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IT-4-P-1565 Imaging of nanoparticles in cells with backscattered electrons in a scanning electron microscope

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Scanning electron microscopy (SEM) is an established technique for ultrastructure imaging of cells. Backscattered electrons (BSEs) yield subsurface information and atomic-number contrast [1] and are used in this work to image cellular structures and NPs incubated in cells. Specifically, optimum primary electron energies E_0 for BSE imaging were determined for thin cell sections with thicknesses 100 nm ≤ t ≤ 1000 nm deposited on indium-tin-oxide (ITO-)covered glass slides which are interesting substrates for correlative light and electron microscopy imaging [2]. We also developed a technique to determine the information depth (ID) which denotes the maximum subsurface depth at which an object can be imaged.
Thin cell sections of HT29 colon carcinoma cells incubated with SiO_2 nanoparticles (NPs) of 40 nm size were studied (see [3] for sample preparation). Poststaining was omitted to avoid artifacts. SEM was performed with an FEI Quanta 650 FEG with a low-voltage high-contrast detector (vCD).
Small E_0 were selected to limit the escape depth of BSEs because electrons from large sample depths degrade image resolution and contrast. Fig. 1a shows a 2.5 keV BSE image of a 200 nm section. The SiO_2 NPs, typically contained in vesicles, can be easily detected due to their bright contrast. Cell organelles display high contrast despite the lack of poststaining in Fig. 1b.
Fig. 2a shows a 1 µm section where E_0 up to 7.5 keV can be applied without sample charging. In addition to the incubated SiO_2 NPs, Au NPs with a size of 40 nm are present on the surface and can be distinguished due to their higher BSE intensity. BSE images were taken at different E_0 between 1.5 and 7.5 keV for depth-dependent detection of SiO_2 NPs. With increasing E_0 more NPs become visible corresponding to the increasing ID. The depth of NPs from the surface was determined by tilting the sample and applying a triangulation method. In Fig. 2b the experimentally determined particle depths (dots) are plotted as a function of E_0 and are compared with calculated ID values obtained by Monte-Carlo simulations (triangles). Based on the escape depth $T = f \cdot A \cdot E_0^{1.67}/(\rho \cdot Z^{0.89})$ (Z: average atomic number, A: average atomic weight, ρ: density) proposed in [4], an analytical expression for the ID was obtained by fitting the experimental data with a modified factor f. This expression allows the determination of the ID of BSEs in biological samples. Experiments with entire cells grown on ITO-coated glass are promising with respect to NP detection and are subject of further work.

References

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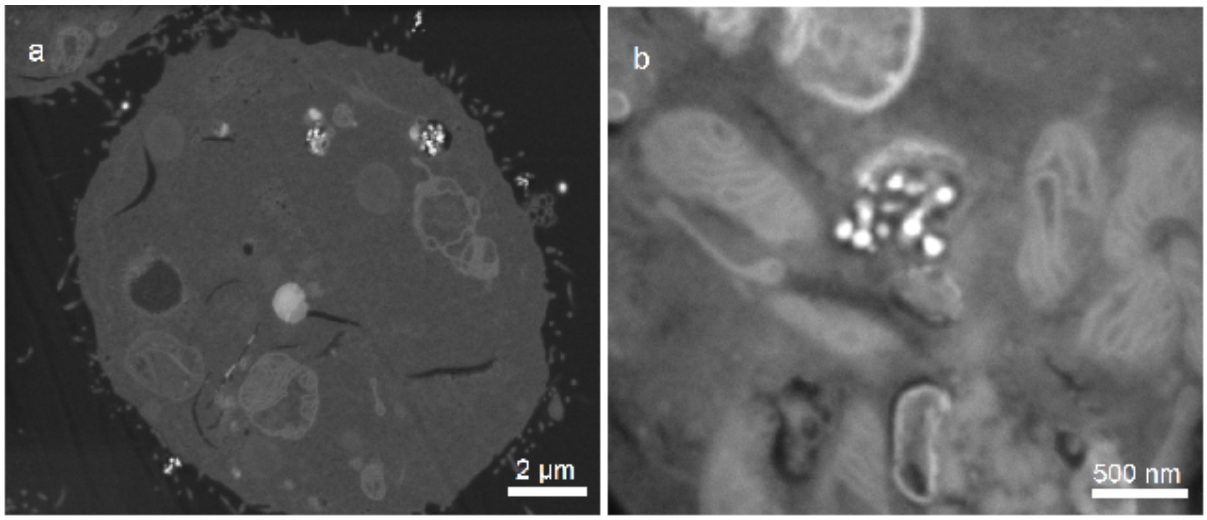

Fig. 1: BSE SEM images of a 200 nm section of an HT29 cell deposited on an ITO-covered glass substrate. (a) Overview image taken at E_0 = 2.5 keV. SiO_2 NPs and organelles are visible in the cell. (b) High-magnification image taken at E_0 = 3.5 keV. NPs and membranes can be well resolved.

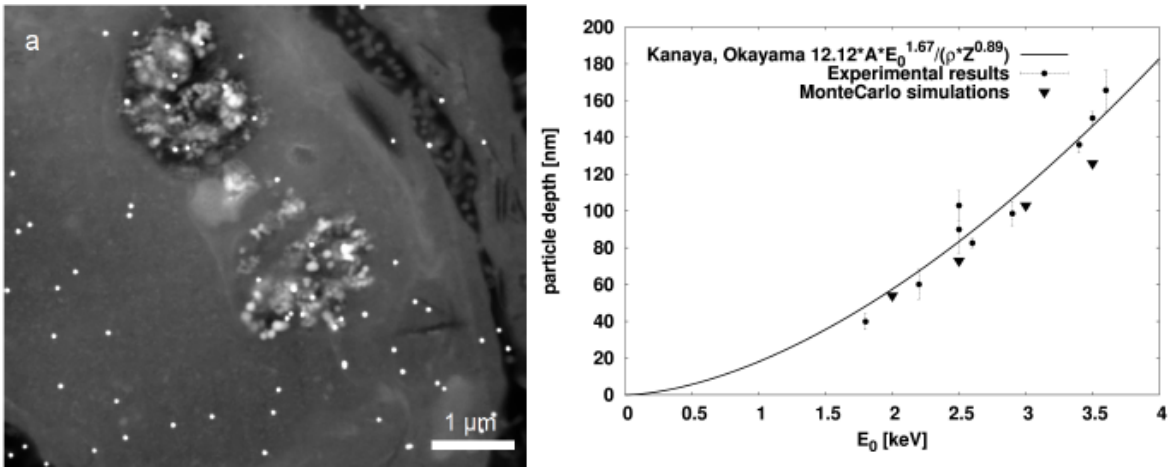

Fig. 2: (a) 7 keV BSE SEM image of a 1000 nm section of an HT29 cell. SiO_2 and Au NPs show different contrast compared to the cell matrix. (b) Plot of the information depth as a function of E_0 with experimentally determined values (dots) and Monte Carlo simulations (triangles). Fit curves are based on the modified Kanaya-Okayama equation [4].