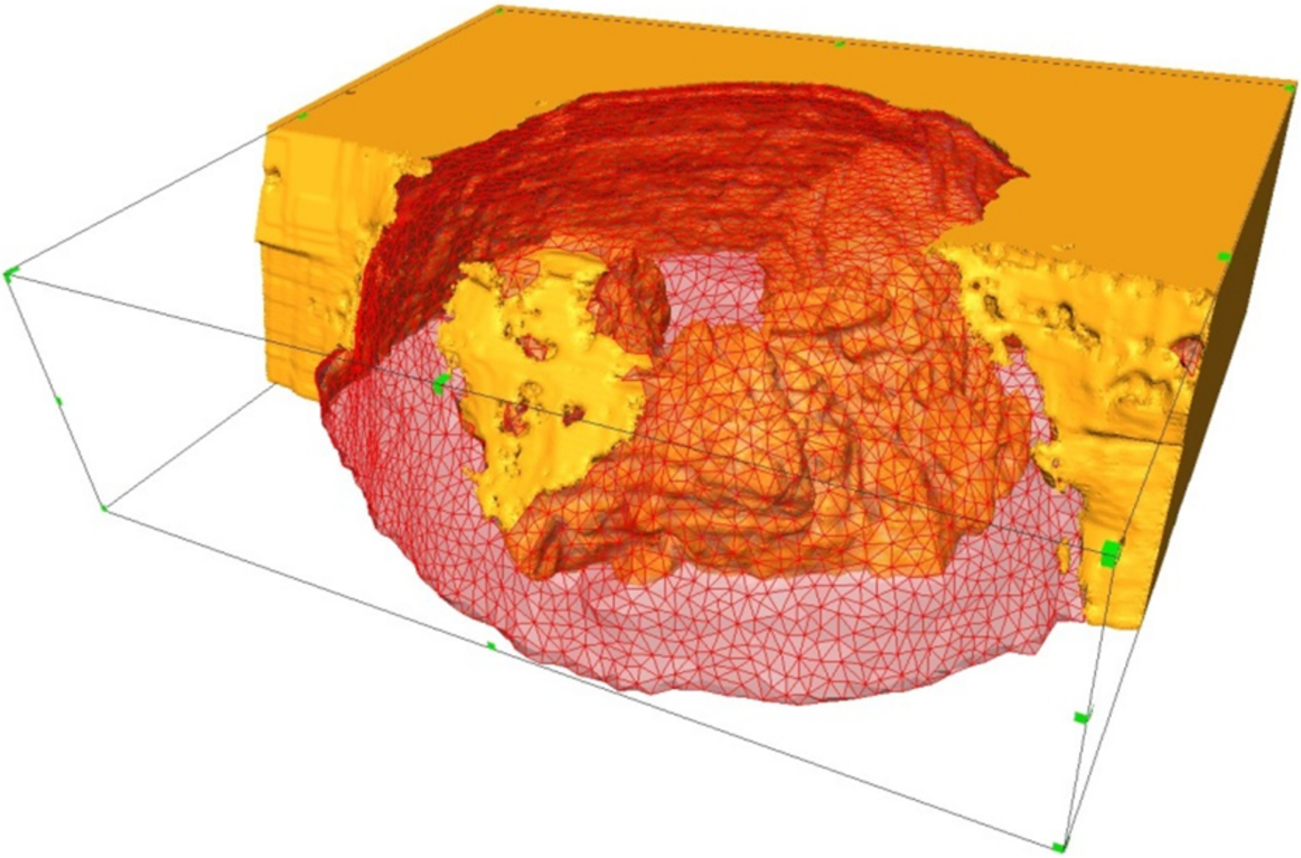
White Paper



The Microstructure of Consumer Products

Including Food & Personal Care Products –
a Cryo-FIB-SEM Investigation

We make it visible.

The Microstructure of Consumer Products

Including Food & Personal Care Products – a Cryo-FIB-SEM Investigation

Author: Caroline Remijn, Marjolein van Ruijven, Gerard van Dalen
Unilever R&D, The Netherlands

Andreas Schertel
Carl Zeiss Microscopy, Germany

Date: March 2014

The study of the microstructure of consumer products is of great importance to understand their properties and how the microstructure relates to texture, sensory characteristics, mouth feel and consumer preferences. It is used in product development to enable optimal conditions for processing and product quality.

Introducing

Combined focused ion beam (FIB) and scanning electron microscopy (SEM) is widely used in material and life science for the characterization of the microstructure in all three dimensions.[1] Up to now, the FIB-SEM has also been applied on a variety of biological samples. Recently volume imaging of the cellular ultrastructure in native hydrated frozen specimens using cryo FIB-SEM microscopy has been presented.[2] Biological samples and consumer products can contain water and have low conductivity and therefore are subject to charge build-up and electron beam damage.

Cryo electron microscopy can be used for preparing and imaging of low melting, beam sensitive or hydrated specimens in the frozen hydrated state.

The preparation starts with cryo-fixation by high pressure or plunge freezing. Under cryo-conditions, the vitrified specimen are transferred in a cryo-preparation chamber for fracturing, sublimation and cryo-sputter coating of a conductive layer on the surface.

Cryo-planing using a cryo-ultramicrotome, is a developed technique that enables 2D cryo-SEM observations of flat sample surfaces in contrast of the rich-textured and topographical surface of a freeze-fracture. A cryo-planed surface is an ideal starting point for a subsequent microstructural 3D cryo-FIB-SEM investigation because the exact region of interest can be easily located on the smooth block face exposing the microstructure. However, there are types of heterogeneous samples for which the method is far from being successful, particularly where there is a mixture of hard and soft material. Cutting can blur the interface between adjacent materials or tear a soft covering from a hard substrate.[3,4]

Some samples cannot be cut by ultramicrotomy because the sample contents may scratch the glass or diamond knife. By focused ion beam milling especially composites containing soft and hard materials can be cut.[5,6] In this White Paper the creation of 3D models of different consumer products by cryo-FIB-SEM microscopy is described.

Instrumentation

The results presented were obtained using ZEISS AURIGA FIB-SEM equipped with a Gatan Alto 2500 cryo preparation system and Oxford EDX detector 80 mm^2.

Experimental materials and methods

The experiments were conducted on different consumer products. A tiny volume of each sample (about 50 µl) was placed on top of a rivet and plunge-frozen in melting ethane. The sample was cryo-planed using a Leica Ultracut UCT EM FCS cryo-ultramicrotome to obtain a freshly cut block face. Cryo-planing was done first with section thickness of 80 nm and a speed of 50 mm/sec using a glass knife and the last sections were made at decreasing thickness, down to 30 nm, with a speed of 2 mm/sec using a DIATOME histo cryo 8.0 mm diamond knife at – 110 °C.

The rivet was mounted onto a Gatan cryo-holder and transferred into a Gatan Alto 2500 preparation chamber.
The sample was sublimated for 60 sec at – 95 °C and sputter coated with platinum for 120 sec at 10 mA current.
The sample was transferred into a ZEISS AURIGA FIB-SEM system and the cooling stage temperature was kept at – 125 °C for the whole time of investigation.

A slice of the material was sequentially removed using FIB milling followed by was sequentially removed using ion beam milling followed by SEM imaging of the freshly exposed surface. The slice thickness was between 25 and 75 nm for the shown examples but can be as small as several nanometers if required. In a completely automated process a stack of images was generated. The serie of 2D SEM images was aligned in x- and y-direction to eliminate any image shift between subsequent slices and aligned in z-direction by taken the slice thickness into account. The resulting 3D data cube can be resliced in any direction and contained structures can be rendered using 3D reconstruction software. A probe current between 500 pA and 10 nA was used for FIB milling. The images of the FIB milled cross-sections were scanned with tilt correction activated in order to compensate for the foreshortening of the y-scale in the tilted view image of the cross-section. Dual Channel Mode was used to record two separate detector signals simultaneously during a single electron beam scan.

The different consumer products: Knorr® bouillon gel, Prodent toothpaste, Dove® shower gel

Results

The first application example is Knorr® homestyle stock concentrated bouillon gel which contains water, oil and salt amongst others ingredients.

The FIB-SEM results of a cross-section through an oil droplet within the aqueous salt phase are shown in figure 1, where the oil is represented in dark grey in contrast to the salt in light grey. Salt has a higher average atomic number compared to the oil phase which gives brighter regions. In total 100 EsB images with a slice thickness of 75 nm are taken. The total milling and imaging time – oder alternativ process time was about 2 hours. An accelerating voltage of 2 kV is used.

In the reconstruction (figure 1C), the oil is shown in red and the aqueous salt phase in yellow. By reslicing of the reconstructed 3D model it can be observed that the salt crystal penetrated inside the oil droplet.

Another application example is the FIB-SEM investigation of a Prodent toothpaste microstructure.

In this case the FIB slice thickness was 20 nm and EsB and SE2 images have been recorded in parallel. An accelerating voltage of 1.5 kV for SEM imaging is used. In total 70 slices are milled. The EsB image provides material contrast, whereas topographical contrast is shown in the SE2 image.
The complementary information supports an accurate image interpretation and analysis.

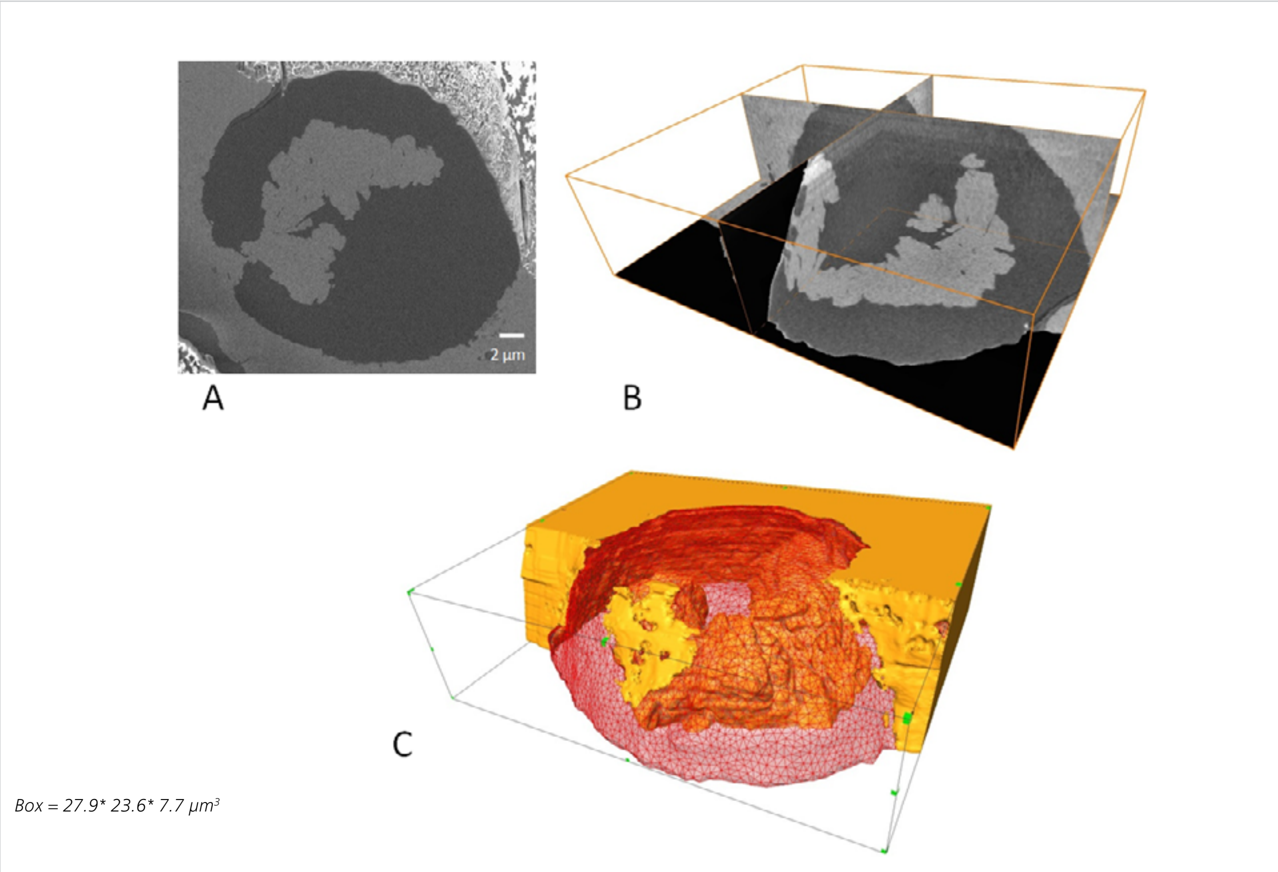
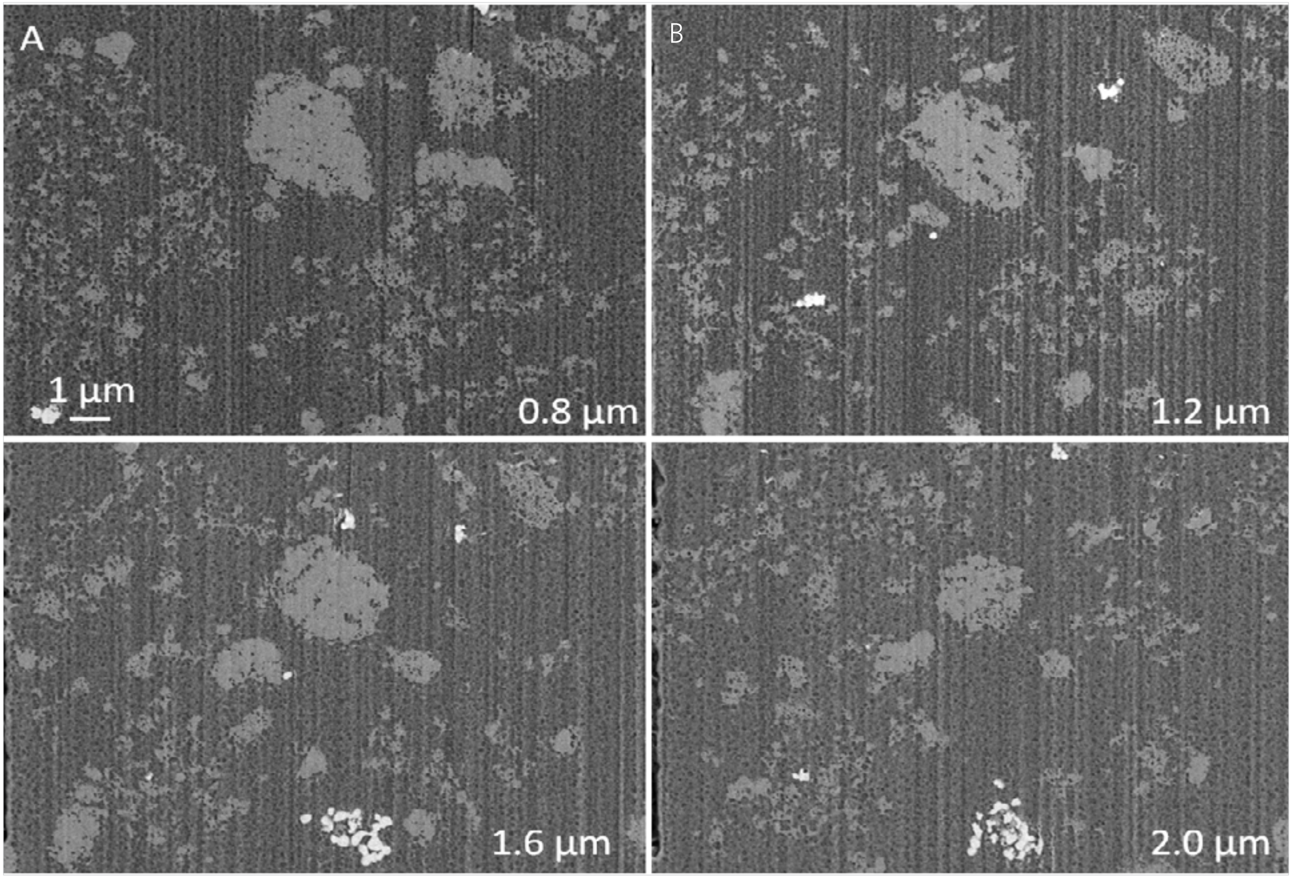


Figure 1 *FIB-SEM results of Knorr® bouillon homestyle stock concentrated.*
(A) Original recorded 2D EsB image of one exemplary slice. The image pixel size is 45 nm and the slice thickness is 75 nm.
(B) Reslicing through the resulting 3D model showing the reconstructed XZ-plane, the reconstructed YZ-plane and the XY-Plane which corresponds to the original recorded EsB images.
(C) Reconstructed 3D model showing in yellow the acqueous salt phase and in red the oil containing phase.

In figure 2 four EsB slices at different z depth are shown as an example.

With energy dispersive X-ray (EDX) analysis, silica is detected in the regions showing a medium grey level scale. The silica originates from the hydrated silica which is present in the Prodent toothpaste. The particles showing the brightest grey level values contain phosphor, probably derived from tetra potassium pyrophos-phate. With 3D reconstruction, the shape, size, orientation, dispersion of particles, and homogenous phases in the heterogeneous product become visible. Knowledge about the microstructure and dispersion of ingredients in toothpaste gives better understanding of stability, foaming behaviour, mouth feel and other product properties.



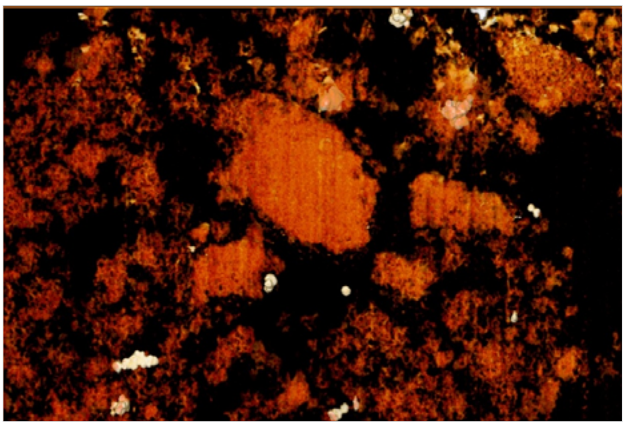


Figure 2 *FIB-SEM images of Prodent toothpaste at different depths (A), the total imaged volume is 11.6*9.4*1.3 µm^3 (pixel size = 12.5 nm). In (B) a topview from the 3D reconstructruction is shown.*

Another good example for the benefits of FIB cross-sectioning is microstructural cryo-investigation of Dove® shower gel. The shower gel contains ingredients with different hardness. For this reason cryo-planing does not always successfully generate a flat and smooth cutting face. FIB milling is well known to generate smooth cross-sections of heterogeneous materials containing soft and hard materials in contrast to smearing, collapsing structures and other preparational artefacts of any mechanical technique.

The data cube consists of in total 100 slices with a slice thickness of 50 nm. In Dual Channel Mode, EsB and SE images are recorded simultaneously with a lateral pixel size of 20 nm. An accelerating voltage of 0.9 kV is used. A set of EsB slices at different depths exemplifies the microstructure of platelets in figure 3A, an exemplary SE image displays the microstructure of vesicles between the platelets in figure 3B. Some curtaining (vertical lines) can be observed in the SE image which is an FIB milling artefact due to particles on top of the sample surface or sample surface topography. The reconstructed 3D model of the microstructure of platelets is presented in figure 4.

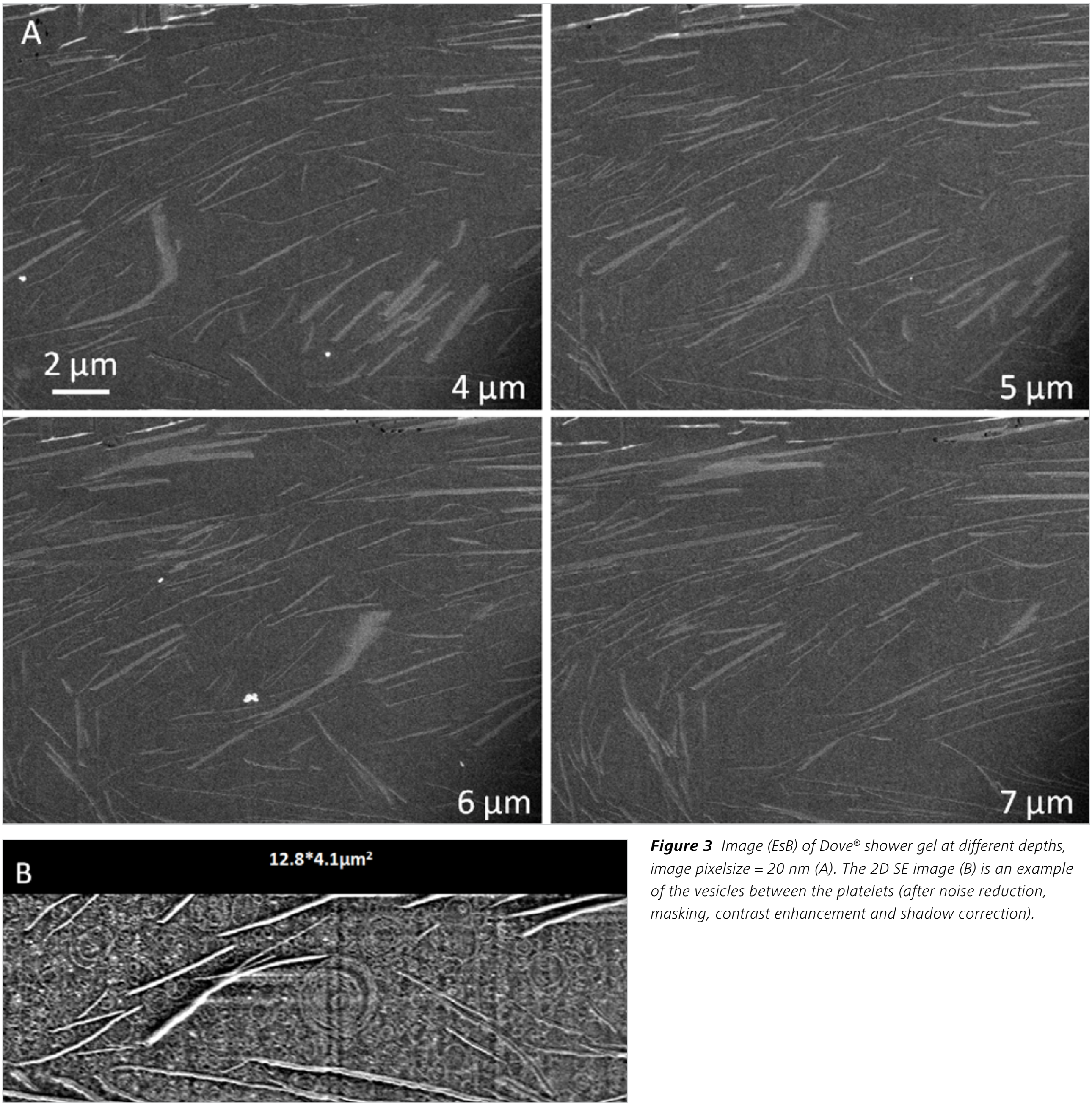


Figure 3 *Image (EsB) of Dove® shower gel at different depths, image pixelsize = 20 nm (A). The 2D SE image (B) is an example of the vesicles between the platelets (after noise reduction, masking, contrast enhancement and shadow correction).*

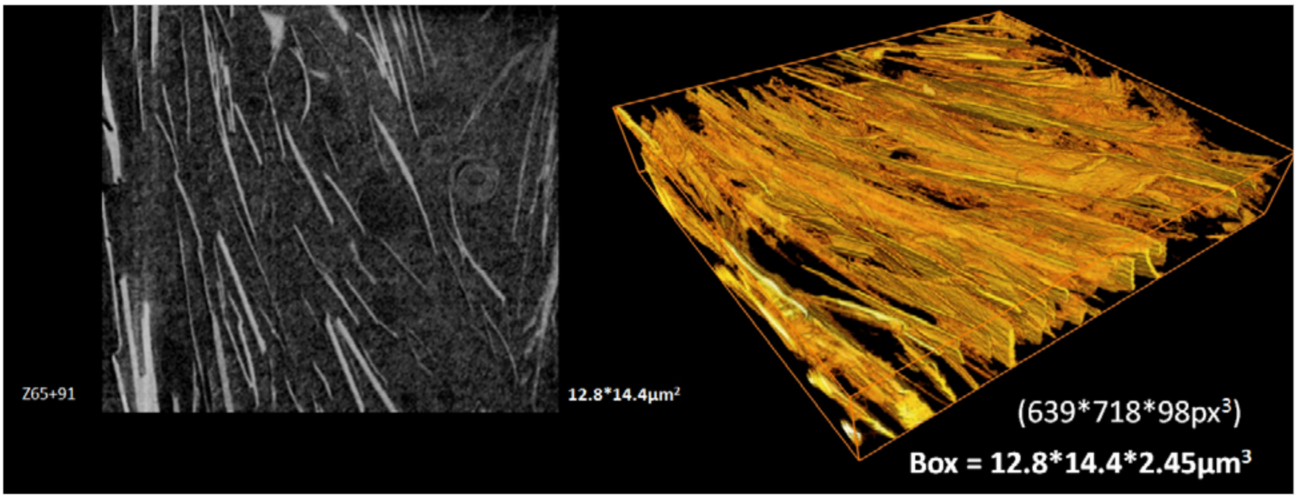


Figure 4 *FIB SEM image (EsB) of one slice (A). The 3D image (B) of the Dove® shower gel, box = 12.8*14.4*2.5 µm³.*

With the use of the EsB detector the material contrast between the lamellar sheets (originated from stearate soap) and the surrounding matrix can be observed and a 3D model of the microstructure can be reconstructed. The detection of the low energetic secondary electrons by the in-chamber SE detector is more sensitive to charging, but the multilamellar vesicles become more clearly visible. These vesicles contain glycerine, sodium lauryl suphate, isethionate and lauric acid and are responsible for the moisturizing of the shower gel. The vesicle microstructure and interaction with the lamellar sheets will affect viscosity. This is relevant in designing formulations and consumer preferred product properties like foaming behaviour and moisturizing properties.

Conclusion

The application and use of cryo-FIB-SEM microscopy for the microstructural 3D study of food and personal care products has been demonstrated. Any other conventional preparation technique for electron microscopy like resin embedding or freeze drying implies dehydration which introduces additional risk of artefacts and is much more time consuming.

Cryo-SEM preparation methods like freeze fracturing and cryo-planing allow the two-dimensional investigation of the microstructure, but cryo-FIB-SEM is the only method for three-dimensional studies.

Cryo-planing gives good opportunities as FIB-SEM sample pretreatment, a flat surface was created which makes it easier to select the region of interest and gives less curtaining due to surface topography. Cryo-FIB-SEM offers a range of new possibilities in electron microscopy of soft materials.

Application area

- 3 Dimensional
- Soft and hydrated material

Recommended instrument type

- ZEISS AURIGA FIB-SEM
- Cryo preparation chamber
- Cryo-ultramicrotome

References

1. McMahon G., Rybczynski J., Wang Y., Gao Y., Cai D., Dhakal P., Argenti N., Kempa K., Ren Z.F., Erdman N., Naughton M.J., Applications of Multibeam SEM/FIB Instrumentation in the Integrated Sciences, Microscopy Today, 2009, 34–38.
2. Schertel A., Snaidero N., Han H.-M., Ruhwedel T., Laue M., Grabenbauer M., Möbius W., Cryo FIB-SEM: Volume Imaging of cellular ultrastructure in native frozen specimens, Journal of Structural Biology 184 (2013), 355–360
3. Nijsse J., van Aelst A.C., Cryo-Planing for Cryo-Scanning Electron Microscopy, Scanning, vol 21, 1999, 372–378.
4. Carey, M., Low Temperature Scanning Electron Microscopy of Cryo-Planed and Ion Milled Block Faces, poster Gatan UK.
5. Hayles M.F., Stokes D.J., Phifer D., Findlay K.C., A technique for improved focused ion beam milling of cryo-prepared life science specimens, Journal of Microscopy, Vol. 226, 2007, 263–269.
6. Stokes D.J., Hayles M.F., Methodologies for the preparation of soft materials using CryoFIB SEM, Scanning Microscopy, 2009.

Carl Zeiss Microscopy GmbH
07745 Jena, Germany
Materials
microscopy@zeiss.com
www.zeiss.com/microscopy

We make it visible.