An Innovative Method for Imaging and Chemical Analysis of Wet Samples in Scanning Electron Microscopes

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Introduction

Electron microscopy (EM) of fully wet samples is a valuable tool for studies in the material, medical and biological sciences. In order to appreciate the natural structures of tissues or materials they should be examined in their native wet state, as opposed to a dry form that incorporates artifacts of sample processing. Viewing and analyzing wet samples at high resolution has undergone a significant improvement only recently due to the innovative WETSEM™ technology developed by QuantomiX.[1,2]

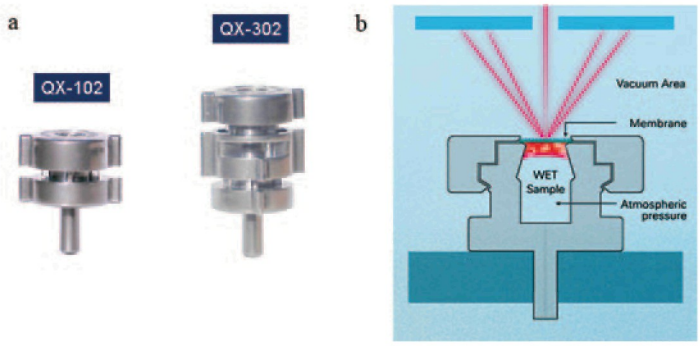

Figure 1 WETSEM™ technology: Fig 1a shows pictures of the Quantomix capsules QX-102 and QX-302. Fig 1b is a schematic representation of WETSEM™.

QuantomiX' capsules for WETSEM™ technology rely on a thin electron-transparent membrane that enables separation, and thereby protection, of the wet sample from the electron microscope vacuum (Figure 1a). Imaging is performed using a standard Scanning Electron Microscope (SEM) combined with a Back Scattered Electron (BSE) detector (Figure 1b). Our investigations into applications of WETSEM™ have highlighted some general advantages of using this new technology beyond its most obvious benefit: the revolutionary capability to image wet samples in an EM. A significant advantage of WETSEM™ over other electron microscopy techniques is the ability to image samples a few millimeters thick without any time-consuming, costly processing such as thin sectioning, embedding, freezing or coating. Our method of imaging is based on detection of BSEs, which result only from interactions of the electron beam with a thin membrane-proximal layer. Accordingly, any material beyond the layer of beam penetration is effectively invisible. The use of a BSE detector results in an image based on material contrast. Image contrast can sometimes be enhanced by staining samples with electron-dense stains. In summary, WETSEM™ combines the simple, rapid sample preparation of light microscopy with the high resolution capacity of EMs. It provides an ideal solution for immediate high resolution imaging of wet samples without drying artifacts. In this article we summarize recent developments and applications of this novel technology relevant to the material sciences.

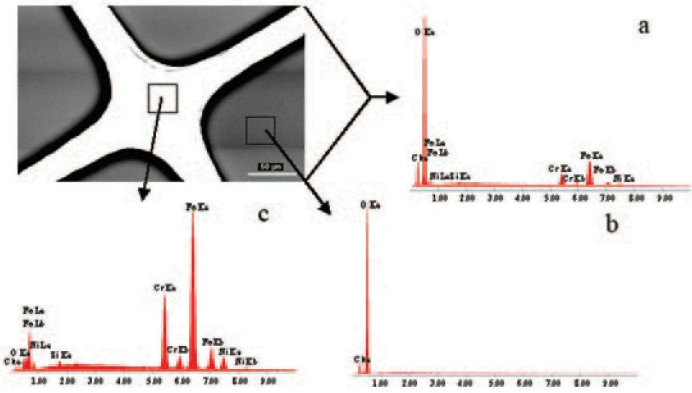

Figure 2 EDS using WETSEM™: Image of a capsule filled with double distilled water. EDS of image full-frame (a). EDS of area outside grid (b). EDS of area within grid (c). Bar: 50μm

Energy Dispersive X-Ray Spectroscopy (EDS) of Wet Samples

Characterization of chemical microstructures is an important application of SEM. However, before the development of WETSEM™ technology this technique could not be applied to samples that are fully wet and under atmospheric pressure. We demonstrate here qualitative elemental microanalysis of samples in their native, fully wet state.

A sample is placed in a sealed QX capsule and analyzed using a conventional SEM equipped with an energy dispersive x-ray spectrometer. In Figure 2 water was placed inside the QX

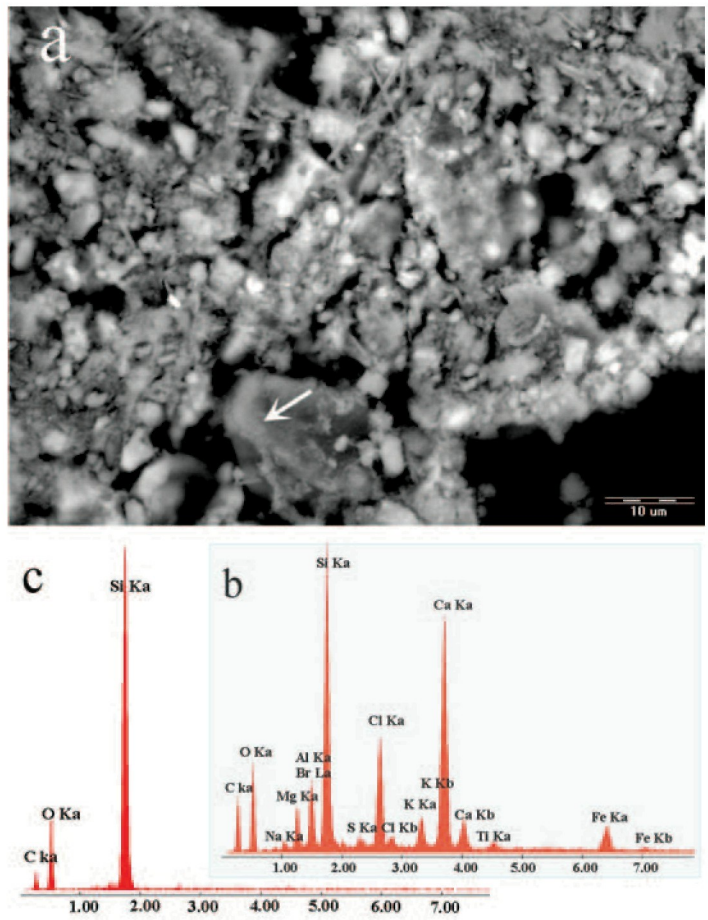

Figure 3 WETSEM™ image and EDS of Dead Sea mud: Image of Dead Sea mud (a). EDS spectrum of image full-frame (b). EDS of area marked by arrow in image 3a (c).Bar: 10 μm

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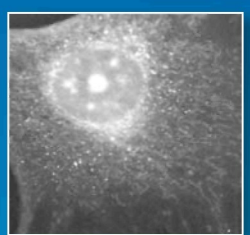
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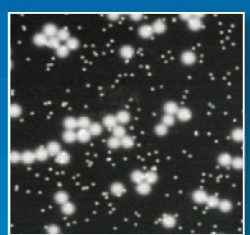
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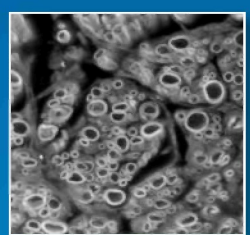
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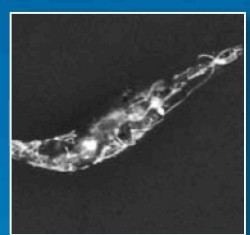
Trypanosoma brucei

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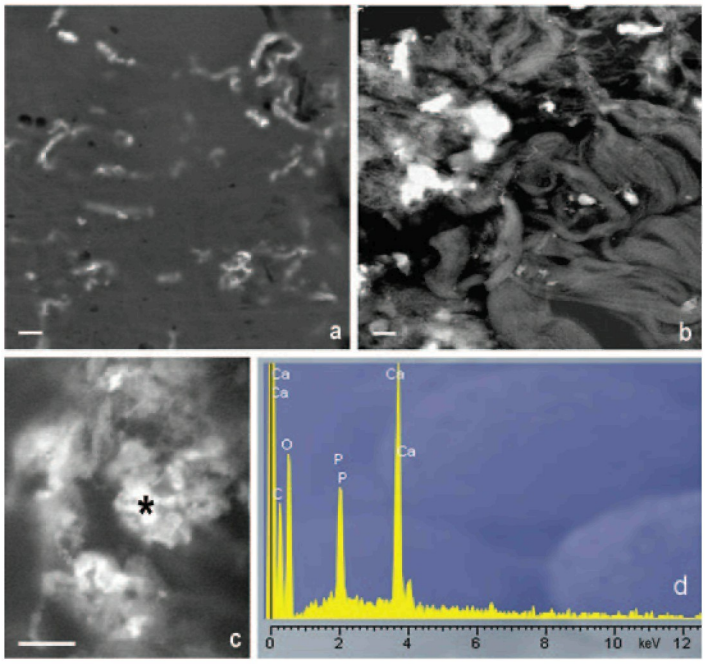


*Figure 4 WETSEM images of PXE dermal biopsies (bar:10 μm): Skin biopsy unfixed and unstained (a), fixed and stained with uranyl acetate (b), high magnification image of mineralized elastin (c) and microanalysis (d) of mineralized area of elastin (denoted by * in 4c). The biopsies were placed in the QX capsule and observed and analyzed by conventional SEM equipped with an EDS detector. (Images and EDS spectrum courtesy of Daniela Quaglino et al.).*

capsule and analyzed by EDS. The oxygen characteristic peak is readily observed (Figure 2b). The contribution to the EDS spectrum of the thin membrane is negligible, a small carbon peak that does not interfere with microanalysis (Figure 2b). The metal grid (bright cross in the image) does generate a large EDS signal (Figure 2c). When the analyzed area contains part of the grid, the spectrum will include the grid signature (Figure 2a) as well as the oxygen and carbon peaks. However, when the analyzed area does not contain the grid, it will not contribute to the elemental signature (Figure 2b).

In Figure 3 we present a WETSEM™ image (Figure 3a) and an EDS spectra of a sample of Dead Sea mud (courtesy of AHAVA cosmetic laboratories). In figure 3b the elemental signature from the area seen in the image is shown ('full-frame' analysis). In figure 3c we demonstrate 'spot' analysis within that area (see arrow in figure 3a) that identifies silicon as the major constituent at that position. When using EDS the natural heterogeneity of the sample is retained and adjacent structures can be directly compared by spot analysis.

The ability to carry out EDS of wet samples has excited the interest of biologists as well as material scientists. Pseudoxanthoma elasticum (PXE) is a genetic disorder affecting the skin, eye and cardiovascular system. The majority of disease alterations can be attributed to degeneration of elastic fibers that undergo progressive mineralization and fragmentation. To date, costly and time-consuming light microscopy and EM analyses of skin biopsies, that require large amounts of material, are the means to identify mineral precipitates and diagnose PXE. A new study has demonstrated that WETSEM™ technology enables rapid, accurate localization and detection of mineralized areas in unfixed and unstained as well as fixed and stained small PXE biopsy specimens (Figure 4).[3] The mineralized areas are easily observed in images of both unstained and stained samples (Figures 4a and 4b). Since the mineralized areas are rich in elements with higher electron density than the rest of the tissue, they appear brighter. Moreover, EDS allowed delineation of the elemental composition of the mineralized areas of elastin (Figures 4c and 4d; note the EDS characteristic peaks of Ca and P) and even the relative ratio of element's deposits.[3] Thus, WETSEM™ may be the means to rapidly and economically diagnose PXE and perhaps other tissue disorders that involve ion precipitation. It is notable that these experiments employed a newly developed QX capsule (QX-302). This capsule is designed to facilitate WETSEM™ of tissues and other soft wet samples. It has a positioning element to place samples next to the membrane (Figure 1A)

WETSEM™ of Native Material Samples

Before the advent of WETSEM™ it was not possible to use EM to image particles in suspension. The samples had to be dried in order to be imaged. However, after drying much of the information on the distribution of particles in the solution was lost since aggregation often occurred during the drying process. The two images of Figure 5 illustrate the different structures seen by SEM depending on the hydration status of a sample. The images show Ag nanoparticles that were synthesized by reduction of silver ions in water and stabilized with a negatively charged cupping agent. The wet sample was prepared by inserting 15 μl of the suspension into a QX-102 capsule with a poly-l-lysine layer coating the membrane. The poly-l-lysine (positively charged polymer) layer is essential in order to assure close proximity of the particles to the membrane. The dry sample was prepared by drying a drop of the Ag nanoparticle suspension on a SEM stub covered with a carbon tape. It is clear that little information on the degree of aggregation or isolation of the particles in the wet environment (Figure 5a) can be gained from the image of the dry sample (Figure 5b).

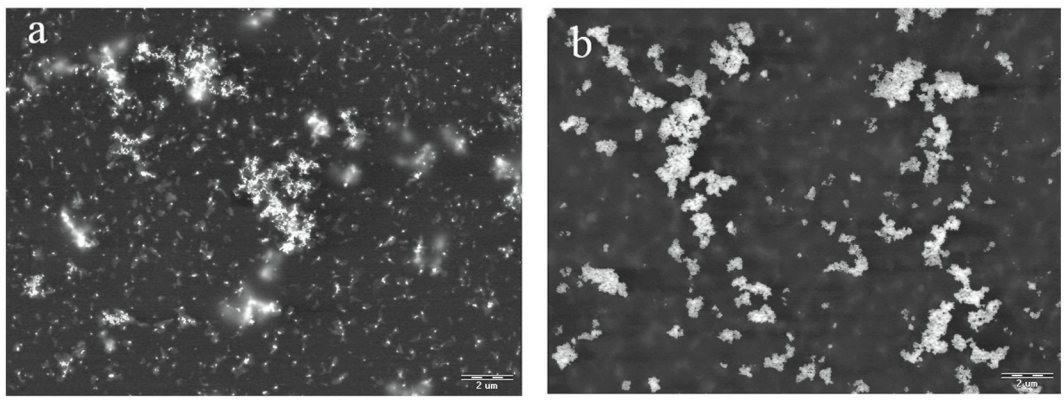
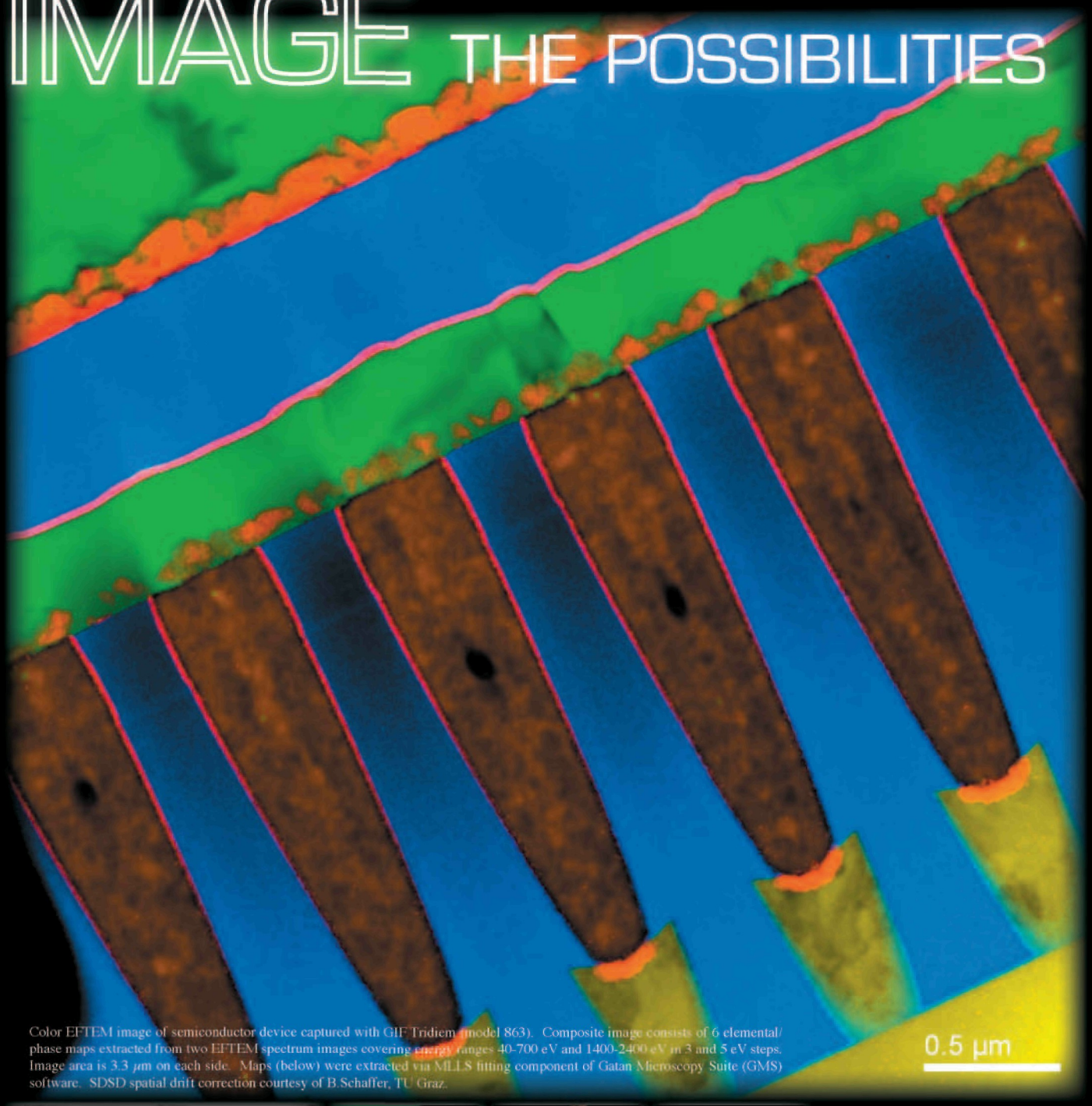
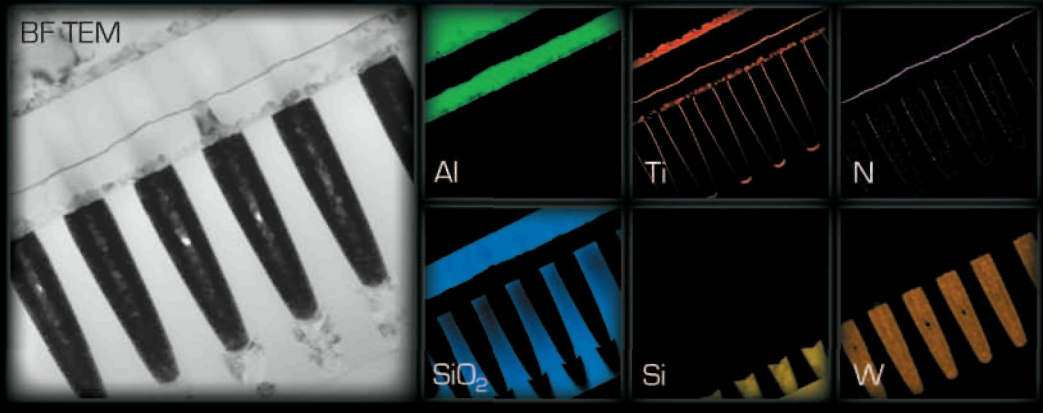


Figure 5 WETSEM™ of Ag nanoparticles: Wet sample (a). Dry sample (b).

IMAGE THE POSSIBILITIES



Color EFTEM image of semiconductor device captured with GIF Tridiem (model 863). Composite image consists of 6 elemental/phase maps extracted from two EFTEM spectrum images covering energy ranges 40-700 eV and 1400-2400 eV in 3 and 5 eV steps. Image area is 3.3 µm on each side. Maps (below) were extracted via MLLS fitting component of Gatan Microscopy Suite (GMS) software. SDSD spatial drift correction courtesy of B.Schaffer, TU Graz.



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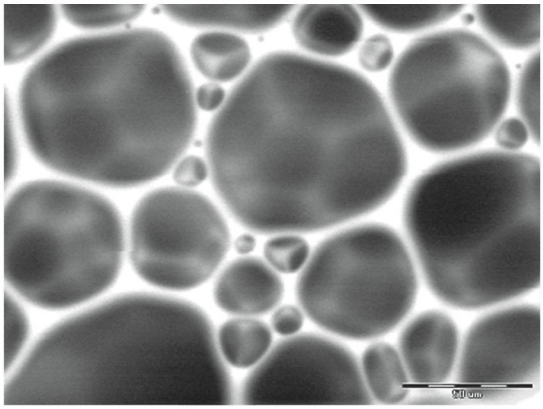

Figure 6: WETSEM™ of Shaving Foam

Examples of wet, or partially wet samples, or samples with unusual consistency that could not be imaged by SEM until now include shaving foam (Figure 6) and deodorant (Figure 7), a volatile material. WETSEM™ technology represents a potentially useful tool for formulation and control processes in commercial and industrial fields. A good example where WETSEM™ technology was used to monitor product quality is illustrated by the images in Figure 8. A sample of pesticide was viewed at lower (Figure 8a) and higher magnification (Figure 8b) to assess the proportion of pesticide contained within capsules designed for controlled release. The two phases can be distinguished easily and it appears that the majority of the active material (gray in the image) is not encapsulated, indicating the need to improve the formulation.

In summary, the innovative WETSEM™ technology facilitates economic, simple, rapid, high resolution imaging and EDS analysis of fully wet samples in a way that has not been possible before. Potential applications for the material sciences are only just beginning to be realized and tested.

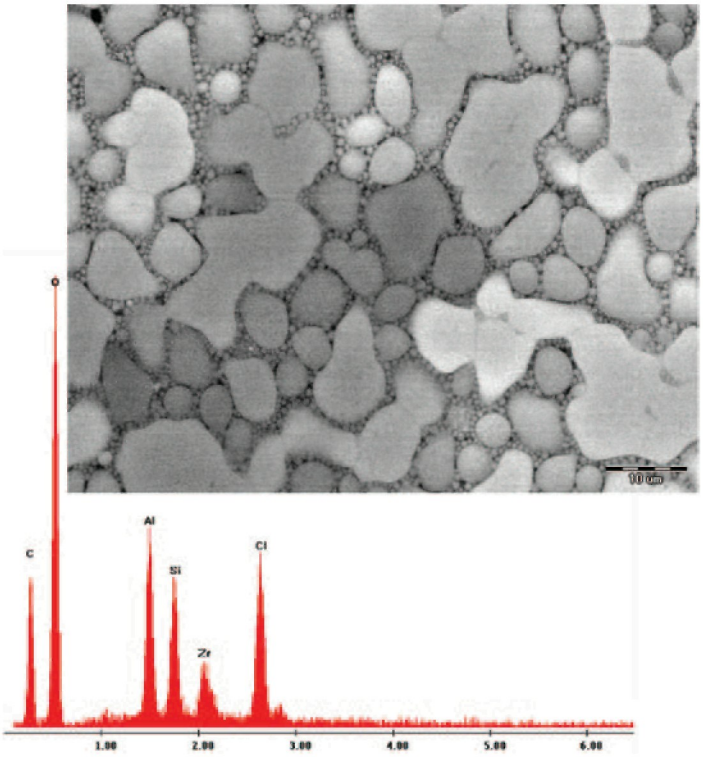

Figure 7: WETSEM™ image and EDS of deodorant. Bar: 10 µm

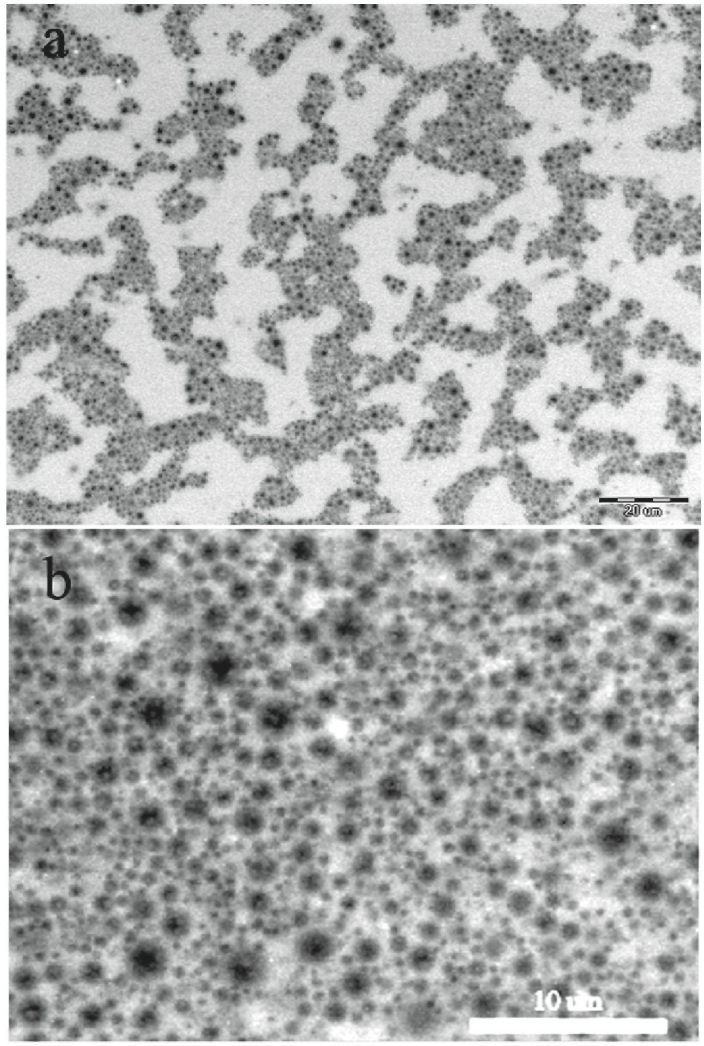

Figure 8 WETSEM™ of pesticide: Lower (a) and higher (b) magnifications

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