Stereomicroscopy: 3D Imaging and the Third Dimension Measurement

Application Note

Jining Xie
Agilent Technologies

Introduction

Scanning electron microscopy (SEM) has been widely used for imaging objects with various dimensions ranging from millimeters to nanometers. Compared with other common microscopies, SEM offers a unique combination of imaging characteristics including high lateral resolution, broad magnification range, and large depth of field. As the working mechanism of SEM, a focused electron beam scans across the surface of a specimen rectilinearly, and the generated electrons (secondary or backscattered electrons) from the beam-specimen interaction are detected synchronically from pixel to pixel with various intensities resulting in the image contrast. Although SEM micrographs appear to be three dimensional, they are in fact purely two dimensional. The grey level of the pixel is not a function of the local height of the point, but rather of materials, morphologies, and certain properties. Additionally, the high depth of field of SEM could obscure the height difference of two objects particularly when both are in good focus. To overcome this limitation, many efforts have been made to recover the third dimension in SEM. Examples include shape-from-shading method [1], Monte Carlo electron transport modeling [2], and FIB/SEM dual beam techniques [3]. One technique, photogrammetry, based on stereo-pair images has been extensively studied and applied to reconstruct three dimensional features. The theoretical description of photogrammetry applied to SEM was first described by Piazzesi [4]. Building on the early work on photogrammetric analysis [5, 6], this technique has become more interesting in recent years partially due to the fast development of powerful software which enables good qualitative and quantitative 3D reconstructions of specimen surfaces. Currently quantitative measurements of specimen at micro- and nano-scales by a truly three dimensional characterization technique are highly demanded in a variety of applications such as high aspect ratio MEMS structures [7], surface roughness determination [8], nanomaterials and nanodevices, life sciences [3], fracture analysis [9], and many others.

In this study, qualitative stereo imaging was demonstrated on Agilent 8500 compact field emission scanning electron microscope by using three different methods based on stereo-pair technique: “lateral shifting”, “individual MCP imaging”, and “sample tilting”. The qualitative imaging creates 3D looking micrographs revealing objects at different levels. In the following, a simplified geometric definition was discussed for quantitative measurement and examples were given by measuring three dimensions on stereo pairs. This quantitative measurement is of importance for investigations of objects with 3D topographic features, especially for uncovering the “hidden” third dimension.

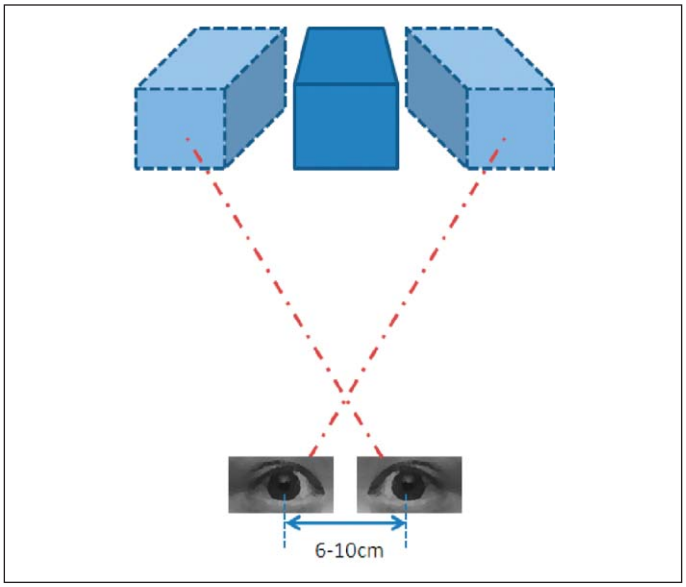


Figure 1. Schematic of parallax obtained by human eyes.

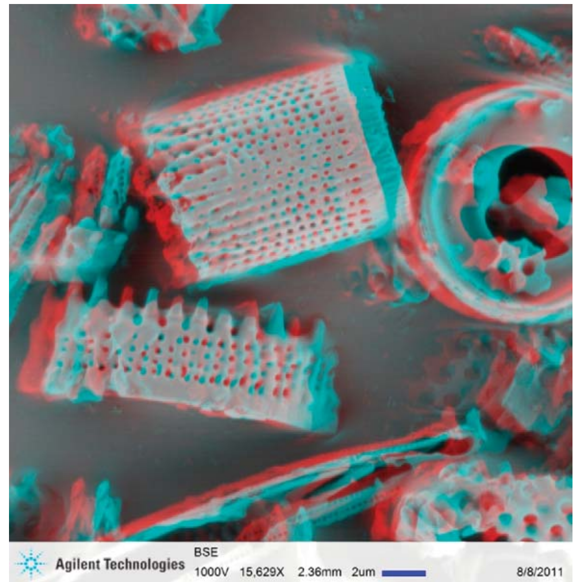


Figure 2. A 3D image of uncoated diatoms obtained by the "lateral shifting" method.

Principles of the Stereo-pair Technique

The human brain is able to derive depth information from data acquired by two eyes. Because our two eyes are laterally separated by a spacing of 6–10 cm, two slightly different views will be seen by the right eye and the left eye, as illustrated in Figure 1. This phenomenon, called parallax, varies with the distance from the viewer to the object of interests. For example, the parallax is larger for a closer object. Our brain processes this parallax information and converts it to depth information instinctively. Methods have been developed to obtain two views of an object with different orientations, termed a stereo-pair. Assuming one image of this pair is obtained by the left eye and the other one is obtained by the right eye, we can produce a 3D image with depth perception. The same principle is used for satellites mapping and measuring the earth's surface. There are a couple of methods available for viewing 3D images. Both parallel viewing and cross-eyed viewing methods work well for two stereo-pair images presented side by side. However, for both methods observing by naked eyes, practice is normally needed to see 3D images, and not everyone can see stereoscopically. Another common, and easier method, is to overlay red and blue false color stereo-pair images by software and observe them by using a pair of red-blue glasses

Stereomicroscopic Imaging

Agilent 8500 compact field-emission scanning electron microscope was used in this study. Its innovative miniaturized all-electrostatic lens and quad-segmented microchannel plate (MCP) detector enable its capability of high resolution imaging at low accelerating voltages (500–2000 V). Samples including diatoms, prickly gold on a copper grid and commercial silica beads were directly fixed on specimen mounts via carbon double tapes. The accelerating voltage for imaging was set at 1000 V. Three methods were conducted on Agilent 8500 to achieve parallax-based stereomicroscopic imaging that mimics the stereo-pair acquisition by human eyes.

1. Lateral Shifting

As a classic method, the lateral shifting usually takes two snapshots with a short lateral displacement so that parallax is created for those features appearing in both images. After loading an uncoated diatom sample into the specimen chamber, a backscattered electron (BSE) image was recorded at one position followed by recording another image after moving the sample slightly in X-direction. Here low voltage BSE imaging significantly minimizes the charging issue. Figure 2 shows the anaglyph grey (Red-Cyan) 3D image overlapped from obtained two images. Apparently, diatoms do not exhibit obvious 3D vision due to its insufficient parallax generated from such a small lateral movement. This method is believed to be only applicable for very low magnification SEM imaging.

2. Individual MCP Imaging

In the Agilent 8500, a quad-segmented MCP detector is used for both SE and BSE imaging, depending upon the applied bias voltage on the detector. Thus a convenient point-and-click switching between SE and BSE imaging mode is realized eliminating inserting and pulling out of a separate BSE detector for regular SEMs. For both SE and BSE imaging modes, signals from all quadrants are summed, while for the topographic imaging mode the signal from one side of detector is subtracted from signals collected on the other side. Additionally, images can be obtained

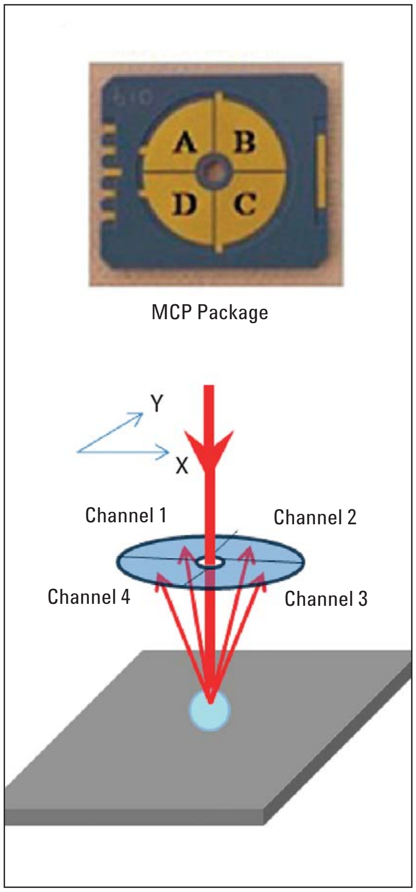


Figure 3. The quad-segmented MCP detector used in Agilent 8500 for individual MCP imaging.

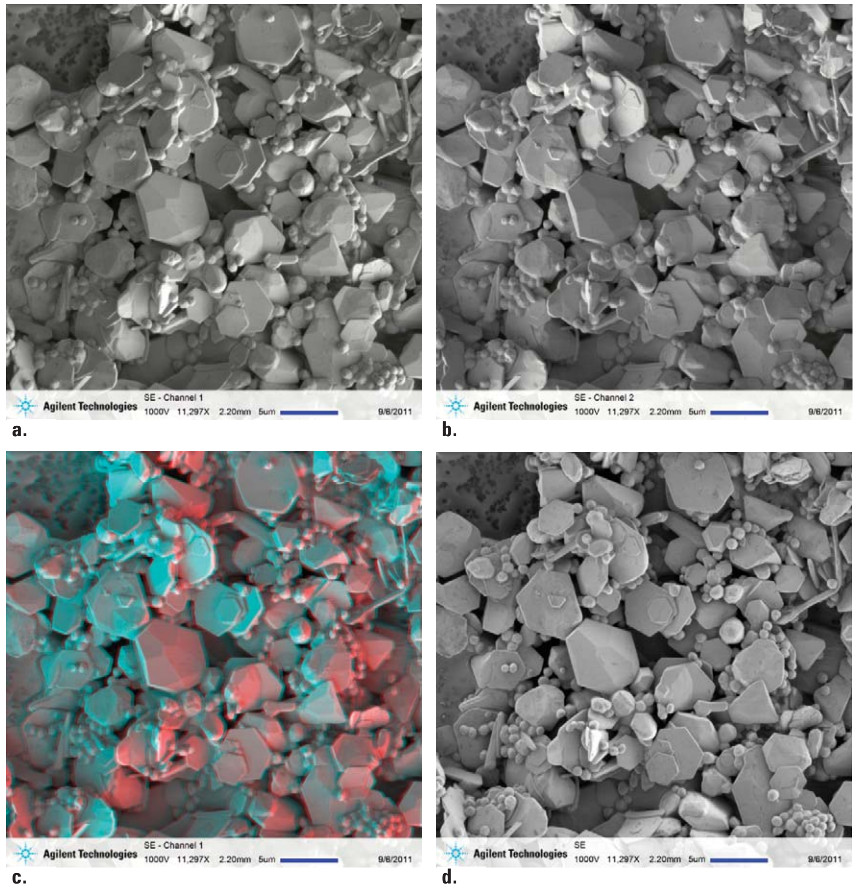


Figure 4. SEM micrographs of prickly gold on a copper grid. (a) SE image derived from only channel 1 detector; (b) SE image formed from channel 2's signal; (c) anaglyph image obtained from channel 1 and channel 2 images: (d) normal SE image with all four channels on.

from individual MCP detectors by just simply selecting particular channels on the software. The signal collection at different angles is analogous to observing an object from different viewpoints, as illustrated in Figure 3.

Stereo-pairs can be formed by either channel 1–2 or channel 4–3 because our eyes are always aligned in horizontal when observing objects. Figure 4a and 4b are channel 1 and channel 2 SE images, respectively. As can be seen from Figure 4c, the anaglyph image made from Figure 4a and 4b clearly reveal 3D features indicating an enough parallax generated from this method. When our individual MCP channel was selected for imaging, an obvious directional illumination was observed: the incident electron beam appeared to be from the side rather than from the top. This is induced by the angled locations of individual MCP plate segments. For comparison, illumination from top is obvious in normal SE mode when signals from all four channels are summed, as shown in Figure 4d. Fortunately, this phenomenon does not affect the 3D imaging formation.

3. Sample Tilting

The most common approach employed in SEM stereomicroscopy is sample tilting. It is well acknowledged that recording two images from the same viewpoint with the sample tilting at an angle is equivalent to recording two images on the still sample from two viewpoints separated with the same angle. Therefore, for SEM 3D imaging, the same area of interests will be scanned twice with the sample tilting between these two scanning. The tilting angle, θ, determines the effect of depth perception. The normal range of θ for average human eyes is about 5–6°. To mimic similar stereo effects, the sample tilting should have the same range as well. In reality, the selection of tilting angle θ is dependent upon several factors such as magnification, working distance, and surface roughness. As a rule-of-thumb, smaller tilting angles are needed for lower magnifications and a smooth surface usually requires larger tilting angles.

To demonstrate this method using Agilent 8500, commercial silica powders were mounted on a variable tilt mount at the untilted (horizontal) position. The sample should be mounted such that the tilt axis is vertical on the screen (Y-direction). Most likely, objects of interest will undergo lateral movements

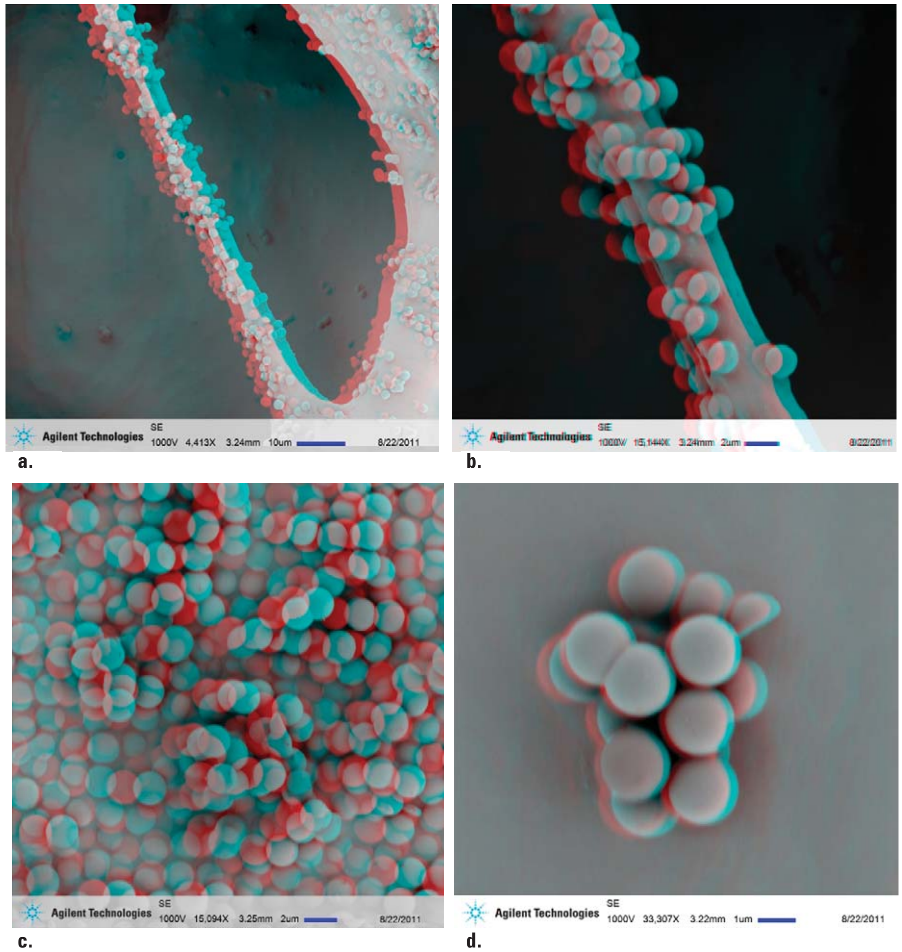


Figure 5. 3D images of silica beads at different magnifications derived by the “sample tilting” method. (a, b) silica beads stick to a fiber hanging over the substrate; (c, d) stacked silica beads.

after tilting, which may cause some problems locating the same area of interest. Therefore, it is necessary to record a low magnification image with a prominent feature which can be used as a fiducial landmark. Ideally, a eucentric stage should be used which enable pure tilting without introducing any lateral translation. After recording an image containing the features of interest, the sample was tilted at 5° followed by finding the same feature and recording at the same magnification and working distance. Since tilting may change the vertical position of the feature slightly, the second image might not be in sharp focus. In order to maintain the same magnification, refocusing should be avoided, because this will cause either failure to match the two images or introduction of inaccuracies in topography determination. To avoid these problems finely adjusting the sample stage in Z-direction to bring the image back into sharp focus was performed. Figure 5(a–d) are representative anaglyph SEM images of silica beads with sufficient parallax effects.

For conventional SEMs, it is the Everhart-Thornley (ET) detector placed on the side above the sample that collects escaped secondary electrons from the surface. To obtain the second image of the stereo-pair, the sample should be tilted toward the ET detector, resulting in an apparent direction of electron illumination. Images similar to Figure 4a and 4b will be obtained. To prevent this phenomenon of illumination orientation, mechanical rotation and image rotation can be used with mechanical tilting [10]. This method is effective but requires a careful adjustment by an experienced SEM operator. Differently, Agilent 8500 uses a quad-segmented MCP detector located symmetrically above the specimen to detect the secondary electrons. As all four segments are used for detection, sample tilting has no effect on illumination orientation. Thus extra effort is not needed to record images from the tilted sample.

Quantitative Measurement: Recovering the Third Dimension

In addition to qualitative stereo imaging, SEM stereomicroscopy is capable of quantitative measurement of 3D features, especially the “hidden” third dimension. Since the “lateral shifting” method creates insufficient parallax, it is difficult to do accurate height measurement. In the case of “individual MCP imaging” method, semi-quantitative measurement is possible with the calculated tilt angle of the individual MCP detector based on its dimension and working distance. Compared with using the former two methods, it is possible to use "sample tilting" to conduct a full-field measurement more accurately, thus it has been widely used for quantitative study of 3D morphologies in SEM stereomicroscopy. The principle of the quantitative measurement in sample tilting is quite simple: surface features of different heights have different lateral displacements in the stereo-pair images, and this disparity, coupled with the tilt angle, can be used to calculate their relative heights in the third dimension.

Ideally, a eucentric tilting is needed, which will simplify the calculation. As illustrated in Figure 6a, orthogonal coordinate axes are established so that the tilt axis is parallel to the Y axis. Assuming the electron beam is focused on the surface region on the Y axis. In the eucentric tilting situation, tilting should not induce displacement of any feature in Y-direction. To further simplify the calculation, parallel projection is assumed which implies that the electron beam is always parallel to the optical axis (vertical to the focal plane) as it scans across the specimen surface. This assumption is regarded as a good approximation for the magnifications and working distances typically used in modern SEMs. Thus an image formed in SEM is geometrically equivalent to projection of the feature onto the focal plane. With reference to Figure 6b, B is a point elevated with respect to point A by an unknown distance L_{BC} before tilting. The projection of line AB on the focal plane will be line AC. After tilting the sample at a degree of α, point B will move to point B′ while keeping point A still. Then the projection of line AB′ on the focal plane will be changed to AC′, which will be observed as the displacement of point B in two stereo-pair images. This is also called parallax movement. From Figure 6b, the geometric relationships based on parallel projection can be derived as follows:

$$\frac{L_{AC}}{\sin\theta} = \frac{L_{AC'}}{\sin(\theta-\alpha)} \qquad \frac{L_{AC}}{L_{BC}} = \tan\theta \qquad (1)$$

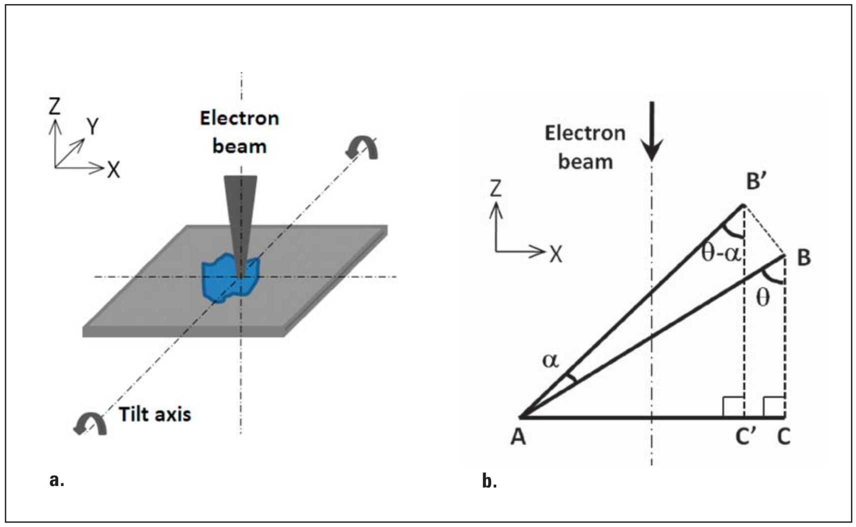


Figure 6. (a) Schematic showing the tilting axis and established orthogonal coordinate axes. (b) geometrical definitions relative to a point B for relative height measurement.

where the lengths L_{AC} and $L_{AC'}$ can be measured on the SEM images, and the tilt angle α is a known value. From above formulas, the relative height L_{BC} can be expressed as:

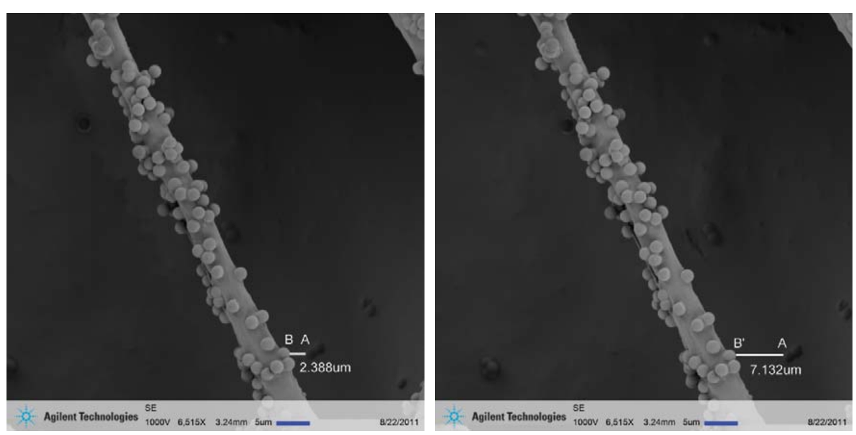
$$L_{BC} = \frac{L_{AC}\cos\alpha - L_{AC'}}{\sin\alpha} \qquad (2)$$

When the tilt angle is small, Equation 2 can be simplified as:

$$L_{BC} = \frac{L_{AC} - L_{AC'}}{2\sin\left(\frac{\alpha}{2}\right)} = \frac{P}{2\sin\left(\frac{\alpha}{2}\right)} \qquad (3)$$

where P is the displacement of point B relative to point A, so-called parallax value. It is the Equation 3 that has been commonly used for the third dimension calculation in SEM stereoscopy.

Let us apply this simply equation to real stereo-pair images. Figure 7 are stereo-pair images of silica beads stick to a fiber hanging over a substrate. Overlaying these two will generate a 3D image. To determine the height difference between a bead and the substrate, we can select an obvious feature on the substrate such as the edge of a substrate defect noted as point A. Then a silica bead is chosen with the same Y coordinate (point B). Before tilting, the distance along X-direction can be measured from Figure 7a, which



a. b.

Figure 7. Stereo-pair images showing the parallax movement of point B relative to point A. (a) image before tilting; (b) image after 5° tilting.

is 2.388 µm. After 5° tilting, point B seems to move to B′ while the line between two points is still horizontal. Apparently, there is no displacement in Y axis indicating a eucentric tilting. The new distance is measured to be 7.132 µm resulting in a parallax value of 4.744 µm. By applying Equation 3, the height difference between point A and point B is 54.379 µm.

It is not always the case in practical measurements that two measured points have the same Y value. And most specimen surfaces are not perfectly flat which implies that those points of interests are not in the same plane at the untilt situation. Considering a more complicated case, the height difference and distance between two arbitrary points, A and B, need be measured using the stereo-pair images. These images can be obtained by different tilting angles θ1 and θ2, and α is the difference in tilt angles. To do so, a reference point O, needs to be selected as the X, Y, Z coordinates (0, 0, 0), as shown in Figure 8. The real coordinates of point A, (X_A, Y_A, Z_A) can be calculated using the measured relative coordinates of point A to the reference point in two images, x_A, $x_{A'}$ (in X-direction) and y_A, $y_{A'}$ (in Y-direction).

$$X_A = x_A - \left(\frac{P_A}{2}\right) = x_{A'} + \left(\frac{P_A}{2}\right)$$
$$Y_A = y_A = y_{A'}$$
$$Z_A = \frac{P_A}{2\sin\left(\frac{\alpha}{2}\right)}$$
$$P_A = x_A - x_{A'} \qquad (4)$$

The real coordinates of point B, (X_B, Y_B, Z_B) can be obtained in a similar way:

$$X_B = x_B - \left(\frac{P_B}{2}\right) = x_{B'} + \left(\frac{P_B}{2}\right)$$
$$Y_B = y_B = y_{B'}$$
$$Z_B = \frac{P_B}{2\sin\left(\frac{\alpha}{2}\right)}$$
$$P_B = x_B - x_{B'} \qquad (5)$$

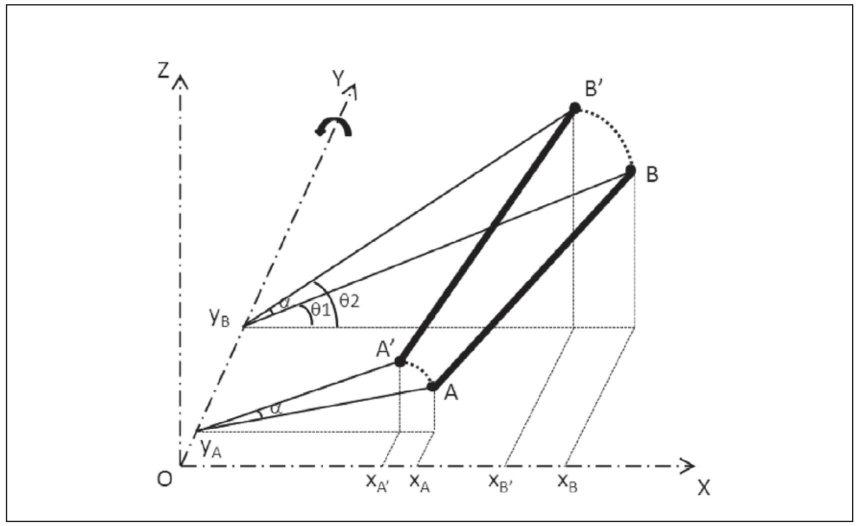
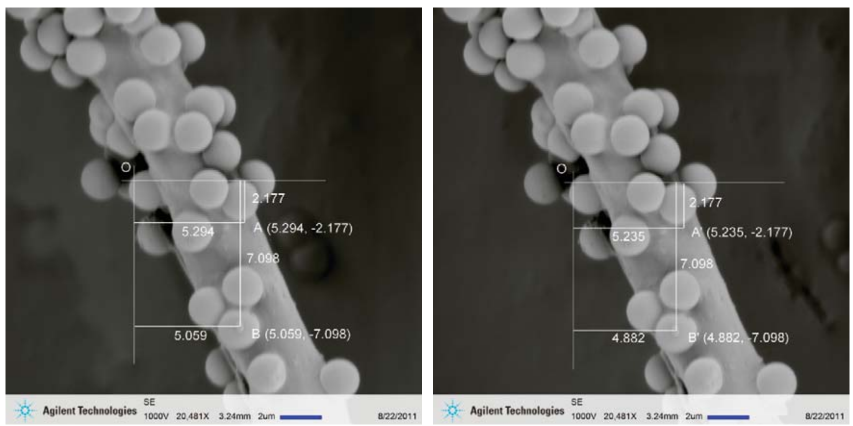


Figure 8. Geometrical definitions to calculate the height difference between two points (A and B) and real length of line AB.



a. b.

Figure 9. Calculation of the height difference and real distance between point A and point B from stereo-pair images. (a) image before tilting; (b) image after 5° tilting.

Thus the height difference between point A and point B is $Z_A – Z_B$. And the distance between two points can be calculated using the following formula.

$$L_{AB} = \sqrt{(X_A - X_B)^2 + (Y_A - Y_B)^2 + (Z_A - Z_B)^2} \qquad (6)$$

Equations 4–6 only stand for eucentric tilting and the tilt angle α, must be small (<10°). At large tilt angles, the assumption of parallel-projection approximation does not apply. The more general equations are beyond the scope of this note.

Figure 9 are another stereo-pair images recorded at a higher magnification. Equations 4–6 can be used to calculate the height difference and real distance between any two arbitrary points, as long as they are obvious in both images. As an example, a small particle on the fiber was set as point A, and a round feature on a silica bead was assigned as point B. With reference to an obvious feature, the origin point O, the coordinates of point A in both images, (x_A, y_A), $(x_{A'}, y_{A'})$ can be determined by measurement, which are (5.294, -2.177) and (5.235, -2.177), respectively. By applying Equation 4, the 3D coordinates of point A are (5.265, -2.177, 0.676), indicating point A is 0.676 μm higher than point O in Z-direction. Similarly, the coordinates of point B can be determined as (4.971, -7.098, 2.029). Thus point B is 1.353 μm (2.029-0.676) higher than point A in Z-direction. And, by applying Equation 6, the real distance between point A and point B is calculated to be 5.161 μm.

It is necessary to point out that the accuracy of quantitative measurement in SEM stereomicroscopy can be affected by a variety of factors, such as tilt angle, magnification, tilt eucentricity, working distance, spatial resolution, and image quality [11, 12]. As an example, imaging at low magnifications could have various magnifications over the whole viewing area: regions farther from the optical axis have higher magnifications than those closer to the axis, so called “barrel type distortion”. This distortion may lead to erroneous measurements on stereo-pair images. Furthermore, as a prerequisite for quantitative 3D measurement in the SEM, calibration procedures based on traceable standards are needed; such as magnification calibration, tilt angle calibration, vertical elevation calibration, and vertical plane calibration [13, 14]. Accurate 3D measurement does require further research efforts to develop reliable and metrologically correct techniques.

Conclusions

SEM stereomicroscopy not only provides qualitative stereo imaging but also enables quantitative measurements, especially recovering the “hidden” third dimension of 3D structures. In this study, three methods were conducted on Agilent 8500 FE-SEM to generate stereo-pair images for 3D imaging. Results show that the “lateral shifting” method does not generate obvious stereo effect while “sample tilting” is able to create sufficient parallax. The Agilent 8500 FE-SEM, which is equipped with quad-segmented MCP detector, offers a simple but effective “individual MCP channel imaging”, to create 3D images without any sample lateral shifting or sample tilting. Quantitative measurement was also carried out on stereo-pair images to determine the third dimension of a 3D structure. These measurements have been demanded for a variety of applications.

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