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Slice Thickness Optimization for the Focused Ion Beam-Scanning Electron Microscopy 3D Tomography of Hierarchical Nanoporous Gold

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Abstract

The combination of focused ion beam (FIB) with scanning electron microscopy (SEM), also known as FIB-SEM tomography, has become a powerful 3D imaging technique at the nanometer scale. This method uses an ion beam to mill away a thin slice of material, which is then block-face imaged using an electron beam. With consecutive slicing along the *z*-axis and subsequent imaging, a volume of interest can be reconstructed from the images and further analyzed. Hierarchical nanoporous gold (HNPG) exhibits unique structural properties and has a ligament size of 15–110 nm and pore size of 5–20 nm. Accurate reconstruction of its image is crucial in determining its mechanical and other properties. Slice thickness is one of the most critical and uncertain parameters in FIB-SEM tomography. For HNPG, the slice thickness are caused by various microscope and sample parameters, e.g., converged ion milling beam shape, charging effects, beam drift, or sample surface roughness. Determining and optimizing the actual slice thickness variation appear challenging. In this work, we examine the influence of ion beam scan resolution and the dwell time on the mean and standard deviation of slice thickness. After optimizing the resolution and dwell time to achieve the target slice thickness and lowest possible standard deviation, we apply these parameters to analyze an actual HNPG sample. Our approach can determine the thickness of each slice along the *z*-axis and estimate the deviation of the milling process along the *y*-axis (slow imaging axis). For this function, we create a multi-ruler structure integrated with the HNPG sample.

Keywords Focused ion beam · Scanning electron microscopy · Tomography · Hierarchical nanoporous gold

1 Introduction

Focused ion beam (FIB) and a scanning electron microscope (SEM) can be combined in a way that both beams coincide at their focal points to locally section a bulk sample by ion milling, and subsequently obtain high-resolution images of the new block faces using the electron beam [1]. Also called FIB-SEM tomography [2], this technique is widely used for the 3D analysis of inhomogeneous micro- and nanostructures and in biology [3, 4], geology [5, 6], investigations of ceramics [7, 8], and semiconductor materials [9, 10].

The use of FIB-SEM tomography to examine nanoporous metals has recently attracted interest [11-13]. One example

Alexander Shkurmanov alexander.shkurmanov@tuhh.de of this type of material is nanoporous gold, which has a uniform network of nanoscale pores and solid metal ligaments and shows distinct mechanical properties [14]. Adding another structural hierarchy level to nanoporous metals can enhance their mechanical behavior. Hierarchical nanoporous structures with nanosized pores exhibit better stiffness and strength than geometrically similar structures with only one characteristic length scale [15]. For the investigation of hierarchical nanoporous gold (HNPG), 3D FIB-SEM for volumetric reconstruction can be used with other measurement techniques for material correlative analysis [16].

Despite being established as a high-resolution 3D measurement tool, slice thickness variations or errors in FIB-SEM tomography have been reported [17, 18]. These inaccuracies can directly affect the volumetric reconstruction and resulting material parameters. Slice thickness errors are caused by converged ion milling beam shape [19], charging effects [8], beam drift [20], and sample properties, such as high surface roughness [21]. As a solution, a reference

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structure can be milled and imaged simultaneously with an actual HNPG sample during FIB-SEM tomography to provide information about the thickness of each milled slice for quantitative reconstruction and further analysis.

Jones et al. [17] proposed a ruler that can provide information on slice thickness. This ruler has two trenches parallel to each other on the Si wafer and another two trenches inclined at a certain angle with respect to the parallel ones. All trenches are filled with a Pt/C deposition layer, to ensure that their outlines (markers) are visible in the cross-sectional image. After every slice, the markers of the inclined trenches change their position in the x-axis, then move toward each other until they intersect, and finally move sideways from each other. Meanwhile, the markers of the parallel trenches maintain a constant position. The real thickness of the milled slices can be computed by the difference in distance between the inclined and parallel trench markers of slices N and N+1. Similar measurements were performed using identical rulers [22, 23]. According to these reports, the ruler preparation is technically simple, the measurements are reproducible, and the results are reliable for the thickness calculation of milled slices. This ruler can also be integrated into the "real structure", i.e., into the HNPG, which will be analyzed by FIB-SEM tomography.

2 Results

2.1 Design of Experiment

In our setup, the substrate was a Si single crystal wafer with a size of $10 \text{ mm} \times 10 \text{ mm}$ polished on one side. A FEI Helios NanoLab G3 FIB-SEM with the ASV4 software for the automatic control of the slice-and-view process was used in the 3D nanotomography measurements. The SEM image stack was analyzed with Avizo software. A corresponding ruler was prepared for each measurement. All lines were milled approximately 1.5 µm deep at an acceleration voltage of 30 kV and an aperture of 40 pA. Two parallel 4-µm-long lines were milled at a 7-µm distance from each other, and two inclined lines were tilted at a 30° angle with respect to the parallel ones. A 2-µm-thick Pt layer was then deposited using a gas injection system at an aperture of 0.43 nA.

All SEM images have the same size of 3072×2048 pixels. The pixel size of approximately 3.3 nm was chosen at a 10-µm horizontal field of view. All measurements were conducted under immersion mode with an acceleration voltage of 2.0 kV, a beam current of 50 pA, and a dwell time of 10 µs. Backscattered electrons were detected using a through-the-lens detector.

Before the ruler was integrated into the HNPG structure, a series of measurements were performed to identify the optimal values of the ion beam imaging parameters influencing the slice thickness. Nine imaging setups with three different pixel sizes of the ion beam image (4.3, 8.4, and 16.9 nm, using 6044 × 4096, 3072 × 2048, and 1536×1024 pixels) were chosen at approximately 25-µm horizontal field of view. Three different dwell times (1 µs, 500 ns, and 250 ns) were selected for each pixel size. These measurements were conducted by milling 100 slices each with a depth (y-axis) of approximately 5 µm and a target thickness of 10 nm. Two markers were analyzed for each slice, i.e., 200 slice thickness measurements were collected. The values typically ranged between 1 and 19 nm, 50% of which were between 6 and 14 nm. For each measurement, an extreme value (z-score) analysis was performed to detect outliers, i.e., slices that are thinner than 1 nm or thicker than 20 nm. Approximately 8% of the data were considered as outliers and removed prior to further analysis.

The mean and standard deviations of the slice thickness were calculated for each ion beam image parameter and are presented in Fig. 1 as a function of dwell time (A and B) or pixel size (C and D). According to the plots, the mean slice thickness increases from 9.25 to 10.50 nm with the increase in dwell time. Standard deviation also increases from approximately 4.30 to 5.25 nm.

Another situation can be stated for the ion beam parameters in comparison with the pixel size. The mean slice thickness slightly decreases from approximately 9.60 to 9.30 nm. However, the standard deviation does not exhibit a linear behavior – it decreases from around 5.0 to 4.30 nm at the small pixel sizes (< 8 nm/px) and then slightly increases up to 4.50 nm at the large pixel sizes (8–17 nm/ px) of the ion beam image.

According to the plots, the target thickness of 10 nm can be obtained by using a pixel size of 8.4 nm/px and a dwell time of 750 ns. Thereby, an additional measurement using these parameters was performed to confirm this hypothesis. The values were presented on the same plots, and a mean slice thickness of 10.05 nm with a standard deviation of 3.90 nm was achieved.

Figure 2 shows the mean thickness and standard deviation values as heat maps and functions of dwell time and pixel size. According to these plots, the mean thickness increases from the left, predominantly in the bottom corner, to the top right corner. The lowest standard deviation is located on the top left corner and the center of the map, and the highest can be found on the right top and bottom corners.

The results serve as the basis to optimally adjust the parameters of the dual-beam FIB-SEM device to achieve the desired mean and standard deviation of slice thickness. The optimum dwell time and pixel size can be applied to tomographic measurements of actual HNPG structure. Fig. 1 Mean slice thickness and its standard deviation from the initial measurement series as a function of dwell time (**A** and **B**) and pixel size (**C** and **D**). *Gray lines* serve as a visual guide



Fig. 2 Slice thickness mean value (**A**) and its standard deviation (**B**) from the initial measurement series as a heat map of pixel size (vertical scale) and dwell time (horizontal scale)

2.2 HNPG Sample Preparation

The HNPG sample was prepared following a "dealloyingcoarsening-dealloying" protocol in three stages [15]: (i) Ag93Au7 master alloy was electrochemically dealloyed in 0.01 M H_2SO_4 , ii) the obtained material was coarsened by vacuum annealing, and iii) the remaining Ag was removed by electrochemical dealloying in 0.01 M H_2SO_4 . In the first stage, surface diffusion resulted in the formation of the nanoporous material. The second stage established the upper hierarchy level, and the last stage generated the lower hierarchy level by creating a small porous structure inside the large struts. Finally, an HNPG structure with a ligament

size of 15–110 nm, a solid phase of approximately 30%, and a residual Ag content of less than 5 wt% was obtained. For the second phase, the HNPG structure was filled with epoxy resin to provide additional support during FIB milling [24] and reduce the background signal from the subsequent layers during SEM imaging for further image segmentation of the data [25–27].

2.3 FIB-SEM Tomography of the HNPG Structure

Given the non-uniformity of the ion beam shape, a multiruler structure similar to that in [17] was prepared. Eight platinum and carbon layers were vertically and alternately arranged to etch marker lines inside the layers. These markers can be used to estimate the slice thickness from each layer and observe the non-uniformity of the milling process along the *y*-axis.

A carbon layer with a spatial size of 10 μ m ×6 μ m and thickness of 1 μ m was deposited onto the HNPG sample surface and internally etched with four marker lines (two parallel ones and two inclined ones) as mentioned above. A platinum layer with a thickness of 1.3 μ m was then deposited over the carbon layer with the marker lines. This process was repeated four times. For the SEM images, a relatively larger horizontal field of view of 17.3 μ m was chosen to include the whole ruler structure and the HNPG sample. Figure 3 presents a cross-sectional image of the final structure.

For the measurements on the Si wafer, 100 slices with a target thickness of 10 nm were milled from the prepared structure and 200 marker distances were analyzed from each layer. Instead of 5 μ m, 40 μ m was considered as a large milling depth. The final mean slice thickness is presented in Fig. 4A as a function of the distance from the HNPG surface. For each obtained mean slice thickness, a corresponding



Fig. 3 SEM cross-sectional image of the multi-ruler structure prepared on the HNPG sample infiltrated with an epoxy resin

standard deviation was calculated as mentioned above. As shown in Fig. 4B, the standard deviation decreases with the increasing distance from the HNPG surface.

Measurement analysis shows that with the increase in distance, the mean slice thickness linearly grows from approximately 9.3 to 9.75 nm, and its standard deviation decreases from around 5.5 to 4.75 nm. Therefore, the best results with respect to target thickness and lowest uncertainty were obtained from the top ruler. Meanwhile, for the bottom structure, a decrease in mean slice thickness and an increase in its standard deviation were observed.

The obtained mean and standard deviation of slice thickness are respectively lower and higher than the values defined for the ruler on the Si wafer. This phenomenon might be explained by the large depth of the milled slices that consequently require a long time to mill a single slice. The increase in milling time is reflected by high ion beam deflection and undesired sample charging [8, 17, 27]. Instead of a semiconductor silicon wafer, the use of a conductive gold material as a substrate might have also changed the charge balance of the sample.

Given that the mean slice thickness linearly decreases with the depth of the milling slice, this value can be used to estimate the real thickness of the HNPG material at each point along the y-axis. In this case, a large HNPG material was enclosed between two ruler structures, following a process similar to TEM lamella preparation [28-30] as schematically shown in Fig. 5A. The HNPG sample was covered using a Pt layer with a spatial size of 12 μ m \times 6 μ m and a thickness of 1 µm and then rotated to ensure that the ion beam was milling a 15-µm-deep trench inclined at 22° with respect to the surface normal. Afterward, the specimen was rotated around its normal axis in 180° and another inclined trench that was identical to the first one was milled from the other side of the specimen. Finally, the two trenches formed a trigonal prism, which was then removed from the sample by using a lift-out needle and welding Pt on one prism side.

Fig. 4 Mean (**A**) and standard deviation (**B**) of slice thickness as a function of the distance from the sample surface measured after the analysis of the multi-ruler structure prepared on the HNPG sample infiltrated with epoxy resin





Fig. 5 Schematic of the preparation of HNPG lift-out step (**A**). Crosssectional schematic (**B**) and SEM image (**C**) of the final double-ruler structure with a 4 μ m-thick HNPG material in the middle. **D** Approximation of the mean slice thickness by assuming its linear behavior as a function of the distance from the Si wafer surface

The prism was transformed into a $10-\mu$ m-long, $4-\mu$ m-wide, and $4-\mu$ m-high parallelepiped by holding it in a space within the FIB chamber and adopting a cleaning cross-section pattern. This parallelepiped was then placed on a ruler prepared on the Si wafer, similar to the one used for the initial measurements. After the needle was cut, a 2- μ m-thick carbon layer was deposited on the HNPG material. A new ruler structure with parallel and inclined lines was etched in this carbon layer and filled with 2- μ m platinum layer. Its cross-sectional schematic and real SEM image are shown in Fig. 5B C, respectively.

Slice thickness was measured using the two rulers located approximately 8.4 μ m from each other along the *y*-axis. The mean slice thickness from the top ruler was 9.28 nm with a standard deviation of 4.90 nm, and that from the bottom was 9.10 nm with a standard deviation of 4.85 nm. These data can be applied to measure the slice thickness in any

space point of the HNPG structure for further reconstruction (Fig. 5D).

3 Conclusions

FIB-SEM is a powerful 3D nanotomography tool used in a wide range of disciplines. This effective technique is currently employed to describe the nano-morphology of materials and collect training data for machine learningbased segmentation algorithms [31].

The structural and geometric characteristics of the investigated materials define the parametric prerequisites for FIB-SEM tomography. For the HNPG sample, the slice thickness should not exceed half the pore size, i.e., 10 nm. Variation in slice thickness caused by various microscope and sample parameters should be considered.

In this work, we proposed the use of a ruler or calibration structure in 3D measurement to define the real slice thickness of HNPG along the *z*, and *y*-axes. For this goal, we prepared a parallelepiped HNPG sample, which was enclosed by two rulers. We found that the mean slice thickness depends on the ion beam imaging parameters. Under the assumption of a linear decrease along the cross-section depth, the slice thickness for each point of the investigated material can be estimated. Accurate 3D reconstruction is then realized using the vector of all slice thicknesses [32].

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Declarations

Conflict of interest There is no conflict of interest.

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