

3D Cryo-DualBeam™

Double your CRYO-data

A special thank you to Dr. George McKerr, University of Ulster, Department of ABS, Northern Ireland, for detailed collaboration and samples.

Three-dimensional analysis of cryogenically frozen samples using Focused Ion Beam cross-sectioning and SEM imaging.

Imaging of cryogenically frozen samples which have been fractured to reveal internal structures is a well established microscopy technique. The use of cryo-temperatures is the preferred method for obtaining information from biological materials, from some polymers and from low k semiconductor materials in their native state. These sensitive samples offer particular challenges to conventional preparation methods for biological materials such as chemical fixing, critical point drying and impression techniques (replica). The preparation techniques are open to criticism about the

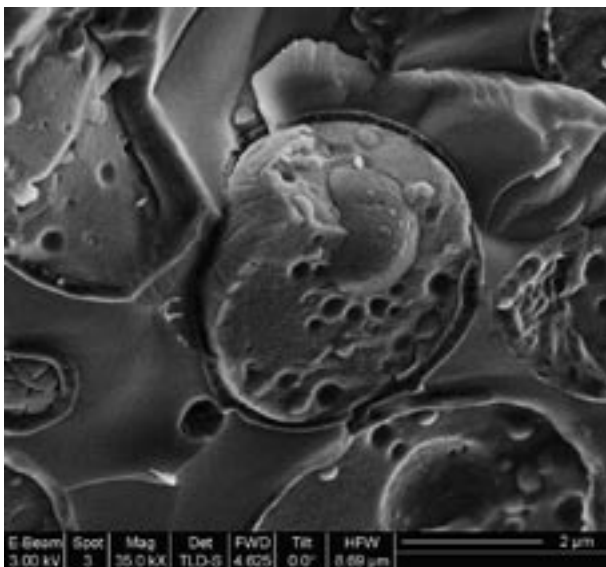


Image 1: SE image of a cryo-fractured yeast cell. The nucleus is clearly visible, but most of the vacuoles have been pulled out during fracture.

quality of the data they produce, because of the effects they have on the original sample. Cryo-preparation produces the highest quality results, but also has its own limitations.

A key part of cryo-preparation is the fracturing of the frozen sample. The physical process of breaking a sample into two pieces involves the weakest structures and therefore the breaking often follows the bi-layer in the cell membrane. So there is no real control over the position of the viewing surface that is available. This fracturing process therefore dictates the limitation of the structural investigation.

This simple problem can now be overcome by applying a FIB column for milling at dedicated, well defined locations. By the addition of cryogenic sample preparation techniques to FEI's innovative DualBeam instruments it is now possible to perform *site-specific* cross-sectional analysis of biological samples without the loss of any data or artifacts introduced by the preparation technique or due to the cryo-fracturing technique.

For the first time, true 3D data can be directly obtained from the exact location required, thus maximizing the structural information available from the sample and removing the dependence on the fracturing process.

All the techniques that are used with conventional SEM cryo-microscopy are very much still applicable to the new cryo-DualBeam technique, but for the first time, the preparation and direct 3D inspection of a single cell organism / feature by simple freezing, cleaving, FIB

sectioning and electron beam microscopy is now possible.

The traditional problems expected from ion beam machining of biological material and soft materials such as polymers, have been removed with the use of a new FEI procedure (patent pending).

The sample is frozen, then placed in a transfer module. This is then placed into a microscope loadlock and pumped down. The sample is fractured and immediately transferred into the microscope. Once the temperature and vacuum levels have stabilized (a few seconds) the sample can be inspected using a low kV electron beam. The exact parameters (accelerating voltage, beam current, sample field, signal collection method) can be varied to produce best results.

Once a feature of interest has been located, the ion beam is used to cut a cross-section through the site. Many sensitive materials have been subjected to this technique and so far no beam damage artifacts have been observed. The ion beam is used to remove material like a scalpel and the electron beam is used to image the feature without induced effects. The ion beam remains parallel to the cross-section face at all times and so does not expose the sample directly.

After the cross-section has been made, the face of the section is so smooth that no topographic information is immediately visible.

The sample is then sublimed inside the chamber to reveal and extenuate the desired features. This process drives off a small amount of water from the surface of the sample to show the internal morphology which can be readily imaged in the microscope using the electron beam.

The sample may also be transferred back into the loadlock where a fine metal coating may be added (few nm thick, gold, gold palladium or chromium). The sample can then be inserted for further examination. The process of re-locating the feature is immediate due to the fully integrated nature of the DualBeam instrument.

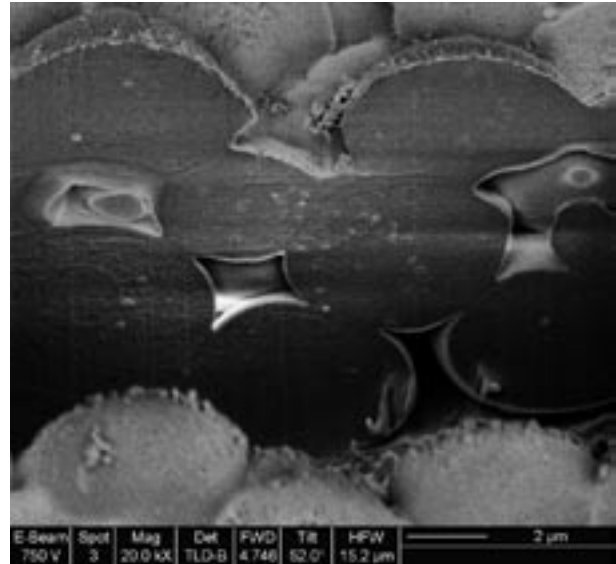


Image 2: SE image of an un-treated ion beam milled cross section through a cryogenic yeast sample. Limited internal structure is visible.

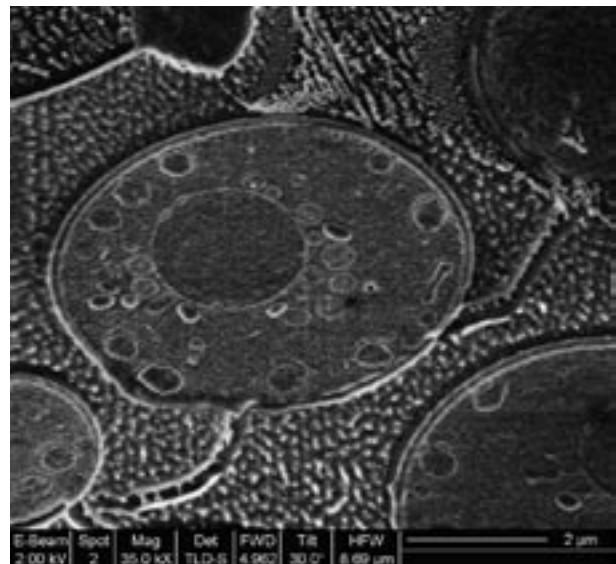


Image 3: SE image of a Cryo-FIB prepared yeast cell. Both nucleus and vacuoles are clearly visible.

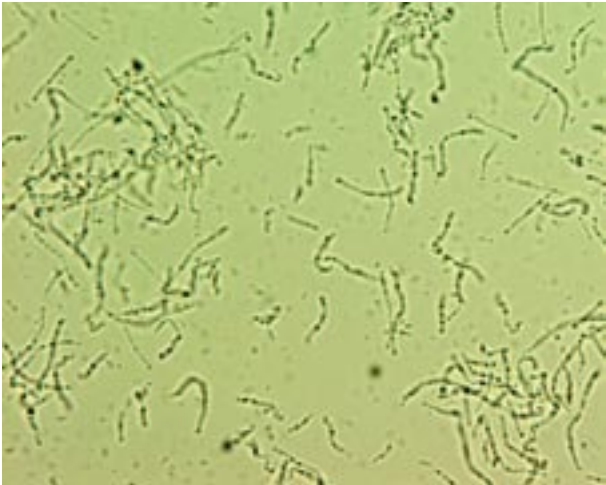


Image 4: Optical transmission micrograph (bright-field) of sporulating cells of the bacterium *Bacillus cereus*. Optically dense areas represent the rounded spores which contain condensed bacterial DNA (x60 objective lens).



Image 5: Fluorescence photomicrograph of spores within cells of the bacterium *Bacillus cereus* (x100 objective lens).

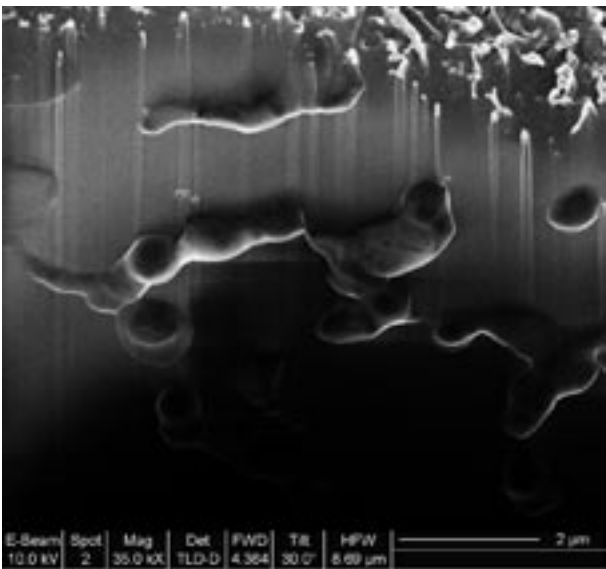


Image 6: Electron beam SE image of *Lactobacillus* spp. Imaged beyond the "ice wall" which has been created by Cryo-FIB cross-section. Spatial detail of several rod shaped bacterial cells can be seen at high contrast.

Many internal features are evident in the images taken using this technique that would not have been seen relative to a fracture site alone. The preparation of the samples is in principle the same as for the conventional cryo-microscopy method, but now multiple (up to a dozen) individual sets of results can be obtained from a single sample due to the FIB capability of site specific access. Many sites can be examined from different samples within a single day.

There is significant scope for further combination of techniques.

These images (images 4, 5 and 6) of bacteria show that the organisms can be prepared using conventional optical techniques, and then stained and imaged so interesting sites can be pre-localized by using fluorescence microscopy, which is a standard technique to do this.

Once located, the navigation to, and preparation of, site specific sections to reveal the interesting nuclear material within the bacteria is immediately possible.

Other techniques that are immediately applicable for use on the exposed features would include backscattered electron imaging and Energy Dispersive X-ray analysis for automatically quantifying compositional differences in the sectioned area such as may be relevant for bone or bio-compatible materials.

For further reading see:

The use of a SEM/FIB DualBeam applied to biological samples – H. Mulders, GIT Magazine Imaging and Microscopy Volume 5, May 2003

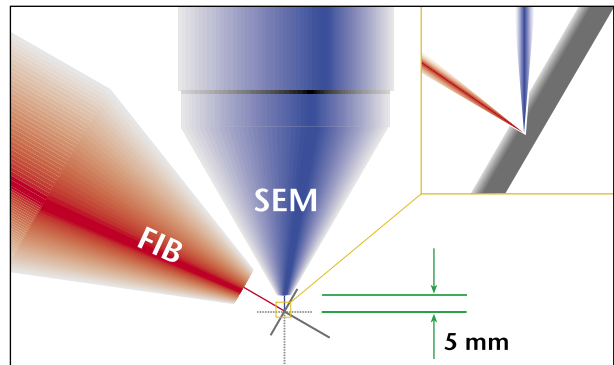


Image 7: The FEI DualBeam concept utilizes Focused Ion Beam technology and Scanning Electron Microscopy simultaneously at the same point on a sample. For Cryo DualBeam applications the full benefit of FIB site specific cross-sectioning normal to the sample surface, and direct SEM imaging from a high tilt angle are the unique capabilities that make this application possible.

FEI Company

Lloyd Peto
 FIB & DualBeam Business
 Development Manager
 The Aztec Centre, Aztec West,
 Almondsbury
 BS32 4TD, United Kingdom

Tel: + 44 (0) 1454 207940
 Fax: + 44 (0) 1454 201885

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

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

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

