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From 2D and Single Particle to 3D and Batch Analysis as a Routine Quality Check Procedure for the Morphological Characterization of Core-Shell Microparticles

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In recent years, core-shell (CS) particles have been increasingly used for a wide range of applications [1-3]. CS particles show unique properties by merging individual characteristics of the core and the shell materials. Selecting the materials of the inner core and the outer shell layers influences the functions and properties of the designed particles. An alteration particularly in their surface roughness affects the final performance of the particles in the targeted application [4]. Quantitative evaluation of the roughness of CS microparticles is, however, a challenging task employing microscopic techniques being scarce and showing large differences in terms of methodology and results.

In our previous work, we have reported a systematic study with a reliable analysis tool, which evaluates profile roughness quantitatively, for individual core-shell microparticles using electron microscopy (EM) images of both types, Scanning Electron Microscopy (SEM) and transmission mode SEM (or TSEM) [5, 6]. The SEM images contain two-dimensional (2D) information, therefore, provide profile roughness data only from the projection in the horizontal plane (in other words, from the "belly") of a spherical particle. The present study offers a practical procedure to give access to more information by tilting the sample holder and hence allowing images of a single particle to be recorded at different orientations under the same view angle. From the analysis of these images, extended information on surface roughness of the particle can be extracted. Thus, instead of obtaining 2D information from a single SEM image, three-dimensional (3D) information is obtained from 2D projections recorded at different particle orientations.

Three types of home-made particles having various roughness values were investigated in this work [5, 6]: i) bare polystyrene (PS) particles, ii) PS particles decorated with a first magnetic iron oxide (Fe_3O_4) nanoparticle shell forming CS microbeads, and iii) PS/Fe_3O_4 particles closed with a second silica (SiO_2) shell forming core-shell-shell (CSS) microbeads. The most suitable sample preparation procedure prior to microscopy and analysis was considered as follows: the particles have been suspended in ethanol with ultrasonication for 5 min and samples for analysis have been prepared by drop-casting on conventional carbon TEM grids. The samples on a thin film TEM grid were placed on the sample holder. Images of single particles were taken at different tilt angles by an SEM with high-resolution and surface sensitive SE InLens® mode.

The image analysis workflow is depicted in **Figure 1**. Image acquisition consisted of taking a series of SEM images of a single particle with stepwise tilted sample holder up to 10° (a). For tilting experiments only SEM images (b) were recorded, which were automatically analysed by the newest version of a self-written Python script [7]. First, the software applies IsoData thresholding algorithm to segment the images, the contour of the particle is identified, and the center of the particle is calculated by minimizing

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the standard deviation (SD) of the distances between the iterated center point and each contour point (c). The lateral profile and the mean radius (R_{mean}, **Figure 1**d) of the particle are obtained from these calculations. Finally, the software outputs the root mean squared value of the roughness (RMS-R_Q, **Figure 1**d) from the particle projection.

Figure 2a and b show SEM images of the same single PS/Fe_3O_4/SiO_2 CSS particle ('p1') recorded at 2 kV accelerating voltage without tilting (p1@T_0°) and with tilting at 10° (p1@T_10°), which represents the maximum stage tilting angle attainable with our SEM. The lateral profiles extracted from these two SEM images are presented in **Figure 2**c. Both profiles showed large waviness due to the quite rough surface of the particle. When the particle was tilted to 10°, it was observed that the high waves in the profile remained roughly the same. On the other hand, a slight rise and fall were observed in the low waviness regions. Nonetheless, no significant difference was examined between the roughness values calculated from the projection without tilting (RMS-$R_{Q(T_0°)}$ = 37.0 nm) and with tilting at 10° (RMS-$R_{Q(T_10°)}$ = 37.2 nm). **Figure 2**d and e show SEM images of a second particle ('p2') randomly selected from the same particle batch recorded at identical conditions as particle p1. However, the surface of the second particle was smoother than that of particle p1. The lateral profiles extracted from these two SEM images of the particle p2 recorded with and without tilting showed smaller waviness compared to particle p1 and the waviness stayed almost unchanged (**Figure 2**f), when the particle was tilted. Calculated roughness values from the SEM images without tilting and with tilting at 10° were 20.6 and 20.2 nm, respectively, which were considerably lower than those of particle p1. These results clearly showed that, there was no dependency of roughness value on the projected particle section. In addition, particle p1 and particle p2 could clearly be differentiated due to their roughness values.

The created image analysis tool accurately quantifies a particle's roughness value not only from one image but from a set of projections providing a quasi-3D overview of the surface, which can be used to analyse several single particles in a batch to evaluate the homogeneity. This approach was also used to determine the variation in roughness from batch to batch as a routine quality check procedure, independently of the image resolution. The software yields additional information about the size of an individual microparticle, within a few seconds by pressing only the run button. Concurrently, having sufficient statistical data, measurement uncertainties of the determined roughness value associated to various orientations were estimated. Moreover, flow cytometry measurements provided complementary data to the roughness derived by the present image analysis approach.

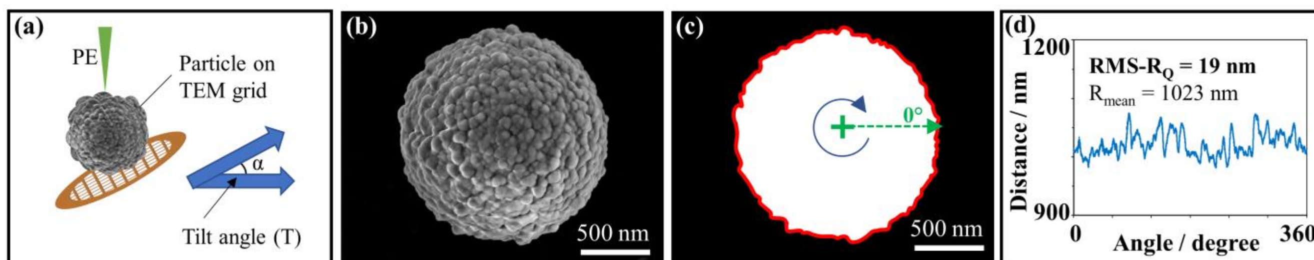

Figure 1. Illustration of tilting of the samples with SEM setup (a), an exemplary SEM/InLens image (b), automatically segmented image, identified contour (in red) and center of the particle ('+') with the custom Python script (c), and calculated mean radius (R_{mean}) and root mean squared roughness (RMS-R_Q) of the particle (d).

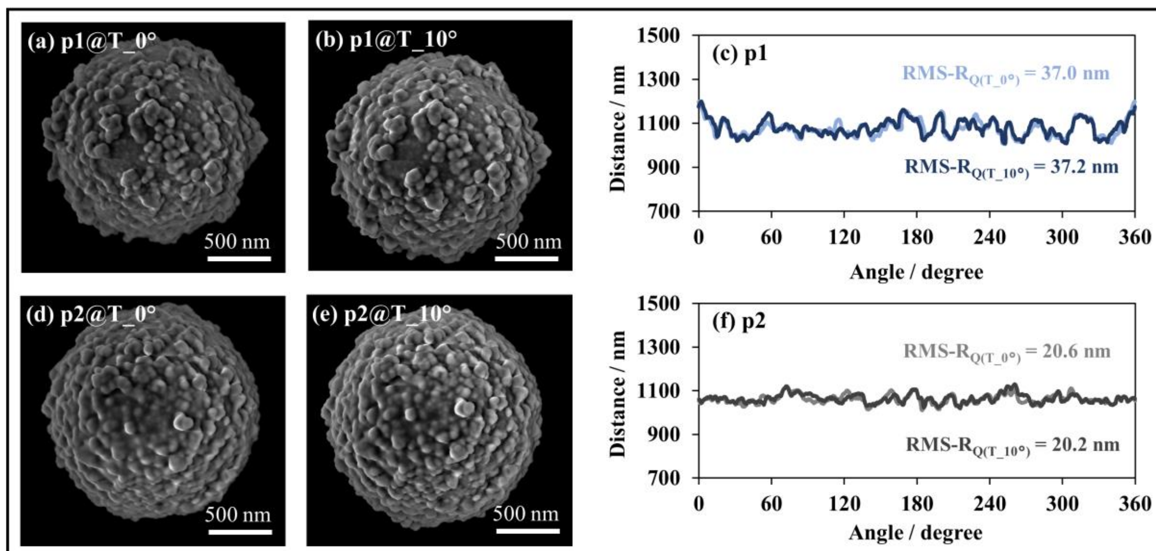

Figure 2. SEM/InLens images of a PS/Fe_3O_4/SiO_2 CCS microparticle recorded (a) without tilting and (b) at 10° tilting, (c) lateral profiles and the root mean squared roughness values (RMS-R_Q) calculated from images a and b. SEM/InLens images of another PS/Fe_3O_4/SiO_2 CCS microparticle from the same batch recorded (d) without tilting and (e) at 10° tilting, (f) lateral profiles and the root mean squared roughness values (RMS-R_Q) calculated from images d and e (*T = tilting angles in degrees, p1 = particle 1, p2 = particle 2*).

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