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Biological Scanning Electron Microscopy

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Scanning electron microscopy (SEM) has, to a great extent been regarded as the Cinderella of the electron microscopy world. The glamour and attraction of high resolution working at the limits of the technique have most often been associated with the transmission electron microscope (TEM). The SEM is most often regarded as being an instrument to produce low-resolution images for the role of illustrating some other, more high-powered, technique.

The development of the field emission gun scanning electron microscope (FESEM) enabled researchers to explore more fully the potential of this kind of microscopy to serve the needs of the research community. The increased resolution, offered by the use of a field-emission electron source has opened up a number of interesting possibilities to gain better information about biological samples.

Because the interaction between the beam electron and the specimen occurs at the atomic level then the emitted signal will reflect the consequences of such interactions. Traditionally, SEM imaging has been associated with the conversion of secondary emitted electrons (SE), from the specimen surface, into imaging information. To optimise such imaging it is necessary to coat the specimen with a thin coating of metal – by sputter coating. This process, in itself, will limit the spatial resolution available from the image.

Imaging using the backscattered electron (BSE) emission from the specimen – being the original beam electrons deflected out of the specimen by the atomic nuclei of the material in the upper layers of the material. The amount of deflection will depend upon the atomic number of the material. Thus, atomic-number contrasted images can be obtained. This has proven to be especially useful when performing immunocytochemistry studies.

By definition, the BSE signal level will be much lower than that of the SE. This means that more beam electrons are required. The FESEM provides such a facility and enables BSE imaging at a level that would not be possible with an ordinary SEM.

Another feature of BSE imaging is that, due to the higher energy of the electrons, the imaging signal can be emitted from considerable depths within the specimen – and not just from the surface layers, as is the case for SE imaging. We have exploited this feature in a number of ways, mainly involved with observing biological components stained or labelled with heavy metals. Such information can come from several microns within the specimen. Some interpretation is called for in order to understand the nature of what is observed in the images.

By 'tuning' the energy of the incident electron beam, by adjustment of the accelerating voltage, it is possible to control the depth from which the information is gathered. By reduction of the energy to a sufficiently low level it is also possible to ensure that unstained biological material will be sufficient to absorb the electron beam and hence create contrast. If the specimen is then placed upon a surface that is an efficient emitter of BSE and imaging is restricted to the BSE signal then, at the correct beam energy, that material can be imaged in the FESEM. Gold or Gold Palladium provides such a surface, but most other metals will also perform similarly.

Using this technique images of the cytoskeleton and bacterial biofilm have been produced, at high resolution, without resorting to either chemical fixation or staining of any sort.

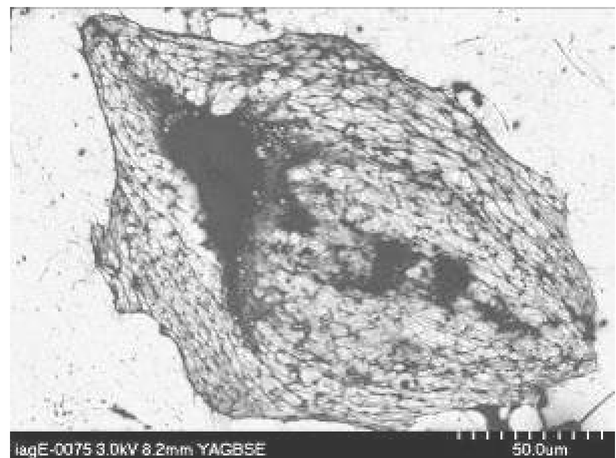

Fig 1. The cytoskeleton imaged without fixation or staining using BSE imaging in the FESEM.

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