Contribution of Metal Layer Thickness for Quantitative Backscattered Electron Imaging of Field Emission Scanning Electron Microscopy*

Hyonchol Kim  
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan

Hiroyuki Takei  
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan and  
Department of Life Sciences, Faculty of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Oura-gun, Gunma 374-0193, Japan

Tsutomu Negishi and Masato Kudo  
JEOL Ltd., 1-2 Musashino 3-Chome, Akishima, Tokyo 196-8558, Japan

Hideyuki Terazono  
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan.

Kenji Yasuda†  
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan and  
Department of Biomedical Information, Division of Bioengineering, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan  
(Received 29 December 2011; Accepted 19 April 2012; Published 30 June 2012)

The contributions of metal thickness and the diameter of metal shell particles to quantitative backscattered electron (BSE) imaging in field emission scanning electron microscopy (FE-SEM) were studied to evaluate the potential of using these particles as simultaneously distinguishable labels of target molecules in FE-SEM studies. Gold spherical shells were fabricated with 200 or 300 nm diameter and 5, 10, 15 or 20 nm Au shell thickness, placed on silicon substrates, respectively, and observed in BSE imaging mode by FE-SEM. Flat Au films of the same thicknesses were formed on a Si substrate to evaluate the contribution of shell diameter to BSE imaging. The relationship between relative BSE intensity, which was calculated by setting the intensities of the Si substrate and 20 nm-thick Au layer as standards, and Au layer thickness was studied for all samples. With increasing Au layer thickness, BSE intensity also proportionally increased for all samples ($R^2 > 0.93$) in the range of these thicknesses. Gradients of the increase were 1.5 times different between the flat film and metal shells, which was caused by the presence of voids in shell particles. The difference in gradients for increasing shell thickness between 200 and 300 nm particles was 15%. This result indicated that 1.5 times difference in shell diameter contributed to the increase of BSE intensity against the increase of shell thicknesses as a 15% error, and strict control of both metal shell diameter and thickness in the fabrication process is essential when using these shells as labels of BSE measurements in FE-SEM. [DOI: 10.1380/ejssnt.2012.301]

Keywords: Electron–solid interactions, scattering, diffraction; Reflection electron microscopy (REM); Scanning electron microscopy (SEM); Nano-particles, quantum dots, and supra-molecules; Nano-films, stacks, and other nano materials; Metal nano-cup; Biological label; Adaptive SEM technology

I. INTRODUCTION

Field emission scanning electron microscopy (FE-SEM) is widely used for the observation and element analysis of nano-scale objects. Secondary electron (SE) imaging is usually applied for observations of detailed structures on sample surfaces. Various methods are for the analysis of elements, attaching additional detectors to the FE-SEM. One general method uses energy dispersive spectroscopy (EDS). In EDS analysis, quantitative evaluation of element components on a surface is performed with measurements of characteristic x-rays; however, the lateral resolution is 30 to 80 nm even if a low acceleration voltage is used to reduce noise signals from the substrate layer under the target samples [1].

Another method is backscattered electron (BSE) measurement. BSE is a beam electron that is reflected from the specimen by elastic scattering. Because the intensity in BSE images is strongly related with the atomic number of samples, BSE images reflect the distribution of elements in samples [2–4]. In our previous studies, we found that at least six kinds of thin film elements can be simultaneously discriminated as more than 1.3 times difference of BSE intensity in the image [5], and this analysis can also be applied to double-layered thin films, which are fabricated with the deposition of thin Au layers on other element films [6]. In previous studies, BSE analyses were also applied to spherical metal shells, which
can be used as target labels, as same as colloidal gold in the usual immunoelectron microscopy [7–9], and the same relationship between BSE intensity and the acceleration voltage of the incident electrons as results for thin films was obtained under general observation conditions in FE-SEM. The lateral resolution of BSE images depended on the size of the incident electron beam, which is relatively higher than that in EDS; therefore, BSE measurement is suitable for the identification of nano-scale elements, such as nano-particle mixtures composed of different elements spread on the substrate [10].

In this paper, contribution of metal layer thickness to the BSE intensity was quantitatively studied. In a previous BSE study using double-layered thin films, the suitable condition for element identification depended on the film thickness [5], which indicated that the thickness of metal films contributed to BSE observation; therefore, the contribution of both Au thin film layers and Au spherical shells was quantitatively studied in this study.

II. EXPERIMENTAL

A. Fabrication of flat Au film layers

Flat Au film layers, 5, 10, 15 or 20 nm thick, were formed on a Si substrate using a metal grid as a mask. A grid with 0.5 nm argyle holes was placed on a Si substrate, and a 5 nm Au layer was formed on the substrate through the grid mask by thermal deposition. Next, the grid mask was manually rotated about 30° and another 5 nm Au layer was formed, and areas on which Au was deposited at 0, 5 or 10 nm were formed on the Si substrate. This procedure was repeated 4 times and 0, 5, 10, 15 or 20 nm Au thin film layers formed on the Si substrate.

B. Fabrication of Au shell particles

Gold shell particles were fabricated in accordance with a method reported in previous studies [11, 12]. Polystyrene beads (STADEX SC-020-S, SC-030-S and SC-051S, nominal diameter 196 and 309 nm, respectively; JSR Co., Tokyo) were used as casts of the particles and placed on a Si substrate. Next, 5, 10, 15 or 20 nm thick Au layers were formed on the beads by thermal deposition. Au-deposited particles were placed in an UV-ozone cleaner (UV253HR; Filgen, Inc., Aichi) and UV-ozone ozidation was performed to remove polystyrene casts from the particles [13, 14]. Gold shell particles were then obtained, suspended in water with dislodgment from the Si substrate with an application of ultrasound using a desktop sonicator, and placed again on a clean Si substrate for FE-SEM observation.

C. FE-SEM observation

Both Au flat films and Au shell particles were observed in BSE detection mode using an FE-SEM (JSM 6701F; JEOL Ltd., Tokyo) with a BSE detector (SM-74071; JEOL). Observation was performed under the following conditions: 5 kV acceleration voltage, 300× (films) and 20,000× (shell particles) magnification, 200 pA probe current, 8 mm working distance, 20 sec capture time, 1280×1024 pixels, and BSE detection mode. An incident electron beam was vertically applied to the sample surface and BSE was monitored with a scattered angle ranging between 16° and 60° from the axis of the incident electron beam. Images were taken with maximum contrast using the intensities of a 20 nm-thick layer and Si substrate (i.e., 0 nm-thick) as the brightest and darkest standards, respectively. The emission current was reset during the last minute of taking pictures to standardize the capture conditions.

D. Quantification of BSE intensities

Intensities in BSE images were quantified using image analysis software (Image J 1.44m; National Institute of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). Intensities in BSE pictures were quantified as 8-bit gray scale values. The largest possible area was selected for each sample in each picture, and the mean value of the intensity was calculated using software. To compare the intensities of Au layers with different thicknesses, the intensities of the 20 nm Au layer and Si substrate (0 nm) were used as standards of maximum intensity, and minimum intensity, respectively.

\[ I_{Z} = \frac{(I_{max} - I_{min})}{I_{max} - I_{min}}. \]

Where \( I_{Z} \) was calculated for all samples, and the relationship between \( I_{Z} \) and Au layer thickness was plotted.

III. RESULTS AND DISCUSSIONS

A. BSE observation of Au flat film layers

For standard evaluation of BSE intensity and metal layer thickness, Au thin films were formed on a flat Si substrate with thicknesses of 0, 5, 10, 15 and 20 nm. Figure 1(a) shows a summary of the film formation. A metal grid mask was placed onto the Si substrate, a 5 nm Au layer was formed by thermal deposition through the grid mask, and the deposition was repeated 4 times with sequential rotation of the grid mask. Figure 1(b) shows the result of BSE observation of the fabricated Au flat layers with 5 kV acceleration voltage of incident electron. According to the increase of film thickness, BSE intensity also increased, as shown in Fig. 1(b). This result can be explained by the increase of BSE from the Au layer, because the amount of BSE generally increased following the increased atomic number at the sample surface. In this specimen, the Au layer, which is a relatively heavier element than Si, formed on the Si bulk specimen as thin film less than 20 nm thick; therefore, BSE contrast was improved by setting the FE-SEM observation condition with a low acceleration voltage for incident electron, because incident electrons having relatively low energy.
FIG. 1. Summary of BSE observation of Au flat thin films. (a) Schematic image of film formation. A metal grid mask was placed on a Si substrate and Au was deposited through the mask. After rotation of the mask, the deposition was repeated. This procedure was repeated 4 times and Au film layers 5, 10, 15 and 20 nm thick were formed on the Si substrate. (b) BSE image of fabricated Au films. Thicknesses of Au films are indicated in the picture. Bar, 50 μm.

FIG. 2. BSE observations of Au spherical shell particles. (a) Schematic image of the shell particle. (b-i) BSE images of particles 200 nm in diameter with 5 nm (b), 10 nm (c), 15 nm (d) or 20 nm (e) thickness, and 300 nm in diameter with 5 nm (f), 10 nm (g), 15 nm (h) or 20 nm (i) thickness. Bar, 500 nm.

FIG. 3. Relationships between $I_Z$ and Au layer thickness for 200, 300 nm particles, and film. Line fittings were performed for all results, and the gradients (%/nm in unit) and $R^2$ values are indicated on the graph.

B. BSE observation of Au shell particles

Next, 200 and 300 nm Au shell particles were observed in BSE imaging mode in FE-SEM to evaluate the potential of using these shell particles as simultaneously distinguishable target labels in FE-SEM observations. BSE intensity also depended on the thickness of the Au layer even in the case of spherical shell particles, which is fundamentally the same principle as for flat film (Fig. 1); therefore, evaluating the contributions of both shell thickness and particle curvature (i.e., diameter) to BSE intensity is essential for quantitative discrimination of Au shell particles in BSE observations. These spherical Au shell particles were fabricated using polystyrene spheres as casts and the diameters of the fabricated particles were uniform, with less than 5% coefficient of variation (CV) [14]. Moreover, the thicknesses of Au shell layers were strictly controlled by using thermal deposition for layer formation; therefore, fabricated particles are ideal for the above evaluation. Figure 2(a) shows a schematic image of the Au spherical shell structure. The metal shell particle was composed of metal (Au) with thermal deposition and its layer thickness was decided as the deposition thickness of the metal. The shell particle also has a void that appeared with removal of the polystyrene cast. Figure 2(b-i) shows BSE images of fabricated Au shell particles of 200 nm diameter with 5 nm (Fig. 2(b)), 10 nm (c), 15 nm (d) or 20 nm (e) thickness, and 300 nm diameter with 5 nm (Fig. 2(f)), 10 nm (g), 15 nm (h) or 20 nm (i) thickness. Observation was performed with 5 kV acceleration voltage of incident electrons, and according to the increase of shell thickness, BSE intensity also increased gradually for both 200 and 300 nm particles.

C. Contribution of particle curvatures to BSE intensity

As shown in Fig. 2, background intensity (Si) also varied with the observation conditions, even if observations were carried out at a common acceleration voltage; therefore, normalization of BSE intensity was carried out by calculating relative intensity, $\tilde{I}_Z$, using Eq. (1) [5]. This calculation was also performed for the film sample in Fig. 1 to compare the tendency of BSE increase with that in spherical shells. Figure 3 shows a summary of the relationship between $\tilde{I}_Z$ and Au thickness. As shown in Fig. 3, BSE intensity increased almost proportionally for all samples by increasing Au layer thickness ($R^2 > 0.93$) in the range of measured film thicknesses, and these gradients were 2.24%/nm for 200 nm shell, 2.58%/nm for 300 nm shell, and 3.61%/nm for film. The difference in gradients between the average of shells and film was about 1.5 times, which was caused by the presence of voids in shell particle structures. BSE intensity represents the averaged components of elements on which incident electrons were scattered. For void shell particles, void volume was also included as a component of the element, which yielded a relative reduction of BSE intensity of these particles in comparison with that of film. Next, the difference in gradients between 200 and 300 nm particles was 15%, caused by the contribution of particle curvatures, and the in-
crease of diameter for 1.5 times contributed to the 15% error in BSE intensity. This result indicated that strict control of the metal shell diameter is essential for precise identification of these particle types, using BSE intensity as an index. In the method of particle fabrication introduced in this study, the particles were fabricated using polystyrene bead casts whose CV values were less than 5% diameter [14], and layer thicknesses were precisely controlled by thermal deposition in nanometer order, which indicated that our method achieved ideal particle fabrication with uniform diameter and metal layer thickness at less than 15% error, and these particles can be used as simultaneously distinguishable labels using BSE observation mode in FE-SEM.

IV. CONCLUSION

In this study, the relationship between BSE intensity in FE-SEM and Au metal layer thickness was studied in detail. The study was carried out for both flat thin film and spherical shell particles of 200 and 300 nm diameters. The particles were designed to be used as simultaneously distinguishable target labels in BSE observations. Film layer thicknesses varied, being 5, 10, 15 and 20 nm, and BSE intensity was observed for each thickness in all samples. In result, BSE intensity increased almost proportionally for all samples according to the increase of Au layer thickness. The difference in gradients between particles and film was about 1.5 times, which was caused by the presence of voids in the shell particle structures. Moreover, the difference in gradients between 200 and 300 nm particles was 15%, which indicated that particle curvatures contributed to the 15% error in BSE intensity with the 1.5 times difference in diameter. These results indicated that strict control of both metal layer thickness and particle diameter is essential for the fabrication of spherical labels suitable for BSE observations in FE-SEM. Our method of particle fabrication, which was also introduced in this study, can fabricate ideal particles with uniform diameters with less than 5% CV and uniform metal thicknesses with nanometer-order control; therefore, we concluded that these spherical shell particles can be used as simultaneously distinguishable labels in BSE observation mode in FE-SEM.

ACKNOWLEDGMENTS

We gratefully thank Ms. A. Kusano, Ms. M. Sasajima, Ms. Y. Abe, Ms. H. Mikami, Ms. M. Murakami, Ms. Y. Suganuma and Ms. K. Aso for technical assistance. This work was financially supported by Kanagawa Academy of Science and Technology.