Supplementary Material:

Direct Imaging of Single-Walled Carbon Nanotubes in Cells

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SUPPLEMENTARY METHODS

Low loss spectra of carbonaceous materials

A comprehensive understanding of the human toxicity of single-walled carbon nanotubes (SWNTs) requires measurement of their spatial distribution within cells exposed to them. Direct observation of SWNTs within cells has previously been considered impossible due to the difficulty of differentiating between the SWNTs (~1nm) and the predominantly carbonaceous cell. To achieve contrast between carbonaceous nanoparticles and the cell, energy loss techniques can be applied. Graphite-like carbonaceous material such as fullerenes and nanotubes exhibit a higher bulk plasmon energy ($E_p$) than amorphous carbonaceous material such as in the cell. The measured low-loss spectrum depends on many factors: for example for thin specimens additional surface plasmons occur at $E_p/\sqrt{2}$ according to a simple Drude-like model, which results in an apparent shift to lower plasmon energies and for graphitic carbon an additional transition at 6eV, the $\pi\rightarrow\pi^*$ transition, resulting from the excitation of the $\pi$-bonded electrons. The $\pi\rightarrow\pi^*$ transition is very weak or absent in cellular materials. The difference in volume plasmon energies between the cell and fullerene C$_{60}$ enables a ratio of energy-filtered images recorded at around 5eV, 20eV and 26eV to be used to differentiate the two carbonaceous materials and has been extended to 3 dimensions by Porter et al. Differentiation between carbonaceous materials has been extended to 4-dimensional volume-spectroscopy for mutli-walled nanotubes in nylon by Gass et al. We hypothesised that differences in plasmon energies between the cell and SWNTs will also enable us to differentiate between these carbonaceous materials. Further, from the same data it should be possible to extract the signal from the iron M$_{2,3}$ edge to identify the position of iron catalyst particles. Areas of interest can then be investigated using high-resolution bright field to confirm the presence of SWNTs.

Sample preparation for electron microscopy
Standard sample thicknesses for transmission electron microscopy (TEM) sections can vary from 70nm to several hundred nanometres resulting in the acquisition of a 2-dimensional image containing 3-dimensional information which in turn can result in the “blurring” of data or lack of information. To increase contrast from cell organelles heavy metal stains are often employed, however, these stains can obscure visualisation of SWNTs at high resolution. For these reasons, we used a range of samples thicknesses to optimise each technique employed. For all work carried out on the SuperSTEM (electron energy loss spectroscopy (EELS), high angle annular dark field scanning transmission electron microscopy (HAADF-STEM) and high resolution lattice imaging), we used ultra-thin sections of around 20nm in thickness of both stained and unstained cell sections. The rational for this was that 2-dimentional slices through cells are obtained, therefore minimising the 3-dimensional information, allowing for high-resolution imaging of individual SWNTs within cellular structures previously deemed not possible. For energy filtered TEM (EFTEM) we used 70 nm sample thickness as high resolution imaging was not required and to enhance stability and contrast from samples. For electron tomography we used 300 nm thick samples to increase 3-D volume information.

**EELS spectrum imaging**

EELS spectrum imaging on the SuperSTEM allows for a data set from a user defined area and pixel density \((x, y)\) to be collected, where each pixel contains an energy loss spectrum over a defined energy range \((E)\). Post acquisition, the 3D data set \((x, y, E)\) can be fully analysed for example: removal of plural scattering events, Kramers-Kronig analysis to extract the real and imaginary parts of the dielectric function and extraction of elemental edges to provide a spatially resolved map with the relevant information.

**Energy-filtered transmission electron microscopy**
With EFTEM two-dimensional, elemental maps using electrons with an energy loss characteristic of a core level, interband transition, or plasmon resonance energy are acquired. Unlike EELS data, where the energy loss information is acquired in parallel, for EFTEM the energy-loss information is acquired in series. The user therefore predefines the energy loss window over which the image is to be acquired. EFTEM allows for faster acquisition of larger sample areas compared to EELS, which is beneficial for reducing the electron dose and subsequent beam damage however there is less sample information that can be extracted from the data as the spectral information is limited.
**Supplementary Figure 1.** Aberration-corrected STEM of SWNTs in lysosomes (20 nm thick section).  a) HAADF image of SWNTs piercing through a membrane. b) Individual SWNTs within the cytoplasm; SWNT ends highlighted in yellow. c) Low-loss EELS spectra taken from points 1 to 4. The $\pi$ to $\pi^*$ transition at $\sim6$ eV is seen clearly only in spectrum 3, from the SWNT bundle, it also exhibits a higher bulk plasmon energy (22.2eV SWNT vs 21.8eV cell). The apparent lower plasmon energy observed here compared to EFTEM results is due to the contribution of the surface plasmon resulting from the ultra-thin nature of the specimen$^8$.

**Supplementary Figure 2.** Low-loss EELS spectra extracted from an EFTEM series of SWNTs within a cell (70 nm thick section). (a) SWNTs inside the nucleus of Figure 2f. (b) Cell not exposed to SWNTs. Box denotes position of 2eV slit used to achieve optimal contrast from the SWNTs. The origin of the double plasmon in (a) arises from the contribution from the two individual plasmon responses from SWNTs and the amorphous cell.

**Supplementary Figure 3.** Energy-loss images from an extended low-loss EFTEM series taken from 0-78 eV. a) 12 eV, b) 18 eV, c) 24 eV, d) 30 eV, e) 36 eV, f) 42 eV, g) 48 eV, h) 54 eV, i) 60 eV, j) 66 eV, k) 72 eV, l) 78 eV loss images using a 6 eV slit of SWNTs within a cell. SWNTs have bright contrast in all energy-loss images with highest contrast at 24 eV around the energy of the $\sigma^+\pi$ bulk plasmon of the graphitic SWNTs. pm - plasma membrane, cy - cytoplasm.
References


Equipment and Settings:

Figure 2. (a-b) Aberration corrected VG501 STEM operating at 100kV, convergence angle; 24mrad, collector angle 19mrad of 20nm thick unstained sections, image resolution 1024x1024 image acquisition time:19micro seconds per pixel (a) HAADF detector angle 70 – 210mrad, EELS energy dispersion 0.1 eV/channel, acquisition time 0.1s/pixel. (b) Bright field image. (c-f) EFTEM images of unstained 70 nm thick sections. Images taken on the Philips CM300 FEG-TEM at 300 kV using a 10 μm objective aperture and a 3 eV slit width. Resolution 1024x1024. Acquisition time: 0.1 seconds for 0eV images and 4 seconds for low-loss images. (c) 26eV image. (d) 75 eV image. (e) 26eV image. (f) Zero-loss bright field image.

Figure 3. (a) A 70 nm thick cell section stained with uranyl acetate and lead citrate. Image taken on a Philips CM30 LaB₆ TEM. (b) EFTEM acquired on the Philips CM300 FEG-TEM. Resolution 1024x1024, acquisition time 0.1s, slit width 20 eV. (c-d) HAADF-STEM acquired on a Technai F20 at 200kV, L =150 mm. Acquisition time: 30 s. Resolution 1024x1024.

Figure 4. Cell sections osmicated and post-stained with uranyl acetate and lead citrate. Resolution 1024x1024. (a, b) 70 nm thick cell section. HAADF-STEM image . L =150 mm. Acquisition time: 30 s. (c and e) 20 nm thick cell sections. STEM images taken using an aberration corrected VG501 STEM operated at 100 kV convergence angle; 24mrad, collector angle 19mrad, image resolution 1024x1024, acquisition time: 19micro seconds per pixel (c and e) bright field image, (e) HAADF image. (d) Confocal microscope image taken in reflectance mode. Cells stained with DAPI.

Figure 5. 300 nm thick cell section osmicated and post-stained with uranyl acetate and lead citrate. (a-d) a series of horizontal slices through a HAADF-STEM reconstruction acquired on a Technai F20 operating at 200kV. L=150 mm. Acquisition time: 30 seconds per image. Tilt range -70° to +70°. The slices are 10 nm apart in the z-direction. Three-dimensional reconstruction was carried out using the simultaneous iterative reconstruction technique (SIRT) using Inspect 3D image processing software.
Orthoslices were taken through specific discrete intervals of the reconstructed data using Amira™ 3D visualization software (Mercury Computer Systems Inc., Mérignac Cedex, France).

Supplementary Figure 1. Aberration corrected VG501 STEM operating at 100kV, convergence angle; 24mrad, collector angle 19mrad of 20nm thick unstained sections, image resolution 1024x1024 image acquisition time:19micro seconds per pixel. (a) HAADF detector angle 70 – 210mrad, (b) Bright Field image, (c) EELS energy dispersion 0.1 eV/channel, acquisition time 0.1s/pixel.

Supplementary Figure 2. EELS spectra extracted from EFTEM images of unstained 70 nm thick sections. Images taken on the Philips CM300 FEG-TEM at 300 kV using a 10 μm objective aperture and a 3 eV slit width.

Supplementary Figure 3. EFTEM series of images of unstained 70 nm thick sections. Images taken on the Philips CM300 FEG-TEM at 300 kV using a 10 μm objective aperture and a 6 eV slit width.