

STEM in an SEM: Towards high-throughput imaging and analysis of metal nanoclusters

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ABSTRACT

Accurately characterising metal nanoclusters is critical for advancing catalysis and energy conversion. While aberration-corrected scanning transmission electron microscopy (AC-STEM) achieves unmatched spatial resolution, its overall workflow throughput is often limited by instrument setup, alignment, and acquisition requirements. In contrast, scanning electron microscopy (SEM) offers higher throughput, but conventional SEM lacks sufficient spatial resolution for ultrasmall clusters of atoms. Here, we introduce a correlative imaging approach combining AC-STEM with high-angle annular dark-field (HAADF) STEM performed within an SEM (STEM-in-SEM), in particular using advanced in-lens field-emission SEM systems which offer the simplicity and ease-of-use of an SEM platform while retaining very high spatial resolution, to enable efficient and scalable metal nanocluster imaging and quantification workflows. Using platinum nanoclusters supported on graphene oxide as a model system for validation, identical-location imaging was employed to benchmark STEM-in-SEM against AC-STEM, eliminating the influence of sample polydispersity. Both techniques rely on the same incoherent HAADF imaging principle, and despite lower acceleration voltage and different detector geometry, STEM-in-SEM yields comparable intensity distributions. A comparison between STEM-in-SEM and AC-STEM demonstrated consistent intensity scaling. A reproducible and calibratable relationship between the number-of-atoms and image intensity can be established under STEM-in-SEM conditions. In this work, STEM-in-SEM is used to reliably quantify nanoclusters containing as few as ~45 Pt atoms or nanoclusters with effective diameters down to ~0.95 nm. Accordingly, a calibrated STEM-in-SEM image can be employed as a quantitative reference for other datasets imaged under the same conditions, provided that variations in specimen thickness and background contrast are sufficiently small to preserve consistent intensity scaling. By combining adequate spatial resolution and operational flexibility, STEM-in-SEM represents a practical and cost-effective complement to AC-STEM. Continued optimisation of imaging conditions, detectors, and automated analysis pipelines will further advance the broader applicability of this methodology in large-scale catalyst screening and nanomaterials discovery.

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1. Introduction

Heterogeneous catalysis, a cornerstone of the chemical industry, relies heavily on the use of metal particles. While nanoparticles of a few nanometres in diameter are typically used, precious metal nanoclusters, defined here as aggregates of tens to four hundred atoms, smaller than conventional nanoparticles, have been gaining increasing attention in industrial processes, such as electrocatalysis, energy storage and conversion, in addition to heterogeneous catalysis due to their high fraction of surface atoms [1–7]. In this work, we focus on catalyst systems prepared via on-surface assembly of atoms into nanoclusters, which enables rapid and scalable generation of nanoclusters directly on electron-transparent supports, thereby facilitating high-throughput characterisation [8–10]. For example, we have previously developed a nanocluster-based catalyst in which metal atoms self-assemble on nanotextured carbon supports, demonstrating enhanced catalytic performance compared to conventional nanoparticle-based systems [11].

Conventional nanocluster synthesis methods, including wet-chemical reduction and atomic layer deposition, are widely used but often require multistep processing and are limited in scalability [12–14]. Magnetron sputtering provides an alternative route via the direct deposition of individual metal atoms onto a support, thereby forming nanoclusters with improved scalability and cleanliness [8,15,16]. This capability is particularly important in the context of high-throughput microscopy workflows, where rapid sample generation is required to match the speed of data acquisition and analysis. Nevertheless, key magnetron sputtering parameters, such as deposition time, applied power, and substrate temperature, require optimisation for each distinct metal/support combination, underscoring the necessity for rapid feedback between nanocluster characterisation and synthesis. Spectroscopic techniques prove inadequate for this purpose, as nanoclusters in this size range generally do not provide sufficiently distinct size-dependent signatures for reliable structural quantification, while diffraction methods cannot offer meaningful structural information at cluster sizes of 1–2 nm, the size range considered optimal for catalytic activity.

To quantitatively resolve nanoclusters (i.e. accurately determine the number of atoms in each), aberration-corrected scanning transmission electron microscopy (AC-STEM) is typically employed and remains the most useful analytical approach. However, conventional AC-STEM is intrinsically low-throughput. Traditional scanning electron microscopy (SEM) is limited in its ability to observe nanoclusters. Its electron-sample interaction volume, and the characteristics of secondary/back-scattered signals in typical SEM configurations, do not provide reliable sub-nanometre mass-thickness contrast. To overcome these limitations, recent advances in high-performance SEM instruments, including field-emission SEMs and FIB-SEM platforms, enable high-angle annular dark-field (HAADF)-STEM imaging within an SEM chamber (STEM-in-SEM) with probe sizes close to atomic resolution, while offering lower accelerating voltages and more flexible and cost-effective operation. This opens new possibilities for quantitative nanocluster characterisation across diverse catalyst systems and supports using accessible instrumentation.

In conventional implementations, STEM imaging is typically achieved by incorporating transmitted electron detectors in standard SEM chambers, which can provide useful nanoscale contrast but are often limited in resolution and signal-collection efficiency [17]. This transmission-mode SEM approach, often referred to as TSEM or STEM-in-SEM, has been extensively reviewed in the literature, where its imaging principles, detector configurations, and broad applications across materials systems have been systematically discussed [18]. Dedicated in-lens STEM-in-SEM systems, in which the sample is positioned within the objective lens region, offer improved probe formation, enhanced detector collection efficiency, and greater control over scattering angles. These features enable significantly improved spatial resolution and signal-to-background performance. Several studies have demonstrated the feasibility of STEM-in-SEM for nanoscale

characterisation [19,20], and commercial systems (e.g., Hitachi SU9000) now provide dedicated in-lens STEM-in-SEM capabilities. However, quantitative validation of STEM-in-SEM for atomically precise nanocluster analysis, particularly through direct comparison with aberration-corrected STEM at identical locations (IL), remains limited. This motivates the present study. In addition, recent developments have extended STEM-in-SEM beyond imaging to diffraction-based approaches such as 4D STEM-in-SEM [19,20], enabling the acquisition of spatially resolved diffraction datasets for crystallographic and orientation mapping at high throughput. These studies further demonstrate the versatility and growing capabilities of SEM-based STEM methodologies. In addition to improved probe formation and detector collection efficiency, recent studies have demonstrated that advanced in-lens field-emission SEM systems are capable of resolving lattice fringe features under STEM imaging conditions at accelerating voltages around 30 kV. For example, bright-field STEM imaging of multi-wall carbon nanotubes in an ultra-high resolution in-lens FE-SEM revealed lattice spacings down to ~ 0.34 nm, supported by FFT analysis of the recorded images. These results highlight that, beyond conventional contrast-based imaging, SEM-STEM—particularly in-lens configurations—can access structural information at the nanoscale, further bridging the gap between SEM and dedicated TEM/STEM platforms [21].

In this study, we demonstrate that STEM-in-SEM is capable of reliably quantifying nanoclusters comprising as few as ~ 45 atoms, resolving and quantitatively analysing nanoclusters with an effective diameter, d , as small as ~ 0.95 nm. The range of 1–2 nm studied in this work corresponds to tens to four hundred metal atoms that are highly useful for catalysis [22,23].

2. Materials and methods

2.1. Sample preparation

Magnetron Sputtering was conducted using a custom-built ATC series AJA sputtering system, using a platinum target (Fig. S1). The deposition time was 2 s with an applied power of 37 W at a working distance of 90 mm, resulting in an atomic loading of 10.8 ± 0.5 Pt atoms per nm². For the Au sample, magnetron sputtering was conducted using a custom-built AJA sputtering chamber equipped with a gold target. The working pressure was maintained at 3 mTorr, the flow rate at 10 sccm, the working distance at 90 mm, the applied power at 10 W, and the deposition time at 2 s. Holey carbon-coated Au finder grids (Agar scientific, H7 finder grids) coated with graphene oxide (0.2 mg/L) by drop-casting to provide a thinner support were secured onto a flat disc for insertion into the chamber before the magnetron sputtering.

2.2. Transmission electron microscopy

Bright field (BF) TEM images were acquired at 200 kV accelerating voltage on JEOL 2100F FEG TEM with a Gatan Model 1027 K3-IS direct-detection camera to assess the sample and to confirm suitability for further analysis and consistent particle deposition (Fig. S2). STEM observations were performed at 60 kV acceleration voltage on a Nion UltraSTEM 100 at the SuperSTEM Laboratory, Daresbury, UK. This microscope is equipped with a fifth-order probe aberration corrector enabling a probe size of ~ 0.09 nm at 60 kV with a convergence semi-angle of 31 mrad and a probe current of 30 pA in the conditions used for these experiments. High-angle annular-dark-field (HAADF) images were recorded using a classical scintillator and photomultiplier tube combination ADF detector with a semiangular range of 85–195 mrad. Consecutive images were acquired at lower magnifications to facilitate returning to the identical location using correlative imaging, with SEM serving as the navigation tool. The dwell time was 60 μ s per pixel for AC-STEM.

2.3. Scanning electron microscopy

Correlative STEM in an SEM was conducted using a Hitachi ultra-high resolution field emission SU9000II T-SEM at the SuperSTEM Laboratory, Daresbury, UK. The high coherence cold-field emitter, immersion lens design and TEM-like sample holder enable this instrument to offer higher imaging resolution than conventional field emission SEM. This microscope was operated in STEM mode at 30 kV with the electron optics adjusted to form a ~ 0.26 nm probe with 21 mrad semi-convergence angle [24]. The microscope is equipped with a solid-state HAADF detector with an adjustable collection angle. The images were recorded with a semi-angular collection range of 500–900 mrad. The dwell time was 8.1 μ s per pixel for STEM-in-SEM imaging. The probe current was approximately 10 pA for STEM-in-SEM imaging. A representative analysis of pixel-level intensity variation and cluster size (in terms of pixel count) is provided in the Supporting Information (Fig. S3), illustrating the statistical basis for reliable detection at the smallest cluster sizes. Recent studies have demonstrated the feasibility of HAADF STEM-in-SEM imaging for nanoscale characterisation using advanced SEM platforms [19,20,25–28], further supporting the applicability of this approach. The Hitachi SU9000II T-SEM used in this work is an ultra-high-resolution SEM-STEM platform equipped with a cold field-emission source, an optimised probe-forming column, and a HAADF detector with large collection angles [29]. These features enable improved probe size, beam stability, and signal-to-background ratio at low accelerating voltages compared to conventional SEM-STEM implementations. In particular, the in-lens geometry allows the electron beam to be formed and controlled in a manner more analogous to TEM, while retaining the flexibility of an SEM platform. Therefore, the workflow demonstrated here relies on general conditions such as sufficient probe stability, detector collection angle, and signal-to-background ratio. To further assess the transferability of the approach, preliminary identical-location measurements performed on a conventional SEM platform are provided in the Supporting Information (Fig. S4). STEM in a SEM of Au/GO was conducted using a Zeiss Crossbeam 550 operated in dark-field STEM mode at 30 kV with a probe current of 200 pA. The dark-field STEM collection semi-angles for the Crossbeam range from 20–150 mrad, while the convergence semi angle used was 3.936 mrad as reported by the manufacturer.

2.4. STEM image analysis

The AC-STEM and STEM-in-SEM data were processed and analysed with a custom Python script (see SI). The images were smoothed by applying a Gaussian filter, and a threshold was applied to identify the cluster's core areas. We defined the cluster footprints as these core areas expanded by a perimeter of a single pixel width. For the AC-STEM data, a global threshold was used, whereas for the STEM-in-SEM data, a locally varying threshold was used to ensure that the threshold applied to the boundary of a given cluster scaled with the maximum intensity of the cluster. This difference reflects the convolution effect associated with the larger probe size in STEM-in-SEM, which leads to an apparent extension of intensity beyond the atomic footprint. While this affects the integrated intensity to some extent, limiting the integration to a slightly expanded footprint provides a consistent estimate and reduces sensitivity to background uncertainty. In the analysis of the IL data, to ensure consistent quantitative analysis, as a preprocessing step, the STEM-in-SEM image was resampled to match the AC-STEM image's pixel size prior to further processing. As exact matching of pixel size during acquisition is generally not practical across different instruments due to discrete magnification settings and calibration differences, this resampling step ensures consistent spatial scaling between datasets. The AC-STEM image was mirrored and suitably rotated and translated to ensure the consistent IL alignment of the two images. The average AC-STEM single Pt atom intensity was used to convert each cluster's integrated AC-STEM intensity to the number of atoms, N_{at} , in the cluster.

The thresholding parameters for the analysis of the STEM-in-SEM data were determined based on visual consistency of the delineation of cluster boundaries with the image contrast, ensuring physically meaningful segmentation. These parameters were not optimised using AC-STEM measurements as ground truth. The subsequent identical-location comparison, therefore, serves as independent validation of the segmentation approach.

2.5. Effective diameter in Pt nanoclusters

The effective diameter, d , can be deduced from the footprint area, S , of the nanocluster. For the purpose of the calculations, we assumed that there is a perfect circle with the same S of the cluster (Fig. S6). The d can be calculated using Eq. (1).

$$d = 2 \sqrt{\frac{S}{\pi}} \quad (1)$$

3. Results and discussion

The Pt metal was dispersed into atoms by magnetron sputtering, and the charge-neutral free atoms at room temperature were deposited directly onto the graphene oxide support (Pt/GO) (Fig. S1). AC-STEM imaging of the as-fabricated Pt/GO reveals the assembly of metal atoms into nanoclusters (Fig. 1A). The cluster size is governed by the surface density of deposited atoms, defect density of the support, temperature, and metal-support bonding energy, as shown in kinetic theory predictions [16]. Quantitative image analysis of AC-STEM data allows determination of the number of atoms comprising each nanocluster (N_{at}), its footprint area (S) (Fig. S5), and its d (Fig. S6), which can be deduced from S . HAADF-STEM imaging performed in SEM of the same sample also reveals the clear presence of nanoclusters (Fig. 1B). The pixel size of the AC-STEM image is 0.029 nm pixel⁻¹, whereas that of the STEM-in-SEM image is 0.016 nm pixel⁻¹. In both cases, the pixel size used is significantly smaller than the instrument resolution, in particular, this ensures that this is not the limiting factor in its achievable resolution for STEM-in-SEM. Notably, this technique is based on the same HAADF imaging principle as conventional STEM, differing only in instrumental parameters such as accelerating voltage, probe-forming optics, and detector geometry. Our result highlights the remarkably high level of structural detail accessible with modern SEM instrumentation. Image analysis of STEM-in-SEM data enables quantitative measurement of nanoclusters' S and the corresponding d . Traditionally, sample sizes are averaged and correlated with the macroscopic properties of the sample. For instance, the nanoclusters' average d measured by HAADF-STEM-in-SEM was 1.40 ± 0.36 nm, while the corresponding AC-STEM measurement at a different location of the same sample gave an average d of 1.39 ± 0.37 nm, considering only clusters with a d larger than 0.5 nm. This cutoff was applied because the SEM-based image analysis is expected to be reliable down to approximately this size. We emphasise that this threshold does not represent the resolution limit of the SEM measurements but rather the lower limit of the specific analysis approach employed. The consistent d values between AC-STEM and STEM-in-SEM techniques obtained from different regions of the support confirm the uniformity of the sample.

To assess the ultimate capability of STEM-in-SEM for nanocluster characterisation, we performed IL imaging of the same atoms and nanoclusters within a given local nanoenvironment using both AC-STEM and STEM-in-SEM imaging techniques. This approach enables direct comparison of the two imaging methodologies without interference from sample polydispersity. Through this identical-location correlation, we can unambiguously determine whether STEM-in-SEM can quantify the number of atoms in a cluster, as verified against the corresponding AC-STEM data. Based on AC-STEM image analysis, the N_{at} was calculated from intensity values, I , [30–33] allowing us to correlate N_{at} with the nanocluster determined from STEM-in-SEM. This relationship

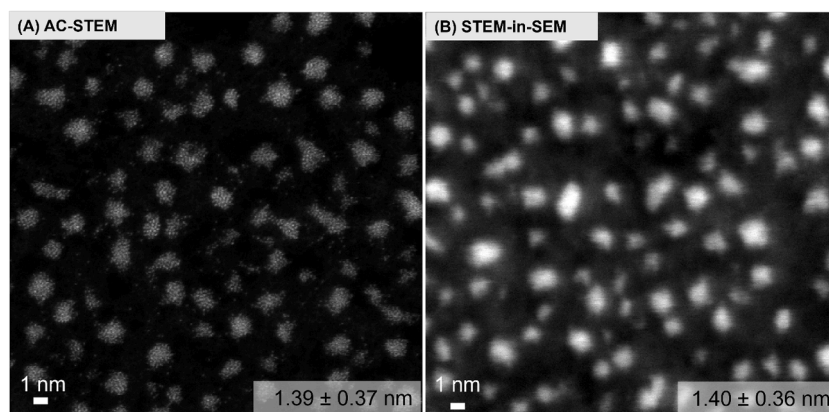


Fig. 1. Non-identical locations comparison of AC-STEM (A, left) and STEM-in-SEM (B, right) images of Pt/GO with average d .

effectively serves as a calibration curve, enabling the conversion of STEM-in-SEM intensity into an estimate of N_{at} . Specifically, the calibration is established by correlating the integrated intensity of individual nanoclusters in STEM-in-SEM with the atom counts independently determined from AC-STEM at identical locations. Once

established, this calibration can be applied to other STEM-in-SEM datasets acquired under comparable conditions, provided that the background intensity can be reliably estimated and remains approximately uniform within each image or dataset.

The paramount characteristic of the identical-location imaging

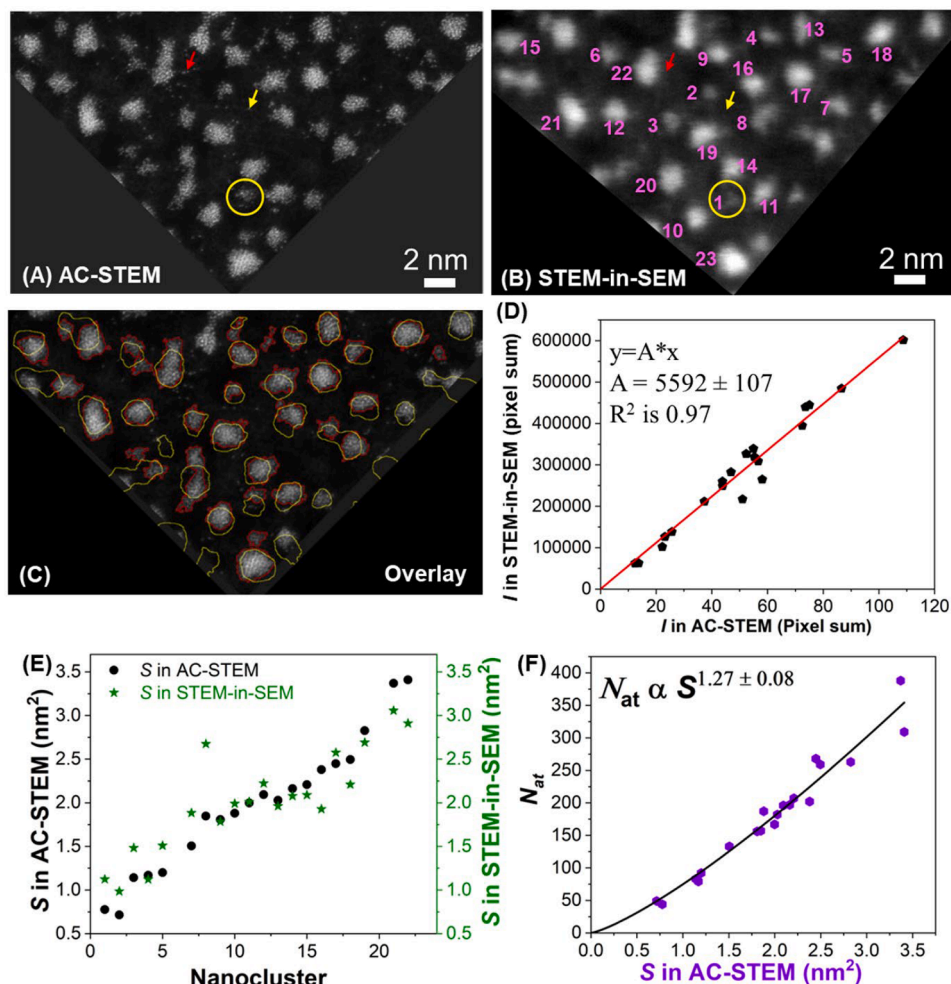


Fig. 2. (A) AC-STEM image of Pt/GO. (B) STEM-in-SEM image of the same region as in (A). (C) Overlaid STEM-in-SEM (yellow) and AC-STEM (red) images of the same region. (D) Intensity of Pt nanoclusters measured by STEM-in-SEM versus AC-STEM. The intensity, I , is defined as the integrated HAADF signal (pixel sum) within the cluster footprint after background subtraction. A small number of clusters (3 out of 23, clusters 6, 20, and 23) exhibited morphological changes and were excluded from the quantitative calibration analysis. The red fitted line corresponds to $y = A \cdot x$, where A is 5592 ± 107 , R^2 is 0.97. (E) Distribution of the footprint area, S , of Pt nanoclusters measured by STEM-in-SEM and AC-STEM. (F) Correlation of the N_{at} and S of Pt nanoclusters under AC-STEM. The estimated N_{at} carries an uncertainty of approximately $\pm 10\%$, primarily due to uncertainty in the single-atom intensity calibration.

approach is its capacity to collect and compare information from the same individual nanoclusters. If the nanocluster remains in an identical position on the support between different analyses, it can be pinpointed and analysed in detail under both AC-STEM and STEM-in-SEM. Using an identical-location imaging approach, 23 nanoclusters have been found correlatively in AC-STEM (Fig. 2A) and STEM-in-SEM techniques (Fig. 2B). This IL approach provides paired measurements of the same individual nanoclusters, enabling robust calibration. In this context, the reliability of the calibration is primarily determined by the consistency of the paired measurements rather than the total number of clusters, hence mitigating the need for a large dataset. Clusters exhibiting morphological changes were excluded, and the remaining dataset shows consistent behaviour across both imaging modalities, supporting the validity of the correlation. In the analysis, we also excluded nanoclusters whose footprints overlapped in SEM but were distinct clusters in the STEM. The overlaid STEM-in-SEM and AC-STEM images (Figs. 2C and S5E) show a nearly perfect overlap of the two techniques over the same region. From the identical-location comparison between AC-STEM and STEM-in-SEM images, we found that free-standing single atoms could not be resolved unambiguously in STEM-in-SEM in the conditions used (Fig. 2A and B, yellow arrow).

Both AC-STEM and STEM-in-SEM employ the same incoherent HAADF imaging, where the detected intensity approximately follows a Z-dependent mass-thickness contrast, as established in quantitative HAADF-STEM theory describing Z-dependent scattering and mass-thickness contrast [30,34–37]. At lower accelerating voltages, the increased electron scattering cross-section enhances the contribution of multiple scattering and thickness effects. In addition, the relatively large inner detector collection angles used in the present instrument further modify the effective ADF scattering conditions. As a result, the HAADF intensity deviates from a simple linear mass-thickness dependence, leading to increased sensitivity to variations in cluster height. In the present SEM configuration, the collection angles are relatively large (≈ 500 – 900 mrad), and the solid-state detector efficiency may be lower than that of the dedicated STEM system. The exact response of these detectors has not been studied in this work, but it is also a factor that merits future investigation to optimise this technique. However, the consistent IL correlation suggests that the detector response is sufficiently consistent within the relevant intensity range. Quantitative atom counting requires careful calibration of image intensity under the specific imaging conditions employed. At the low acceleration voltage (30 kV), the contribution of the support film becomes more pronounced, collectively leading to a modest reduction in overall contrast. Nevertheless, the obtained signal-to-background ratio remains sufficient for quantitative nanocluster analysis. Under these varied operating conditions, the HAADF intensity observed in STEM-in-SEM was found to be consistent with a proportional dependence on that obtained in AC-STEM (Fig. 2D), enabling reliable correlation between the two techniques for nanoclusters with d down to 0.95 nm, determined primarily by signal-to-noise and background contributions under the present imaging conditions. The effective resolution in STEM-in-SEM, with the probe size ~ 0.26 nm, may be additionally influenced by beam spreading and interaction volume effects at low accelerating voltage. In this work, an analysis cutoff of ~ 0.5 nm was applied, below which segmentation becomes unreliable. The associated uncertainty arises mainly from segmentation of low-intensity features [38], while registration uncertainty is negligible under identical-location conditions. Overall, these results demonstrate that STEM-in-SEM can offer sufficient signal-to-background ratio and spatial resolution for quantitative nanocluster characterisation [25,28]. The applicability of the present approach can be extended to other material systems, given the general Z-dependent mass-thickness contrast in HAADF imaging, noting that the method is best suited to thin and uniform supports (e.g., 2D materials), where background contributions are minimised, and reliable quantification can be achieved.

As our strategy for preparing heterogeneous catalysts is based on the

on-surface assembly of metal nanoclusters from atoms directly on any surface, [8] with no additional thinning or complex preparation steps, the analysis throughput is primarily determined by the imaging stage. Compared to AC-STEM, which typically requires more extensive alignment procedures, longer stabilisation times, and relatively slower image acquisition to achieve a high signal-to-noise ratio, STEM-in-SEM enables faster navigation, reduced instrument setup time, and more efficient data acquisition over larger sample areas. In addition, SEM platforms offer further opportunities for throughput automated imaging workflows and flexible navigation across large sample areas. In some conventional SEM systems (e.g., FIB-SEM platforms such as Crossbeam instruments), multi-sample holders or automated stage routines may further increase throughput. Conventional TEM can enable rapid low-magnification imaging for overview purposes; however, it does not provide the same quantitative Z-contrast required for atom-counting analysis of ultrasmall nanoclusters, and therefore is not directly comparable for this application.

Based on IL imaging and STEM image analysis, we can compare S (Fig. 2E, Table S1) and d (Fig. S7, Table S2) obtained from both AC-STEM and STEM-in-SEM for the same individual nanocluster. The STEM-in-SEM results show comparable values and a consistent trend with those from AC-STEM measurement. These results suggest that STEM-in-SEM can capture quantitative S and d . Based on AC-STEM image analysis, N_{at} was calculated from the I allowing us to correlate N_{at} with the S obtained from STEM-in-SEM. The minimum N_{at} resolved in STEM-in-SEM is approximately 45 (as indicated in the yellow-circled area in Fig. 2A–B), and STEM-in-SEM can resolve and statistically analyse nanoclusters down to a d of approximately 0.95 nm. By plotting N_{at} against S (Figs. 2F and S8), insights into the three-dimensional morphology of the nanoclusters can be obtained, as applied in our previous study [11], although alternative explanations cannot be fully excluded. In AC-STEM, the data follow a power-law relationship with an exponent of 1.27 ± 0.08 (Fig. 2F), indicating that the nanoclusters adopt shapes between flat and hemispherical. In contrast, in STEM-in-SEM, a higher exponent of 1.59 ± 0.20 is observed (Fig. S8). The difference may arise from systematic deviations in the determination of the d in STEM-in-SEM, where small clusters tend to be slightly overestimated and larger clusters slightly underestimated, leading to a steeper apparent scaling relationship in the $N_{at} - S$ analysis. The practical detection limit of ~ 45 atoms observed in this work should be treated as an approximate value, with an estimated uncertainty of $\sim 10\%$ [38]. This uncertainty arises primarily from the determination of the single-atom reference intensity used for intensity-based atom estimation, rather than from signal-to-noise limitations alone. As this approach differs from conventional atomic column counting methods, such as the number of atoms derived from the integrated HAADF intensity of entire nanoclusters, the reported detection limit reflects a practical, method-dependent threshold under the present imaging and analysis conditions, rather than a sharply defined fundamental sensitivity limit. Indeed, some faint features observed near the detection limit (Fig. 2A and B, red arrows) may correspond to much smaller clusters (e.g., dimers or trimers), suggesting that, with further optimisation of the observation conditions for the faintest objects, the detection limit can be pushed even further [39].

To exclude potential artefacts from microscope calibration or support-film deformation during transfer, we compared the inter-particle distances of identical nanoclusters measured by AC-STEM and STEM-in-SEM (Table S3). The two measurements were performed on different days: AC-STEM first, then STEM-in-SEM on the second day. We measured several distances between nanoclusters that remain very similar in both imaging modes (Table S3), indicating that the microscope calibrations are matched within measurement uncertainty and the TEM grid did not bend or distort during handling. Therefore, the observed variation in cluster size between AC-STEM and STEM-in-SEM is not due to calibration errors or mechanical deformation of the support. The cross-correlation of structural features also confirmed

registration accuracy between the two datasets. The measured inter-cluster distances and feature separations were consistent across the two imaging modes, demonstrating that STEM-in-SEM preserves the ability to provide quantitative geometric measurements of cluster sizes, with good fidelity compared to the higher-resolution AC-STEM data, despite its lower beam energy and larger probe size. These results validate the quantitative reliability of STEM-in-SEM for spatially resolved nanocluster analysis when properly calibrated. Even though the average distances between nanoclusters remained consistent across the two techniques, some local variations were observed. In particular, the distance between clusters 15 and 21 appeared slightly reduced in the STEM-in-SEM image compared with the corresponding AC-STEM image.

Moreover, nanocluster 20 exhibited signs of ripening after AC-STEM imaging (Fig. 3); in the subsequent STEM-in-SEM measurement, it appeared to have transformed into a smaller, more circular shape. These morphological changes indicate the metastable nature of some nanoclusters in this size range, and thus, one should be mindful of these effects when conducting correlative measurements and ensure that a sufficient number of clusters are compared if the data is used for calibrating additional STEM-in-SEM analysis. In the current set, 3 out of 23 nanoclusters (clusters 6, 20, 23) exhibited noticeable morphological differences between AC-STEM and STEM-in-SEM images and were excluded from the quantitative correlation analysis (Fig. 3). This emphasises the importance of our IL correlative approach, as such subtle

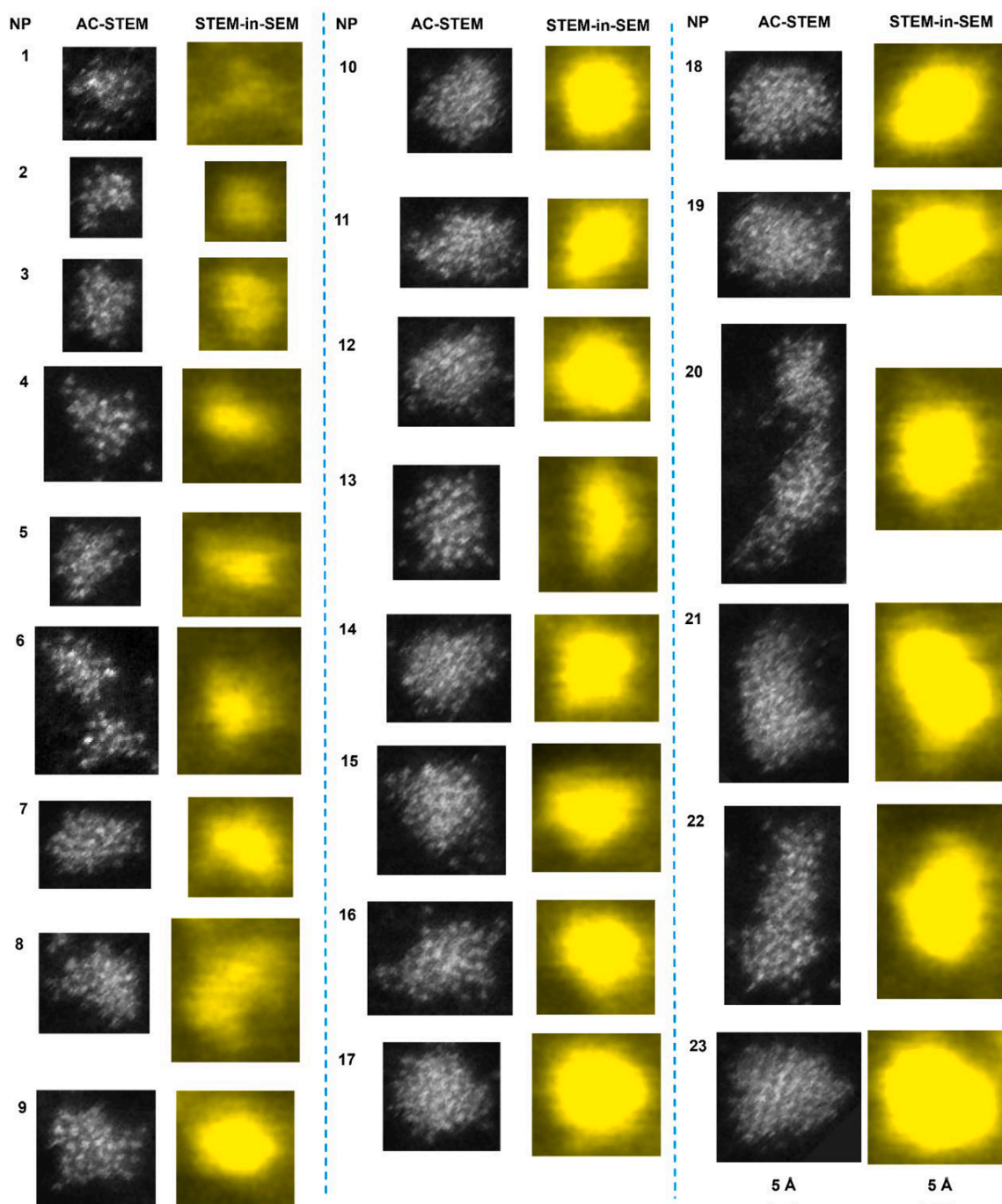


Fig. 3. Comparison of AC-STEM (left) and STEM-in-SEM (right, processed with enhanced contrast) images of individual Pt nanoclusters. The STEM-in-SEM image is saturated to clearly illustrate the edge of the clusters.

yet important local changes would be masked in large, ensemble-averaged datasets, increasing uncertainty of the measurements, particularly for metastable ultrasmall nanoclusters.

4. Conclusion

This study establishes a correlative imaging and quantification methodology based on STEM-in-SEM as a powerful and practical approach for high-throughput imaging and quantitative characterisation of ultrasmall metal nanoclusters benchmarked against conventional AC-STEM. Using identical-location correlative imaging with AC-STEM, we demonstrate that STEM-in-SEM, can resolve and quantitatively analyse nanoclusters down to a projected diameter d of approximately 0.95 nm, or quantify nanoclusters comprising as few as approximately 45 atoms. Minor differences in contrast in our data are believed to arise from lower acceleration voltage, larger collection angles, differences in detector response, and substrate contributions at low voltage, yet the technique delivers reliable intensity-based measurements of cluster size and footprint area. The consistent intensity scaling between STEM-in-SEM and AC-STEM indicates that the N_{at} - I relationship can be reliably calibrated under STEM-in-SEM conditions. Therefore, within this methodology, a STEM-in-SEM image calibrated through identical-location comparison can be used as an internal reference based on the IL-derived N_{at} - I relationship to quantify other samples imaged under the same conditions, provided that the specimen thickness and background are uniform. The combination of sufficient spatial resolution, robust performance under realistic imaging conditions, operational flexibility, and cost-effectiveness makes STEM-in-SEM a valuable complement to AC-STEM. This methodology can be extended to more widely available SEM-STEM systems, including designs implemented across different manufacturers [40], although the achievable resolution and quantification limits may depend on instrument-specific parameters. With further optimisation of hardware, imaging conditions, and automated analysis software, this methodology holds significant promise for scalable, routine workflows in catalyst screening, nanocluster synthesis studies, and broader nanomaterials research.

CRediT authorship contribution statement

Yifan Chen: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Michael W. Fay:** Writing – review & editing, Methodology, Formal analysis. **Wolfgang Theis:** Writing – review & editing, Methodology, Formal analysis. **Michael Dixon:** Investigation. **Christopher D. Parmenter:** Data curation. **Emerson C. Kohlrausch:** Methodology. **Jesum Alves Fernandes:** Methodology. **Paul D. Brown:** Supervision, Funding acquisition. **Quentin M. Ramasse:** Writing – review & editing, Funding acquisition. **Demie Kepaptsoglou:** Writing – review & editing, Investigation. **Andrei N. Khlobystov:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ultramic.2026.114400.

Data availability

Data will be made available on request.

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