Focused ion beam ablation tomography

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In a scanning electron microscope only surface features of a sample are visible. However, by adding a second beam composed of accelerated gallium ions, a Focused Ion Beam (FIB), it is possible to strip away layers of material and see beneath the surface. If performed at very low temperatures (-130°C) the technique is known as cryogenic-FIB-SEM (Cryo-FIB-SEM).

During sample-milling, columns of material often remain, an artifact referred to as curtaining [1]. The observation of these columns is well documented and they are often considered undesirable as they detract from clear images. Curtaining arises from the difference in resistance to the path of the ions as they pass through the sample and approaches to minimize the phenomenon have previously been the subject of papers [2, 3].

Having noticed that some areas of biological cells were removed more rapidly by the beam than others (Fig 1), our idea was to use this difference in susceptibility, often perceived as a weakness of Cryo-FIB-SEM, to obtain information about 3D structure inside biological samples. By imaging the cut face of the sample between each sweep of the ion beam, features created by the differential ablation rate within the sample were plotted and the material removed per sweep calculated as an ablation vector. By pooling these values for a 3D data set it was hoped that internal structures of differing susceptibility to the ion beam would be revealed. To aid with image processing, a software analysis plugin was developed for ImageJ, an open-source image analysis package, to extract and reconstruct ablation susceptibility values as 3D datasets. Several cell types were successfully vitrified for cryo-SEM (cardiac, fibroblast, retinal epithelial cells), providing beautiful 3D images of these various tissues. Protocols were adapted to include platinum shadowing and tungsten deposition to enhance grounding and minimise artifacts. Cultured cells reacted heterogeneously with delicate features appearing obvious to the human eye during milling but which proved difficult to extract using automated image analysis.

A semi-automated approach was chosen where the milled features were delineated manually using a touch-pad before an automatic algorithm extracted susceptibility vectors and reconstructed the data set in 3D. Segmentation between “hard” and “soft” areas was thus obtained by differential susceptibility between these regions, effectively calculating an erosion rate along each column of the sample which represents susceptibility to erosion by the beam. Gradually stripping away layers of the sample away allowed us to build a picture in terms of its resistance to the ion beam (Fig 3).

The method was applied to other samples of various material properties including “woody” onion cells and “hard-soft” Diatoms from the University of Nottingham lake (Fig 2.). These creatures proved to be excellent imaging subjects as they are composed of biological material which incorporates a silicate structure which is hard with a soft cell inside. We were able to see a difference in the ion beam susceptibility and to reconstruct details of the Diatom bodies including internal anatomy.

This investigation explored the potential of a novel tomographic imaging method to reveal 3D nanoscale information from biological samples. The potential of Focussed Ion Beam Ablation Microscopy (FIBAT) has been demonstrated in a range of samples and it is believed that the FIBAT method, used in combination with Confocal Microscopy or other 3D imaging modality, can be used to realise the 3D tomography of cells and supply a novel contrast mechanism which may provide information on internal nano-structure within a range of sample types. A number of technical considerations have been identified and work is ongoing to improve the technique. Further development of the methodology should allow this novel contrast mechanism to be exploited in cryo-SEM imaging of a variety of samples.

References

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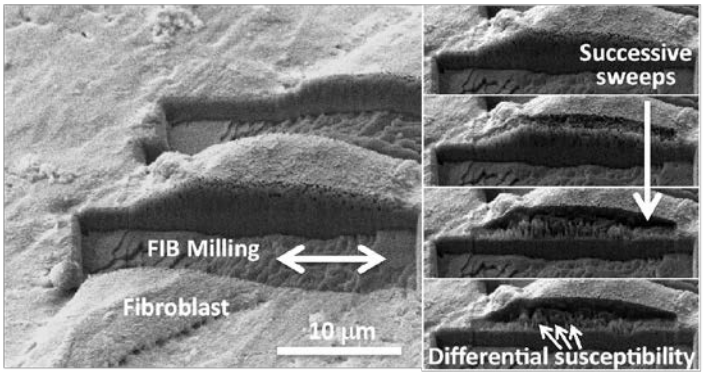

Figure 1. *Human Osteoblast cells showing differential susceptibility with successive sweeps of the Ion Beam*

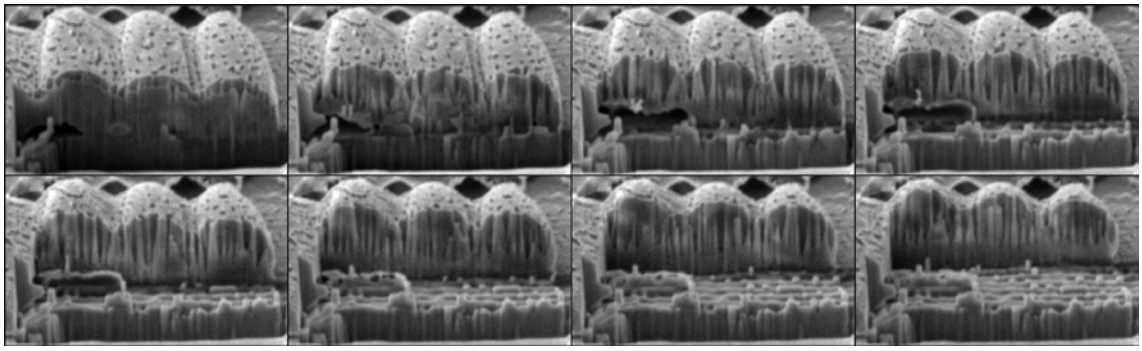

Figure 2. *A selection of images through an entire cyanobacteria sourced from the University of Nottingham lake. The columns can be clearly seen, each is tracked and analyzed using the ImageJ plugin.*

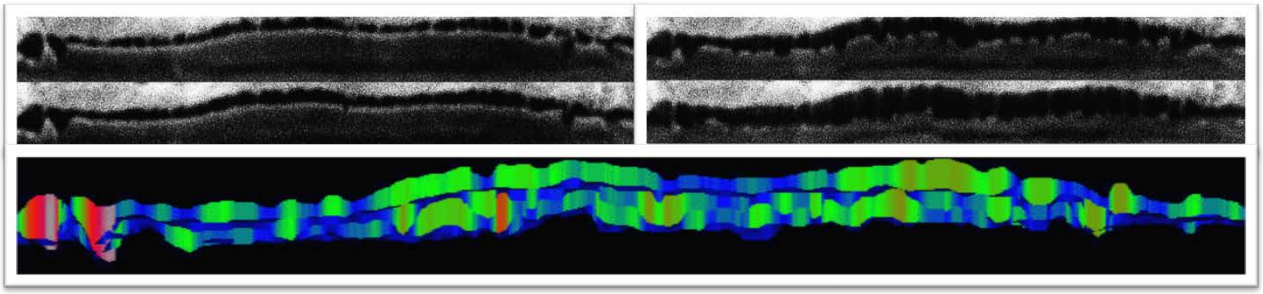

Figure 3. *A series of images of T3T cells with the corresponding FIB Susceptibility plot. Red shows high susceptibility, blue shows more resistive material.*