Development of Geometrical Analysis in SEM

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BIOGRAPHY
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ABSTRACT
How to measure coordinates in two- or three-dimensions with SEM, what is required for such analysis? This contribution gives a view on the development of geometrical analysis in the wider context of microscopy, analysis, and tomography; and it clarifies the key concepts necessary to navigate and select between the different techniques already available.

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Microscopy and Analysis 34(6): 14-16 (EU), December 2020

INTRODUCTION

Electron microscopes are often described as versatile tools because they provide answers to a truly impressive range of scientific questions. An expected consequence of using the same tools for a wide range of research fields is the development of a diversity of ways to use them, i.e. techniques.

Whilst it may be possible to argue that there are at least as many techniques as there are users, a few core concepts and common patterns may be discerned. From this perspective, the main two groups of techniques are as obvious, as they are popular – microscopy and analysis. How does this work?

Techniques can be classified together when they share common concepts, they have similar workflows, and they produce similar outputs. From this perspective, it may be argued the main shared concept in electron microscopy is the realisation that electron-based instruments provide information well beyond the spatial resolution of traditional light-based instruments. It could then be described that initial focus has been on structural characterisation with various types of microscopes, hence the classification 'microscopy', where the output is photographs that have gained the name of micrographs.

A further focus has then developed around chemical analysis with high spatial resolution, hence the classification 'analysis', where the output is spectra. Such 'microscopy and analysis' grouping of techniques in electron-based instruments remains true to its foundation in light-based instruments, and at such it retains the production of the same type of outputs, i.e. photographs and spectra.

A further focus point of several techniques could be understood as originating from X-ray based instrumentation, that were developed to resolve complex internal structures. These techniques rely on surface imaging of sections, e.g. serial block-face SEM and FIB tomography, or transmission imaging of lamellas over a range of rotation angles, e.g. electron tomography in TEM and SEM, followed by algorithms for image registration and three-dimensional reconstruction. Output from these techniques is three-dimensional volume data that may be reduced to two-dimensional images at planes of interest, which are named 'tomograms'.

GEOMETRICAL ANALYSIS

There is a distinct group of techniques within electron microscopy that rely on alternative ways of understanding and using the instrument, and for which the outputs are neither photographs, spectra, nor volume data.

This is the result of the need to produce two- or three-dimensional maps or models of surfaces with high spatial resolution, which are typically constructed from points with two- or three-dimensional coordinates.

Such output could be given a new name, perhaps 'micro-map' or 'micro-model', however it is perhaps better to extend the meaning of 'micrograph' to include these in addition to the traditional coordinate-less photographs.

One could find a logical path from the most complex three-dimensional models all the way back to the drawings in Hooke's *Micrographia* and thus claim a natural progression, however this use of electron microscopes also inherits the basic geometrical concepts and techniques of land surveying. For this reason, the term Geometrical Analysis is proposed and used here.

The need for geometrical analysis techniques has origins in a range of practical issues, including efficient, non-destructive analysis, and modelling. It is possible to reduce three-dimensional questions to conventional imaging, for example measurement of height or depth can also be done with conventional sectioning and imaging, however it is more efficient to avoid the additional sample preparation steps and perform the measurements in three-dimensions.

Similarly, the angle between two planes can be obtained by imaging a section, however the sample is destroyed because of sectioning. Even more so, serial sectioning and imaging is not only impractical for the purpose of a complete three-dimensional surface model, but also lacks depth resolution and adds sample preparation artefacts. Stepping down to two-dimensions, it is more efficient to find a position on the sample by navigating in sample coordinates, rather than relying on a series of annotated images with increasing magnification.

The top three distinctive features of geometrical analysis techniques are emphasis on three-dimensions, separation of shape from composition, and use of sample coordinates. Measurement in three directions (X, Y and Z) with a focused electron beam that scans in two-dimensions is enabled by automated image processing, which is usually termed reconstruction, and which is an integral part of the minimum toolkit required for geometrical analysis. Separation of composition and shape is not required for conventional microscopy, where interpretation is based on visual interpretation, however it is essential for geometrical analysis. Use of sample coordinates requires registration of the sample position and orientation on the sample stage, which then allows for automatic transformation between stage and sample coordinates.

Having described the need for, and the distinctive features of geometrical analysis, what are the main techniques available, and how do they relate?

SEM TOPOGRAPHY

Perhaps the first technique to step away from two-dimensional imaging is SEM topography. It is based on the realisation that angular distribution of electrons backscattered from the surface of the sample is determined by the surface orientation[1, 2].

Therefore, if this angular distribution is measured, then the sample orientation and backscattered intensity may be calculated directly at each point on the sample.

Thus, the technique provides the much-desired separation of shape and composition. The height of every point in the scan is reconstructed from its three-dimensional orientation, and therefore this is a 2½D technique, horizontal position plus height. Spatial resolution in height is on the same range as lateral resolution and, given that the technique is based on BSE signals, dependent upon material density.

Typical workflow consists of a single scan where signals at different azimuthal directions are recorded simultaneously (Figure 1a). Surface height and backscatter intensity calculations are made live during image acquisition and provide an immediate feedback in three-dimensions.

Data may be saved in topographic file formats originating from scanning probe techniques, or three-dimensional file formats originating from three-dimensional scanners (Figure 1b). Quality of measurements in all directions is assured by inspection of results from three-dimensional calibration samples[3].

Equipment required is first a calibrated and segmented detector BSE detector, so that azimuthal distribution can be measured. Calibrated electronics for amplification and digitisation are then required to quantify backscatter intensity in physical units. The technique relies heavily on the reconstruction algorithm, and therefore requires dedicated software for electron microscopy. Measurements of distances and height require software for either topographic or three-dimensional data.

Key advantages are speed and ease of use. Because the topographic reconstruction algorithm is highly efficient, three-dimensional information is calculated live and the sample may be navigated directly in three-dimensions. Because all data is acquired during a single scan, errors in feature identification and coordinate transformations are prevented, therefore the entire reconstruction is fully automated and easy to use. Key disadvantage is its 2½D nature, which limits the application to continuous surfaces without all surface points visible from a single direction.

3D SCANNING

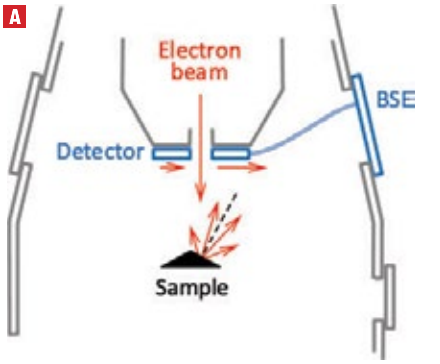
The core concept for 3D scanning in SEM is extraction of three-dimensional data from two-dimensional images using photogrammetric reconstruction. Early implementations of this technique have attempted stereo reconstruction[4], i.e. two images only, however these had limited success, and current approach is to employ multiple images over rotation and tilt series.

The reconstruction algorithm searches for same features in different images taken from around the sample, and uses the positions found in each image to determine sample points in three dimensions. Since sample stages lack accuracy, it must register the position of the sample in each image and then refine three-dimensional calculations. Three-dimensional surface polygons are calculated from the cloud of positions, and texture is obtained by analysing pixel intensities in the input images.

Typical workflow consists of recording a series of images in a range of tilt and/or rotation angles, followed by image processing for reconstruction and three-dimensional analysis (Figure 2a). Experimental data consists of images, preferably with stage information. Reconstructed data may be a three-dimensional point cloud, but preferably a surface mesh with texture from image intensity (Figure 2b). Data format is same as light-based 3D scanners.

Tilt and rotation may be performed using the standard SEM sample stage, either manually or automatically using a script or external stage controller. SE detectors should be generally avoided as they introduce complex shadowing into the image, and therefore provide poor separation of shape from composition.

BSE detector are better in this regard, and they do provide robust data for reconstruction, however they are limited in resolution. Highest



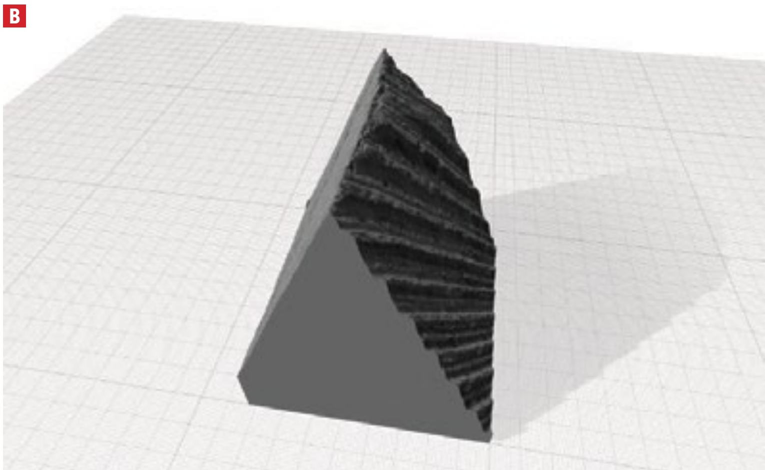
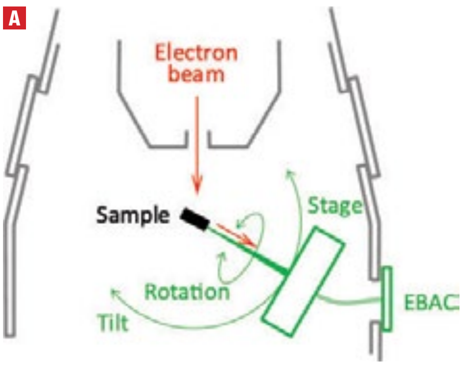


FIGURE 1 *For SEM topography, angular distribution of backscattered electrons (BSE) is detected and used to determine surface orientation and height (a) A rather extreme aspect-ratio data example, showing 2½D measurements on a curved cutting edge displaying smooth and ridged surfaces (b) The 3D model has 42 x 42 x 48 µm.*



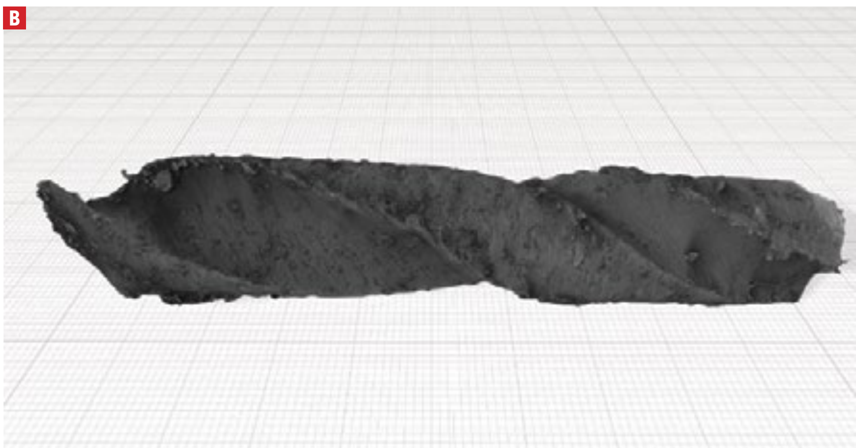


FIGURE 2 *For 3D scanning in SEM, sample is imaged using Electron Beam Absorbed Current (EBAC) signal over a tilt and rotation range, then minute features are automatically detected and referenced across images to reconstruct a 3D model (a). Example of 3D measurement showing a complete drill piece. The 3D model has 0.5 x 0.5 x 2.5 mm.*

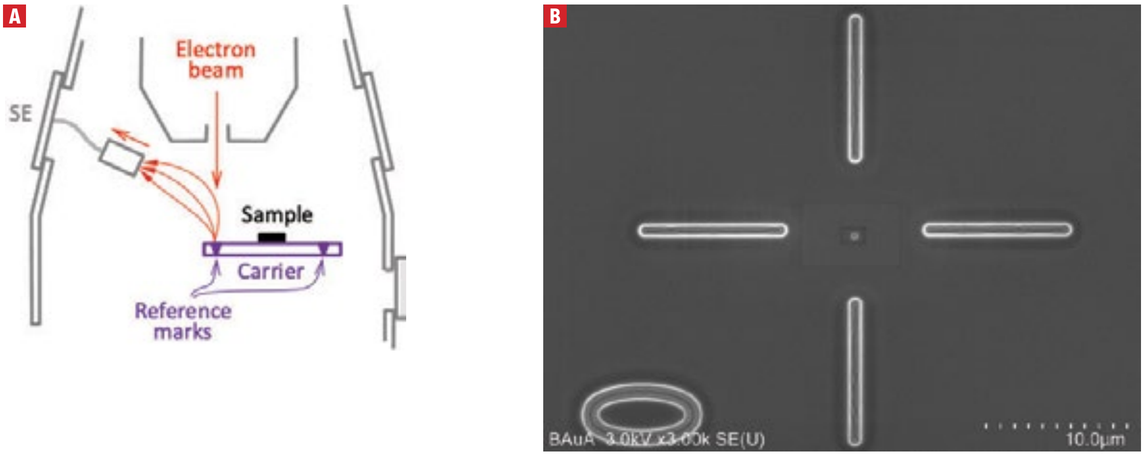


FIGURE 3 *For navigation in sample coordinates, the sample is mounted on a carrier with spatially distributed registration marks, which are then registered by reading the stage coordinates and thus enable automatic coordinate transformation (a). Typical Secondary Electron (SE) image of registration mark (b).*

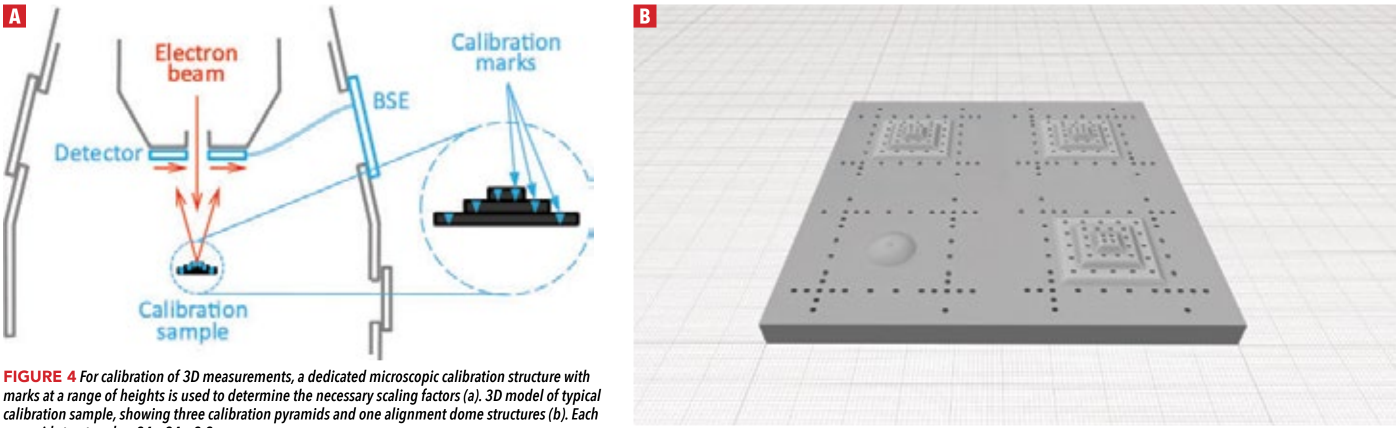


FIGURE 4 *For calibration of 3D measurements, a dedicated microscopic calibration structure with marks at a range of heights is used to determine the necessary scaling factors (a). 3D model of typical calibration sample, showing three calibration pyramids and one alignment dome structures (b). Each pyramid structure has 24 x 24 x 2.8 µm.*

resolution and most practical approach rely on Electron Beam Absorbed Current (EBAC) signal[5-6]. General purpose optical reconstruction software is compatible with SEM data, including auto detection of view angles and image distortions.

Key advantages of 3D scanning stand in its ability to manage complex structures, as rotation and tilt range can be set up in such a way to acquire images from every feature of interest, and make no assumptions about the sample or the microscope. Main disadvantages are the need for complex acquisition of a rotation and tilt series, and the requirement for samples with texture suitable for point identification.

SAMPLE NAVIGATION

This is a keystone technique, because it provides an essential bridge between conventional imaging and the need for sample coordinates. The key concept is to enable navigation to the same position on the sample in different microscopes, or overlay datasets from different microscopes, which need not be only electron microscopes. This concept applies equally in two and three dimensions, and relies on registration of sample on the stage, and transformation between stage and sample coordinates[7].

Typical workflow relies on mounting the sample on a carrier with registration marks, which is then loaded onto the sample stage (Figure 3a). If the sample presents adequate unique features, then these may also be used for registration, and then any sample carrier may be used.

Navigation to the registration marks is used to obtain position from the stage controller, and thus determine the transformation parameters between sample and stage coordinates (Figure 3b). In order to navigate to required location, sample coordinates may then be input manually, or imported from saved data. Recorded images have their coordinates preferably embedded into metadata or stored in independent registration files.

Assuming that the stage is motorised and available for control, the minimum hardware required is a supply of sample carriers with registration marks. Advanced automation may require an external stage controller with motion corrections. Whilst the hardware requirements are relatively standard, the technique relies primarily on specialised software for registration and coordinates transformation. For data processing, a software aware of spatial coordinates is required in order to enable geometric operations such as overlaying and stitching, input of further geometric data such as points, lines or polygons.

Key advantage of sample navigation is the ability to add spatial awareness to microscope data, which is essential in bringing together datasets from different images, microscope sessions, and microscope types[8].

THREE-DIMENSIONAL CALIBRATION

Two-dimensional calibration of electron microscopes is a relatively complex technique, as it deals with acceleration voltage, working distance, magnification and scan rotation, but it may still be performed as a series of manual steps using a calibration sample with known two-dimensional structures[9].

Three-dimensional calibration adds significant complexity, and therefore the key concept is that it must be performed automatically by recognition of spatial distributed markers on a known three-dimensional structure[10]. Measured and expected coordinates are automatically compared in three dimensions, and corrective measures are introduced when necessary. The corrective action taken may be hardware changes, such as scan gains and offsets, or software changes, such as rescaling coefficients.

Equipment required is a three-dimensional calibration sample that has been measured with a metrological instrument, such as a Scanning Probe Microscope (Figure 4). Calibration software is required for automated feature recognition and determination of correction coefficients[11-12].

SUMMARY AND CONCLUSIONS

Given the recent advancements in SEM electronics and software, it is now possible to use a collection of techniques for geometrical analysis. Most of the techniques are well developed and easy to use, and therefore it is now straightforward to obtain topographical or complete 3D surface data, as well as to navigate in sample coordinates.

Whilst further development is likely to add more automation and ease of use, there is a convincing case that the overall technology for geometrical analysis in SEM is now in place.

Article, references along with the 3D objects are available online at: analyticalscience.wiley.com/publication/microscopy-and-analysis

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