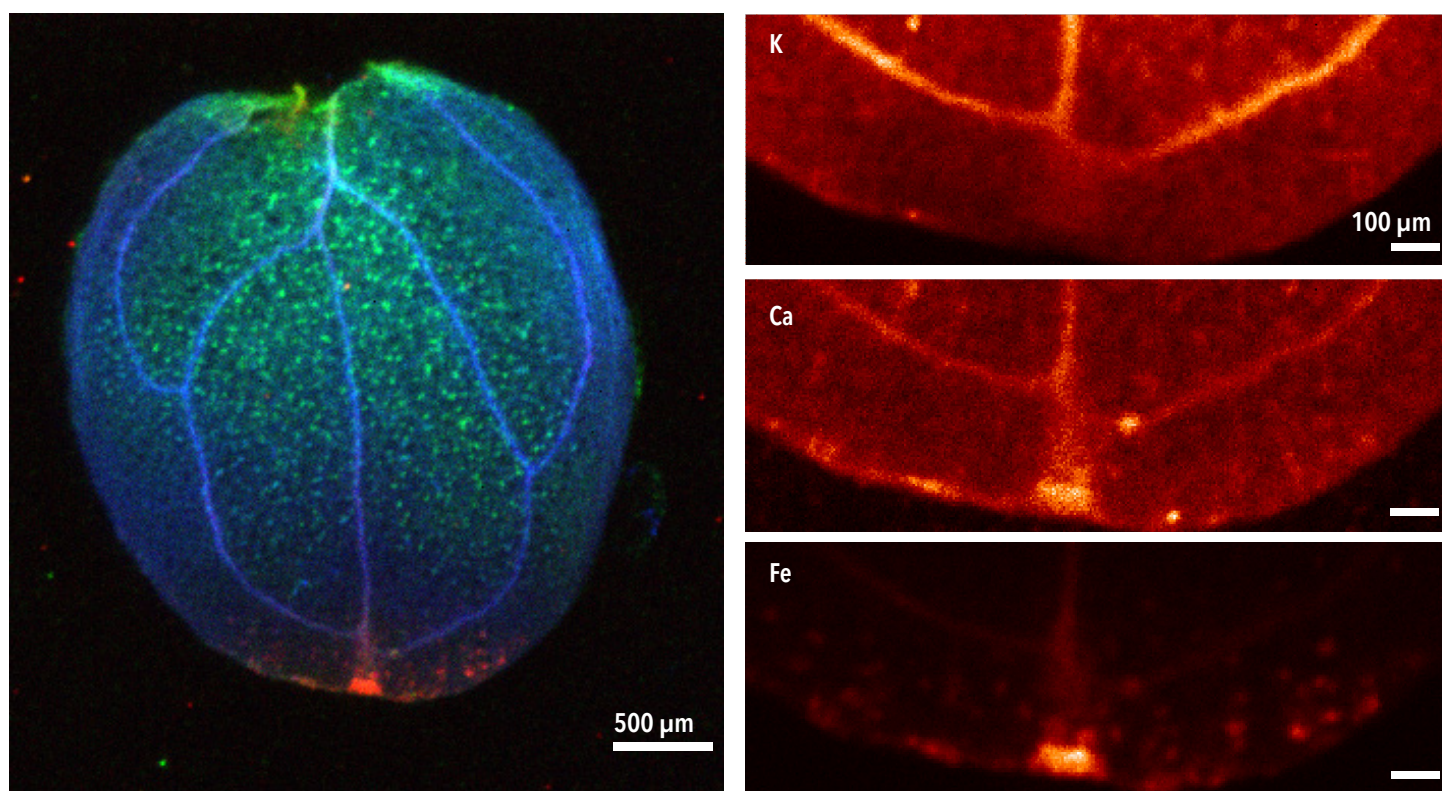
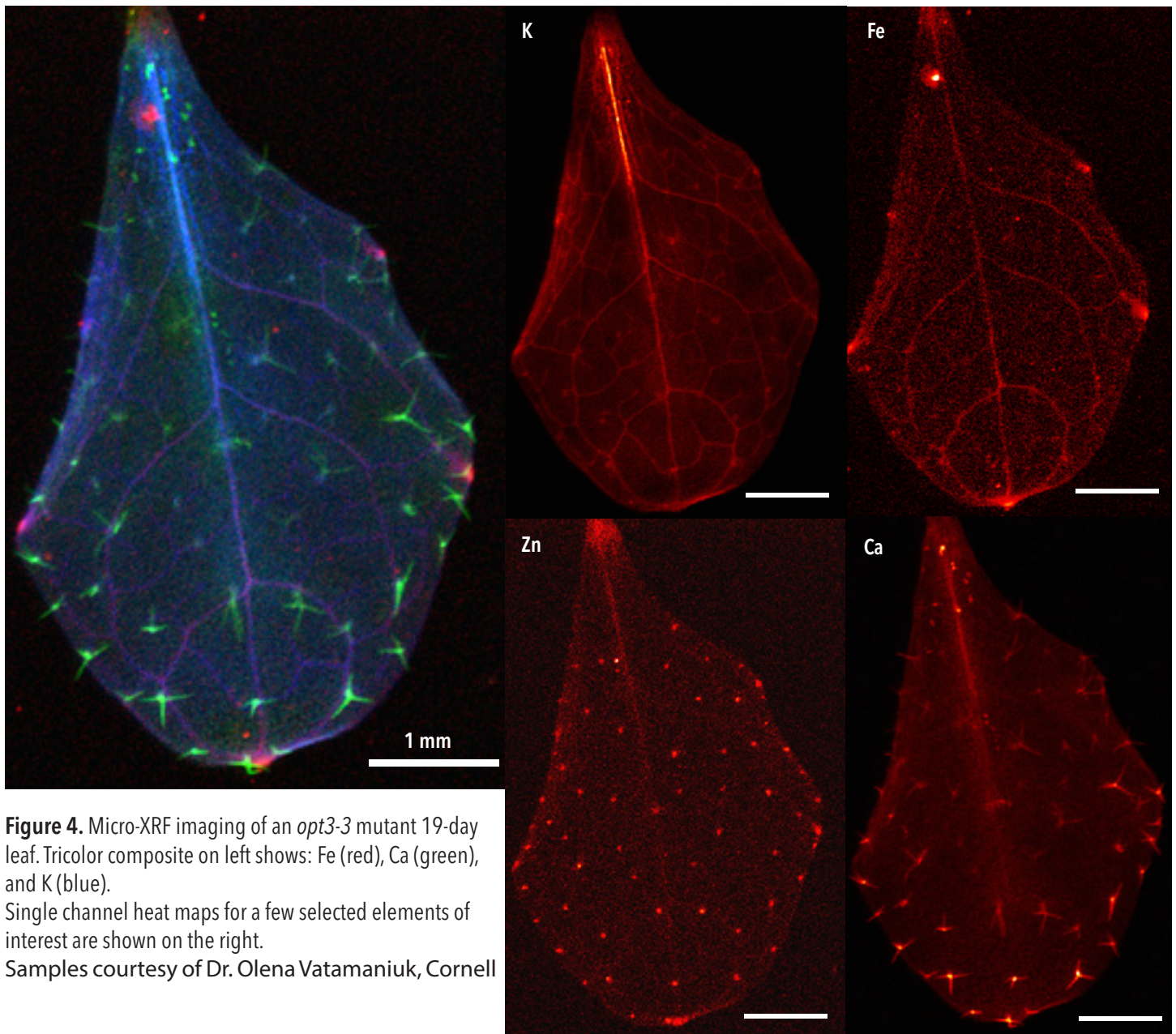


Figure 3. Left: tri-color composite of an *opt3-3* mutant 16-day leaf: Fe (red), Ca (green), K (blue). Right: single-channel distribution maps of selected elements of interest.

Samples courtesy of Dr. Olena Vatamaniuk, Cornell University



RESULTS AND DISCUSSION

The results revealed picogram-level anomalies in trace Fe distribution in the knockout *opt3-3* plant. In both the 16-day and 19-day leaves, Fe concentration was primarily found in the minor veins, near the hydathodes (leaf pores) and along the leaf blade periphery. Increased Fe accumulation was observed in the central minor veins of the older leaf. Since these are locations where OPT3 is preferentially expressed, the findings suggest that OPT3 is crucial for loading Fe back into the phloem—the vascular tissue responsible for conducting sugars and nutrients from leaves downward to the stems, supporting plant development. In contrast, the wild-type leaf exhibited significantly lower Fe distribution, with accumulation seen only at the outermost edge.

Studies by Prof. Olena Vatamaniuk on other elements involved in the transport of water and solutes, such as potassium and calcium, found no statistically significant differences between the wild-type and *opt3-3* distributions. These findings suggest that the overall loading and transport of other nutrients remain unaffected, supporting the hypothesis that OPT3 specifically regulates Fe-specific pathways.

SUMMARY

This study demonstrates that with advancements in laboratory microXRF technology, plant-related trace (parts-per-million) element analysis is now possible outside of the synchrotron. In this study, the AttoMap provided picogram-scale measurements at sub-cellular ($<10\ \mu\text{m}$) resolution. Interestingly, the AttoMap laboratory system appeared to have greater sensitivity for elements such as Ca (3.7 keV) and K (3.3 keV) compared to previously obtained synchrotron results. This is likely due to the AttoMap's polychromatic beam, which provides improved cross-sections compared to the 11 keV monochromatic synchrotron beam used. This finding aligns with previous studies suggesting that "white light" beams are far preferable to standard synchrotron configurations for environmental samples [3]. The quantification of signal gain for lower atomic number elements using the AttoMap has been reserved for follow-up studies.

The AttoMap not only provides elemental distribution imaging but can also be used to quantify the relative amounts of each element. Future possibilities for the system include *in-vivo* studies, in which elements in growing and living plants or roots can be monitored. This is made possible by the large working distance (source-sample focusing distance), which allows for the characterization of roots in soil and/or uneven surfaces such as leaves.

References

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2. Z Zhai, et al. "OPT3 is a Phloem-specific iron transporter that is essential for systemic iron signaling and redistribution of iron and cadmium in *arabidopsis*." *The Plant Cell* 26 (2014): 2249-2264.
3. SR Barberie, et al. "Evaluation of different synchrotron beamline configurations for x-ray fluorescence analysis of environmental samples." *Analytical Chemistry* 86:16 (2014): 8253-8260.



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