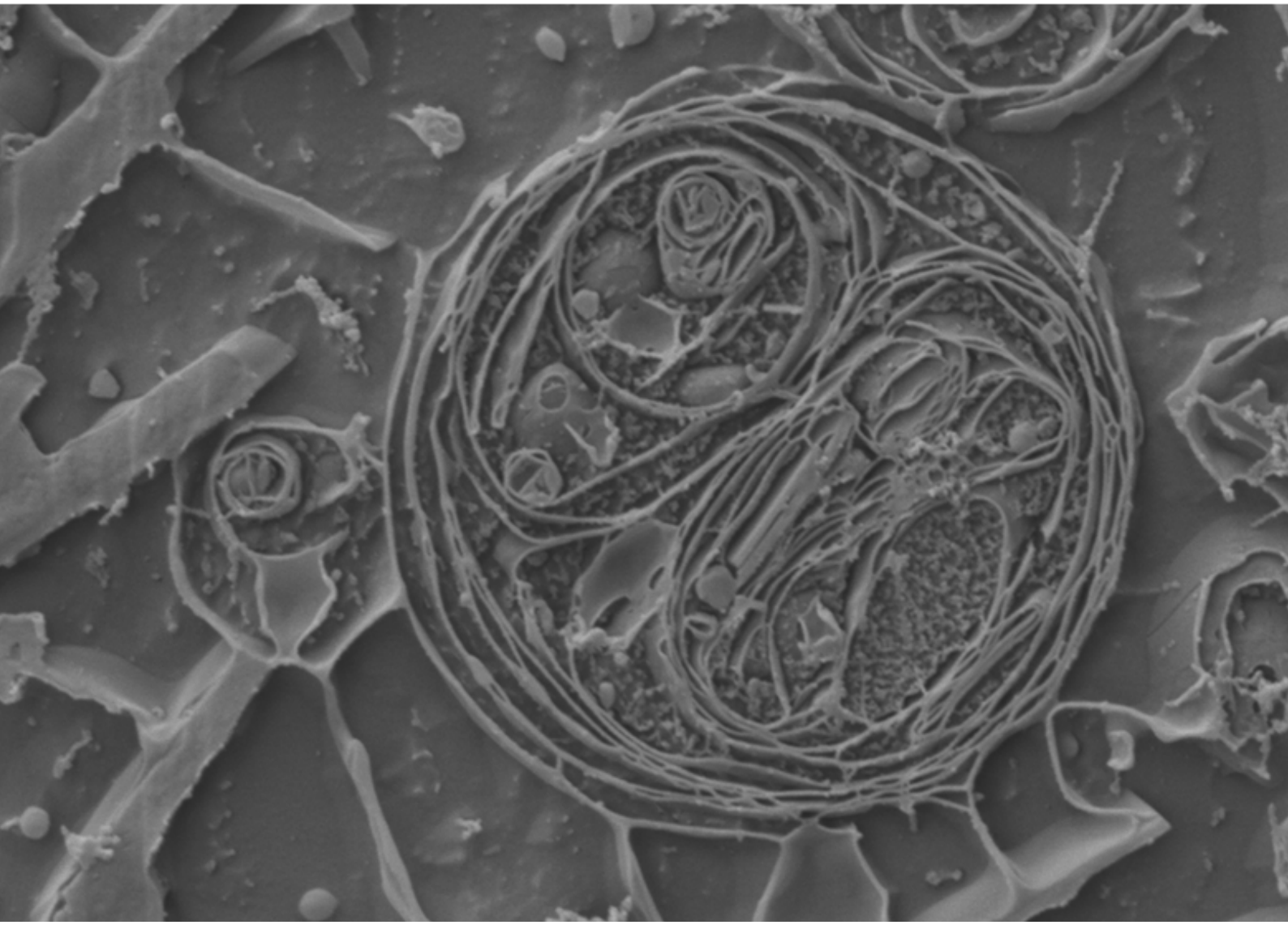
White Paper



Microstructure of Skin Cream Using Cryo-Planing and Cryo-FIB-SEM

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Microstructure of Skin Cream Using Cryo-Planing and Cryo-FIB-SEM

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The processing and formulation of home & personal care products are more controllable if their microstructure is understood. The microstructure can also be related to important properties like texture, sensory performance and consumer preferences for example. Combined focused ion beam (FIB) and scanning electron microscopy (SEM) is widely used in material and life science for the characterization of the 3D microstructure.

Because of the high vacuum compatibility required in a SEM, soft materials should be either fixed, dehydrated and embedded in a resin or studied at the fully hydrated frozen state using cryo-(FIB)-SEM. The internal structure of a frozen sample can be exposed by freeze fracturing through the well frozen part of the sample. Recently volume imaging of the cellular ultrastructure in native hydrated frozen specimens using cryo FIB-SEM microscopy has been shown.[1]

Cryo-planing is another well-developed technique enabling observations of flat sample surfaces at cryo-conditions. Contrast is enhanced by carefully raising the temperature to sublime water generating a gentle surface topography.[2,3,4]

In this application note, freeze-facturing and cryo-planing are compared as different sample preparation methods for Cryo-FIB-SEM volume imaging. Cryo-FIB-SEM is used to create 3D models of the internal skin cream structure.[5,6]

Instrumentation

This study was carried out using AURIGA FIB-SEM, equipped with a Gatan cryo preparation system (Alto 2500) and the Leica cryo-ultramicrotome Ultracut
UCT EM FCS.

Experimental method

The experiments were conducted on the Unilever skin cream product Fair & Lovely.

A tiny volume of the sample (about 50 µl) was placed on top of a rivet and was plunge-frozen in liquid ethane.

One sample was cryo-planed using a cryo-ultramicrotome (Leica Ultracut UCT EM FCS), to obtain a freshly prepared flat surface. Cryo-planing was started using a glass knife with a section thickness of 80 nm and a speed of 50 mm/sec at 110 °C. After switching to a diamond knife (Diatome histo cryo 8 mm) the section thickness was continuously decreased down to 30 nm for the last sections with a speed of 2 mm/sec. Under liquid nitrogen, the rivet was mounted onto a cryo-holder and transferred into a Gatan Alto2500

preparation chamber. The sample was sublimated and sputter coated with platinum for 120 sec at 10 mA current.

For comparison of the different pre-treatment methods another sample was first freeze-fractured in the Gatan Alto2500 preparation chamber and then sputter coated.

The cryo-planed sample and the freeze-factured sample were imaged using AURIGA at – 125°C and an accelerating voltage of 3 kV. After Cryo-SEM observation Cryo-FIB-SEM volume imaging was performed in order to generate a 3D model of each sample.

In a sequential manner slices of material were removed by ion beam milling and each freshly exposed slice surface was imaged. A FIB probe current of 500 pA for FIB slicing and up to 10 nA for coarse milling was used. After FIB milling of each slice an Energy Selective Backscattered (EsB) and Secondary Electron (SE) image were recorded simultaneously using Dual Channel mode. The images, taken after 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.

In a completely automated process a stack of 100 images was generated. Avizo Fire software was used to align the stack of 2D SEM images and to create a 3D-model of the internal skin cream structure.

Results

In figure 1 SEM images of cryo-planed surface are shown, whereas in figure 2 a freeze-fractured and cryo-planed surface

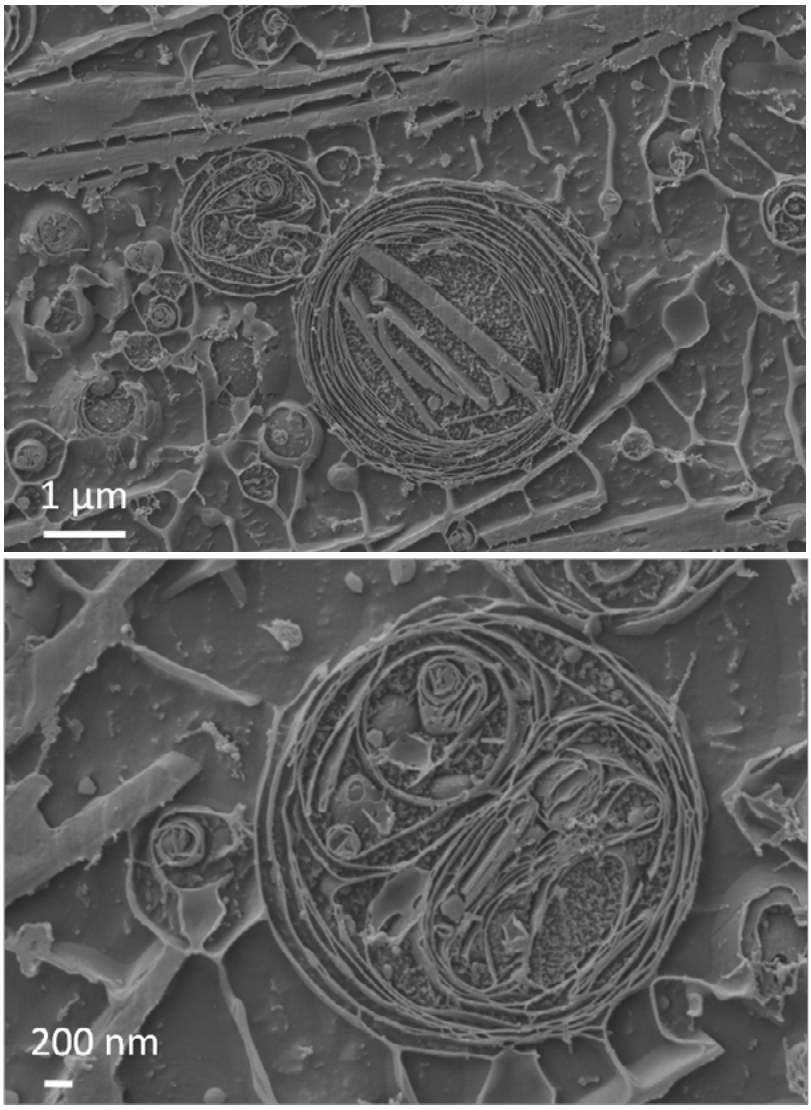

Figure 1 *SEM images of Fair & Lovely skin cream showing a cryo-planed surface (SE detector, 3 kV).*

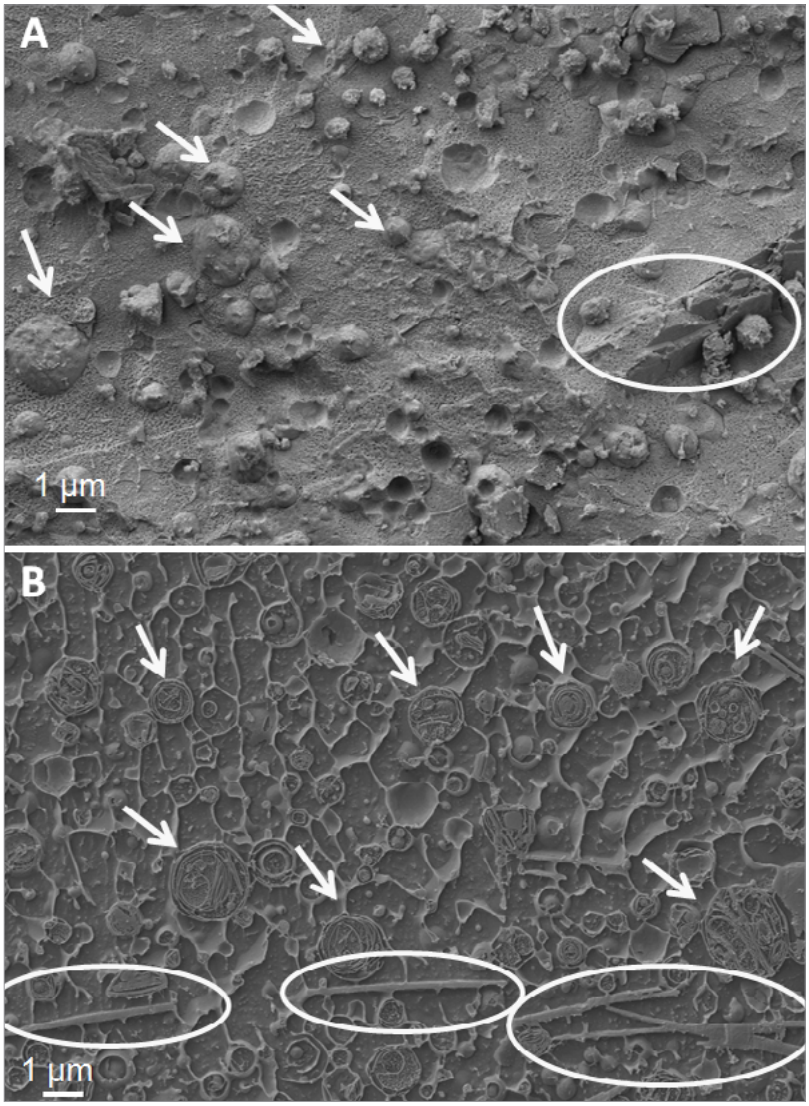

Figure 2 *SEM images of Fair & Lovely skin cream (SE detector, 3 kV) comparing (A) a fractured surface and (B) a cryo-planed surface (→vesicles, ⬭platelets).*

are compared. By using cryo-planing (figure 1 and 2B), the internal structure of the vesicles is exposed and shown in cross-section. The distribution of vesicles is easily seen. Platelets are nicely cross-sectioned and easily observed.

Freeze-fracture planes generally propagate through the weakest points of a sample and therefore the freeze-fracturing is considered to be relatively uncontrolled and random. Therefore the freeze-fracture of vesicles is along the different internal shells of the vesicles exposing their surface. Depending of the location of fracture concave or convex surfaces are observed. The distribution of vesicles is shown, but platelets were more difficult to be seen. Platelets are sometimes removed because of breaking out and being part of the mirror freeze-fractured sample part. To visualise the internal structure cryo-planing was the much better approach, because all constituent parts were cut in a single plane.

In order to reconstruct the shape and the internal structure of the vesicles in all three dimensions a set of serial SEM images was recorded by Cryo-FIB-SEM volume imaging. An accelerating voltage of 2.3 kV and a lateral pixel size of 16 nm were used for SEM imaging and the slice thickness was 20 nm for FIB milling. The 3D model is shown in figure 3.

In the 3D reconstruction it can be observed that the vesicle consist of different layers and small vesicles are observed as well. Understanding of the internal microstructure of the skin creams gives information about the stability of the products which is necessary during product development.

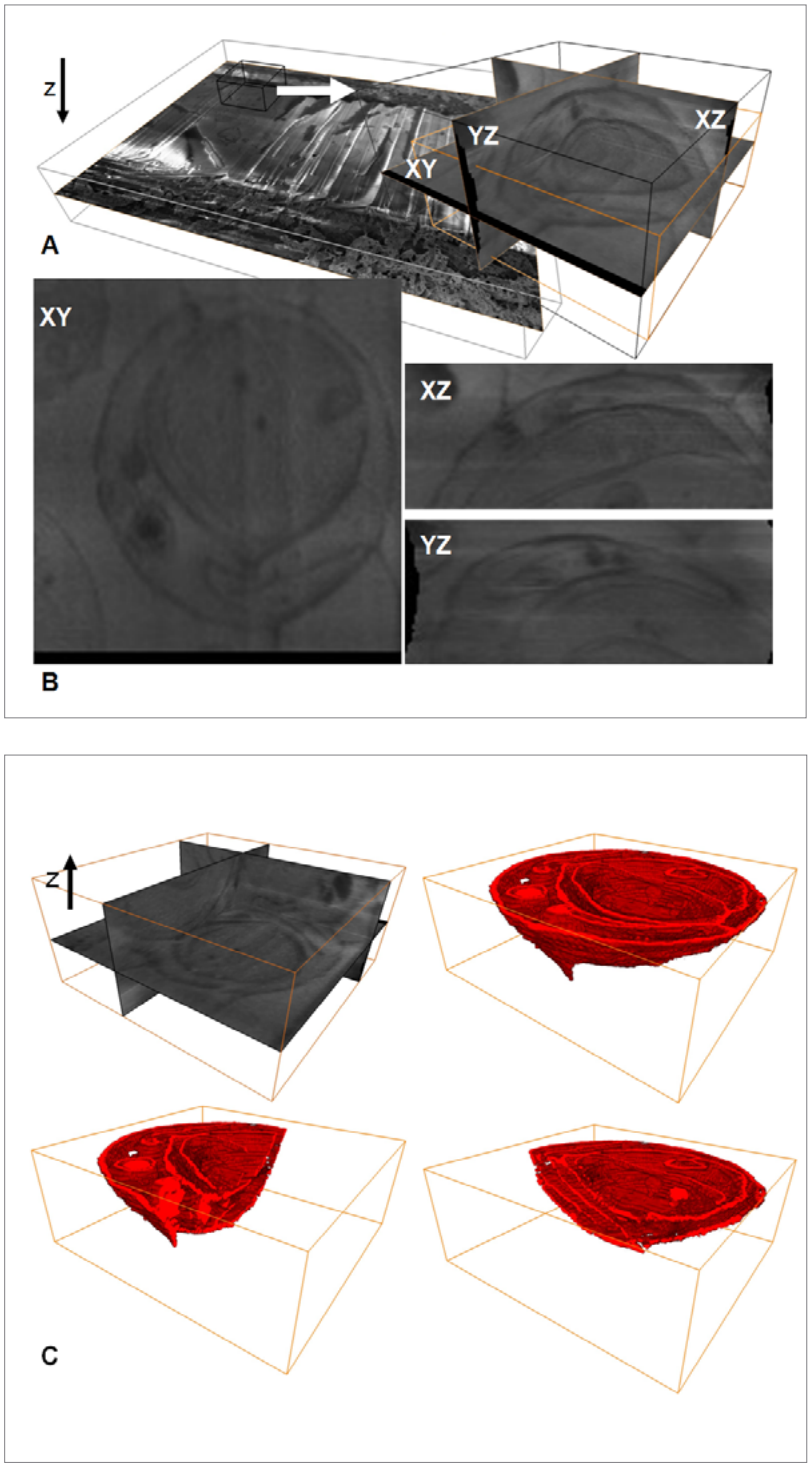

Figure 3 *(A) FIB-SEM images of Fair & Lovely skin cream showing an imaged volume of 2.4*2.4*1.2 µm³. (B) The 2D images are examples of XY, XZ and YZ planes (ortho slices) cutting through the 3D volume. (C) The reconstructed 3D model of a vesicle is shown. The volume is 2.4*2.4*0.7 µm³) and the voxel size is 16*16*20 nm³.*

Conclusion

For cryo-SEM observation of skin cream and similar specimen cryo-planing is the preferred sample preparation method to visualize the internal microstructure in a planar cross-sectional view.

Also for subsequent cryo-FIB tomography cryo-planing gives good opportunities. The generated flat surface makes it easy to choose an appropriate location for FIB tomography all-over the cross-sectioned sample area. Due to flat cryo-planed surface much less curtaining by FIB milling occurs as in case of the topography rich freeze-fractured surface.

Cryo-FIB-SEM milling allows to perform accurate homogeneous serial slicing at very specific sites of interest and to visualize the microstructure of specimen in all three dimensions.

Application area

- 3D
- Soft material

Recommended instrument type

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

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