

Nanostructures in the Cryo FEG SEM

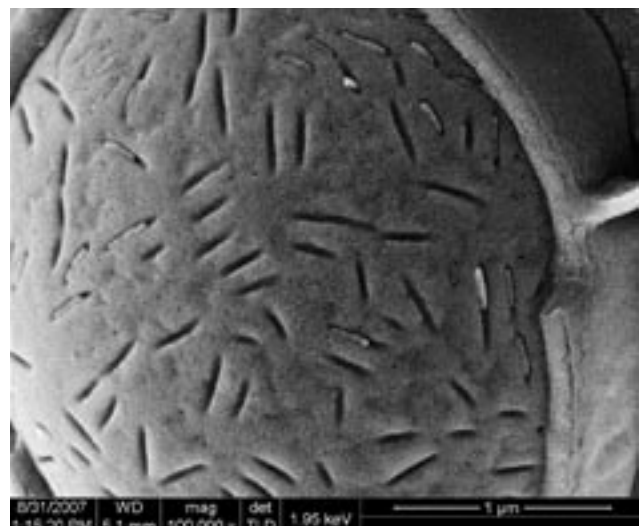
Any sample, all data, anywhere

Key benefits:

- *Short preparation time*
- *Freeze fracture*
- *Magnetron sputter at 1 to 2 nm, Au/Pd, Pt, W*
- *Sublime/etch samples in the prep or SEM chamber*
- *Rapid temperature control*

Cryo nanostructure imaging

High resolution imaging of nanostructure in biological material has usually been observed in the TEM using cryo-sectioning or freeze fracture replication methods. These techniques are recognized as being complex and at least time consuming. The FEG SEM with a cryo transfer system provides a high resolution capability for SEM cryo samples. This compliments TEM methods by providing higher resolution on SEM prepared samples while still retaining the ease of use of the SEM. The FEG SEM with cryo-transfer facilities provides a tool for imaging the nanostructure of beam sensitive or 'wet' samples common to life sciences. Cryo fracturing the frozen sample prior to entry into the specimen chamber opens up the sample for examination. Observation of cryogenically prepared biological samples in the FEG SEM can yield inter-membrane information from such fractured surfaces of the sample.



10 nm transmembrane particles on the inner membrane of yeast.

Sample preparation could not be easier

Sample preparation time is very short due to the simple processes employed. The sample is mounted on the transfer device, then plunge frozen in the cryogen unit. It is then transferred under vacuum in a low temperature state to the preparation chamber of the cryo system. Here it can be sublimed or fractured and sputter coated. Finally it is transferred at a known temperature to the specimen chamber of the SEM for observation.

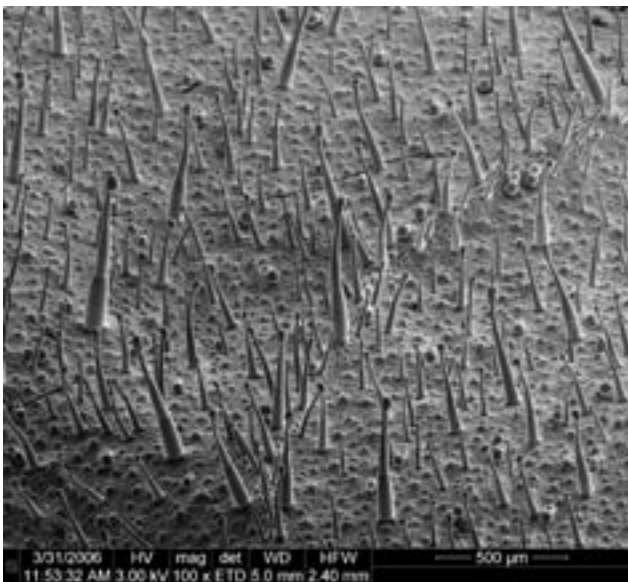
Structural information from the natural surface

Samples that show extensive water containing structures on the surface such as plant leaves and petals can be cryogenically frozen to retain the structures intact whereas with other techniques collapse or dehydration can occur. Plunge freezing with liquid nitrogen is sufficient for this type of sample as faster freezing with high pressure will not provide a better sample surface preservation due the bulk of the sample and the thick membranes within. This type of sample is generally sublimed for 2 minutes in the preparation chamber while held at low temperature to remove natural surface ice and then sputter coated for conductivity with a fine metal before entering the specimen chamber of the SEM.

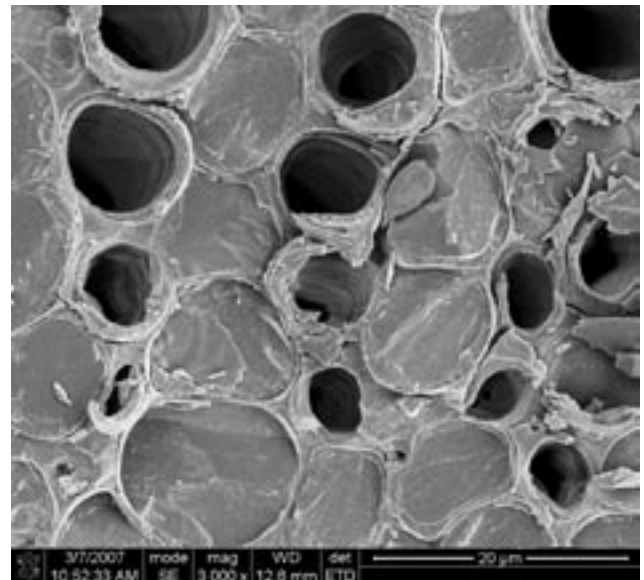
Structural information from cryo fracture

Cryo fracture is a technique whereby the sample is frozen and fractured at a chosen temperature. The nature of the fracture can be determined by the temperature chosen before fracturing. Therefore the fracture temperature for most life science samples can be critical and with the Quorum PP2000T one has the ability to optimize this parameter.

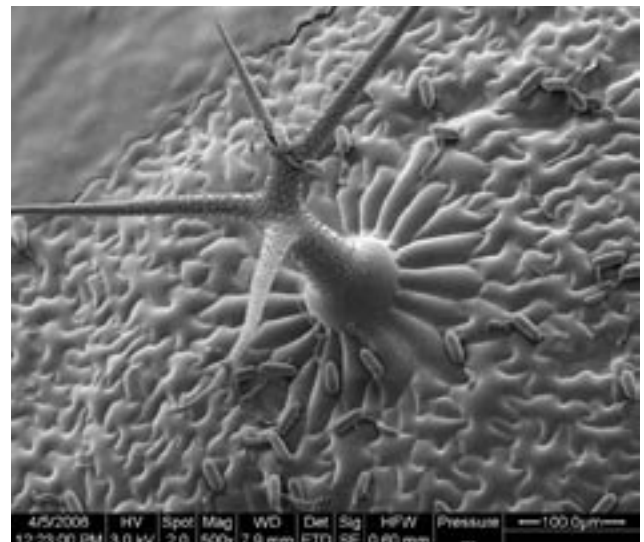
Cryo fracturing a sample exposes structural form providing another dimension to observe internal detail relating to surface structures. There is no need to sublime this type of preparation, just chose the temperature, fracture with fracture tool provided and sputter coat before entering the specimen chamber.



Tobacco leaf surface.



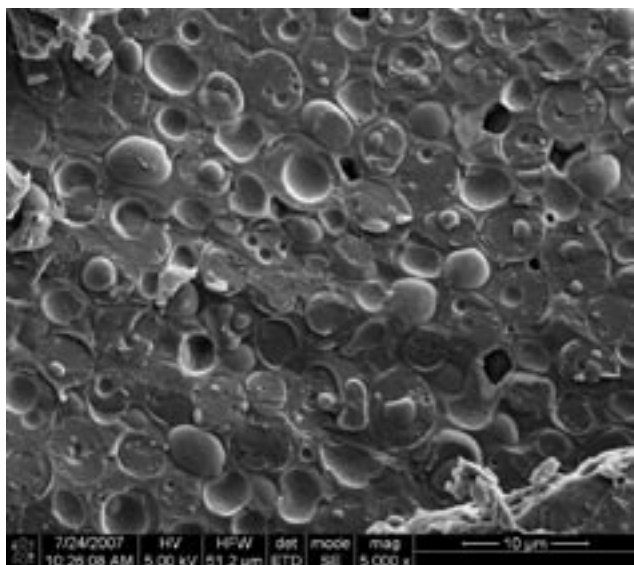
Dandelion, spiral core of the stem.



Trichome on Arabidopsis leaf.

Cryo sublimation

The internal structure of cooled specimens can be revealed by freeze fracture. Even greater detail can often be revealed by carefully raising the temperature of a freeze fractured hydrated specimen to a point at which water begins to sublime at a controlled rate (-90 °C to -100 °C). This etching process is arrested by rapidly re-lowering the temperature. This technique can either be controlled in the specimen chamber or in the cryo chamber. The advantage of using the specimen chamber is that one can observe more accurately the process.

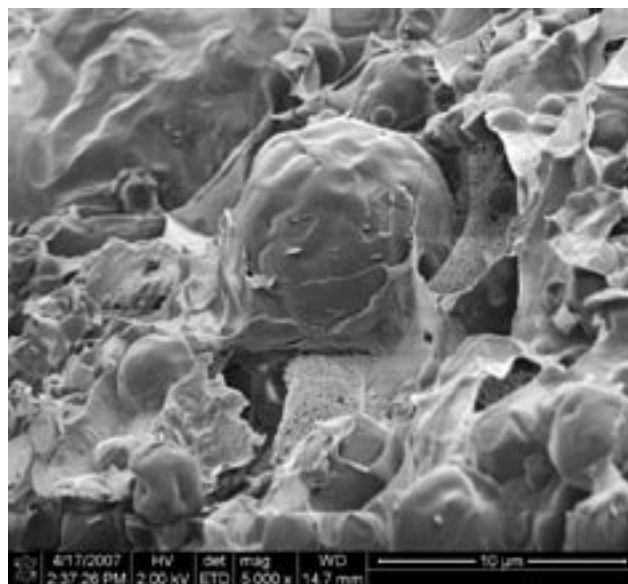


Sublimed surface of yeast cell fracture.

Stabilization of Low Melting Point Solids

Suspensions, oils, fats, waxes, plastic polymers and emulsified products are often either damaged by the electron beam or are not stable in the vacuum environment of an SEM.

Such samples can be cryo stabilized by being frozen and transferred to a cryo preparation chamber under vacuum. There they can be fractured; sputter coated with a conductive layer and examined on a cold stage in the SEM. Fracturing such samples gives information on the distribution and size of the various phases and components within an individual formulation or product. Since specimen preparation and examination can be completed within a short time, productivity is an added bonus.



Hydrolyzing cream showing large liposome.

Dehydration within the specimen chamber

As with sublimation to etch the sample of water (ice) dehydration can occur if the sample is left long enough at a slightly higher temperature relative to the anticontaminator. The result is a completely dried sample, may be with some contraction of the outer surface but still showing important structure.

A more advanced technique is then to remove the dried sample from the FEG SEM and float the sputtered coating off the sample as one would do with a TEM replica.

The result is a metal sputtered replica of the original surface floating on a water surface. This can then be collected by a support grid and placed in a FEG STEM holder for higher definition observation.

Advantages of Cryo FEG

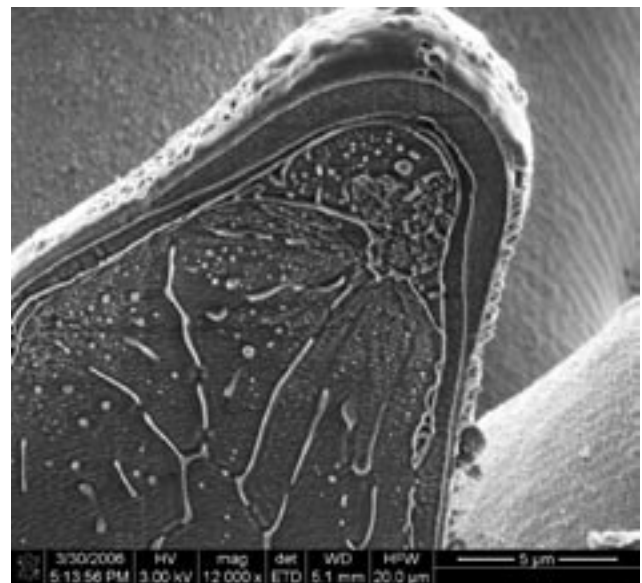
A distinct advantage of the cryo sample preparation procedure is that it does not include critical point or freeze drying techniques. This minimizes the chances of artifactual structures.

With the Cryo FEG combination an improvement in operating conditions applies, where beam damage (local heating) can be effectively controlled. Etching and condensation

(ice recrystallisation) are not generally observed because of parallel control of temperature at the sample and the cold trap within the specimen chamber. Even if condensation occurs the sample does not have to be retracted into the cryo transfer unit, but can be sublimed within the microscope 'in situ'. Long observation times are possible on the sample without deterioration of the sample or constant correction of the cryo conditions. The FEG SEM with the Schottky emitter and a vacuum buffered column can be run at very low beam currents. This along with fine continuous control of the kV (200 v - 30 kV) allows precise setting of the instrument to suit the material being observed. It is now possible, in conjunction with a cryo system such as the Quorum Cryo Transfer system, to achieve high resolution imaging on cryo samples at accelerating voltages of less than 6 kV. Such low voltages allow excellent topographical imaging without precluding the use of magnifications of the order 100,000 x or higher.

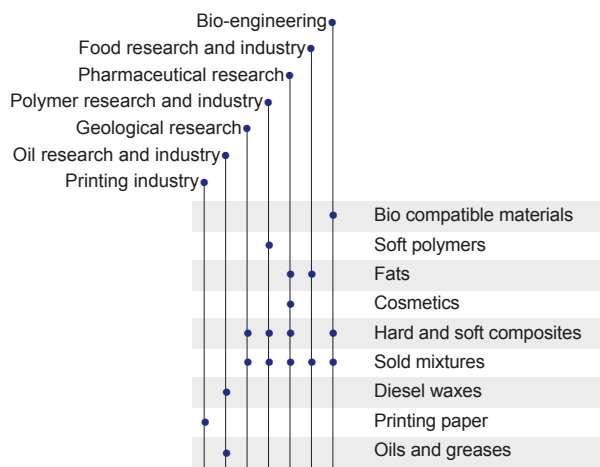


Arabidopsis leaf, showing fungal bacteria.



Tobacco petal cross-sectioned sublimed and coated with PtPd.

Market target list



WORLD HEADQUARTERS
5350 NE DAWSON CREEK DRIVE
HILLSBORO, OREGON 97124 USA
PH: +1.503.726.7500

FEI JAPAN
PH: +81.3.3740.0970

FEI ASIA PACIFIC
PH: +86.21.6122.5988

FEI EUROPE
PH: +31.40.23.56000

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