STEM mode in the SEM for the analysis of cellular sections prepared by ultramicrotome sectioning

N Hondow, J Harrington, R Brydson and A Brown

Institute for Materials Research, School of Process, Environmental and Materials Engineering, University of Leeds, Leeds, LS2 9JT, UK.

N.Hondow@leeds.ac.uk

Abstract. The use of the dual imaging capabilities of a scanning electron microscope fitted with a transmitted electron detector is highlighted in the analysis of samples with importance in the field of nanotoxicology. Cellular uptake of nanomaterials is often examined by transmission electron microscopy of thin sections prepared by ultramicrotome sectioning. Examination by SEM allows for the detection of artefacts caused by sample preparation (e.g. nanomaterial pull-out) and the complementary STEM mode permits study of the interaction between nanomaterials and cells. Thin sections of two nanomaterials of importance in nanotoxicology (cadmium selenide quantum dots and single walled carbon nanotubes) are examined using STEM mode in the SEM.

1. Introduction

The increasing presence of nanoparticles in various aspects of our lives (healthcare, energy, consumer products, etc) has led to studies examining their potential health risks and the development of the field of nanotoxicology [1]. In vitro investigations in this field comprise cyto- and geno-toxicity testing of nanomaterials, with electron microscopy proving to be an excellent technique to study uptake through a combination of imaging and spectroscopy. However, to enable imaging in the TEM extensive sample preparation is required, involving resin embedding fixed cells with thin sections being cut using an ultramicrotome. As with all sample preparation methods, valid concerns have been raised regarding the potential for the sectioning process to cause the formation of artefacts [2].

For nanotoxicology studies it is vital to be able to confidently state the location of a nanomaterial in relation to an exposed cell. Nanomaterials that have been pulled out of the resin and dragged along the surface of the thin section by the action of the microtome knife could easily lead to misidentified nanomaterial–cellular interactions. One method to determine the three-dimensional location of a nanomaterial is through electron tomography, which has been used extensively in the chemical, biological and materials sciences [3]. In the nanotoxicology field, electron tomography and consequent reconstructions have been used to locate single walled carbon nanotubes (SWCNTs) within cell organelles after in vitro exposure [4]. Electron tomography can provide definitive confirmation as to the location of nanomaterials, however it is an involved technique, requiring much time for data collection and post-acquisition analysis, and it not necessarily suited towards the rapid screening of samples.

We have suggested an alternative technique in which we have used the combination of transmitted and secondary electron imaging in the SEM to determine whether nanomaterials are present in or on a thin section [5]. The dual imaging capabilities of a SEM fitted with a transmitted electron detector allow for projection images to be produced using the transmitted electrons (similar to those in STEM conducted in a TEM) while the same area of sample can be imaged using the secondary electrons at a
low accelerating voltage giving a surface sensitive signal. STEM mode in the SEM has been used since the 1970s [6], however there is not many reports in the literature which have recorded images in both secondary and transmitted electron modes, and until recently no examples of using both of these modes of images for the study of thin sections [5]. The inclusion of a transmission detector has become a common addition to a SEM, and in the study of samples of nanotoxicological importance it has numerous benefits of wide field of view, high throughput of samples and ease of analysis, in addition to the capability to identify surface artefacts using the secondary electron signal.

2. Experimental
Scanning electron microscopy (SEM) was conducted on a Leo 1530 Gemini FEG-SEM fitted with a KE Developments scanning transmission electron microscopy (STEM) detector. SEM imaging using the in-lens detector was conducted at an accelerating voltage of 3 kV and at a working distance of 3 mm. STEM imaging was conducted at an accelerating voltage of 20 kV and at a working distance of 8 mm (resolution ~ 13 nm [5]). Using this specimen-detector configuration we estimate the acceptance angle of the bright-field detector to be about 17 mrad and the dark-field detectors to range from ~ 120 mrad up to ~ 1000 mrad. The Bragg angle for 20 keV electrons diffracted by 0.35 nm lattice planes is 12.3 mrad, indicating that imaging with the dark field (DF) STEM mode in the SEM corresponds to a medium angle annular dark field configuration. For further details please see [5].

Results from two nanotoxicology related samples are discussed in this paper. The first consists of MCL-5 human lymphoblastoid B (suspension) cells that were exposed to polyethylene glycol coated cadmium selenide-zinc sulphide quantum dots (Invitrogen); the second of adherent BEAS-2B normal human bronchial epithelial cells that were exposed to single-walled carbon nanotubes (SWCNT; 1-3 µm length, diameter 1-2 nm, kindly supplied by Prof. A. Barron, Rice University). The samples were prepared using standard techniques for electron microscopy analysis and from the resulting resin block silver sections (~ 70 nm thick) were cut using an ultramicrotome (Leica Ultra-cut E) and placed on a copper grid (Agar Scientific Ltd). No conventional heavy metal stain (uranyl acetate or lead citrate) was employed on the thin sections. For further experimental details regarding the cell culture and nanoparticle dosing, and sample preparation for electron microscopy please see [5].

3. Results and Discussion
A large field of view can be imaged using the STEM mode in the SEM, as shown by the nanotoxicological sample of interest, lymphoblastoid B-cells exposed to CdSe/ZnS quantum dots (figure 1(a)). It is very easy to increase the magnification to image an individual cell (figure 1 (b)), and then to identify collections of quantum dots that are present at higher magnifications (figure 1 (c)), without the changes in lens settings (and therefore focus) that is experienced in the conventional TEM. We have previously shown that it is possible to identify the elemental composition of nanoparticles using energy dispersive X-ray spectroscopy in this mode of imaging [5].

![Figure 1. STEM mode in the SEM DF images (a) low magnification image showing more than 30 lymphoblastoid B-cells exposed to CdSe/ZnS quantum dots, (b) image of a cell that is highlighted in the previous image by a white box, (c) higher magnification image of quantum dots present in and surrounding the cell in the previous image.](image-url)

In addition to being able to easily increase the magnification from imaging individual cells to collections of quantum dots, it is also possible to image the surface using the secondary electrons. A change in conditions is required (in our instrument this is primarily a change in voltage from 20 kV to
3 kV and a decrease in working distance) however it is relatively simple to image the same area of the sample (figure 2). The corresponding secondary electron image in general has very little contrast, and although general features of the cell can be identified, the collections of quantum dots cannot be distinguished from the surrounding cell. What is obvious in the secondary electron image is the artefact that is present in the centre of the cell (figure 2 (b)), as it is on the surface of the section. The lack of contrast in the higher magnification secondary electron image (figure 2 (d)) corresponding to the quantum dots located within the cell in the transmitted electron image (figure 2 (c)) confirms that they are within the thin section and that any interpreted nanomaterial–cell uptake is therefore genuine.

![Figure 2.](image)

We have also examined the uptake of SWCNTs by human bronchial epithelial cells by TEM and STEM mode in the SEM [5]. The SWCNTs, which tend to form bundles, are dispersed through the resin section and can be seen in the DF STEM mode in the SEM image (figure 3 (a)). However in this case, extra information can be gathered from the corresponding bright field (BF) STEM mode in the SEM image; along with the SWCNT bundles the knife marks from the sample preparation can be seen especially in the BF STEM image. Of most interest however is the corresponding secondary electron image (figure 3 (c)) in which the bundles of SWCNTs are clearly seen to have been pulled out and dragged along the surface of the section. In some cases the bundles of SWCNTs may be dragged
across the surface of the section near a cell (figure 3 (d) (e)). This type of analysis allows identification of artefacts and prevention of discussion of false nanomaterial uptake.

4. Conclusion
The combined use of STEM mode in the SEM with secondary electron imaging allows for the examination of thin sections and identification of genuine nanomaterial-cellular interactions whilst avoiding the misinterpretation of any sample preparation derived artefacts. The location of CdSe/ZnS quantum dots was easily identified in dark field STEM imaging, and surface particulate contamination was noted by examining the corresponding in-lens SEM image. STEM mode in the SEM in conjunction with in-lens SEM imaging identified locations where carbon nanotubes had been pulled out and dragged across the surface of a thin section. This technique can be used ensure that cellular uptake of nanomaterials is correctly identified and is ideally suited to the rapidly screening of specimens due to the large field of view that can be imaged.

Acknowledgments
This work has been supported by the EPSRC (EP/E059678/1). The authors wish to thank Dr Shareen Doak and Dr Bella Manshian (Swansea University) for samples and Dr Alice Warley, Dr Anthony Brain and Mr Ken Brady (King’s College London) for expert sample preparation.

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