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Standards and software for automated 3D calibration

Introduction

Three-dimensional (3D) microscopes are critical tools in scientific research, assessment, and quality control, allowing for the visualization and measurement of microscopic structures with high resolution and accuracy. However, achieving accurate 3D measurements requires precise calibration of the microscope system. For fast and easy-to-handle geometric calibration, an automated approach is essential.

In this whitepaper, we will discuss the importance of 3D microscope calibration, the advantages of automated calibration with integrated feature recognition and different types of 3D calibration samples and their application.

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Importance of 3D calibration

The accuracy of 3D measurements obtained from a microscope is highly dependent on the calibration of the system. 3D calibration not only verifies the spatial scales, it also must cover the determination of perpendicularity and further systematic errors of the microscope. Appropriate geometric calibration ensures that the 3D measurements acquired with the microscope are accurate and repeatable. Without correct calibration, there is a risk of errors and inaccuracies in the measurements, leading to incorrect data and potentially flawed research results.

Automated 3D calibration

3D calibration requires artefacts with lateral and vertical structures, with known size for controlling the spatial scales (figure 1).

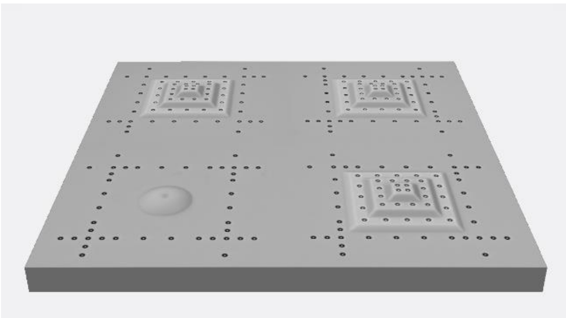

Figure 1: 3D reference sample with fiducial marks for automated calibration.

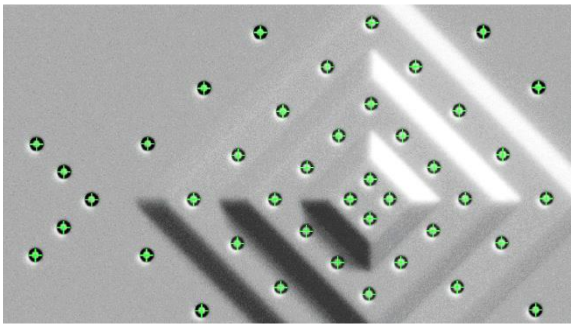

Figure 2: 3D calibration sample with automatically measured reference marks.

Separate calibration of position and height is time-consuming and holds the risk for erroneous measures. Therefore, the key concept for 3D calibration is that it must be performed automatically through the recognition of spatially distributed features on a known three-dimensional structure (figure 2).

Marker-based techniques enables advanced, flexible, and fast calibration: Coordinates measured are compared automatically with known coordinates, in three-dimensions, and correction parameters are obtained. The corrective action taken may be hardware changes, or software changes, such as rescaling coefficients.

Equipment required is a three-dimensional calibration sample with known reference measures, that have been certified upon request. Calibration software is required for automated feature recognition and determination of characteristic geometric microscope parameter, primarily lateral scales (x, y), height scales, shearing and further systematic device parameter.

Advantages of automated marker-based 3D calibration

Better accuracy

- Full parameter approach: Automatic determination of scales in x, y, and z. In contrast to separate lateral and height calibration, squareness deviations can also be determined, also in z-direction (Figure 3).
- Calibration is independent of leveling of the calibration sample, due to coded marker design and 6-degree-of-freedom (6DoF) approach.
- Automated recognition of a high number of features leads to better reliability and enables statistical evaluation for accuracy estimation and outlier detection.

Higher efficiency

- 3D calibration in one step: A single calibration standard is sufficient instead of using separate standards for position and height.
- Time-saving calibration by applying only one calibration standard and dedicated software for feature recognition and parameter estimation.
- Automated generation of reports and data logs for long-term monitoring and quality management.

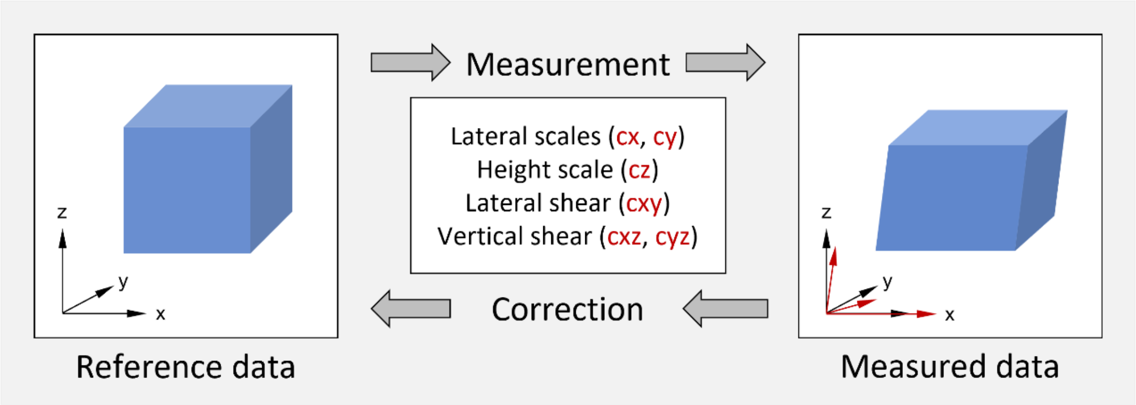

Figure 3: Linear geometric errors in 3D measurements, determined with 3D calibration and applied as correction values for device and data misalignment.

More flexibility

- Versatile application for different 3D microscopes, e.g., CLSM, 3D-SEM, SPM/AFM.
- Adaptable concept for different applications and Field of Views (FoV).
- API and data interface for integration in customized workflows.

Available types of 3D calibration standards

3D calibration standards consist of step pyramids with spatially distributed reference marks. A typical calibration structure contains four step pyramids that can be used as an array or individually to cover various magnifications. However, some structures have a spherical element instead of a pyramid, which is e.g., applied for SEM detector adjustments.

A common structure for SEM application covers three pyramids and a spherical element on an area of 100 µm x 100 µm. Each pyramid has three steps, with a nominal height of 1 µm for each step (figure 4). For optical 3D microscopes, bigger structures are available.

Depending on the manufacturing process, the structures consist of different materials. Established FIB-deposited structures consists of a composite of carbon and platinum. For bigger structures and larger quantities, structures are produced in Silicon-based substrates and have dedicated coatings (figure 5).

Besides default 3D calibration samples, e.g., products for SPM/AFM and 3D-SEM, custom-based design and prototypes are available on request. This includes structures with a higher number of pyramids and varying arrangements, which allow for calibration across different magnifications or larger areas.

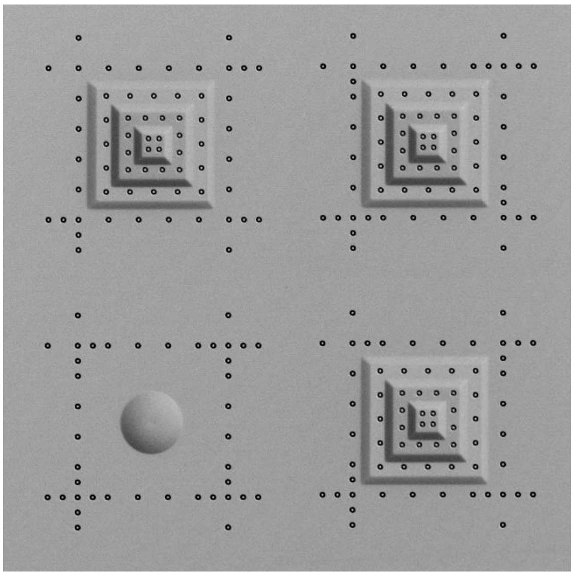

Figure 4: 3D calibration standard with multi-step pyramids. Several height steps enable determination of nonlinear errors in z-direction (SEM image, Field of View = 92 µm).

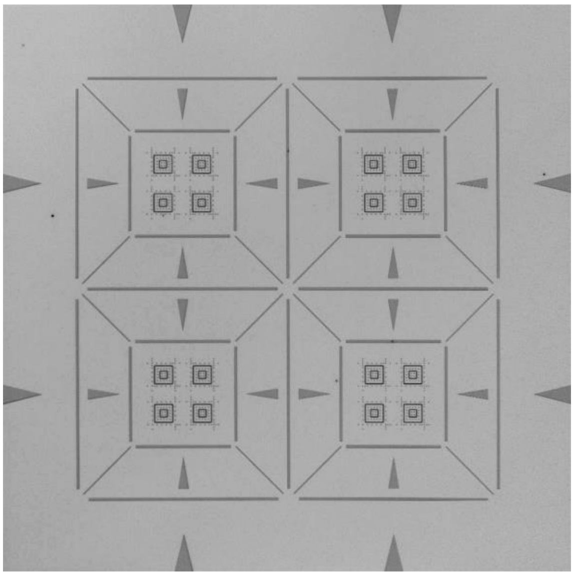

Figure 5: 3D calibration sample with repetitive structures for calibration of larger areas (CLSM image, Field of View = 645 µm).

Application examples

Routine calibration for 3D fractography

For automated and quantitatively determination of fracture characteristics and mechanisms, besides SE images, measurement of surface topography is required. Since samples can have different materials and properties, it is essential to select specific SEM parameters and perform regular calibration of the height measurements.

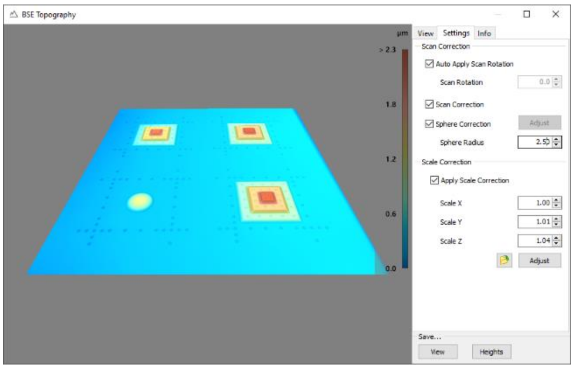

Figure 6: The DISS6-Topo SEM topography measurement system includes integrated 3D calibration, allowing for control and adjustment of the z-scale under varying SEM parameters.

Therefore, 3D calibration was integrated in the topography measurement application, which utilizes a standard SEM 3D calibration sample (figure 6). This fast and easy-to-use approach enables the acquisition of a large amount of quantitative height maps, which are used for AI-based evaluation of a variety of fracture samples (figure 7).

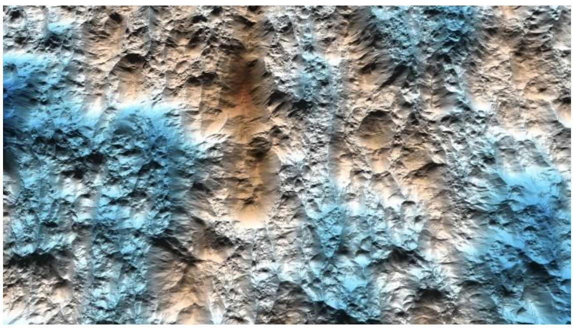

Figure 7: Topographic data of fractographic sample, measured with SEM (rendered height map, approx. Field of View = 960 µm)

Reference measurement of hardness indenters

Vickers hardness indenter diamond tips are used to determine the macro-range of Martens hardness. For traceability, they are measured by scanning probe microscopy (SPM, figure 8). Accuracy estimation of the determination of the tip geometry, especially the apex angle, suggest that a previous dimensional calibration of the SPM is necessary.

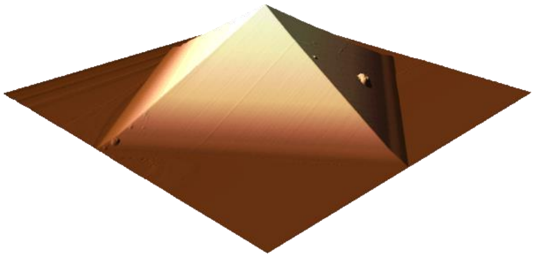

Figure 8: SPM measurement data of Vickers indenter (3D view, height approx. 10 µm)

When comparing uncalibrated, conventionally calibrated, and 3D-calibrated SPM data, the resulting apex angle leads to different results for the Martens factor (table 1). The results show most precise results when applying 3D calibration. Therefore, it was applied as a valuable enhancement of existing calibration strategies.

Parameter	Conventional calibration	3D calibration
Apex angle	135,75°	135,81°
Martens factor	26,12	26,20

Table 1: Results for Apex angle and the Martens factor for conventional and 3D calibrated Vickers indenter.

Long term stability of confocal laser scanning microscope (CLSM)

CLSMs enable fast and reliable topography measurements for different applications. However, for verification of the accuracy and traceable measurements conform to quality standards, 3D calibration is required. To cover the long-term stability and verification after maintenance, a standard calibration sample was used for determination of lateral and height scale corrections and shearing corrections between all spatial axes.

During a 1.5-year period, variations of the resulting parameter were recorded and used for corrections, and thus enabled the accurate and traceable measurement of further investigated samples (figure 9).

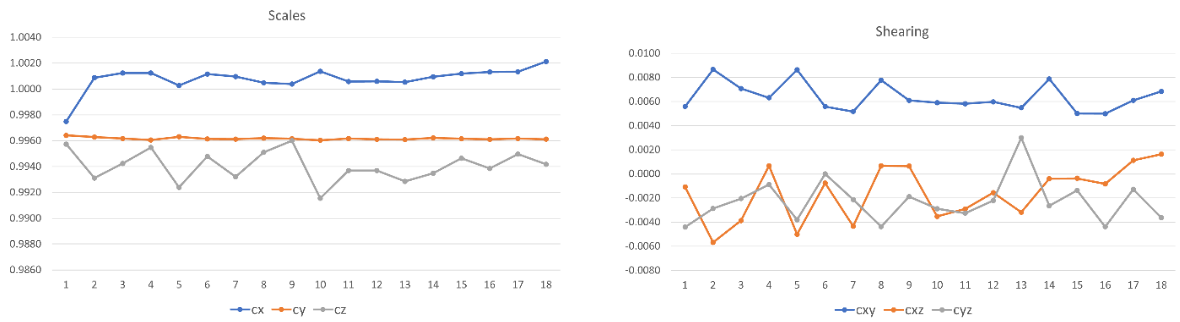

Figure 9: 3D calibration of CLSM with a traceable 3D calibration standard. X-axis represents the series of measurements in the mentioned period, which is not equivalent to the time axis. Left: Scale correction factors in x, y, and z. Right: Shearing correction between coordinate axes.

Conclusion

3D calibration is a fast and easy-to-handle method for the verification and correction of measurements obtained with 3D microscopes. It reveals and quantifies geometric inaccuracies of the applied microscope and guarantees precise and reliable measurement results.

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