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Development of standard measurement protocol for the size analysis of SiO_2 nanoparticles using a scanning electron microscopy

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The size and size distribution of materials are important factors for understanding the characteristics of nanomerterials. Although transmission electron microscopy (TEM) is a powerful tool for determining the nanomaterial size, the nanoparticle cast on carbon-film for the TEM sampling exhibits limitation in size measurement due to agglomeration during the solvent elimination. By using scanning electron microscopy (SEM), such limitation can be overcome by modifying the surface of substrate for sampling. Here, we show that the size measurement of well-defined SiO_2 nanoparticles could be performed with minimized sampling issues. The experimental conditions such as a working distance, an accelerating voltage, a filament current and a metal coating were adjusted to optimize imaging condition. Experimental uncertainties were also derived from the consideration of uncertainty factors and measurement of CRM for SEM imaging.

Keywords: SEM, SiO_2, nanomaterial, size, working distance, accelerating voltage, metal coating

1. INTRODUCTION

The unique large surface area of nanoparticles is promising for applications such as electrical and thermal insulators as well as biocompatible nano-bio sensors and diagnostics [1-4]. In the case of a cell viability test, their potential harmful effects as changing the size of the nanoparticles have reported in some articles. [5-7] With making the SiO_2 nanoparticles with narrow size distribution [8], accurate size measurement is prerequisite for an evaluation of toxicity. SEM is a useful and easy accessible method to characterize a nanostructure. Basically, we have been carried out to find the standard measurement condition for a negligible size change during imaging the nanoparticles in SEM. The controlled conditions are following: accelerating voltages (5 kV and 10 kV) and filament currents of an electron beam (5 μA and 10 μA) as well as the working distance (5.1 mm and 9 mm) and with Pt coating and without.

2. EXPERIMENTAL DETAILS

Si wafer cut to 1 x 1 μm^2 were prepared via piranha process to make clean and hydrophilic surface. 60 nm SiO_2 nanoparticles dissolved in deionized water were dropped on Si substrate and dried naturally. All images for Pt coated (100 s at 15 mA) or uncoated SiO_2 nanoparticles were taken in the experimental condition of an aperture size of 50 micrometer, a secondary electron detector in an upper mode, SE(U) with Field Emission Scanning Electron Microscopy, FE-SEM, (HITACHI S-4800 model). The image resolution was fixed to 1280 x 960 pixels with the magnification of 100,000 for SiO_2 nanoparticles and 200,000 for the 1D grating CRM, which of the pixels were converted to the 1 nm / pixel and 0.5 nm / pixel, respectively. The 1D grating CRM of the mean pitch of 80 nm with an uncertainty of 0.5 nm was used to calibrate the magnification of SEM. The image J 1.45s software program (available from the National Institutes of Health, freeware) was utilized to carry out the image analysis of 1D grating and nanoparticles. The analysis for the nanoparticles was described in Fig. 1. The processes are following; 1) make the binary image digitized by a white and a black, 2) set the threshold range carefully by overlapping between raw and binary image. The highest length in the particle rounded by an image process stands for the feret diameter. The number of nanoparticles more than 13 was analyzed for the statistical analysis.

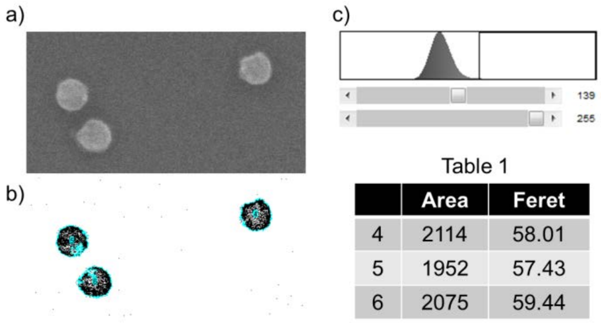


Table 1

	Area	Feret
4	2114	58.01
5	1952	57.43
6	2075	59.44

Figure 1. a-b) The SEM image for SiO_2 nanoparticles and the binary image set to a threshold region as shown in c. The feret diameters and area of particles analyzed in image J represent in table 1.

3. RESULTS AND DISCUSSION

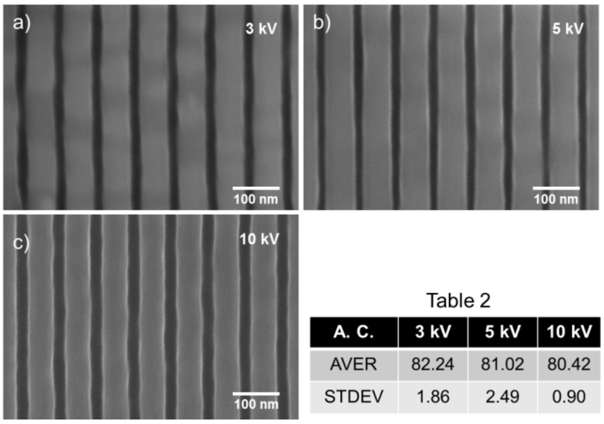


Table 2

A. C.	3 kV	5 kV	10 kV
AVER	82.24	81.02	80.42
STDEV	1.86	2.49	0.90

Figure 2. a-c) SEM images for 1D grating CRM upon the accelerating voltages at the working distance of 6 mm. The measured mean pitch sizes were summarized in table 2.

The CRM for the calibration has been used to find the optimal accelerating voltage of an electron beam for measuring the size of the SiO_2 nanoparticles. The images were analyzed with sixteen lines for two image frames using image J 1.45S at 3 kV, 5 kV and 10 kV, respectively. The values are 82.24 nm, 81.02 nm and 80.42 nm. The size of the mean pitch was reduced as increasing the accelerating voltage. Although the value at 10 kV shows the better resolution, we select the base experimental imaging condition to 5 kV to minimize the charging effect.

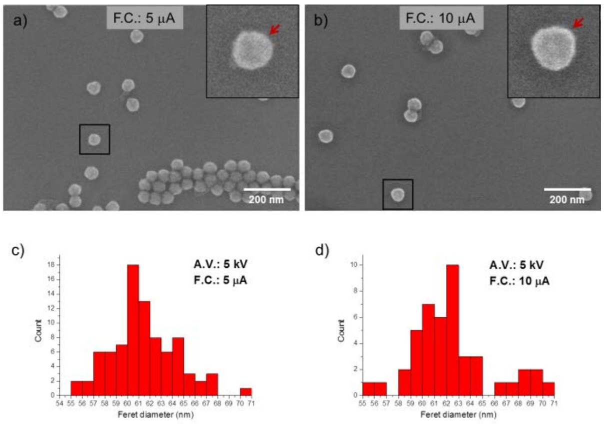


Table 3

A. V.	5 kV		10 kV	
F. C.	AVER	STDEV	AVER	STDEV
5 μA	61.39 (n = 76)	2.68		
10 μA	62.41 (n = 45)	3.36	59.59 (n = 26)	2.51

Figure 3. a-b) SEM images for SiO_2 nanoparticles dispersed on Si substrate at the filament current of 5 μA and 10 μA, the accelerating voltage of 5 kV and working distance of 5.1 mm. The particles in square area of images were enlarged, which show the bright colours at boundary's edges of nanoparticles, marked by red arrows. c-d) Histograms for nanoparticles counts versus feret diameter with 1 nm interval at each conditions. Table 3 shows the average sizes and its standard deviations of particles for each condition.

In Fig. 3, we observed the size change of NPs depending on the filament current of 5 μA and 10 μA. The distinct edges of nanoparticles, marked by the red arrows in inset of Fig. 3b, represent the charging effect by the electron beam scanning in SEM. In table 3, the average sizes and its standard deviations of particles upon the accelerating voltages and the filament currents were summarized with the statistical analysis for the number of 86, 52 and 28 particles. At 5 kV, the average size of 61.39 nm in 5 μA is smaller than that of 62.41 nm in 10 μA, which is due to an artefact by the charging effect of the high beam current. On the other hands, the size at 10 kV shows the lowest value to 59.59 nm. Such an enhancement of the resolution depends on the increment of the accelerating voltage. Unlike such a change in the accelerating voltages and the filament currents, there have not been found in the distinct difference at the working distance of 5.1 mm and 9 mm. We realized that the boundary regions of nanoparticles were manifested in the higher beam current and the higher accelerating voltage.

Table 4

W. D.	5.1 mm		9 mm	
F. C.	AVER	STDEV	AVER	STDEV
10 μA	59.59 (n = 26)	2.51	59.96 (n = 43)	2.79

Table 4. Feret diameter for SiO_2 nanoparticles observed in the working distance of 5.1 mm and 9 mm.

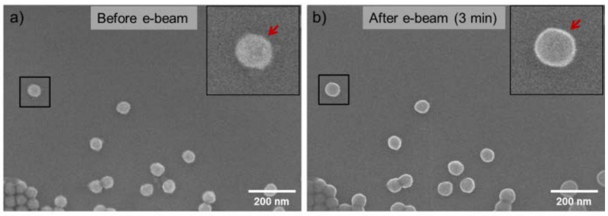
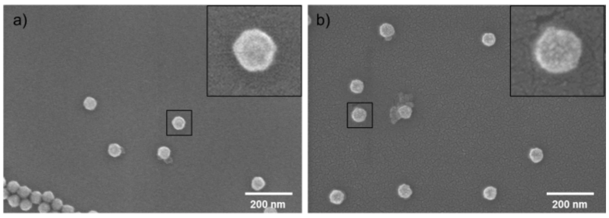


Table 5

Before e-beam		After e-beam	
AVER	STDEV	AVER	STDEV
60.05	2.94	67.57	3.23

Figure 5. a-b) SEM images for SiO_2 nanoparticles before the irradiation of the electron beam and after. The current and the irradiation time of electron beam are 4.1 μA and 3 min in the working distance of 5.1 mm and the accelerating voltage of 5 kV, respectively. The size change of the nanoparticles under the irradiation of the electron beam was analyzed for the thirteen particles, which was tabulated in table 5.

The size change of nanoparticles as increasing the electron beam irradiation time were found at the accelerating voltage of 5 kV and the beam current of 4.1 μA in Fig. 5. The average values (standard deviations) of 60.05 (2.94) nm and 67.57 (3.23) nm for the number of nanoparticles of 13 make the size difference of 7.52 nm by the electron beam irradiation for 3 min. Bigger sizes and distinct bright boundary edges of nanoparticles appear in the enlarged area of Fig. 5b. The image acquisition time of one frame is 15 s for the image resolution of 1280 x 960. It could be approximated that the increment of the particle size for one frame image is ca. 0.63 nm. To make the uncertainty less than 1 nm at the experimental condition of 5 kV and 5 μA, the image acquisition time should be minimized as short a time period as possible, or have to find the mild imaging condition like lower filament current.



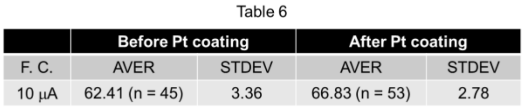


Table 6

	Before Pt coating		After Pt coating	
F. C.	AVER	STDEV	AVER	STDEV
10 μA	62.41 (n = 45)	3.36	66.83 (n = 53)	2.78

Figure 6. a-b) SEM images for SiO_2 nanoparticles before Pt coating and after, observed at the accelerating voltage of 5 kV, the working distance of 5.1 mm and the filament current of 10 μA. The Pt coating for 100 s at 15 mA makes the rough SiO_2 nanoparticles and Si surface as shown in enlarged areas. The size change of the nanoparticles by the Pt coating was described in table 6.

To avoid the charging effect, the insulating nanomaterial such as SiO_2 was generally coated by a conducting material. The Pt coated SiO_2 nanoparticles on Si substrate were compared with the Pt uncoated ones in Fig. 6a,b. Pt coated SiO_2 nanoparticles as well as Si substrate have become the rough surface with a small grain boundary. The manifest edge line of the nanoparticle due to the charging effect seems to be diminished as shown in an inset of Fig. 6b. The size increment of 4.42 nm by the Pt coating appears in table 6. The thickness of the coating is crucial for retracking the exact particle size before the metal coating.

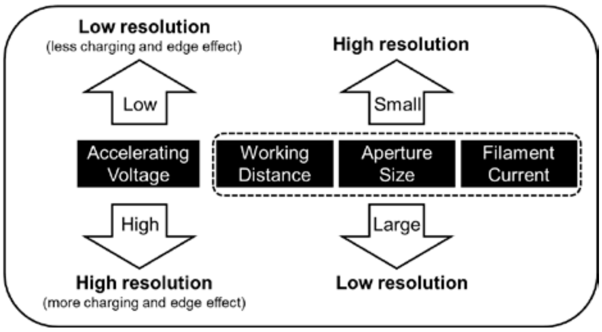


Figure 7. Schematic diagram for SEM experimental conditions determining the resolution as well as the charging and edge effect.

The SEM imaging condition should be optimized in considering following parameters: the accelerating voltage, the working distance, the aperture size and the filament current. Those determine the image resolution that is displayed in the schematic diagram of Fig. 7. Although a higher accelerating voltage is good for the high resolution, it shows more charging and edge effect. On the other hand, the smaller values of the working distance, the aperture size and the filament current also make a poor quality image by the lower signal to noise ratio, but higher resolution. We suggest that the optimal conditions for imaging SEM are following: $\geq$ 10 kV accelerating voltage, $\leq$ 5 μA filament current, $\leq$ 50 μm aperture size and $\leq$ 5.1 mm working distance.

4. CONCLUSION

We have conducted the size measurement of well-defined SiO_2 nanoparticles dispersed on the hydrophilic Si substrate with SEM. Based on the image condition for the 1D grating CRM, the imaging conditions of the SiO_2 nanoparticles were optimized as controlling the accelerating voltage, the filament current, the working distance and the metal coating. We found that the higher accelerating voltage than 5 kV and the lower filament current than 5 μA were needed to precisely measuring the SiO_2 nanoparticles in SEM.

5. REFERENCES

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