I. INTRODUCTION

Over the last few decades multiple tomography techniques have been developed to work at various length scales. Serial sectioning in conjunction with optical or electron microscopy has been shown to provide valuable three-dimensional information. This has traditionally been accomplished using mechanical polishing techniques to remove material, followed by surface imaging using a scanning electron microscopy (SEM) or a reflective optical microscope. In 1962, branching of pearlitic ferrite and cementite lamellae was shown by Hillert, thereby contradicting the prevailing theory of sideways growth. Hull et al. performed serial sectioning to obtain the prior-beta grain volume distribution in Ti alloys and assembled the information present in each slice into a three-dimensional (3D) volume using a reconstruction algorithm. Mangan et al. used mechanical polishing in combination with SEM, while Kral and Spanos employed optical microscopy, in order to study the Widmanstatten structure in high manganese steels. Fiduciary marks made using the focused ion beam (FIB) were used to align the images, while the thickness of the slices removed by polishing were estimated in some cases by following the shape of the indentation marks made on the surface of the sample, or in other cases by Ugelstad beads. It was estimated that slices as thin as 300 nm could accurately be removed. Imaging and alignment resolution was determined by the size of the electron probe used in the SEM.

In addition, electron tomography is a well-established technique for use with biological systems to recover 3D structure with high resolution (e.g., Ref. 9). This has proved particularly useful for imaging systems such as viruses or cell organelles. Recently, electron tomography has been extended to materials science, primarily utilizing bright field imaging. This technique works in a way similar to x-ray projection-based tomography with the primary difference being that in electron tomography a much higher resolution (~1 nm) can be obtained. Two-dimensional projection images are acquired at small angular increments to obtain 3D information. More recently, a method of spectroscopic electron tomography has been demonstrated using energy-filtered transmission electron microscopy (EFTEM). In this case, only signals from inelastic electron-matter interactions are considered. These are proportional to the local concentration of the selected chemical component. Energy dispersive spectroscopy (EDS) mapping in TEM can also be used as a useful tool for 3D reconstructions with resolution in the range of 1–5 nm. It should be noted that for a single tilt axis tomography experiment the tilt range should be as close to 180° as possible in order to avoid artifacts and obtain acceptable isotropic resolution. In practice, however, due to the TEM holder designs and limited pole piece space, the tilt range can be far below 180°, which can result in insufficient data to complete a 3D reconstruction. Sample preparation must also be carefully performed to avoid artifacts arising from changes in thickness while tilting. The available field of view on a sample is typically on the order of 100 nm–1 μm, taking into account sample preparation procedures and microscope limitations.

The 3D analysis of inhomogeneous microstructures at microscopic and nanoscopic scales is of great importance to the field of materials science. The FIB allows investigation into systems that have critical feature sizes varying from a few tens of nanometers to tens of microns and has been used to perform three-dimensional reconstructions. Dunn et al. and Tomiyasu et al. found that the achievable lateral resolution varied between 25 and 50 nm when using the FIB to perform tomography via secondary electron imaging and secondary-ion-mass spectroscopy (SIMS). It was noted, however, that the final resolution depends heavily on factors such as differential sputtering in the material, ionization yield of chemical species, and alignment of the slices. These
problems make it difficult to study features that have dimensions on the order of 10 nm. Some of these problems were addressed by making slices with the beam parallel to the surface\(^{19,20}\) to avoid the effect of differential sputtering; however, additional errors were introduced while aligning the images as the stage had to be moved after every slice. In all cases, it was found that the smallest probe size (8–10 nm) that could be used in the FIB was only one of the factors limiting reconstruction resolution.

Present state-of-the-art SEMs have an ultimate imaging resolution of 1–1.5 nm;\(^{22-25}\) therefore, if the high-resolution imaging capabilities of a dedicated SEM are combined with the precision machining capabilities of the FIB, a potential for significant improvement in reconstruction resolution exists. The advent of dual-beam (DB) integrated focused electron-ion and electron-beam systems will make the application of the techniques described in this article easier to implement in practice. While the ultimate imaging resolution of a DB system may be somewhat lower than that of a dedicated SEM instrument, the sample will not have the alignment issues associated with multiple machine exchanges.

II. EXPERIMENT

A. Samples

For this research, the features to be reconstructed needed to be sufficiently small and to have significant topology to explore and demonstrate the resolution limits of this technique. Keeping these requirements in mind, two different material systems were studied. The first example is a multilayered structure grown by molecular-beam epitaxy (MBE) with alternating layers of Si and Ge\(_{0.4}\)Si\(_{0.6}\) grown on a (100) Si wafer with a bilayer pitch of 28 nm. The second example is a bulk sample containing \(\theta^{'}\) Al\(_{2}\)Cu precipitates existing as plates oriented along the (100) directions in an Al matrix. Typically, plate size primarily depends upon the thermal history of the sample. In the present study, the alloy Al\(_{0.95}\)Cu\(_{0.05}\) was homogenized at 600 °C and aged at 250 °C for 24 h.

B. Sample preparation

Preparation of both the Al–Cu and Si–Si/Ge reconstruction samples was performed starting with mechanical polishing to bring the area of interest close to the edge of the sample. The samples were then cleaned using acetone and methanol in an ultrasonic cleaner to remove the mounting adhesive and other contaminants from the sample surface. Cross-sectional TEM samples, used to provide complementary information, were prepared using conventional methods. A schematic of the geometry used to obtain 3D information about the samples using FIB serial sectioning is shown in Fig. 1. The samples were introduced into the FIB chamber and Pt was deposited at the area of interest. The size of the deposited Pt block was 10 \(\mu\)m long \(\times\) 2 \(\mu\)m wide \(\times\) 500 nm thick and was deposited using a beam current of 70 pA. This was done to protect the surface from ion irradiation damage while milling subsequent slices, especially for cases where the area of interest was close to the surface, such as for the Si–Ge multilayered sample. The edge of the sample tended to be rough on the nanoscale, following cleaving and polishing. In order to obtain a flatter edge, a section of material was milled away by the FIB. A reference line was milled into the Pt using a beam current of 1 pA to help determine the distance from the sample edge during sample slicing. The sample was then rotated 90° such that the edge of the sample faced upwards. Alignment marks (shown by rectangular trenches in the sample) measuring 150 \(\times\) 150 nm\(^2\) were created to serve as fiduciary marks for subsequent image alignment using a 4 pA beam current. Similar trenches were also milled in pairs at an angle of 10° relative to the surface normal and were later used to accurately calculate the distance between slices. The distance from the reference line was recorded before milling and was used to monitor the stage drift, if any, during the milling process. A beam current of 11 pA was used to mill away the final section of the sample. Processing duration was determined by both the desired slice thickness and the required milling depth, and in this study tended to be on the order of 3–4 min. SEM imaging was performed at an accelerating voltage of 5 kV and a 3 mm working distance. A moderate probe current (~10 pA) was used during imaging to achieve an acceptable signal-to-noise ratio while utilizing a small diameter probe.

Once satisfactory images were acquired, the sample was removed from the SEM and inserted back into the FIB chamber to mill the next slice. This process was repeated between the FIB and the SEM until the desired number of slices was obtained. The images were aligned using the fiduciary marks and the region of interest (ROI) was cropped from the entire set of images. Routines developed in MATLAB™ were then used to concatenate the slices in three dimensions and the information obtained from the slices was interpolated into three dimensions.

C. Slice alignment

Cross-sectional transmission electron microscopy (XTEM) was performed to independently study the structure and morphology of the samples studied and to calculate the
distance between slices. Samples were attached to a Cu ring to provide additional handling support. The sample was then mounted vertically on a sample holder and inserted into the FIB chamber. Figure 2 shows a schematic of the technique and the TEM membrane showing the alignment trenches made using the FIB. An area of interest was chosen in the center of the ring and an electron transparent TEM membrane was made via FIB etching, as shown in Fig. 2(b). A high beam current (1000–6500 pA) was used to remove the bulk of material near the edges of the sample while lower beam currents (70–350 pA) were used near the TEM membrane. The thickness of the membrane was maintained at around 200–300 nm. The trenches were milled to span the entire thickness of the membrane. Square trenches were milled by the FIB to be used as fiduciary marks and their profiles were studied by cross-sectional TEM. The angle of the sidewall with respect to the direction of milling (θ) was calculated by measuring the length and width of the trench, as shown in Fig. 3(a). It was found that the sidewall of the trench was not perfectly linear and the maximum deviation from linearity was measured (denoted by ΔL+ΔR). Measurements were made on six different trenches that were milled using a 4 pA beam current for 25 s over a 150×150 nm² area and are listed in Table I. The slope of the sidewall with respect to the milling direction can be calculated from the average W/L ratio, to be 0.175, where W represents the change in width (W1–W2), and L is the distance between slices. The maximum deviation from linearity (ΔL+ΔR) was found to be 6.35 nm over a depth (L) of ~650 nm. Using the SEM, it was determined that the alignment mark could be measured with 3 nm accuracy, defined as δ(W). Therefore if δ(L) and δ(W) represent errors associated in measuring L and W, respectively, where δ(L) = δ(W) × L/W ratio, then δ(L) = 3 nm × 5.7 = 17.1 nm. These results provide a fundamental limit in the accuracy with which we can predict the distance between slices, L, by measuring widths of alignment trenches in cross-sectional images. While this method is a significant development, the method fails to reach the resolution goal for this work of predicting the distance less than 10 nm accuracy. Since the value of δ(W) is governed by several factors, such as the resolution of the SEM, it was realized that in order to improve the accuracy of these measurements, the ratio of W/L needed to be increased. This was achieved by milling additional trenches on an angle (termed

Table I. Measurements taken to determine the profile of the alignment trenches where (W) refers to the changes in width as a function of length (L), (θ) represents the angle of the vertically FIB milled sidewalls, and (ΔL+ΔR) represent the maximum measured deviation from linearity.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>W (W1–W2) (pixels)</th>
<th>L (pixels)</th>
<th>W/L</th>
<th>θ (°)</th>
<th>ΔL+ΔR (nm)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>932</td>
<td>0.170</td>
<td>4.85</td>
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<tr>
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<td>0.178</td>
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<td>5.74</td>
</tr>
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</tr>
<tr>
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<td>0.178</td>
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</tr>
<tr>
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<td>162</td>
<td>922</td>
<td>0.176</td>
<td>5.02</td>
<td>5.74</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.175</td>
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<td>6.35</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td>0.006</td>
<td>0.18</td>
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</table>

Fig. 2. (a) Schematic illustration of the technique used for making alignment trenches on TEM membrane. (b) SEM micrograph showing a perspective view of the membrane.

Fig. 3. Bright field TEM images taken (a) along the (110) zone axis showing the vertically FIB milled alignment trenches, (b) along the (110) zone axis showing the profile of the z-spacer trenches, and (c) along the (110) zone axis showing a pair of z-spacer trenches along with the measurements made to determine their profile.
z-spacer trenches) in the vicinity of the area under investigation for the 3D reconstruction. Figures 3(b) and 3(c) illustrate the method by which the trenches were made on a TEM membrane in order to study the trench profiles. The trenches were made in pairs (labeled “A” and “B”) on a TEM membrane. All the A trenches were milled after tilting the sample by 10° along the y axis. The B trenches were milled after tilting in the opposite direction by 10° along the y axis. In this way, we obtain pairs of trenches as shown in Fig. 3(b) with an angle \( \phi \) between the inner walls. In the XTEM image shown in Fig. 3(b) the A and B trenches are immediately adjacent to each other and the inner walls appear to intersect. On the actual samples, the A and B trenches are milled at a distance of about 50–100 nm from each other. The profiles of the trenches are not affected as a result of this change. The profile of the z-spacer trenches was studied from the XTEM images, as shown in Fig. 3(c). Measurements from this geometry are listed in Table II. It can be observed that the angle \( \phi \) between the inner walls is greater than 2\( \theta \), which governs the ratio \( W/L \) and hence the level of accuracy in predicting the distance between slices. The average value of \( W/L \) was found to be 0.577, while \( \Delta(W) \) was on the order of 3–4 nm, yielding a \( \Delta(L) \) of 5.2–6.9 nm, a greater than twofold improvement.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>( W(W2-W1) ) (pixels)</th>
<th>( L ) (pixels)</th>
<th>( W/L )</th>
<th>( \phi/2 )</th>
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<td>923</td>
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<tr>
<td>Average</td>
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<td>0.577</td>
<td>16.1</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td>0.010</td>
<td>0.25</td>
</tr>
</tbody>
</table>

D. Three-dimensional interpolation

1. Intensity-based interpolation

The information obtained by serial sectioning exist as discret two-dimensional images and must be rendered into three dimensions for tomographic interpretation. This requires interpolation to reconstruct the region between slices. Image interpolation for gray scale images generally falls into two broad categories: intensity based and shape based. In this work, intensity-based interpolation was used for the Si–Ge multilayered sample. This process involves analyzing each image as a two-dimensional array with each node representing a pixel of the image where the value at each node is translated into a gray scale pixel value (0–255, 8 bits). The two-dimensional (2D) arrays were then concatenated to form a 3D array while taking into consideration the interslice distance and the pixel gray scale values are interpolated to obtain a continuous 3D data set.

2. Shape-based interpolation

While intensity-based interpolation works adequately for geometrically simple structures with relatively low curvature along the sectioning direction, it fails to accurately recreate the data in cases involving geometrically complex features. In 1990, Ray and Dupe described a shape-based interpolation technique and showed its applicability to 3D medical imaging. This technique has since been extensively used in the physical sciences. In the present work, images from the Al–Cu sample were reconstructed using the shape-based interpolation technique. Features were identified on the image and it was converted to a binary image (black and white) by performing image segmentation. The edges of the features were delineated and each pixel represented by its distance from the nearest edge. This value was positive if the pixel was inside the feature, and negative when outside. These two-dimensional arrays of values were then interpolated into a volume.

III. RESULTS AND DISCUSSION

A. Si–Ge multilayered sample

This sample comprised a multilayered structure with alternating layers of Si and Si0.6/Ge0.4 grown on a (100) Si wafer. Each Si and Si0.6/Ge0.4 layer had a thickness of ~14 nm, and a total of ten bilayers were grown. Figure 4(a) shows a cross-sectional TEM image of the sample showing the alternating Si–Ge and Si layers grown on the Si substrate. It can also be seen that the Si–Ge layers vary in thickness. This thickness variation can be attributed to the strains induced through lattice mismatch between the Si and Si–Ge
layers. The formation of islands on the film is one of the strain relieving mechanisms and follows the Stranski-Krastanov growth mode. The thickness of the layers was measured from the image and the bilayer thickness was measured to be $28 \text{ nm}$. Figure 4 shows a cross-sectional SEM image of the Si–Ge multilayered sample acquired at 5 kV with a probe current of $10 \text{ pA}$ at a magnification of $100k\times$. These conditions were subsequently used for acquiring 2D images for 3D reconstructions. The multilayered structure is clearly visible where Si$_{0.6}$Ge$_{0.4}$, generating a higher secondary electron (SE) yield, appears brighter than the Si layers. The layers of varying thickness correspond to those observed in cross-sectional TEM images.

1. Contrast normalization and three-dimensional reconstruction

The process of acquiring multiple sections requires working alternately on the FIB and the SEM. During each session the brightness and contrast settings for image acquisition must be adjusted and it was found that it was difficult to achieve the same setting for all of the slices. This is important because the gray scale intensity value of each pixel is used for the 3D interpolation of the Si/GeSi data set. Figure 5(a) illustrates an as-acquired image of a slice showing the cropped area of interest along with its respective gray scale histogram (b). To accommodate variations in imaging conditions, contrast normalization was performed on every image. This was accomplished by stretching the histogram to cover the entire gray scale spectrum (0–255). A representative image obtained following normalization is shown in Fig. 5(c) along with its respective gray scale histogram (d). This operation was performed using the image processing software SIS ANALYSIS™. This software has the ability to overlay images and alter the opacity of the images such that features from both images can be seen simultaneously, allowing for image alignment using the fiduciary marks. An image of each slice was added as a new layer and aligned with the previous image. When all the images were aligned the area of interest was chosen and cropped from all layers. The cropped component of each layer was then saved as an individual image and 3D interpolation and visualization was then performed. This was carried out using functions and routines.
written in the image processing toolbox of MATLAB™. Visualization was performed using “slice” (MATLAB reference function) and orthogonal planes in the \(x-y-z\) direction were displayed at predetermined positions. For this study, the program was used to display both the slices stacked prior to interpolation and the edge slices representing the interpolated volume.

It was attempted to make slices uniformly 20 nm apart, but it was observed that the actual distance between the slices varied significantly (Fig. 6). In order to concatenate these images into 3D space, the interslice distances were rounded off to the nearest even number of nanometers, as 2 nm is the smallest unit in the grid (along the \(z\) axis) created for interpolation. This number can be changed to any desired value but was kept at 2 nm to maintain a balance between resolution and computation time for interpolation. Figure 7(a) shows a perspective view of the 2D slices concatenated per the interslice distance. A perspective view of the 3D interpolated volume of the Si–Ge multilayered structure is shown in Fig. 7(b). The interpolation was performed in the \(z\) direction and the slice shown in the \(x-y\) plane in the figure is generated as a result of interpolation. As expected, the layers of Si and Si–Ge were found to be aligned in the \(x-z\) plane which proves the accuracy of the data acquisition and alignment procedures. A modulation of the thickness of the Si–Ge layers in the \(z\) direction was expected as these structures are known to form roughened surfaces.

### B. Al–Cu alloy sample

In the Al–Cu system, the Al\(_2\)Cu \(\theta'\) precipitates form disk-like structures along the orthogonal \(\langle 100 \rangle\) directions when aged.\(^{32,33}\) Figure 8(a) shows a TEM image acquired along the \(\langle 100 \rangle\) zone axis showing Al\(_2\)Cu \(\theta'\) precipitates in Al matrix. (b) SEM image of an electrochemically polished Al–Cu sample acquired at 5 kV accelerating voltage. (c) SEM image of a section of the Al–Cu alloy milled using the FIB showing the \(\theta'\) precipitates and alignment marks. (d) 3D rendering showing how the Al–Cu slices were concatenated prior to interpolation.
This suggests that the surface of this grain is also oriented along the (100) plane. Figure 8(c) shows a SEM image of a FIB milled section of the same sample. Fiduciary alignment marks are also shown in the image. In this case the section was oriented such that the precipitates along all three orthogonal directions would be seen in the cross section as rods. It was found that the $\theta'$ precipitates could clearly be seen in the FIB sectioned images; however, a distinct difference can be noticed as compared to the electropolished section images: the edges of the precipitates appear blurred. A careful study of the images and an understanding of the sample preparation technique utilized can offer a possible explanation for this observation. Precipitates have been classified as A, B, or C depending on their orientation in Fig. 8(c). It can clearly be seen that the A set of precipitates appear discreet while in the case of the B and C precipitates the contrast seems to fade as the distance from the precipitate increases. It can also be seen that the gradual fading of contrast appears preferentially on only one side of the B and C precipitates. After studying multiple sections, it was found that the B and C orientations of precipitates intersect the surface at low angles. Since the SE signal originates from a depth of 1–10 nm below the surface, a gradual fading of contrast is thus geometrically expected.

1. Three-dimensional reconstructions

Shape-based interpolation was performed using a code written in MATLAB™. The input to the program was two sets of binary images. The first set contained a 1 pixel wide continuous white border along the edge of the precipitates, where all of the other pixels were black. The second set showed all precipitates as solid white pixels. A 3D perspective view showing the concatenated slices is shown in Fig. 8(d). A 3D isosurface reconstruction is shown in Fig. 9 from two different viewing angles. The platelike structures can be observed in Fig. 9(a) oriented in three different directions. The viewing direction in Fig. 9(b) has been adjusted to show the orthogonal nature of these precipitates. Two of the orientations appear as rods while the third can be seen face on. It should be noted that while the orthogonal nature of these precipitates was not apparent from the 2D cross-sectional images, it can clearly be seen from the perspective view of the 3D reconstruction. The faces of the precipitates in Fig. 9 appear to have a distinct rippling morphology, which we believe is due to rendering artifacts.

IV. SUMMARY

In this research, sub-20 nm features were reconstructed in Si–Si/Ge and Al–Cu systems, using focused ion-beam nanomachining, combined with high-resolution secondary electron imaging. A procedure was developed to estimate the interslice distance with high accuracy by studying the profile of spacer trenches, which further improves the resolution and accuracy of the technique. It was found that the interslice distance tended to be unequal from slice to slice even though equidistant slices were attempted while milling using the FIB. While this could be partially improved by letting the FIB stage drift stabilize for longer periods of time, it would be difficult to get exactly equidistant slices since this distance is comparable to the resolution of the FIB for the probe current used (11 pA).

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