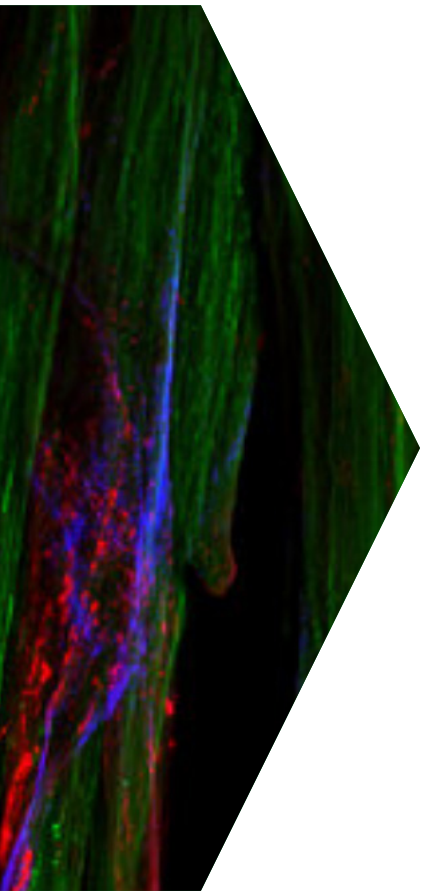
Applications Note

AttoMap MicroXRF



Analysis of Trace Elemental Distribution in Plant Specimens

Localizing and quantifying metals at sub-cellular resolution (5-10 µm) in plants provides critical insights into transportation mechanisms and signaling pathways that influence element uptake. One key application in crop science is analyzing elemental distribution to provide feedback on genetic modifications made to improve uptake of trace elements (e.g., Cu, Fe, and Zn) for increasing micronutrient content and crop yield.

This white paper will review plant sciene applications of the AttoMap XRF microscope.

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Trace Elemental Distribution in Plants with the **AttoMap™ Series XRF Microscopes**

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Background: Understanding the spatial distribution of inorganic content in plant specimens is crucial to various agricultural and environmental disciplines, including:

- **New phytoremediation techniques**, in which plants are designed to remove toxic contaminants and recover the expanding amount of polluted land;
- **Phytomining**, in which "hyperaccumulating" plants can harvest precious minerals in an economic and environmentally-friendly way; and
- **Agricultural studies**, in which plant uptake of metals is modified to improve crop growth, reduce the absorption of toxic elements, and increase the micronutrient value of the crop[1]

Despite the importance of trace metals in plants, analysis is challenging and typically requires traveling to a synchrotron light facilities, which are capable of providing the intense and focused beams of x-rays needed. The problem is that access to synchrotron microXRF is extremely limited; synchrotron facilities are expensive to build (~$1B) and operate (often over $100MM per year) and are consequently limited to a small number around the world.

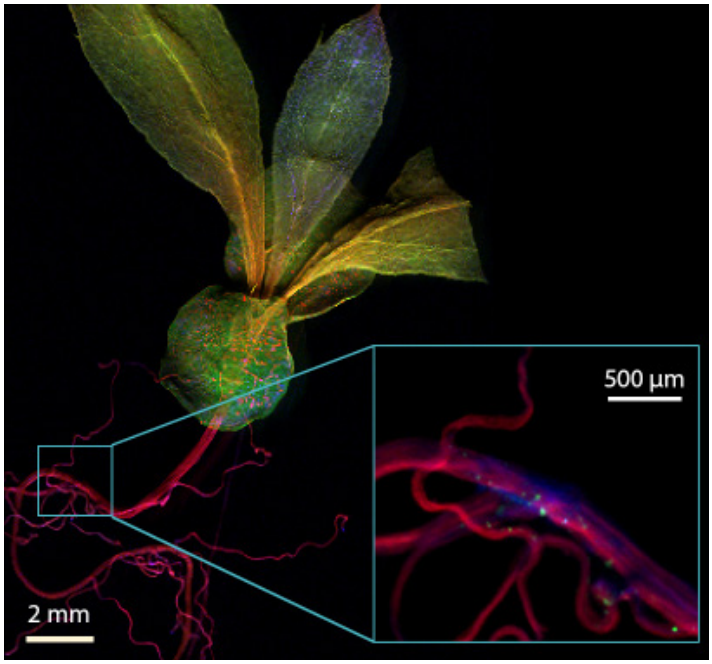

Figure 1: *AttoMap Micro-XRF mapping of a hyperaccumulating seedling. Larger view is a tricolor composite of K (red), Ni (blue), and Cl (green). Zoom-in of roots shows trace uptake of Mn (green). Courtesy of Dr. Antony van der Ent and Dr. Peter Erskine, University of Queensland, Australia.*

Novel Approach: Sigray AttoMap XRF Microscope

Sigray has developed the AttoMap microXRF system through patented breakthroughs in x-ray source and x-ray optic technologies to bring synchrotron-like microXRF capabilities within a laboratory setting. The system achieves sensitivity levels orders of magnitude higher than electron-based techniques such as SEM-EDS and provides quantitative and resolution advantages in comparison to LA-ICP-MS.

AttoMap was originally designed for biological applications and funded through several National Institutes of Health (NIH) grant proposals. As a result, key system innovations are optimized for biological applications and include:

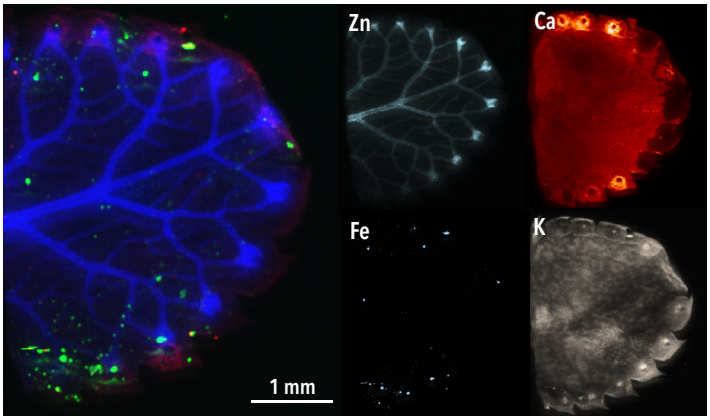

Figure 2: *AttoMap Micro-XRF provides elemental imaging for multiple elements simultaneously. Left: tri-color composite of Zn (blue), Fe (green), and Ce (red). Right: individual channels for elements of interest. Courtesy of Cerege, CNRS, Aix-Marseille University.*

1. A patented **multi-target x-ray source** that allows users to optimize fluorescence signals of interest and detect trace elements at the sub-ppm level
2. Sub-cellular resolution reaching down to **3-5 µm**
3. **Goniometer stage in the AttoMap-310 model** to allow varying incident measurement angles to maximize x-ray interaction volume and sensivity in thin biological samples.
4. Integrated **optical microscope** for correlative analysis
5. **Advanced software tools**: Suite of tools including an easy-to-use GUI interface to provide standardless fundamental parameters (FP) quantification and editable Jupyter python notebooks. Open-box software allows extensibility of algorithms and collaboration.

Applications Overview

The Sigray AttoMap was used to measure the uptake and partitioning of iron (Fe). This is one of the most challenging applications for microXRFs due to extremely low Fe concentrations (10-12 picogram-scale), which require parts per million (ppm) sensitivity for accurate measurement.

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

Method

This study analyzed leaves from 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 investigate the potential role of the OPT3 protein. Leaves from the plants were sampled at various growth stages: one leaf was collected from the same plant at 16 days of growth and another at 19 days of growth. All elements were simultaneously analyzed with Attomap microXRF, to characterize the distribution of key, plant growth-related minerals, including Ca, Zn, Mg, Fe, and K.

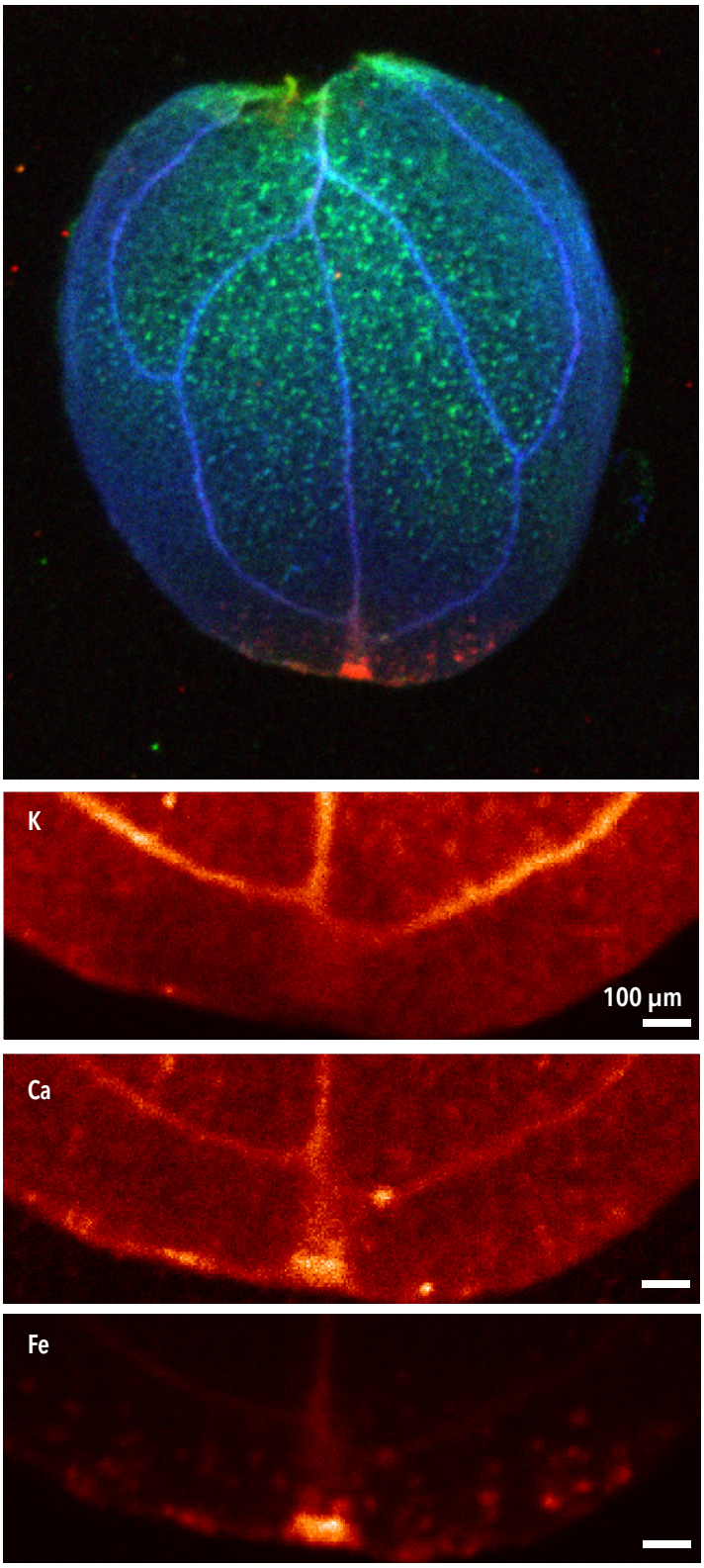

Figure 3: *Left: tri-color composite of an opt3-3 mutant 16-day leaf: Fe (red), Ca (green), K (blue). Right: single-channel distribution maps of selected elements of interest.*
Samples courtesy of Dr. Olena Vatamaniuk, Cornell University.

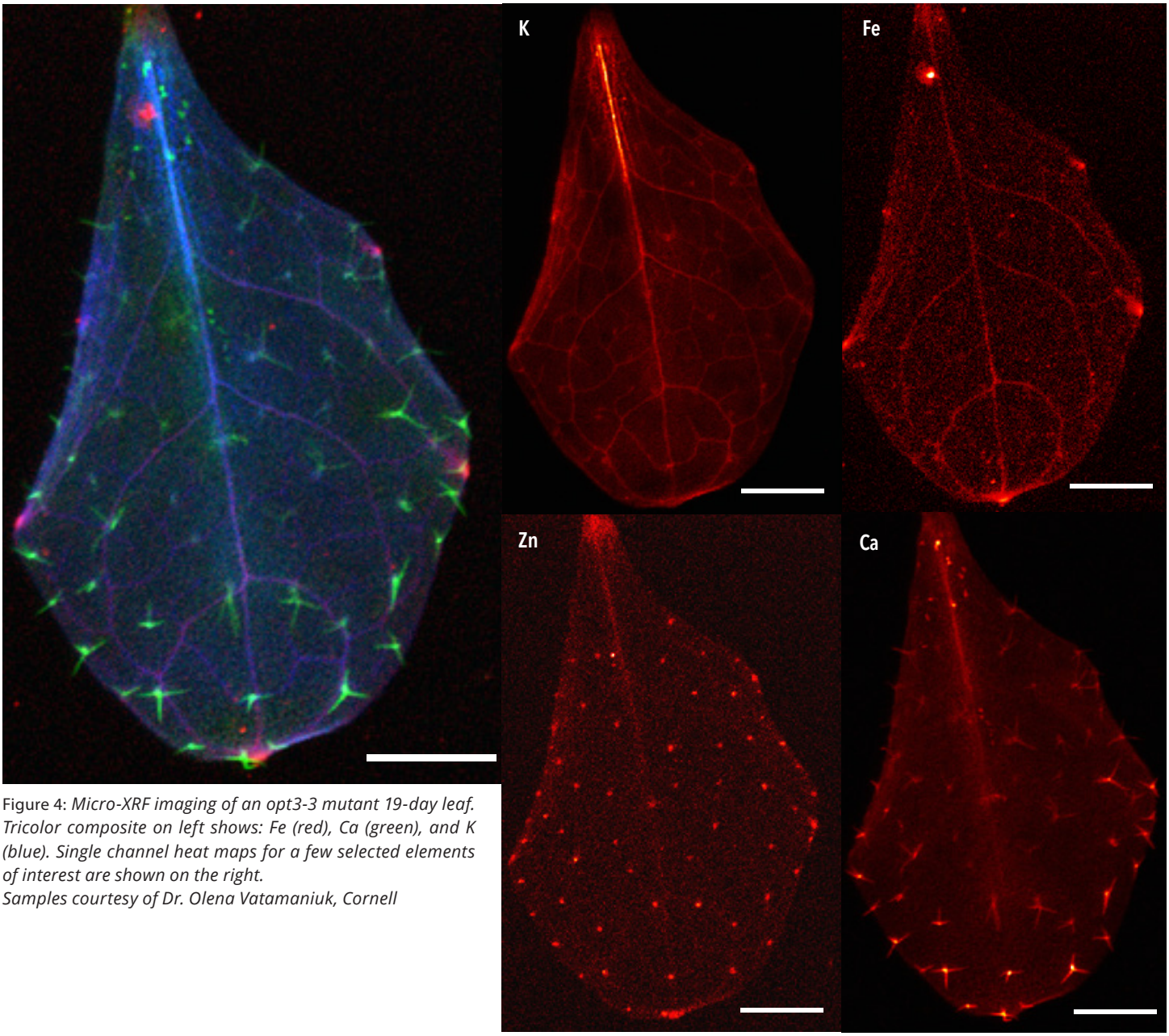

Figure 4: *Micro-XRF imaging of an opt3-3 mutant 19-day leaf. Tricolor composite on left shows: Fe (red), Ca (green), and K (blue). Single channel heat maps for a few selected elements of interest are shown on the right.*
Samples courtesy of Dr. Olena Vatamaniuk, Cornell

For the 16-day leaf (Fig. 3), an area of 3.5 mm x 3.8 mm was mapped using a 10 µm spot size and a 10 µm 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 its broad elemental sensitivity, a copper (Cu) target would be optimal if Fe (6.4 keV) were the sole element of interest. Follow-up studies aimed at improving Fe sensitivity can be uniquely achieved in the AttoMap using its patented multi-target x-ray source.

For the 19-day leaf (Fig. 4), the scan area was 4.0 mm x 8.3 mm, with a 10 µm spot size and a 15 µm step size. The source settings were kept the same as the 16-day leaf.

Results and Discussion

The results showed picogram-level anomalies in trace Fe distribution in the knockout *OPT3-3* plant. In both the 16-day and 19-day leaves, Fe was primarily concentrated in the minor veins of the leaves, located near the hydathodes (leaf pores) and toward the leaf blade periphery. Additionally, the

older leaf (19-day) exhibited increased Fe accumulation in the central minor veins. Since elevated Fe levels were found in locations where OPT3 is preferentially expressed, the results indicate that OPT3 plays a crucial role in Fe reloading into the phloem—the vascular tissue responsible for conducting sugars and nutrients from leaves downward to support plant development. By comparison, the wild-type leaf exhibited significantly lower Fe distribution, with accumulation restricted to a small outermost edge.

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

Summary

Biological trace-elemental studies at sub-ppm sensitivities can now be conducted outside of the synchrotron facilities. In this study, the AttoMap achieved picogram-scale measurements at sub-cellular (<10 µm) resolution. Due to the selection of an x-ray target material (W) with a strong polychromatic spectrum, the AttoMap laboratory system demonstrated greater sensitivity for elements such as Ca (3.7 keV) and K (3.3 keV) compared to the synchrotron results previously obtained. This aligns with previous studies suggesting that "white light" beams are preferable to monochromatic synchrotron configurations for environmental samples[3].

Beyond elemental distribution imaging, the AttoMap can quantify the relative amounts of each element. Future applications include in-vivo studies, allowing researchers to monitor elemental uptake in living plants or roots. This capability is faciliated by AttoMap's large working distance (source-to-sample focusing distance), which enables analysis of roots in soil and uneven surfaces, such as leaves.

1. HH Chu, et al. "Successful reproduction requires the function of Arabidopsis YELLOW STRIPE-LIKE1 and YELLOW STRIPE-LIKE3 metal-nicotianamine transporters in both vegetative and reproductive structures." Plant Physiology 154 (2010): 197-210.
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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