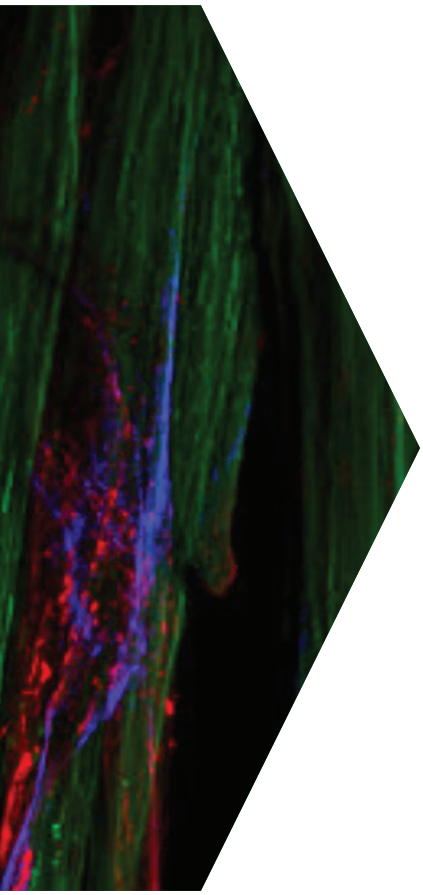
Applications Note

AttoMap MicroXRF



Analysis of Trace Elemental Distribution in Plant Specimens

Localizing and quantifying metals at sub-cellular resolution (5-10 µm) in plants provides important insight on transportation mechanisms and signaling pathways that determine uptake. One application example in crop sciences is using 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 of critical importance to a number of 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.

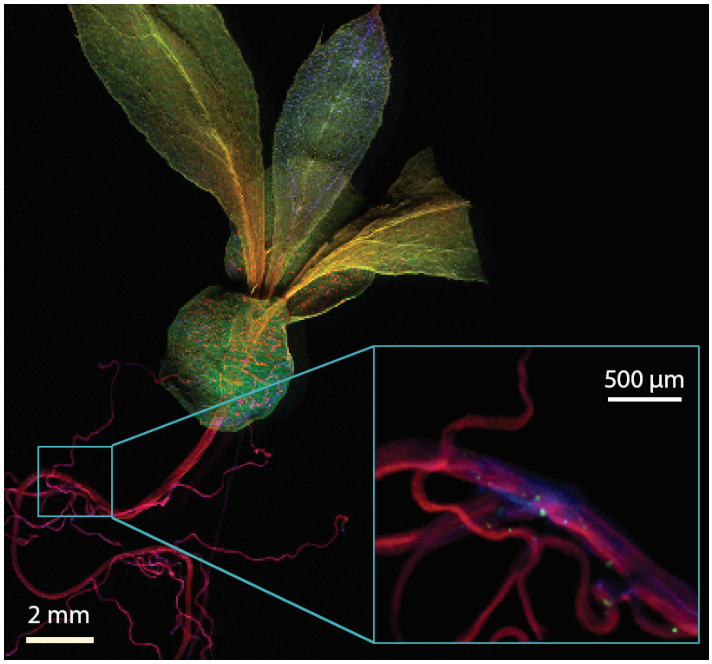

Figure 1: *AttoMap Micro-XRF mapping of a hyperaccumulating seedling. Larger view is a tricolor composite of K (red), Ni (blue), and Cl (green). Zoom-in of roots shows trace uptake of Mn (green). Courtesy of Dr. Antony van der Ent and Dr. Peter Erskine, University of Queensland, Australia.*

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 to within the laboratory. The system can achieve sensitivies that are 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:

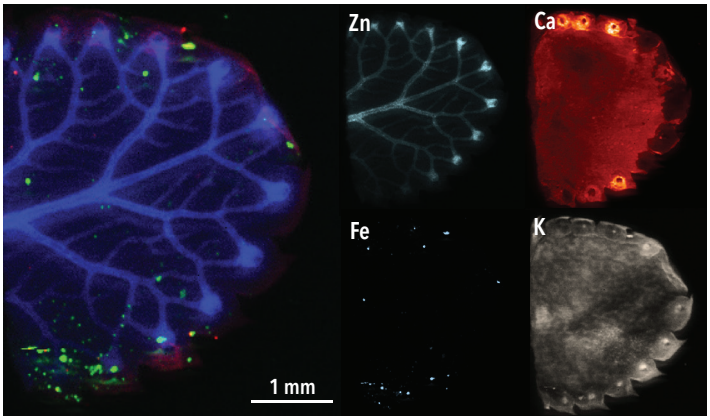

Figure 2: *AttoMap Micro-XRF provides elemental imaging for multiple elements simultaneously. Left: tri-color composite of Zn (blue), Fe (green), and Ce (red). Right: individual channels for elements of interest. Courtesy of Cerege, CNRS, Aix-Marseille University.*

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 use cases for microXRFs due to extremely low (10-12 picogram-scale) Fe concentrations, which require parts per million (ppm) sensitivity to measure.

Iron is critical to the growth of plants and plays a major role in respiration and photosynthesis reactions; around 30% of the world's arable lands are considered iron-limited for plant growth2. The study results indicate that the transporter protein, OPT3 (Oligopetide Transporter 3), mediates Fe-loading into developing leaves which suggests OPT3 proteins regulate Fe demand signaling from shoots to roots.

Method

This study analyzed leaves of a genetically modified arabidopsis knockout (*OPT3-3*), courtesy of Prof. Olena Vatamaniuk (Associate Professor of Soil and Crop Sciences, Cornell University), and a control (wild type) sample to determine the potential role of the OPT3 protein. Leaves from the plants were sampled at various stages of growth: one leaf was removed 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 important, plant growth-related minerals Ca, Zn, Mg, Fe, and K.

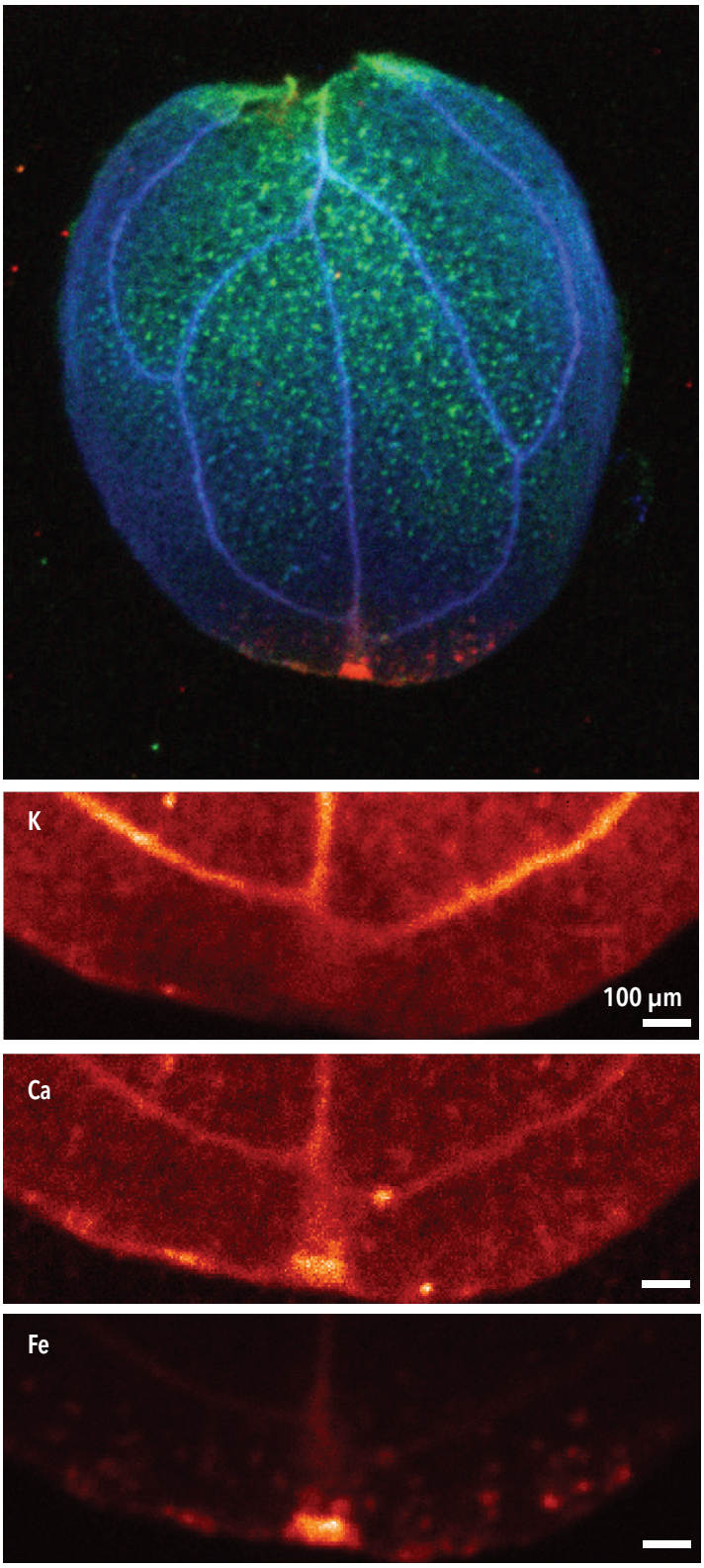

Figure 3: *Left: tri-color composite of an opt3-3 mutant 16-day leaf: Fe (red), Ca (green), K (blue). Right: single-channel distribution maps of selected elements of interest.*
Samples courtesy of Dr. Olena Vatamaniuk, Cornell University.

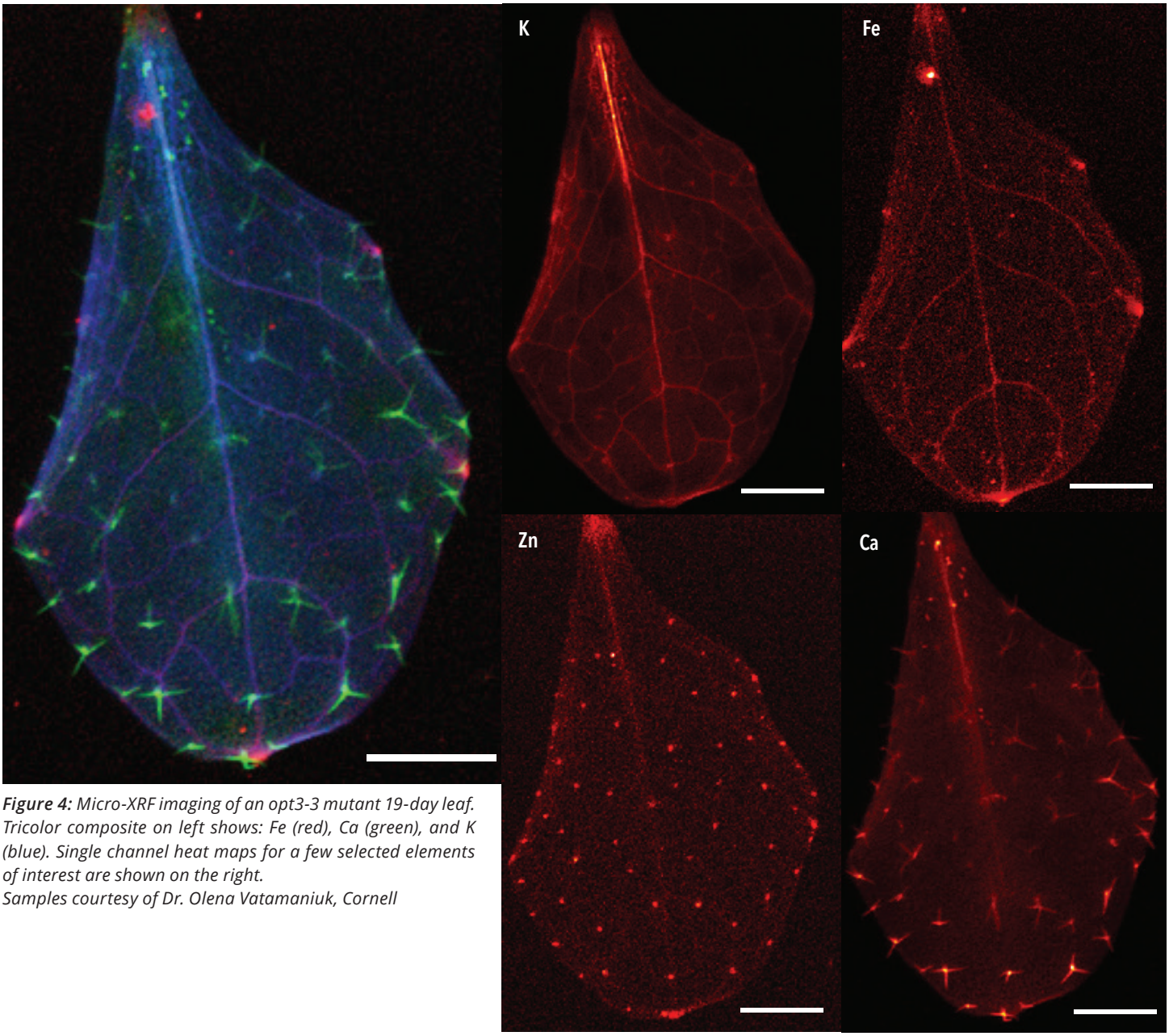

Figure 4: *Micro-XRF imaging of an opt3-3 mutant 19-day leaf. Tricolor composite on left shows: Fe (red), Ca (green), and K (blue). Single channel heat maps for a few selected elements of interest are shown on the right.*
Samples courtesy of Dr. Olena Vatamaniuk, Cornell

For the 16-day leaf (Fig. 3), an area of 3.5 mm x 3.8 mm was mapped at 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. Note that although tungsten (W) was selected because of the interest across the broad range of elements, a copper (Cu) target is optimal if Fe (6.4 keV) is of sole interest. Follow-up studies for even better Fe sensitivity can be uniquely achieved in the AttoMap using its patented multi-target x-ray source.

The 19-day leaf (Fig. 4) scan area was 4.0 mm x 8.3 mm at a spot size of 10 µm and a step size of 15 µm. 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. The Fe concentration in both the 16-day and 19-day leaves was found primarily at the minor veins of the leaves, located near the hydathodes (leaf pores) and toward the leaf blade periphery, with increased

Fe accumulation in the older leaf's central minor veins. Because increased Fe was found in locations where OPT3 is preferentially expressed, the results indicate that OPT3 may be crucial for loading Fe back into the phloem, the vascular tissue that conducts sugars and nutrients from the leaves back downward to the stems to support plant development. In comparison, the wild type leaf showed significantly lower Fe distribution in the leaves, with accumulation seen only at a small outermost edge.

Studies by Prof. Olena Vatamaniuk of other elements involved in the transportation of water and solutes from leaves, such as potassium and calcium, did not show statistically significant differences in wild type versus opt3-3 distributions. Such findings indicate that overall loading and transportation of other nutrients does not appear to be affected, supporting the hypothesis that OPT3 affects Fe-specific pathways.

Summary

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

The AttoMap provides not only distribution imaging of elements, but can also be used to quantify the relative amounts of each element. Future exciting 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) that allows for roots in soil and/or uneven surfaces, such as leaves, to be characterized.

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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