APPLICATION NOTE

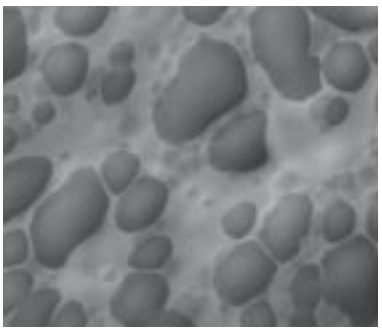
FEI COMPANY™

Unique Imaging of Soft Materials Using Cryo-SDB

Gain more realism, see less artifact.

Key points:

- *FIB a site specific section from an area of the sample*
- *Reveal interfacing between internal structures and membranes*
- *Hard and Soft combined materials can be sectioned*
- *Serial section a specific site to give a 3D tomogram*
- *Gain more realism see less artifact*



An ESEM image of mayonnaise

This application note describes the new and developing Cryo-Small Dual Beam and its impact on Soft Materials research and general understanding of Soft Materials. The Cryo Transfer system has proved its worth with the SEM on fracture study, but now can be utilized in a FIB environment to explore samples in a cryogenic state. FIB milling into their content reveals previously unseen nano-structural existence, providing the Soft Materials industry and research with a new radical technological tool.

Soft Materials

Materials that are composed of either semi-solid and,or liquid base components,constitute a substance that is sometime difficult to realize,when studied by microscopic or analytical means. They are generally called Soft Materials.The use of suspensions, emulsions, oils, fats, waxes, plastics and polymers has increased over the last 2 decades and we now come into contact with many soft materials on a daily basis. With the ever increase in soft material products coming from the Food, Cosmetics and Healthcare industry alone means the pressure is on researchers to fully understand the nature of their product with the purpose of improvement and profitability. Improvement can be at the component level therefore study by volumetric analysis of the product where particulate distribution and relationship become obvious is certainly of great value to the reseacher. The use of the Cryo-SDB provides a unique approach to this form of reseach.

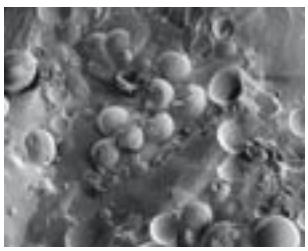
ESEM technique.

For many years FEI has provided a method for imaging soft materials in their natural state, sometimes wet, by means of the Environmental SEM, now known as the Quanta range. This was a gigantic break-though over conventional high vacuum SEM imaging and analysis because some of the soft materials that previously had to be in a dry state could now be imaged wet and 'as is'. The information provided by this method proves to be most useful when the soft material undergoes a dynamic process, such as heating or cooling that can be recorded. Imaging and analysis of wet soft material surfaces, in ESEM, on it's own can leave important infomation hidden.

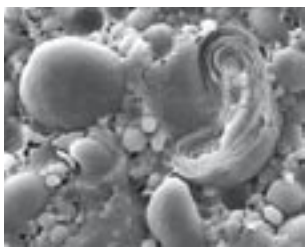
SEM Cryo-Fracture technique.

This is a technique where the wet soft material is fast frozen, fractured using a Cryo system attached to the SEM or SDB, transferred into the specimen chamber and held at a cryogenic temperature for observation.

Cryo Fracture in the SEM provides a chance of finding an item of interest on the fractured **surfaces**. General internal structure of the soft material can be found from the **random** content of the fracture surface. It is therefore a useful surface technique. The ESEM mode and Cryo Fracture tiechniques are complimentary and have made a significant contribution to advancing the knowledge of soft materials for dynamic and mechanical properties as well as component structure and form. Still there is further information to be gained in the form of the internal volumetric relationship of components with the ability to analyse these in an "as is" way. The present day Cryo SDB prepares the soft material so that this goal can be achieved.



Fracture surface of Mayonnaise with the Quanta 3D



Fracture of liposomes used in the Pharmaceutical industry

Advanced techniques with the Cryo-SDB

The combination of the Quorum PP2000T Cryo preparation/ transfer system with the Quanta 3D or the Nova NanoLab small dual beam has allowed the volumetric analysis of soft materials to be investigated from the micro-level (Quanta 3D) to the nano-level (Nova NanoLab). Both instruments have an Ion and Electron column but differ in the application of the instrument to the type work needed.

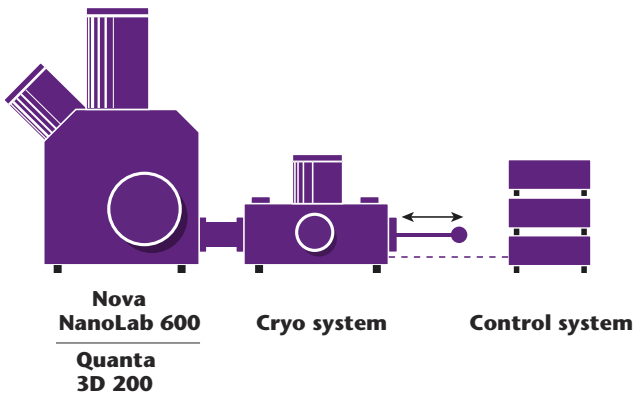
The Quanta 3D small dual beam instrument is unique in respect that it has an ESEM mode for the study of wet samples, a Low vacuum mode for charging samples and a High vacuum mode for general SEM work and Cryo applications.

Once a cryo system is installed on the Quanta 3D samples that have been investigated by the ESEM or Low vacuum mode can be prepared for cryo ion milling with the Ion column in the High vacuum mode, even if they originally were accepted as wet samples.

The Nova NanoLab is a high resolving small dual beam instrument that has a FEG electron column with in-lens SE and BSE capabilities. The Ion column provides a cutting tool for those soft material samples that exhibit nano-particulate components or fine high resolution features. The in-lens detectors operate effectively at very low kV on cryo ion milled samples to image contrast mechanisms not visible by conventional SE detectors.

Soft material samples can be cryo stabilized by freezing in Liquid N_2 or Ethane/Propane, then transferred to a cryo preparation chamber under vacuum where they can be fractured, sputter coated with a conductive layer and finally examined on a cold stage in either the Quanta or Nova small dual beam system.

Fracturing technique provides an information plateau for the distribution and size of the various phases and components within the sample. This enhances the chance of making a site-specific-section successful. Using the cryo-transfer technique also means sample preparation is complete within a few minutes allowing the FIB cutting and the observation time to have priority during the day.



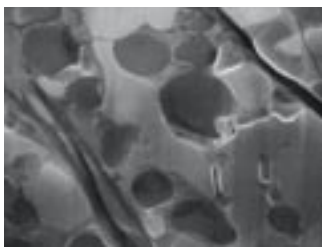
The Site-Specific-Section technique.
Site Specific Sections of difficult soft materials can be obtained by the combination of Cryo techniques and Dual Beam technology. Better understanding of the bulk status can be gained by freezing the sample in time, fracturing to visualize a specific site and then Fib-sectioning the sample for a single shot analysis. This is the Site-Specific-Section technique.

Slice and View technique for 3D reconstruction.
FEI can provide a unique software method for volumetric analysis of soft materials by making consecutive sections at a specific site. A detailed 3D reconstruction of the bulk material through the slices can be made. This Slice and View technique provides valuable 3rd dimensional volumetric information not previously available from bulk soft materials without extensive preparation and time consuming ultra-microtomy techniques.
Cryo-Fib cutting a site-specific-section will reveal interfacing between internal structures and membranes not seen on the fracture surface. Combination materials of hard and soft components can be Cryo-Fib cut without disruption to the structural form of the mixture.

Applications

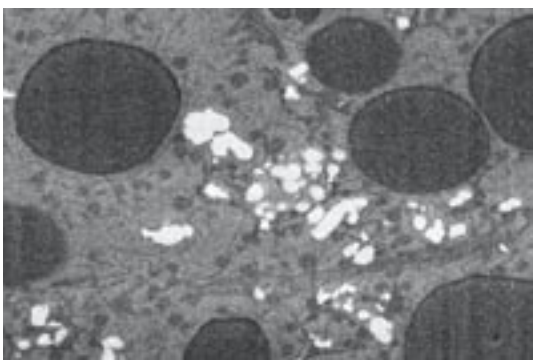
Liposomes
A Liposome is a minute spherical sac of phospholipid molecules enclosing a water droplet, used to carry drugs or other substances into the tissues. They can be found extensively in cosmetics and some pharmaceuticals. Liposomes have been widely investigated by freeze fracture replication. More recently they have been examined by Cryo-transfer in the TEM using the vitrified thin film technique. TEM examination is limited to samples of less than 500 nm (larger liposomes are electron dense). High resolution low voltage Cryo-SEM allows very large liposomal structures to be examined by cryo-fracture techniques. The complex multi-layering of these structures is clearly visible. Freeze fracture replicas and Cryo-SEM fractures are limited to surface investigation. To gain more information on their volumetric distribution in a product Site-Specific-Sectioning using the Cryo-Ion beam milling, in the Dual Beam, will deliver a 3rd dimensional result relating to the real volume distribution character of the soft material at the time of freezing. Changes in shelf life, core content release, structural analysis and other studies can be investigated with the results from the Cryo-SDB.

FIB section of liposomes after sublimation and sputter coating

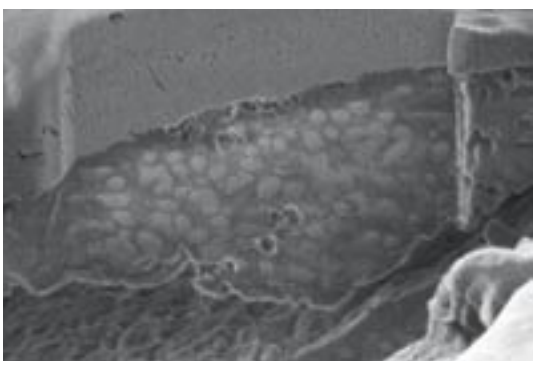


Nova FIB section of Sun Screen cream imaged at 10.0 kV with the TLD-SE detector

Cosmetics and Health Care products
These are predominately nano size component products and therefore need the higher resolution of the Nova NanoLab electron column to image distribution. Some products contain Liposomes so in a site-specific-section contrast can be dramatic at low voltage beam energy.



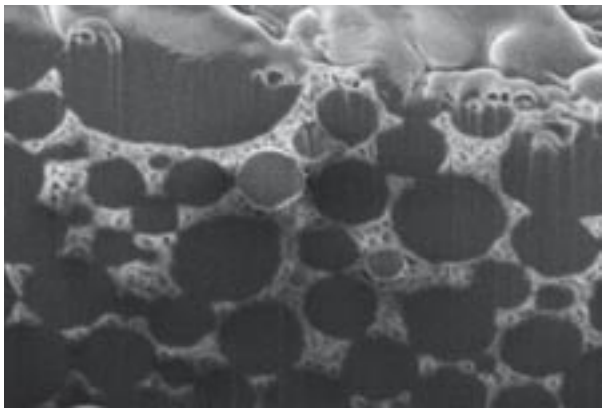
Nova FIB section of foundation cream imaged at 1.5kV with the TLD-BSE detector



Quanta 3D FIB section of Shaving Gel imaged at 3.0 kV with the ETD-SE detector

Food stuffs

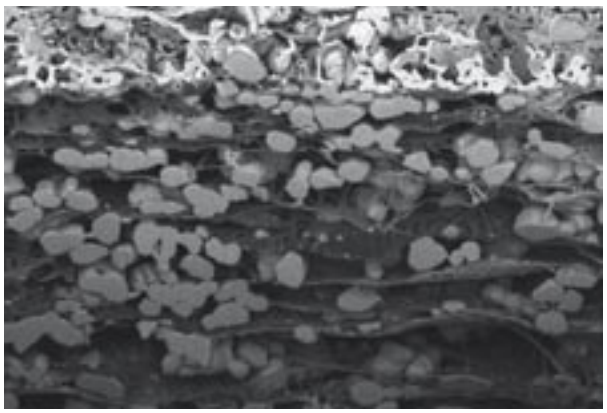
Food stuffs can contain starch, fats, sugars, oils, water and air. Many of these components can be imaged from the fracture to deliver vital information. More information can be gained by Fib milling a site-specific section on the fracture to show the components in real relationship. Many food component are in the micro size range so the Quanta 3D is an ideal instrument for these applications, for Fib milling of cryo sections or using the ESEM capabilities for dynamic experiments. Some components of food stuffs can be pre-stained to enhance the contrast such as starch in products. The enhanced contrast can help with distribution statistics thoughout the sample. Air is a major component in some food stuffs and therefore also contributes to the volumetric analysis when using Slice and View 3D reconstruction.



Quanta 3D FIB section of Mayonnaise imaged at 3.0 kV with the ETD-SE detector

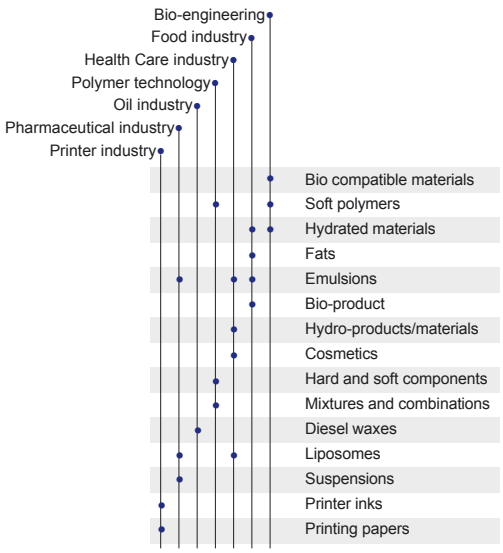
Polymers

Fib milling of polymers at room temperature can be successful if the material is reasonably hard but soft polymers and especially composite polymers of soft and hard components are impossible without a constant cryogenic temperature employed at the time of cutting. The sample of laminated polymer membrane containing ceramic particles is a good example of this. The site-specific-section shows no drift at the cutting face and the section shows no compression of the laminated polymer membrane.



Nova FIB section of laminated polymer with ceramic particles imaged at 2.0 kV with the TLD-BSE detector

Soft materials presence



FEI Company
World Headquarters and
North American Sales
5350 NE Dawson Creek Drive
Hillsboro, OR 97124-5793 USA
Tel: +1 503 726 7500
Fax: +1 503 726 7509

e-mail: sales@feico.com
www.feicompany.com

European Sales
Tel: +31 40 27 66 768
Fax: +31 40 27 66 786

Asia-Pacific Sales
Tel: +65 351 7671
Fax: +65 354 0644

Japan Sales
Tel: +81-3-3740-0970
Fax: +81-3-3740-0975

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