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Serial block face SEM and TEM imaging for quantitative measurement of cellular uptake of semiconductor quantum dot nanoparticles

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There are an increasing number of potential applications for nanoparticles in clinical medicine, including targeted drug delivery and contrast agents for biomedical imaging, which promise faster, less invasive and more precise treatments than those currently available [1]. Current *in vitro* studies are concerned with the biological impact of nanoparticles, with electron microscopy commonly employed to image the intracellular location. It is critical to quantify the absolute nanoparticle dose received in a given exposure, and to understand the factors which affect this. This is difficult, with the complex and varied mechanisms of nanoparticle interactions with cells.

Our aim is to develop a full quantitative description of nanoparticle uptake by an *in vitro* cell line. Imaging flow cytometry is a high-throughput, low-resolution technique useful for measuring the cellular uptake of fluorescent nanoparticles [2], but it cannot measure the dose in terms of a number of particles. TEM of thin cell sections has the required spatial resolution to provide the location and number of cellular vesicles per 2-D cell slice plus the number of nanoparticles per vesicle [3,4]. However this is limited by both the nature of the 2-D thin section, with only a small amount of the cell analyzed, and the time-intensive nature of TEM imaging.

Serial sectioning can provide information across a whole cell, and the increased use of serial block face scanning electron microscopy (SBF SEM) opens avenues to analysis of much larger volumes without the labour- and time-intensive nature of examining serial sections in the TEM [5]. The reduced resolution of SBF SEM as compared to TEM limits examination to nanoparticle filled endosomes rather than individual nanoparticles, but the size, shape and location of these endosomes can be quantified in whole cell volumes.

We will show results from studies where commercially available Qtracker 705 quantum dot nanoparticles were loaded into human osteosarcoma (U-2 OS) cells and at certain time points were fixed and resin-embedded for quantitative electron microscopy analysis [3,4]. The same resin-embedded sample was used for both the production of the TEM thin sections and for SBF SEM. No post-fixation heavy metal staining was required for either analysis as the electron-dense quantum dots are easily identifiable from the cellular features which are still visible due to staining by the osmium fixative. SBF SEM analysis using a Gatan 3-View system allowed collection of a data set containing 3-D information from numerous cells.

SBF SEM data has been used to correlate higher resolution 2-D TEM data to high throughput, low resolution optical imaging of quantum dot nanoparticle loaded cells [3]. After 1 hour of exposure to Qtracker 705 quantum dots, internalized nanoparticles can be identified in both TEM and SBF SEM. The location of quantum dot endosomes can be identified in SBF SEM, and it was found that they are distributed evenly throughout the cell after the short exposure time. This indicates that any one thin section of a cell is potentially representative of the whole cell, allowing for conversion of the quantitative 2-D TEM data to 3-D. This results in the determination of a calibration factor to transform flow cytometry fluorescence intensity data to a nanoparticle dose distribution, in terms of the fundamental unit, the number of nanoparticles internalized per cell [3]. We will also show how the distribution of the endosomal load within cells develops over a further 24 hour period [4].

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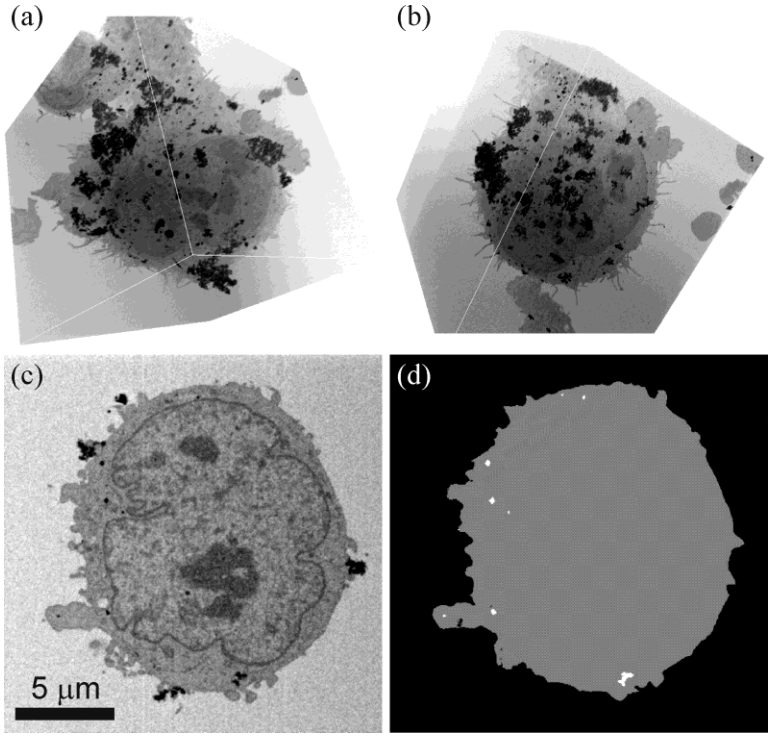

Figure 1. SBF SEM of a U-2 OS cell exposed to Qtracker 705 quantum dots. (a) and (b) Stills from a reconstruction of a cell, (c) contrast inverted SBF SEM image and (d) segmented version of (c).