Determination of the micelle height: detailed description of the “histogram method”

The diameter of the core of the PS-r-DE-b-PI micelles has been determined from the observed height in the AFM images. The height is a more accurate measure of the particle height than particle diameter due to the absence of tip convolution effects. To estimate the average particle height, cross-sections are commonly used. This is not the most accurate method as cross-sections need to be placed over the highest point of the particle (in case of spheres) to determine the diameter. In addition the height of a particle can be determined by using the roughness analysis method (Veeco SPM-software, v5.18). If a small box is selected around a particle the analysis will display besides the RMS roughness also the z-range within the box (the height of the highest point minus the height of the lowest point). The height of a spherical particle can be determined by subtracting the substrate roughness of a flat area from this value. The latter of the two methods is more accurate but, like the earlier one, requires a lot of user interaction (drawing lines or boxes) as well as averaging.

The height of the PS-r-DE-b-PI micelles used in the manuscript was derived from a histogram in which the heights of the pixels within the image are plotted against their relative abundance. For flat surfaces with uniform, flat particles (height $z_1$) the histogram displays two peaks in which the distance between the peaks corresponds to the average height of the particles. For a flat surface with spherical particles (height $z_1$) the histogram also displays two peaks. The distance between the maxima however, is no longer corresponding to the particle height as the highest points of the particles are not the most abundant. Instead, the histogram shows a symmetric peak for the level of the substrate and a tailing peak for the particles (Figure 1). We correlated the average height, found by cross-sections to the histograms and we found that in this case the height of the particles can be determined from the distance between the center of the substrate peak and the bending point of the particle peak.
Figure 1. Histograms displaying the relative abundance of data points.

The method described above has been verified by measuring the micelles height by drawing and averaging the results for numerous cross-sections. The deviation between both methods is below 1 nm for all samples.

In addition the above method has been verified on a sample with spherical gold nanoparticles (Figure 2). The height of the particle indicated by the arrow (13.5 nm) was determined by plotting a cross-section across the top of the particle. The height of the same particle was also determined by displaying the statistical data for a $32 \times 47$ nm box placed tightly around the particle (Figure 3). The height of the particle is obtained by subtracting half of the substrate roughness from the obtained value of the $z$-range within the box. For all particles the average height was determined at 13.6 nm. The error estimated at $\pm 1.1$ nm is dominated by the accuracy at which the roughness of the substrate is determined.
Figure 2. Intermittent contact mode image of gold nanoparticles on mica. The height of the particle indicated by the arrow is determined from a cross-section.

Figure 3. The height of a particle can be derived from the z-range obtained for a box placed tightly around the particle.
Figure 4 displays the histogram that has been obtained from the same image. The histogram in the middle image displays two peaks. The taller indicates the height of the substrate and the smaller one (see close-up image) the height of the particles. It was found that the distance (13.7 nm) between the center of the “substrate-peak” and the bending point of the “particle-peak” corresponds to the average height of the particles as determined by the cross-section method and the z-range method (13.6 nm). The error for determining the height by this method is dominated by the accuracy at which the marker can be placed at the actual bending point and was estimated at about 0.3 nm. Because placing a cross-section across the tops of the particles is not necessary the error is much lower. An additional indication of the accuracy of the method is provided by the AFM image in the top-left corner of Figure 4. All pixels with a height greater then 13.7 nm are colored uniformly red. As to be expected for a good average height about 50% of the particles are not colored red.

Figure 4. Histogram data in which the height (depth) of each pixel is plotted against its relative abundance. The distance between the center of peak indicating the height of the substrate (green line) and the bending point of the peak originating from the presence of the particles (red line, see close up on the right) corresponds to the average height of the particles.