The rate of human population growth has been dramatically increasing. It took us tens of thousands of years to reach one billion people but in the last fifty years, the global population has increased by one billion people nearly every ten to fifteen years. This increase also applies to livestock, whose population keeps increasing to meet the demand for animal protein and dairy products. Throughout history, farming has provided us with what we call the three Fs: Food, Feed and Fiber. Considering a crude oil shortage is on the horizon, agriculture will also have to provide polymers and fuel for future generations. Altogether, these combined factors give us an incentive to make agriculture more productive.

After every harvest, mineral nutrients are extracted from the field. To maintain adequate soil fertility and production yield, they must constantly be replenished. These nutrients can be replenished by fertilizers, applied to the soil, seed, or leaves. However, the intrinsic low absorption efficiency of these nutrients by plants or seeds can result in hyper accumulation in the environment, causing injuries for microorganisms and water contamination. Moreover, as with crude oil, the reserves of some of these nutrients are limited. This means that they must be used as efficiently as possible.

Enhancing the ability of plants to absorb and metabolize nutrients from fertilizers is a fundamental step towards increasing the efficiency of use and decreasing any collateral environmental damages. To reach this goal, one has to understand the mechanisms of nutrient absorption, transport and metabolism of plants. Thus, one can engineer more efficient fertilizers. Plant scientists have employed a pool of techniques that allow investigating plant metabolism, such as enzymatic analysis and gene expression, in addition to quantitative methods to evaluate the content of nutrients like atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).
However, most of these techniques interrogate the plant ex vivo. In this context, X-ray fluorescence (XRF) is a potential tool for investigating plant nutrition while the plants are alive. XRF presents unique features, which fit perfectly for in vivo analysis. For this application, we consider the non-destructive aspect the most appealing one, allowing either a single shot, sample evaluation or temporal behavior studies, e.g. kinetic assays. Furthermore, the multi-elemental and simultaneous qualitative and quantitative analysis, the limited spectral interferences, mg kg⁻¹ limit of detection levels, direct measurement for solid samples, and the X-ray probe depth, should not be forgotten. Furthermore, since XRF is a well-established analytical technique, many quantitative approaches are already available, such as external calibration, standard addition, fundamental parameter, emission-transmission methods, and others. Our group has explored the attributes of microprobe XRF to investigate the mineral nutrition of plants (Figure 1).

Nutrient deficient seeds are not only a poor source of mineral nutrients for consumers, but these seeds also result in less vigorous and low productivity plants². Priming the seeds with micronutrients is a convenient way to circumvent this problem³⁴. Figure 2 shows a Phaseolus vulgaris seed, also known as a kidney bean, which was treated with CuSO₄(aq) at 1,000 mg Cu L⁻¹. The procedure was intended to evaluate the Cu transfer to the seed and ultimately to the plant. The seed was split with a stainless steel blade, the area depicted by the red rectangle was scanned using the 30 μm X-ray beam, matrix of 64 x 50 points and 25 μm Ti filter. To build up the quantitative Cu map, we used the emission-transmission method⁵. The instrumental sensitivity was determined by measuring a Cu Micromatter™ standard and the absorption correction was calculated using a Cu disc irradiator. We checked the trueness of this procedure by measuring a cellulose pellet spiked with a known concentration of Cu. The limit of quantification (10σ) of the method was 5.8 μg Cu cm⁻². The map showed that Cu formed a decreasing concentration gradient towards the seed’s inner region. Nevertheless, most of Cu remained trapped within the seed coat, forming a hotspot in the hilum spongy tissue.

Soybeans (Glycine max) are the most important source of vegetal protein worldwide. In terms of volume, potassium is the second mineral nutrient required by this crop. It is only behind nitrogen, which is naturally supplied to the plant by the rhizobium bacterial community which lives in association with the plant roots. Hence, in practical terms, K⁺ is a key nutrient that farmers must broadcast in the largest quantity in order to produce soybeans properly. Rubidium cations present similar physical-chemical properties to K⁺ and are usually employed as a tracer for K absorption. We have explored in vivo XRF microanalysis to characterize K⁺/Rb⁺ uptake. Roots of soybean plants at the V1 stage were immersed in 15.0 and 7.5 mM RbCl(aq). The plants were loaded into the Orbis PC and the measurements were performed using the 1 mm collimator. We analyzed the stem 30 mm above the root crown, and monitored the passage of Rb as shown in Figure 3(a). Figure 3(b) presents the Rb XRF net counts as a function of time. One can notice that Rb⁺ absorption followed a linear behavior, and the rate of uptake was dependent on the Rb⁺ concentration. This strategy can be employed to investigate the simultaneous absorption of other nutrients.

Foliar fertilization is one of the most common approaches of supplying plant micronutrients. In this procedure the losses due to soil leaching and adsorption can be reduced⁶. Additionally, for some nutrients the foliar absorption takes place faster than through soil fertilization. One of the main challenges consists in developing formulations that enhance the absorption process, while at the same time avoid phytotoxicity. The design of new foliar fertilizers requires an understanding of how nutrients are absorbed and transported. Figure 4 presents a sequence of chemical images in which we monitored the absorption and movement of Zn in a soybean leaf. A 50 μL droplet of ZnSO₄(aq) was deposited and caste on the down side of the leaf.
Then, using the 30 μm X-ray beam, matrix of 32 x 25 points and the 25 μm Ni filter, we mapped the area after three, 24 and 48 hours after the application. The images show that the Zn spot shape was modified as a function of time, demonstrating the Zn transport after absorption into the leaf veins. This approach has allowed us to compare the performance of several nutrient sources, as well as, the effect of surfactants and adjuvants on the absorption and transport of nutrients.

XRF is a formidable tool for investigating nutrient spatial distribution and dynamic processes taking place in different plant organs in vivo. The insights presented here can easily be transposed to other crops beyond kidney beans and soybeans. The sensitivity for lighter elements such as P, S, and K can be increased using a helium flush system to purge the path between the sample and X-ray detector with helium enriched gas. In addition, optimization of primary beam filter selection and X-ray tube current and voltage settings has been useful to enhance the element signal-to-noise ratios for various nutrient studies. Besides plant science, our group is currently working on the development of sample environments to investigate in situ processes taking place in the soil and rhizosphere.

Figure 3. (a) Picture of the stem region were the Rb was monitored, the red circle indicates the location of the X-ray beam. (b) Rb-Kα XRF net counts as a function of time of exposure, the root uptake of Rb⁺ followed a linear function of time and the slope was higher when roots were dipped in 15.0 mM than 7.5 mM.

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Written by Marcos H. F. Gomes, Nádia M. Duran, João G. B. Cassanji, Eduardo S. Rodrigues, Tatiana N. M. da Cruz, Eduardo de Almeida and Hudson W. P. Carvalho, Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil

References

It’s not often I write in the first-person, but my recent webinar, Learn How I Prepare Samples for EBSD Analysis, has given me this opportunity. When putting this webinar together, several different titles were proposed and discussed. One was “Learn How You Should Prepare Samples for EBSD.” I didn’t like this because I’m not 100% sure if my methods are how you should prepare samples, but I did want to share how I prepare samples, and give examples from my experiences.

I mention this because I don’t consider myself a sample preparation expert. I do however have a lot of experience preparing samples for EBSD. As an analytical technique, EBSD is often only as good as the quality of patterns that can be obtained. When we strategize about different ideas we could implement in our software, we assume that a sample will give an EBSD diffraction pattern. It’s not that sample preparation is difficult, but it is meticulous and having the right equipment and consumables can make things easier. My hope is that sharing my thoughts and experiences will make it easier for you to get the EBSD results you want.

**Goal of EBSD Sample Preparation**

My goal when preparing a sample for EBSD analysis is to obtain a representative crystal lattice at the sample surface that is of sufficient quality to produce an EBSD pattern I can index. Decreased lattice quality will manifest itself as a loss of sharpness of the bands within the EBSD pattern, as seen in Figure 1. If the lattice is inherently poor, from deformation for example, then that tells me something about the sample. What I don’t want to do is infer something about the sample from the pattern quality measurements that is actually a sample preparation artifact. It’s because of this, that I recommend using the same preparation procedure when comparing samples to keep as many variables as possible constant.

I like to mount my samples in metallographic mounts for preparation and analysis. This is because it’s easier for me to control the force applied to the sample with the equipment I have using the mounts. Generally, I use 1” mounts, as EBSD requires a 70° sample tilt, and a smaller diameter mount gives me more of a safety margin when analyzing across the entire prepared surface. One trick I use is to offset the sample to one edge, so that it’s easier to position the sample at tilt. Typically, I use a hot mount press with thermoset resin. This allows me to quickly mount the sample for preparation in around 10 minutes. If a sample cannot tolerate the heat or pressure, then I use an epoxy mix. With this approach I plan on an overnight curing time before preparation. I like to use a conductive mounting material, because anything that reduces charging effects will help improve EBSD mapping quality. With the hot mount, I use a conductive thermoset material, while with the epoxy, I mix in a conductive filler. These are not perfect conductors. I’ll often check the conductivity between the sample and the SEM stage using a voltmeter, but if it reduces resistance it will help. In either case, I just follow the recommendations of the supplier for how to use the material.

**Grinding a Sample**

While grinding and polishing are very similar, with both using a sized abrasive to remove material, I typically differentiate grinding as using abrasive papers and polishing as selecting a cloth and adding an abrasive. When we first started polishing samples, we struggled with consistency. To help improve our capabilities, we attended a course on sample preparation from ASM International. Our primary takeaway was that consumables are meant to be consumed, and that the lifetime of the SiC grinding papers is short. When we started using new SiC papers for each sample, our preparation and EBSD pattern quality improved immediately. Our grinding steps are listed in Table 1. The grinding time for each grit size is one minute. Personally, I use two papers for 30 seconds each. I do this in case I’m polishing multiple samples and I want to make sure I am getting good material removal at each step. On our machine, we can prepare a single sample using individual sample force, or a set of samples using a single holder. The force specified in the table is per sample, so you can use the area of the sample to determine the pressure. The wheel direction refers to the direction of wheel rotation relative to sample head direction. Complementary refers to both rotations in the same direction. This reduces the removal rate, but also helps keep the front and back sides the sample parallel. This is important because we want to try and control the analysis plane for improved spatial positioning accuracy relative to the tilt of the sample.

![Figure 1. EBSD Patterns from ZrO2. Blurry bands in patterns (left) are due to sample preparation. After improved final polish, bands are much sharper (right).](image-url)
Polishing a Sample

For polishing, I use Imperial cloths from Allied High Tech. This cloth is described as low-napped, synthetic rayon and a good all-purpose polishing cloth. I then use alumina (Al₂O₃) polishing suspensions. I use pre-mixed formulas, as they are easier and cleaner to store in the lab. I pre-wet the cloth, and then add the abrasive to the wheel. I polish longer (10 minutes each step), with a slightly lighter force and slower wheel rotation, but with the wheel and head in contra directions. I am careful to make sure there is adequate suspension throughout the time, but I am manually monitoring this and adding as necessary. There are automatic dispensing systems available, and I have one on my wish-list. This would allow me to more fully specify my process for repeatability. If you check out the webinar, there are videos that show this process.

Final Polishing

I’m a big believer in a good final polish. Often people are familiar with the initial polishing for general microscopy, and those procedures can produce some EBSD diffraction patterns, but a good final polish can significantly improve your pattern quality and EBSD results. I typically use a vibratory polisher, which provides a continuous gentle final polish to remove any residual deformation from earlier polishing steps. I use this in conjunction with colloidal silica suspensions, which have a 9.8 pH value and provide chemical-mechanical polishing for damage removal. 0.05 µm is the most commonly used abrasive size, but I will also use a 0.02 µm solution for pure metals or heavily deformed materials. I don’t always have a fixed polishing time in mind. With the vibratory polisher, I can leave it on based on my schedule. I’ll often target 2-4 hours for polishing. The one thing I aim for is to keep time constant for samples that I am trying to compare. If I am using the smaller abrasive, then I tend to polish longer. With the vibratory polisher, I again use the Imperial cloth. I try to keep it clean to maximize lifetime. I usually use a cloth and solution until it solidifies, and if I can minimize the dust that acts as solidification nucleation sites, I can prolong the lifetime.

Summary

This is the standard procedure I use for most materials that come into our lab for analysis, and I try it whenever possible. The better the initial EBSD pattern quality, the more useful results you can get through EBSD pattern indexing and mapping analysis.

Written by Matt Nowell with reference to the EDAX “Learn How I Prepare Samples for EBSD Analysis” webinar.

<table>
<thead>
<tr>
<th>Step</th>
<th>Abrasive</th>
<th>Polishing Cloth</th>
<th>Time (minutes)</th>
<th>Force (per sample)</th>
<th>Wheel Speed (RPM)</th>
<th>Wheel Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 µm Al₂O₃</td>
<td>Imperial</td>
<td>10</td>
<td>9 lbf</td>
<td>130</td>
<td>Contra</td>
</tr>
<tr>
<td>2</td>
<td>0.3 µm Al₂O₃</td>
<td>Imperial</td>
<td>10</td>
<td>9 lbf</td>
<td>130</td>
<td>Contra</td>
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2017 Worldwide Events

July 31-August 4
Denver X-ray Conference
Big Sky, MT

August 6-10
Microscopy & Microanalysis (M&M) 2017
St. Louis, MO

August 20-25
International Materials Research Congress
Cancun, Mexico

August 21-25
Microscopy Conference (MC) 2017
Lausanne, Switzerland

September 13-15
Materiallographie
Aalen, Germany

September 17-22
Midwestern Association of Forensic Scientists
Cincinnati, OH

September 24-29
Multinational Congress on Microscopy (MCM)
Rovinj, Croatia

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Materials Science & Technology (MS&T) 2017
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- Weiterstadt#

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- November 13-17
- Tilburg*
- Weiterstadt#

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- Tilburg*

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- November 9-10
- Tokyo
- Osaka

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Jerry Jasso

Jerry joined EDAX as the United States Midwest Sales Manager in 2016. He is responsible for all EDAX’s customers and potential customers in Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Nebraska, Ohio, Wisconsin and West Virginia.

Prior to EDAX, Jerry served as the lone Field Sales Representative at Ted Pella, Inc. from 2009-2015. He oversaw all vacuum evaporation/sputter coating systems sales, training, and troubleshooting in the U.S., Canada, and Mexico. From 1998-2005, Jerry worked at DAKO Corporation, an Agilent Company. As a Technical Service Representative, he learned sales techniques and transitioned into a pure sales position as the company’s Midwest Account Manager. From 1990-98, Jerry served as the Electron Microscopist/Medical Imaging Specialist for the Division of Pathology at the Children’s Hospital Medical Center of Akron, OH.

Jerry became the Core Electron Microscopist/Histotechnologist at the Cleveland Clinic Foundation’s Division of Research in 1983. In 1986, he joined the Cleveland Research Institute as a Photomicroscopist/Photomicrographist. He assisted with the purchase of two SEMs, and EDAX microanalysis equipment.

Jerry earned a bachelor’s degree in microbiology from the University of Akron and later went back to school and received his Master’s in Business Administration from Kent State University in 1996.

Jerry resides in Akron, OH with his wife Cindy and their two long-haired Chihuahuas, Dakota and Rex. The couple has four children, Zack, Adam, Lindsey, and John. In his spare time, Jerry enjoys golf, bicycling, photography, fly-fishing, impressionist art, and music. He is involved with the Boy Scouts of America and participates in Seventh Day Adventists church building.

Zhang Wenfeng

Zhang joined EDAX as a Sales Manager in Southern China in November 2015. His responsibilities include sales and working with customers and key accounts, especially electron microscope manufacturers, in the region.

Prior to EDAX, Zhang was a sales manager at Shengfan Electromechanical Company, where he sold X-ray Fluorescence (XRF), Inductively Coupled Plasma (ICP), microscopes, and other equipment. In 2003, Zhang graduated with a degree in physical optics from Wuhan University in Hubei, China.

Zhang currently resides in Guangzhou with his wife, Toka and their sons, Bobby (eight) and Harry (four). In his spare time, he enjoys mountain climbing and Chinese Kung Fu, including Tai Chi Chuan and nunchucks.
One of six departments in the School of Engineering at Jönköping University, the Department of Materials and Manufacturing focuses on research into manufacturing processes, solidification, materials characterization and properties modeling of cast materials, surface technology, and simulation methodology. New areas, such as polymer or advanced alloy design are also being explored. The department closely collaborates with companies in related industries, including aerospace, automotive, and energy suppliers. The researchers at Jönköping University have a wide range of testing equipment, however their daily activities involving materials characterization focus around the utilization of SEMs.

The department’s PhD students and Postdocs are the main users of the SEMs. In addition to his research and pedagogical duties, Dr. Ehsan Ghassemali is responsible for developing and supervising the analytical microscopy research activities in the department. He also coordinates procurement, installation, training, and usage of the FIB/SEM and sets up workshops and seminars. The department utilizes EDAX Trident EDS-EBSD-WDS Analysis Systems on both their SEM and FIB/SEM.

The main purpose of the Department of Materials and Manufacturing is to develop knowledge about the relation between material composition, melt quality, process, geometry, and defect formation, as well as their relationships to the mechanical and physical properties of materials and components. This information provides the basis for mathematical modeling and simulation of cast components, allowing researchers to examine various phenomena for situations where it is difficult or even impossible to carry out relevant experiments.

The major focus of the research is on the overall and local static (e.g. tensile, compression) and dynamic (e.g. fatigue, creep) properties of components under various conditions, such as room and elevated temperatures, corrosive environments, etc. This information is then used to design and develop new metallic alloys that are durable in harsh working conditions, such as those in the energy, transportation, and aerospace industries.

“Analytical microscopy in 2D and 3D is one of our core activities in the department,” said Dr. Ghassemali. “The EDAX Trident package provides a seamless and user-friendly platform that fulfills our needs in this regard. Failure analysis and alloy development are impossible without analytical microscopy. The EDAX detectors together with the TEAM™ software provide a unique opportunity and proper freedom for our researchers to explore and justify the physical, mechanical, and chemical behavior of cast components.”

For more information about the Department of Materials and Manufacturing at Jönköping University visit: http://ju.se/en/research/research-groups/materials-and-manufacturing.html