Received: 18 October 2023 | Accepted: 19 November 2023

DOI: 10.1002/jemt.24465

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Enhancing SEM positioning precision with a LEGO®-based sample fitting system

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Abstract

Scanning electron microscopy (SEM) is a precious tool in materials science and morphology sciences, enabling detailed examination of materials at the nanoscale. However, precise and accurate sample repositioning during different observation sessions remains a significant challenge, impacting the quality and repeatability of SEM analyses. This study aimed to develop and evaluate a LEGO®-based sample positioning system for SEM analysis. The system was designed to consistently identify and align features across multiple repositioning cycles, maintain accurate positioning along the z-axis, minimize distortion, and provide repeatable and reliable results. The results indicated a high degree of precision and accuracy in the repositioning process, as evidenced by the minimal displacements, deviations in scaling and shearing, and the highly significant results ($p < 0.001$) obtained from the analysis of absolute translations and rotations. Moreover, the analyses were consistently replicated across six repetitions, underscoring the reliability of the observed results. While the findings suggest that the LEGO-based sample positioning system is promising for enhancing SEM analyses' quality and repeatability, further studies are needed to optimize the system's design and evaluate its performance in different SEM applications. Ultimately, this study contributes to the ongoing efforts to develop cost-effective, customizable, and accurate solutions for sample positioning in SEM, contributing to the advancement of materials science research and all SEM analysis requiring overtime observations of the same sample.

Research Highlights

- This study focused on the development and evaluation of a novel LEGO-based sample positioning system specifically designed for SEM analysis.
- One of the standout features of this system is its ability to consistently identify and align features across multiple repositioning cycles, showcasing its precision and reliability.

- To further understand the mechanical aspects of the SEM stage, we employed the Rambold Kontroll comparator, which provided a baseline understanding of its mechanical tolerance.
- The registration process results were particularly noteworthy, as they revealed high accuracy with minimal displacements.
- Furthermore, the consistent outcomes observed across multiple repetitions emphasize the reliability and robustness of the methods we employed in this research.

KEYWORDS

feature registration process, mechanical tolerance evaluation, positioning system, sample repositioning precision, scanning electron microscopy (SEM)

1 | INTRODUCTION

Scanning electron microscopy (SEM) is a fundamental scientific technique enabling the detailed examination at the nanoscale. However, despite its immense utility, SEM analysis presents significant challenges, one of the most critical being the precise and accurate sample repositioning (Liu et al., 2021). This is crucial for conducting repeatable measurements and analyzing different sample regions over time. Traditional methods for repositioning the sample often involve complex mechanical systems, which, while effective, can introduce errors and limit the accuracy of the measurements. These errors can have a cascading effect, impacting the data quality collected and, consequently, the analysis conclusions (Zhu et al., 2022).

Several factors contribute to the challenges associated with sample positioning in SEM. First, the sample must be positioned with high precision and accuracy to ensure the region of interest correctly aligns with the electron beam. This requires a positioning system with fine control over the sample's position and orientation. Second, the sample must be repositioned multiple times during the analysis to examine different regions of the sample or to conduct repeat measurements. This requires a positioning system that can accurately reposition the sample multiple times while maintaining alignment with the electron beam. Third, the sample may need to be removed from the SEM chamber and reinserted later, necessitating a positioning system that can accurately reposition the sample after removal and reinsertion (Maraghechi et al., 2018).

This study aims to address these challenges by exploring a novel approach based on a LEGO system for precisely repositioning the stub within the SEM multiple times while maintaining accuracy in positioning. The LEGO system, renowned for its modularity and precision in construction, is adapted in this study to develop a customizable sample positioning system that can be adjusted for different SEM applications. This innovative approach leverages the inherent advantages of the LEGO system, including its flexibility, precision, and cost-effectiveness, to develop a solution that can overcome the limitations of traditional sample positioning methods.

2 | MATERIALS AND METHODS

The precision of the scanning microscope's stage was first evaluated using a Rambold Kontroll comparator with a precision of 0.01 mm. Measurements were conducted 10 times along the *x*-axis and an equal number of times along the *y*-axis to ensure consistency and reliability in the results. This repetitive measurement approach was adopted to account for any potential variations or anomalies that might occur in individual measurements, thereby providing a more comprehensive understanding of the stage's precision (Figure 1).

Following this, the sample holder was constructed by attaching a piece of LEGO to a stub using epoxy adhesive. Another LEGO piece was placed orthogonally on top of the first one, and another stub was glued on top without its mounting support. A small hole was drilled into the lower stub to thread a small tinned copper wire through, establishing an electrical connection between the upper and lower stubs (Figures 2 and 3).

The sample used was a block of human tibial cortical bone sourced from the Historic collection of the Orthopedic Institute of Brescia. The bone was decellularized using 90 volumes of hydrogen

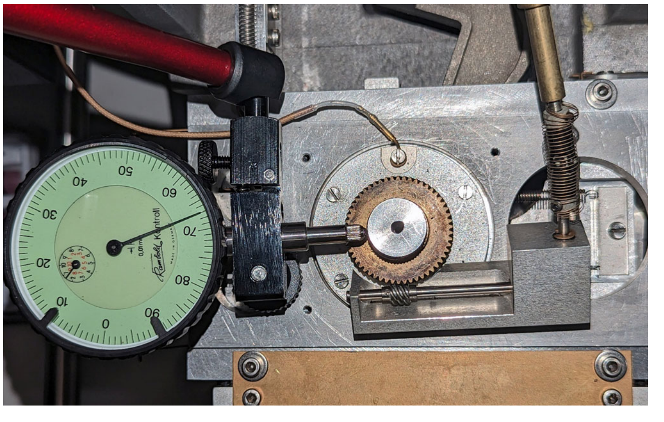

FIGURE 1 The micrometric dial indicator and its magnetic holder.

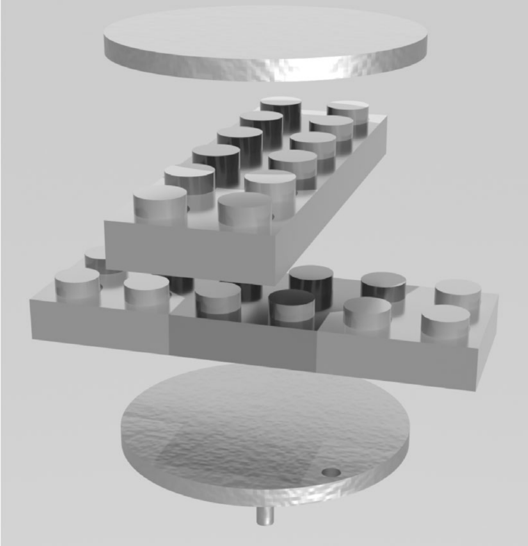

FIGURE 2 Exploded view of the positioning system. This figure illustrates an exploded view of the LEGO-based sample positioning system developed for SEM analysis.

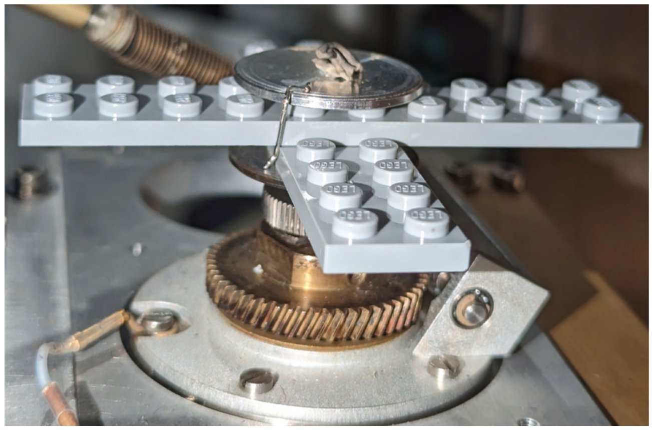

FIGURE 3 Assembled positioning system inside SEM chamber. This figure shows the LEGO-based sample positioning system fully assembled and installed inside the SEM chamber.

peroxide for 30 days. Subsequently, the specimen was mounted on appropriate stubs with a colloidal silver glue, gold coated in an Emitech K550 sputter-coater (Emitech, France) and observed with a FEI XL-30 FEG high-resolution SEM (now Thermo Fisher, USA). Images were directly obtained as 8bpp, 1424 × 968 TIFF files (Zecca et al., 2023).

A Haversian canal with distinct morphological features was identified to ensure the same portion was always photographed at 800× magnification. After the initial photograph, six additional photographs were taken without altering the brightness and contrast, only adjusting the focus (Figure 4).

For each photograph, the HV beam was turned off, and the lower chamber of SEM went through a complete cycle of venting; the chamber was opened, the two LEGO pieces were completely detached and reattached, the electrical bridge was reestablished, and the vacuum in the chamber was recreated, to simulate different observation session in the time accurately.

The obtained photographs were analyzed using a registration plugin of the Fiji software, where a rigid registration with feature recognition was requested (Schindelin et al., 2012).

All the data from the experiments were inputted into the SPSS software for statistical analysis.

3 | RESULTS

The initial results obtained from the Rambold Kontroll comparator provided a foundational understanding of the mechanical tolerance inherent to the SEM stage. Specifically, the comparator showed an average deviation in position along the *x*-axis of 0.003 mm with a standard deviation of 0.012 mm. Along the *y*-axis, the average deviation was 0.004 mm with a standard deviation of 0.021 mm. These measurements serve as a baseline for the mechanical precision of the SEM stage, setting the stage for subsequent evaluations of the LEGO-based positioning system.

In the subsequent analysis, the software's feature registration process identified a total of 2103 corresponding detections across the different images (Grocott et al., 2016). This high number of matched features is indicative of the software's capability in accurately recognizing and aligning features, which is a critical aspect of the registration process.

The quality of the linear registration, as determined by the software, yielded the following results:

Average displacement	0.7742863979931917 px
Minimal displacement	0.5932622392512529 px
Maximal displacement	1.001925325169912 px

These displacements represent the range of pixel shifts observed across all corresponding detections, with the values suggesting a high degree of accuracy in the registration process (Table 1).

These results indicate the degree of scaling and shearing observed during the repositioning process. The scaling values are close to 1, indicating minimal deviation in the *z*-axis positioning. The shearing values, although small, indicate a slight distortion during the repositioning process. These data are crucial for evaluating the precision and accuracy of the LEGO-based sample positioning system.

The absolute translations along the *X* and *Y* axes, total distance, and rotation were further analyzed, with the results presented in Table 2 and summarized in Table 3.

The total distance and rotation analysis revealed a highly significant result ($p < 0.001$). This indicates that the variations observed in the total distance and rotation during the repositioning process were statistically significant, underscoring the importance of these parameters in evaluating the performance of the sample positioning system.

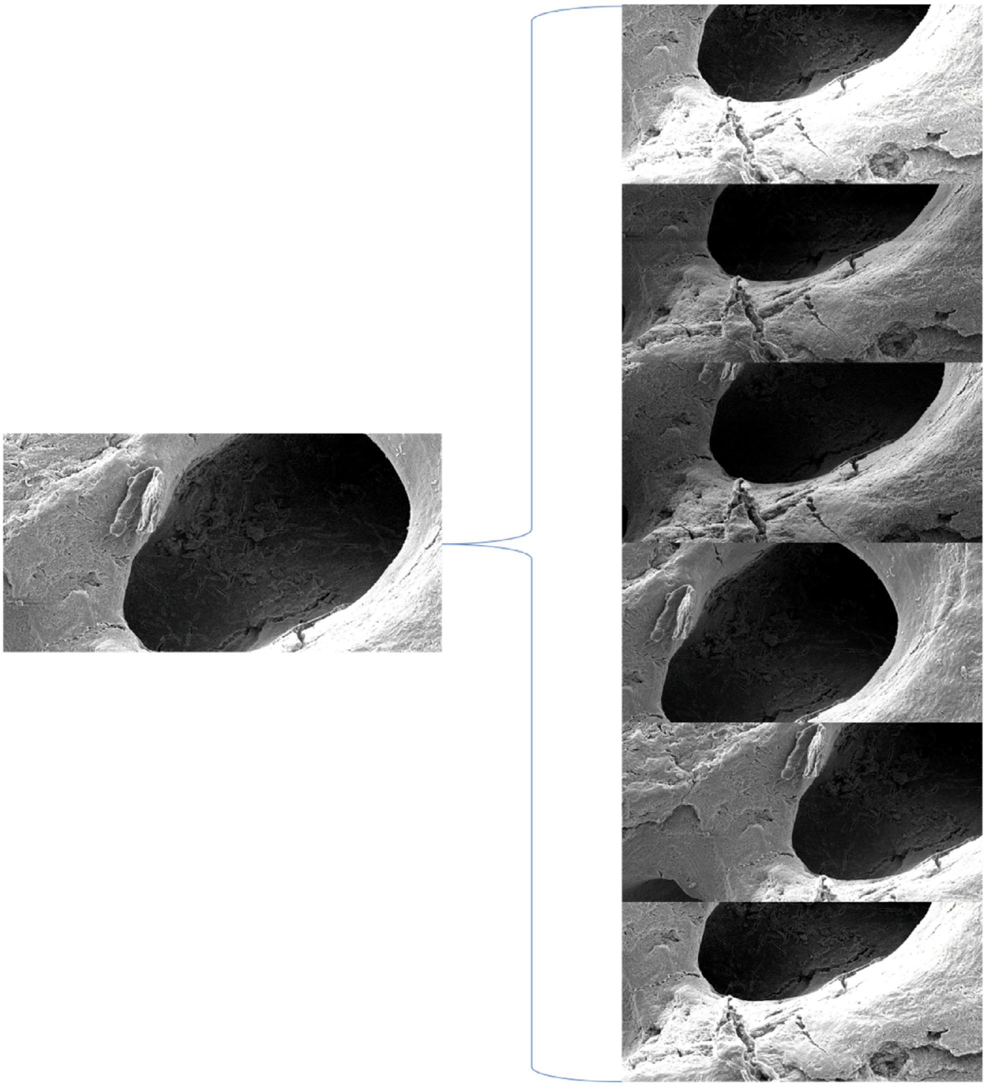

FIGURE 4 Central and surrounding acquired images. The figure displays the initial image acquired at the center, surrounded by all subsequent images obtained during the experiment.

TABLE 1 Raw data of scaling and shearing.

Scaling	Shearing
1.000000000000000	0.000000000000000
0.999986945983207	0.005109585421457
0.999987344755432	0.005030937187057
0.999987574341528	0.004985094035997
0.999985719082453	0.005344308294813
0.999977249372758	0.006745423403526
0.999987751022862	0.004949525657914

Note: This table presents the raw data obtained for scaling and shearing during registration.

4 | DISCUSSION

The study aimed to evaluate the precision and accuracy of a LEGO-based sample positioning system developed for SEM analysis. The results obtained provide valuable insights into the system's performance. The feature registration process identified 2103 corresponding detections, indicating the software's ability to match a significant number of features across different images. This is a crucial aspect of evaluating the registration process's accuracy and effectiveness, as it demonstrates the system's capability to consistently identify and align features across multiple repositioning cycles.

The quality of the linear registration obtained during the registration process was characterized by an average displacement of approximately 0.774 px, with minimal and maximal displacements of about 0.593 and 1.002 px, respectively. These results indicate the degree of displacement observed during the registration process, with the minimal, maximal, and average displacements representing the range of pixel shifts observed across all corresponding detections. The relatively small values of these displacements suggest that the system maintained a high degree of accuracy during the repositioning process.

Furthermore, the results for scaling and shearing during the repositioning process indicated minimal deviation in the *z*-axis positioning and slight distortion, respectively. The scaling values were very close

TABLE 2 Raw data of translations and rotation.

Aboslute traslation *X* axis (in microns)	Aboslute traslation *Y* axis (in microns)	Total distance (in microns)	Rotation (in degree)
0	0	0	0.000000000000000
9.459651064	36.57888109	37.78226489	0.583323038707307
9.222824722	36.37153312	37.5226454	0.602525203981419
30.21135728	11.59419111	32.35971842	0.994818871024354
5.714160747	36.96236368	37.40144332	1.315177101254800
24.38110227	5.960784651	25.09918528	1.111474496225390
4.639002288	27.21497951	27.60752528	0.867234487137890

TABLE 3 Summarized data.

Aboslute traslation *X* axis	Aboslute traslation *Y* axis	Total distance	Rotation
Mean	Mean	Mean	Mean
11.946871196943300	22.097533310538800	28.253254655589100	0.782079028333023
Standard deviation	Standard deviation	Standard deviation	Standard deviation
11.08142294	15.9144786	13.45292756	0.433704152

to 1, indicating minimal deviation in the *z*-axis positioning. It is essential to note that the *z*-axis positioning is directly related to the focus, which was managed manually during the study. This manual management of focus could introduce potential errors, and it is a factor that should be considered when interpreting the results. The shearing values, although small, indicated a slight distortion during the repositioning process. These results are crucial for evaluating the precision and accuracy of the LEGO-based sample positioning system, as they demonstrate the system's ability to maintain accurate positioning along the *z*-axis and minimize distortion during the repositioning process.

The analysis of the absolute translations along the *X* and *Y* axes, total distance, and rotation revealed a highly significant result ($p < 0.001$), indicating that the variations observed in these parameters during the repositioning process were statistically significant. This underscores the importance of these parameters in evaluating the sample positioning system's performance. The mean absolute translations along the *X* and *Y* axes were approximately 11.947 and 22.098 μm, respectively, with a mean total distance of approximately 28.253 μm and a mean rotation of approximately 0.782°. The standard deviations of these parameters were relatively small, indicating a high degree of consistency in the repositioning process.

Moreover, the analyses were consistently replicated across all three repetitions, yielding the same results each time. This consistency underscores the observed results' reliability and the employed methods' robustness, demonstrating the system's ability to provide repeatable and reliable results across multiple repetitions.

In addition to the previously discussed results, the data obtained from the Rambold Kontroll analogue comparator provided further insights into the positioning accuracy of the scanning microscope's stage. For the *x*-axis, the average deviation from the desired position was 0.003 mm, with a standard deviation of 0.012 mm. This indicates a high level of precision with a relatively small spread of values around the mean. On the y-axis, the average deviation was slightly higher at 0.004 mm but with a larger standard deviation of 0.021 mm.

The LEGO-based sample positioning system's performance, as highlighted by the results, is commendable. However, it is essential to note that these results were achieved under specific conditions. One critical condition to ensure the system's precision and accuracy is properly locking the rotation of both the stub and its support. Ensuring that the rotation of these components is restricted is vital to maintaining the desired positioning and alignment during the SEM analysis.

If not controlled, the rotational movement can introduce discrepancies in the acquired images, affecting the overall quality and repeatability of the SEM analyses. By effectively locking the rotation of the stub and support, the system can maintain its high degree of precision, as indicated by the minimal displacements and deviations observed in the results.

In conclusion, the LEGO-based sample positioning system developed for SEM analysis represents a promising solution for enhancing the quality and repeatability of SEM analyses. The system demonstrated its ability to consistently identify and align features across multiple repositioning cycles, maintain accurate positioning along the *z*-axis, minimize distortion, and provide repeatable and reliable results across multiple repetitions. However, further studies are needed to optimize the system's design, evaluate its performance in different SEM applications, and compare its performance with other commercially available systems. Ultimately, this study contributes to the ongoing efforts to develop cost-effective, customizable, and accurate solutions for sample positioning in SEM, ultimately contributing to the advancement of materials science research.

AUTHOR CONTRIBUTIONS

Piero Antonio Zecca: Conceptualization; investigation. **Andrea Brambilla:** Writing – original draft. **Marcella Reguzzoni:** Validation;

writing – review and editing. **Marina Protasoni:** Writing – review and editing; data curation. **Mario Raspanti:** Supervision.

ACKNOWLEDGMENTS

We would like to express our heartfelt gratitude to Prof. Pazzaglia, who kindly donated the bone sample for observation. More importantly, we are deeply thankful for his scientific contribution and his human touch to our laboratory. His support has been invaluable to our research, and we are profoundly grateful for his generosity and collaboration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Zecca, P. A., Brambilla, A., Reguzzoni, M., Protasoni, M., & Raspanti, M. (2023). Enhancing SEM positioning precision with a LEGO®-based sample fitting system. *Microscopy Research and Technique*, 1–6. https://doi.org/10.1002/jemt.24465