3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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Focused ion beam ablation tomography

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Addition of a second beam (Focused Ion Beam or FIB), of accelerated gallium ions to a scanning electron microscope (SEM), it is possible to remove layers of material and see beneath the surface. If performed at cryogenic temperatures (-150°C) the technique is known as cryogenic-FIB-SEM (Cryo-FIB-SEM).

During Cryo-FIB-milling, it was noted that some areas of cells were removed more rapidly by the beam than others (Fig 1), which resulted in columns of material forming on the cut-face, an artefact know as curtaining [1]. It was postulated that the difference in resistance to the path of the ions was responsible for the curtaining, the minimization of which has previously been addressed [2,3].

Our idea was to use this difference in susceptibility, often perceived as a weakness of Cryo-FIB-SEM, to obtain information about 3D structures inside biological samples. By imaging the cut face of the sample between sweeps 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 plug-in was developed for ImageJ, an open-source image analysis package, to extract and reconstruct ablation susceptibility values as 3D datasets. Several cell types (cardiac, fibroblast, retinal epithelial cells) were prepared for Cryo-FIB, which provided a stack of images of the tissues. Protocols were adapted to include metal deposition (Pt and W) to enhance grounding and minimise artefacts. 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 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 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 imaging of a variety of samples.

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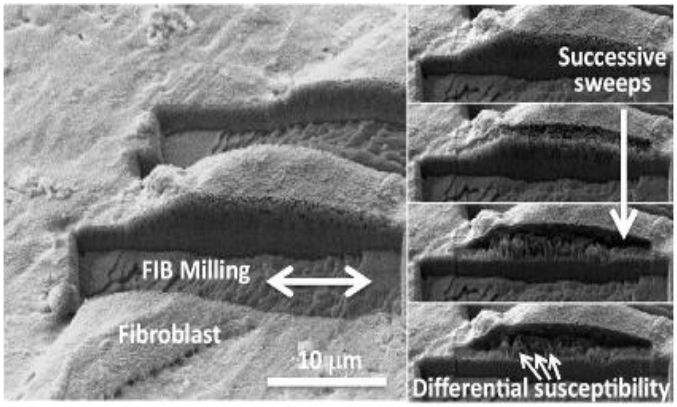

Figure 1. Human Osteoblast cells showing differential susceptibility with successive sweeps of the Ion Beam

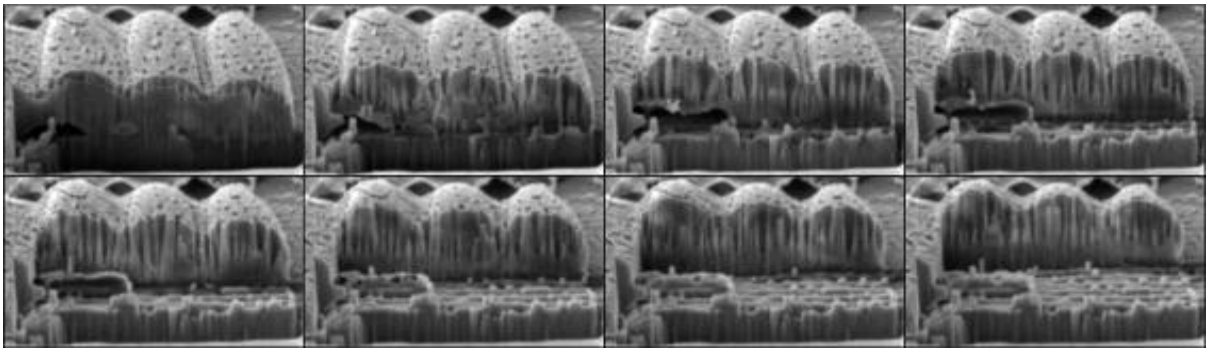

Figure 2. A selection of images through an entire cyanobacteria sourced from the University of Nottingham lake. The columns can be clearly seen; each is tracked and analyzed using the ImageJ plugin.

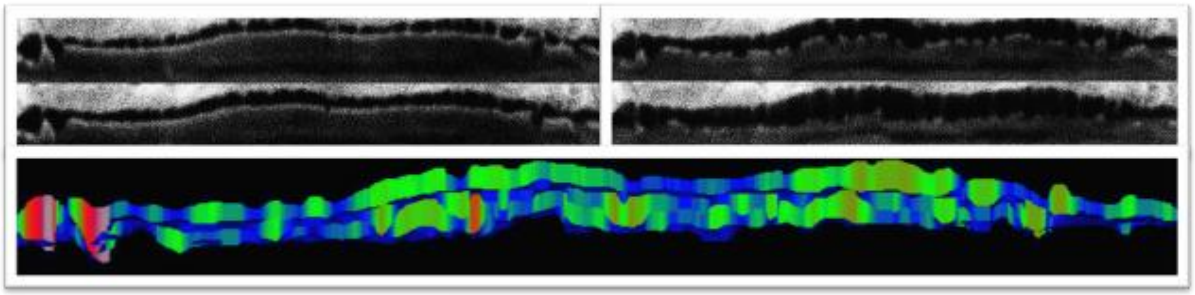

Figure 3. A series of images of T3T cells with the corresponding FIB Susceptibility plot. Red shows high susceptibility, blue shows more resistive material