Identifying single quantum dots using statistical analysis of electron spectroscopic imaging (ESI) series

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Novel fluorescence-based light microscopy techniques offer precise localisation of molecules of interest in biological samples. However, the localisation does not reveal the specific morphology of cellular structures around labelled targets. This information gap can be complemented by transmission electron microscopy (TEM). It allows visualisation of ultrastructural details in the vicinity of target molecules at nanometer resolution. Correlative microscopy so far visualises function with fluorescence light microscopy and ultrastructure with TEM. The possibility to detect fluorescent markers in TEM would allow combination of functional and ultrastructural studies in the same instrument. Conventional TEM, however, does not offer direct detection of e.g. fluorescent quantum dots (QDs), which are electron-opaque fluorescent nanocrystallites. Identifying QDs in electron micrographs is restricted in its applicability. They have to be identified manually by their contrast and shape [1], as it is not their fluorescence that has been so far detected in TEM. With energy-filtered TEM (EFTEM), electron-spectroscopic core-loss images can alternatively be used to detect elemental distributions of QDs [2]. But more importantly, EFTEM is also expected to detect the equivalent of the fluorescent signal by recording selected, inelastically scattered electrons from the low-energy-loss window. This was proven for a chemical, the fluorescent drug doxorubicin, which was imaged at 18 nm resolution within the structural, cellular context [3].

By analysing the spectral distribution of electron energy loss, we show that QDs are distinguishable from the background. We imaged cadmium sulfide (CdS) core-type QDs of 7 nm diameter on amorphous carbon support film (Fig. 1a). Using scanning TEM we showed that their energy-loss spectrum contains a specific absorption signal in the low-loss region (Fig 1b,c), in an energy range corresponding to the excitation of the fluorescent light signal. According to theoretical calculations, their band-gap is expected to be approximately at 2.7 eV [4] (Fig. 1c). Moreover, using electron spectroscopic imaging (ESI), we collected stacks of images of dispersed CdS QDs on carbon support at different energy losses (Fig. 2a,c). From the resulting data cubes, spectra at all image locations were analysed and classified according to different low-loss features. Principle component analysis (PCA) was applied to automatically calculate feature vectors that separate spectra by low-loss variances. This procedure distinguishes QDs from background signal using constraint PCA selection (Fig. 2a,b). By applying a more relaxed PCA selection, the delocalised inelastic signal becomes visible (Fig. 2c,d).

We demonstrate that QDs are indeed identifiable by their fluorescent excitation in the TEM. In light microscopy different QDs are routinely used for multicolor biological imaging [5]. Assuming that different QDs in a mixed sample are also detectable using distinct low-loss features, multicolor TEM can now be envisioned.

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1. B. Giepmans et al., Nat. Meth. **2** (2005), 743-749.
2. R. Nisman et al., J. Histochem. Cytochem. **52** (2004), 13-18.
3. A. Mhawi et al., J. Struct. Biol. **158** (2007), 80-92.
4. P. E. Lippens and M. Lanno, Phys. Rev. B **39** (1989), 10935-10942.
5. V. Biju et al., Anal. Bioanal. Chem. **391** (2008), 2469-2495.

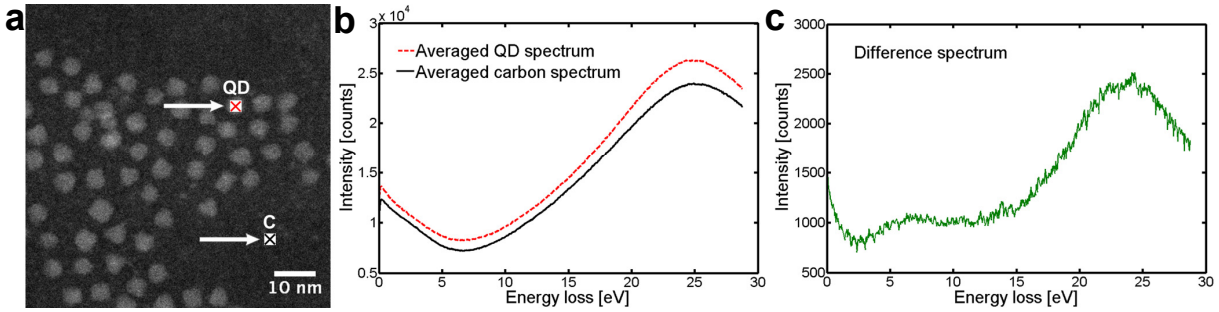

Figure 1. Low-energy-loss signal of 7 nm sized CdS quantum dots (QDs) on amorphous carbon support. **a** - STEM image, recorded with a C_S-corrected TEM Libra 200 MC (Carl Zeiss NTS). **b** - Electron energy-loss spectra of one QD (dashed line) and of carbon support of 30 nm thickness (solid line). **c** - Background subtracted spectrum revealing a shoulder in the low-energy-loss signal. The energy-dispersion is 32 meV/channel, the recorded energy range 0 to 28.76 eV.

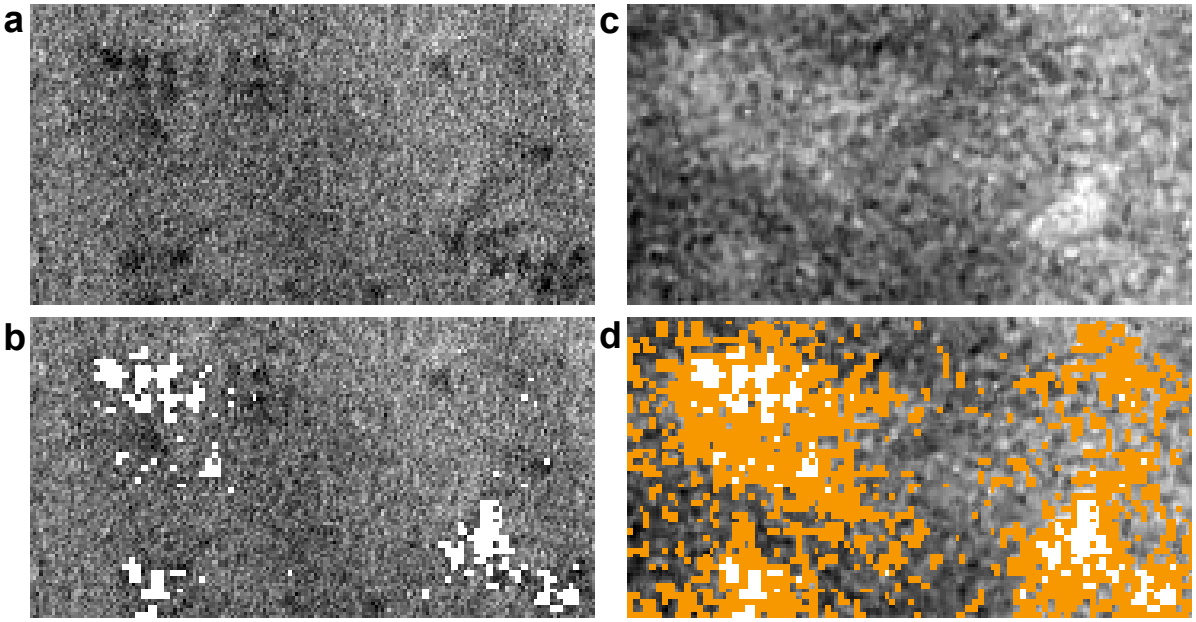

Figure 2. Identified QD pixels in a specified area after image processing and principal component analysis (PCA) of input spectra. **a** - Elastic bright-field (zero-loss) image of 7 nm CdS QDs on amorphous carbon support (30 nm thickness). **b** - Overlay of zero-loss image with pixels classified as QD pixels (white) according to low-loss constrained PCA selection. **c** - Inelastic image of the same area as in (a) with delocalised energy-loss signal. **d** - Overlay of inelastic image with pixels identified as QDs according to low-loss spectra (white) as in (b) and relaxed PCA selection (orange/grey). The ESI stack was recorded with a C_S-corrected TEM Libra 200 MC (Carl Zeiss NTS), with images from 2-14.5 eV in 0.5 eV steps.

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