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Article

A Grazing-Incidence SEM Strategy for High-Contrast Imaging of Multiscale Nanomaterials. MoS₂, a Case Study

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Abstract

Scanning electron microscopy (SEM) is a powerful tool for the morphological characterization of multiscale nanomaterials, including two-dimensional (2D) systems such as graphene and molybdenum disulfide (MoS₂). However, conventional SEM imaging often struggles to resolve nanoscale features due to limited contrast and depth sensitivity, especially when dealing with ultrathin layers. In this work, we propose and demonstrate a simple yet effective strategy to overcome these limitations by exploiting grazing-incidence (radent) observation, achieved through a controlled tilting of the sample close to 90°. This approach significantly enhances the emission of secondary electrons from near-surface regions, thereby increasing image contrast and revealing morphological details, such as edges, ripples, defects, and overlapping layers, that remain hidden under standard imaging conditions. Optical characterization of the prepared MoS₂ colloids further supports the formation of monolayer and few-layer sheets, validating the structural information obtained from SEM. Interestingly, this approach recalls natural strategies observed in living organisms, where grazing-angle vision improved edge perception and surface recognition and therefore it can be considered as bio-inspired. Beyond its application to MoS₂, this biomimetic methodology offers a versatile and broadly applicable solution for improving morphological analysis of 2D nanomaterials and thin films, providing deeper insights into their structural characterization.

Keywords: 2D nanostructures; molybdenum disulfide monolayer; SEM; depth-of-field; tilting; bio-inspired approach

1. Introduction

Morphological analysis plays a fundamental role in the study of materials and nanomaterials [1]. For instance, the morphological analysis of a fractured metallic surface (i.e., fractography) allows to establish if an alloy has a ductile or brittle behavior [2]. Indeed, the alloy has fragile behavior in case sharp or conchoidal fracture surfaces are observed, while it has a ductile behavior if plastically deformed surfaces are generated during the fracturing operation. Consequently, it is of pivotal importance to obtain images of the highest quality so that they may include as much as possible clear and detailed morphological information. Such a requirement is even more stringent in case of nanostructured/microstructured systems, where the material behavior is much more strictly

depending on morphology. However, imaging multiscale nanomaterials, combining micrometric and nanometric dimensions remains particularly challenging [3,4]. This dual size scale makes such nanostructures only partially accessible to conventional techniques, like scanning electron microscopy (SEM). Indeed, due to magnification limits, they often fail to fully resolve nanoscale features. For example, two-dimensional (2D) nanostructures, like lamellas, platelets, single sheets, nanobelts, etc. and monodimensional (1D) nanostructures, like wires with infinitesimal diameter, nanorods, etc. are multiscale nanostructures (i.e., both micro- and macro-dimensions are contained in the nanomaterial) and therefore have such a drawback. Therefore, the morphological features of these nanostructures can be visualized by scanning electronic microscopy and other standard microscopy techniques (e.g., digital microscopy, stereomicroscopy, etc.), but it is quite difficult to understand that the observed objects are 2D or 1D (i.e., they have a Z-extension) because of the limited capability of this technique to resolve the nanometric dimensions. Therefore, to meet this need, if possible, some expedients, optimization procedures, and tricks capable to increase the technique sensitivity for imaging the nanometric size must be adopted.

In this context, the development of alternative observation geometries capable of enhancing surface sensitivity and contrast is becoming increasingly relevant. A particularly promising approach is the use of grazing-incidence SEM, a technique inspired by natural visual strategies and optimized for highlighting subtle topographical and morphological features that are otherwise undetectable. Such an approach is especially useful when dealing with ultrathin coatings, interfaces, or 2D nanostructures, where the interplay between micro- and nanoscale features strongly influences material performance.

Electronic microscopies are widely used in the morphological investigation of all material types [5,6]. In particular, SEM microscopy represents a standard technique in material science largely used also because it allows to observe specimens without requiring complex sample preparation procedures. The simplicity of SEM sample preparation is mainly due to its high depth-of-field, i.e. the ability to focus on points at different planes [7]. This SEM feature is very high at low magnifications (1mm at 100X [7]) and gradually reduces with increasing of magnification (however, it results always extremely high compared to other microscopic analysis techniques like the optical microscopy, stereomicroscopy, transmission electron microscopy, etc.).

This peculiarity of SEM imaging allows optimizing the observation by a sample tilting operation, which is practically impossible with other microscopy techniques. Tilting the sample relative to the electron beam-detector axis enhances the emission of secondary electrons (SE), improving the image quality (contrast). Since image quality is directly correlated with the number of SE reaching the detector, an increase in contrast upon tilting implies a higher SE yield (number of SE per primary electron, PE). This enhancement arises from the geometry of electron-sample interaction, which can be optimized by adjusting the sample inclination within the limits imposed by the depth of field. Beyond improving conventional imaging, this methodology can provide fundamental morphological insights into materials whose function relies on nanoscale organization — such as catalytic surfaces, functional coatings, or layered 2D systems like MoS₂. Its application thus bridges the gap between classical SEM imaging and advanced morphological analysis, offering a versatile tool for materials science and biomimetic engineering.

SE are emitted by direct collision between primary electrons (PEs) and the electron belonging to the atom. Generated SEs have very low energy values (usually less than 30eV [8]) and consequently come out from the sample only if produced close to the surface [9]. In order to generate them as close as possible to the surface, the incident PEs beam must be as grazing as possible, so that the full path of the incident beam in the material is located in an immediately sub-surface region. Indeed, the distance of the deepest point reached by primary electrons is given by the product between penetration depth (d) and $\sin$ of the grazing angle α (which is complementary to the incidence angle, θ). When this quantity is close to zero, the distance of SE from the surface is negligible and therefore all SEs generated in the PEs crossing the material escape from the sample surface. Such a behavior involves the maximum yield of SE; however, since the depth-of-field has always some limitations,

the best sample inclination must necessarily be a compromise between these two quantities (i.e., maximum inclination with completely sharp visual field).

The objective of this work is to demonstrate how a controlled grazing-incidence SEM observation significantly improves morphological contrast and information content in the study of multiscale nanomaterials. We illustrate this approach through the case study of MoS₂, showing how complementary optical analyses confirm the structural features revealed by the SEM observations. The strategy proposed here not only deepens the fundamental understanding of 2D materials but also provides a methodological framework that can be extended to a broad range of coatings and nanostructured surfaces.

In this manuscript, in order to establish the capability of this approach to improve image quality, SEM micrographs obtained by the ~90°-tilting approach have been compared with that resulting from standard SEM observations (that is, performed without any sample tilting). In particular, contrast quality in SEM micrographs obtained in presence and absence of tilting has been quantified by measuring the amount of grey levels involved in these micrographs and according with such image analysis the tilting operation has caused a significant increase of the amount of morphological features visualized in the micrographs.

2. Materials and Methods

2.1. Preparation of MoS₂ Colloidal Suspension

Owing to its layered structure, MoS₂ can be exfoliated by the combined action of mechanical shear and sonoacoustic energy to originate monolayer and/or few layer nanosheets. Commercial powder of MoS₂ (98% by weight, average size of 4-5 μm, Werth-Metall, Grammental, Germany) has been used as starting material for the preparation of colloidal suspensions. Morphology and size of the as-received powder have been investigated by SEM imaging at different magnifications (see Figure 1 a,b,c). The chemical composition of this powder has been established by energy dispersive X-ray spectroscopy (EDS, see Figure 1c). Accordingly, the MoS₂ grains have a layered structure and contains little amount of phosphorus as an impurity.

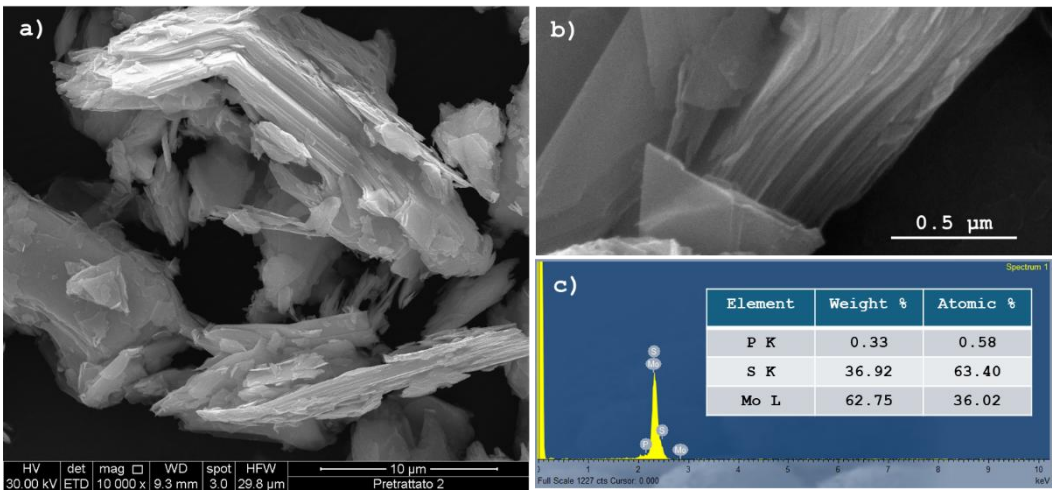


Figure 1. SEM micrographs of commercial MoS₂ powder (a,b) and its EDS spectrum with the elemental composition (c).

In the preparation of colloidal suspension, the as-received MoS₂ powder underwent a preventive mechanical treatment. This mechanical treatment was based on the application of shear stress to the basal planes of the micrometric lamellar crystals to convert them to small platelets. Internal coherency in these small crystalline platelets results much lower than in the starting micrometric lamellas, thus allowing their easy sonoacoustic exfoliation. In particular, the as-received powder was slightly

pressed between two circularly moving surfaces of polytetrafluoroethylene (PTFE). While sandpaper has been previously reported for a similar purpose [10], PTFE was preferred here to minimize contamination. This mechanical pretreatment is of pivotal importance, as sonication alone does not effectively induces exfoliation.

Following mechanical pre-treatment, the powder was dispersed in high-purity acetonitrile (CH_3CN , HPLC grade, J.T. Baker, London, UK) [11], and the obtained suspension was taken for a few hours in a sonication bath (ultrasonic bath with built-in heating SONOREXTTM SUPER, Bandelin, Berlin, Germany) in order to convert the MoS_2 platelets to a mixture of mono-layers and few-layer nanocrystals. Sonication was performed for 2h, taking the liquid sample in a closed glass vial.

To further purify the dispersion and selectively isolate few-layer crystals of MoS_2 , the achieved colloidal suspension was diluted with additional pure acetonitrile and sonicated under identical conditions (see Figure 2a). Then, the diluted suspension was left to settle for a few weeks. The concentrated MoS_2 colloidal suspension was stable due to electrostatic repulsion between charged nanosheets. Dilution increased interparticle spacing and promoted sedimentation of larger particles. After settling, the supernatant was carefully collected by decantation and used for subsequent analyses.

2.2. Sample Preparation for SEM Analysis

Samples for SEM characterization were prepared by drop-casting small volumes of liquid supernatant onto clean glass substrates. The solvent (acetonitrile) was allowed to evaporate under ambient conditions, leaving a thin film of dispersed MoS_2 nanosheets on the substrate surface. The morphological characterization was performed by using scanning electron microscopy (SEM, Quanta 200 FEG microscope, FEI, Hillsboro, OR, USA). Prior to SEM observations, all samples were sputter-coated with a thin Au-Pd alloy layer to increase surface electrical conductivity and adhesion to the substrate.

During the morphological investigation, each sample was examined under two distinct imaging geometries: (i) standard positioning (0° -tilting), and (ii) grazing-incidence configuration ($\sim 90^\circ$ -tilting). For the 0° -tilting observations, a standard aluminum pin stub was used, while for the $\sim 90^\circ$ -tilting observations a stable vertical sample positioning was achieved by using a special aluminum slotted specimen stub (see Figure 2).

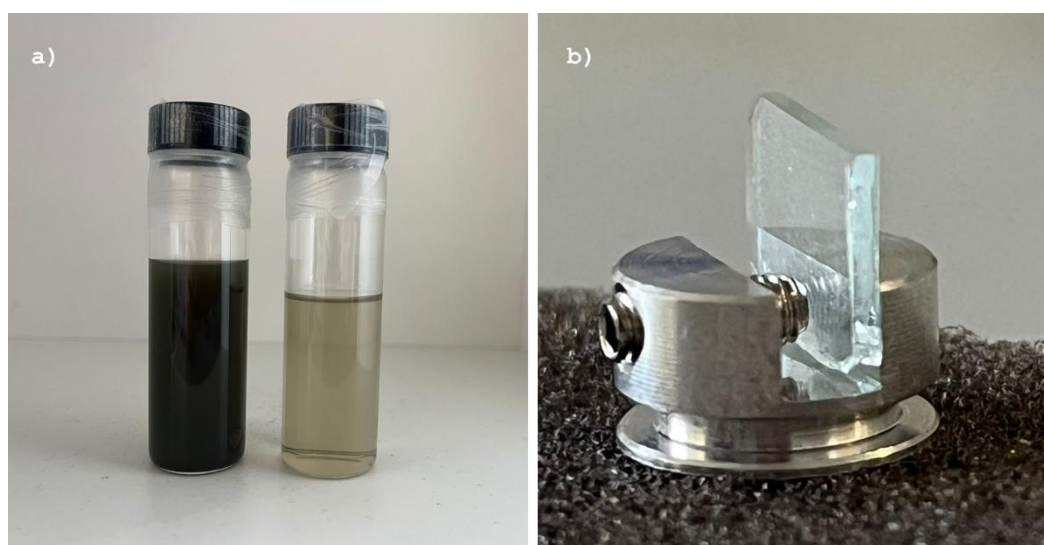


Figure 2. Concentrated and diluted MoS_2 colloidal suspensions in acetonitrile (a); and aluminum slotted specimen stub with a glass slide coated by MoS_2 (b).

SEM micrographs were analyzed by using the National Institutes of Health (NIH) Image J 1.48v software. This software is an open-source image processing application, designed to analyze

multidimensional scientific images, like the SEM micrographs. Image analysis was performed on areas containing an identical number of pixels to ensure quantitative comparability.

3. Results

3.1. Optical Characterization of MoS₂ Colloids

This optical characterization was carried out to verify the presence of MoS₂ monolayers in the prepared colloidal samples. Figure 3a shows the optical absorption spectrum of the colloidal suspension of MoS₂ in acetonitrile. This spectrum reveals the presence of three well-defined peaks centered at approximately 680 nm, 622 nm, and 458 nm, corresponding to photon energies of 1.82 eV, 1.99 eV, and 2.71 eV, respectively. These absorption peaks correspond to well-established optical transitions in mono-layer and few-layers MoS₂ and are generated by interband transitions [12,13].

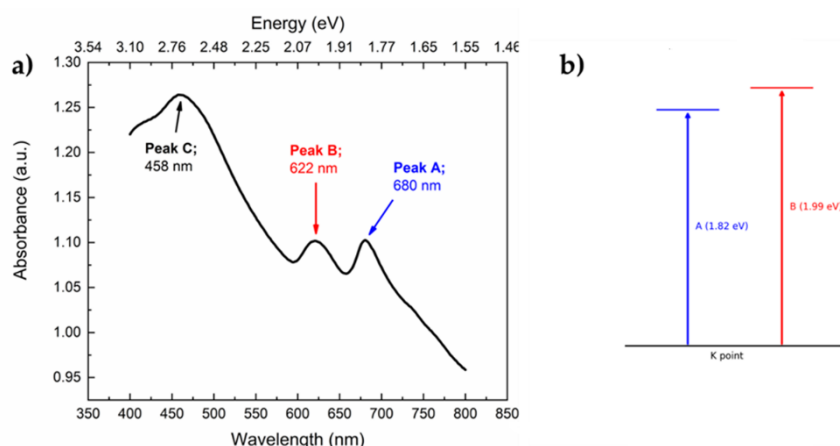


Figure 3. UV-Vis spectrum of MoS₂ colloidal suspension (a) and scheme of the dipole-allowed transitions (b).

Specifically, the absorption peaks at 680 nm (peak A) and 622 nm (peak B) are assigned to the so-called A and B excitons, which originate from the direct transitions at the K-point of the Brillouin zone (see scheme shown in Figure 3b) [14]. These excitons arise from the spin-orbit splitting of the valence band, which is particularly significant in transition metal dichalcogenides (TMDs) due to the heavy transition metal atoms. The A exciton (at 1.82 eV) involves transitions from the upper valence band (with lower spin) to the conduction band minimum, while the B exciton (at 1.99 eV) originates from the lower valence band (with higher spin) to the same conduction band minimum. The energy separation between the A and B excitons in this spectrum is approximately 0.17 eV, which is in agreement with theoretical predictions and previous experimental reports for MoS₂ monolayers [12,15]. The presence of well-resolved A and B excitonic peaks suggests that the MoS₂ flakes in the colloid are predominantly monolayers or few-layers in nature. The third absorption peak, located at 458 nm (2.71 eV), indicated as peak C in Figure 3a, is associated with transitions involving higher-energy states in the Brillouin zone. This peak is attributed to a phenomenon known as ‘band nesting’, which indicates as parallel bands in the electronic structure lead to a high joint density of states and enhanced optical absorption. The observation of this peak further supports the presence of well-dispersed, crystalline MoS₂ nanosheets in the prepared colloid.

A simple linear model can be used to estimate the average number of layers (N) in the nanoparticle from the A exciton energy value (E_A) [16]:

$$E_A(N) = E_{\text{bulk}} + K/N \quad (1)$$

where $E_{\text{bulk}} = 1.29$ eV (bulk MoS₂), $K = 0.8$ eV (empirical constant). Replacing the measured E_A value of 1.82 eV (exciton energy of the A band in our sample), it yields $N \approx 1.5$, thus confirming the presence of MoS₂ monolayers as predominant specie in the obtained colloidal suspension with a minor amount of MoS₂ bilayers [12].

The bandgap energy of the MoS₂ colloid, $E_g = 1.37$ eV, has been optically estimated by using the modified Tauc plot method [17] based on the data in the optical absorbance spectrum (see Figure 4). The Tauc plot was constructed using the absorbance (A) values directly, without conversion to the absorption coefficient, α , since the effective optical path length is unknown for colloidal systems. This approach is particularly suitable for colloidal systems where the exact concentration and path length may not be precisely known or controlled.

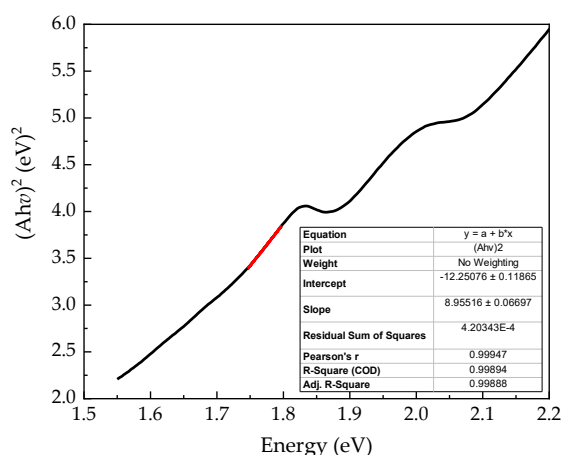


Figure 4. Modified Tauc plot of the MoS₂ colloidal suspension.

According to the literature, the direct allowed electronic transitions model better describes this colloidal MoS₂ semiconductor and the Tauc equation written by using the absorbance for this model is typically expressed as:

$$(Ah\nu)^2 \propto (h\nu - E_g) \quad (2)$$

In more detail, this method assumes that absorbance is proportional to the absorption coefficient (i.e., $A \propto \alpha$), and is valid for a qualitative estimation of the optical bandgap. The vertical axis of the plot was expressed in units of (eV)², as recommended in the recent literature [12], avoiding the incorrect use of arbitrary units or units involving cm⁻¹, which would imply a path-length-dependent α value. The linear portion of the (Ahv)² vs. hv plot was extrapolated to the energy axis to determine the optical bandgap. This optical bandgap value estimated from the modified Tauc plot is approximately 1.37 eV (Figure 4). This value is lower than the A exciton energy (1.82 eV), as expected, since the excitonic peak includes electron-hole binding energy and does not directly correspond to the fundamental bandgap. This is consistent with the A excitonic transition observed at 680 nm (1.82 eV), supporting the presence of monolayer or few-layer MoS₂ in the prepared colloidal sample.

The optical bandgap estimated from the Tauc plot (1.37 eV) is lower than the A-exciton energy (1.82 eV). This apparent inversion arises because the Tauc method, applied to absorbance data of a colloidal system, does not probe the fundamental band-to-band transition but rather an apparent optical onset influenced by excitonic absorption and structural disorder [18]. In colloidal MoS₂, light scattering, sheet aggregation, and exciton-related absorption tails can shift the extrapolated edge toward lower energies. Therefore, the Tauc-derived value should be regarded as an apparent optical onset rather than the intrinsic bandgap, while the A-exciton peak (1.82 eV) more accurately reflects the band-to-band transition reduced by the exciton binding energy.

3.2. Application of the Tilting Method to the MoS₂ Mono-Layer Observation

A comparison between the SEM micrographs of MoS₂ monolayers placed on a flat glass surface and imaged as both non-tilted and ~90°-tilted views is shown in Figure 5. As clearly visible, the contrast in the SEM micrographs obtained by these two types of observations differs significantly.

Standard non-tilted images (see Figures 5a,b) are characterized by a contrast level much lower than that characterizing $\sim 90^\circ$ -tilted views (see Figures 5c,d). The tilted configuration produced images with significantly enhanced contrast, characterized by a broader distribution of grey levels and improved visibility of nanoscale morphological features (e.g., ripples, bendings, fractures, porosities).

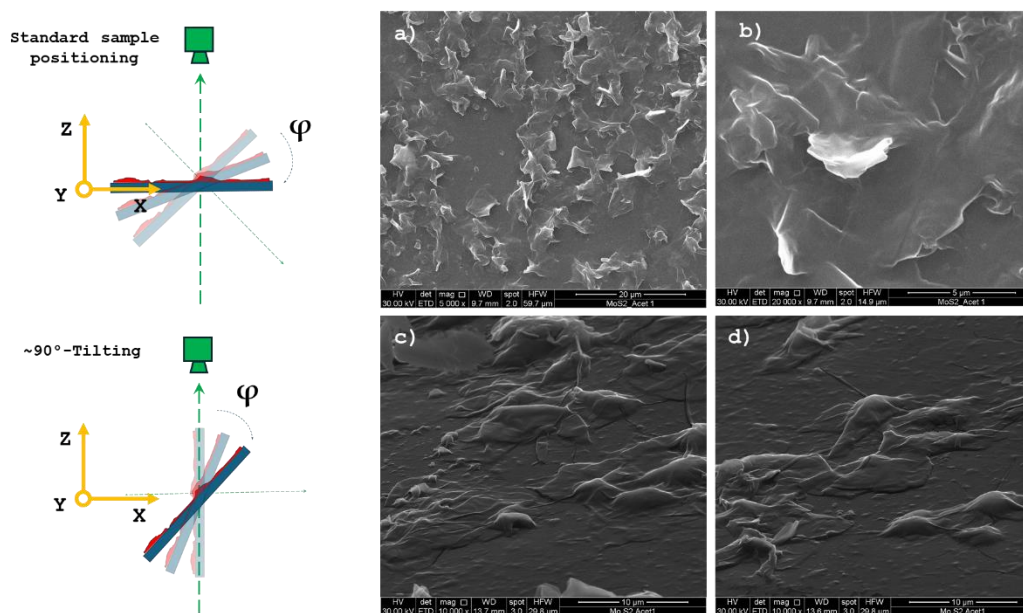


Figure 5. Schematic representation of different sample positioning and related SEM micrographs: standard sample positioning (a,b) and $\sim 90^\circ$ -tilting (c,d).

The limited greyscale range in non-tilted observations makes it difficult to distinguish fine surface features. Differently, high-contrasted images can provide a large amount of topological information on the investigated samples.

Qualitatively, the $\sim 90^\circ$ -tilted micrographs revealed structural details, such as nanosheet edges, overlapping regions, ripples, defects, pores, and fracture sites, that were indistinguishable in standard SEM images. Enhanced contrast also allowed visualization of subsurface features and thickness variations, which are crucial for the structural assessment of ultrathin 2D materials.

The above considerations can be quantitatively confirmed through the image analysis of these SEM micrographs, i.e. by evaluating the different grey levels present in these SEM micrographs. Grey levels in the micrographs of the non-tilted and $\sim 90^\circ$ -tilted samples were analyzed with an image analysis software, and the obtained results are shown in Figure 6. To make comparable the SEM micrographs of the samples obtained with and without tilting, images containing the same number of pixels were analyzed by measuring the percentages of all grey levels present in them. Then, the achieved histograms were normalized with respect to the maximum intensity (the intensity of the most abundant grey level visible in the image). Such normalization allowed for the correct visualization of the number of grey levels contained in each graph. The number of grey levels grew from 169 (Figure 6a) to 237 (Figure 6b), which corresponds to an increase of 40.2%. In addition, in this case, we measured the area below each curve, finding a 30.1% increase in this area. Therefore, it is evident from such results that the $\sim 90^\circ$ -tilting operation significantly increases this image contrast.

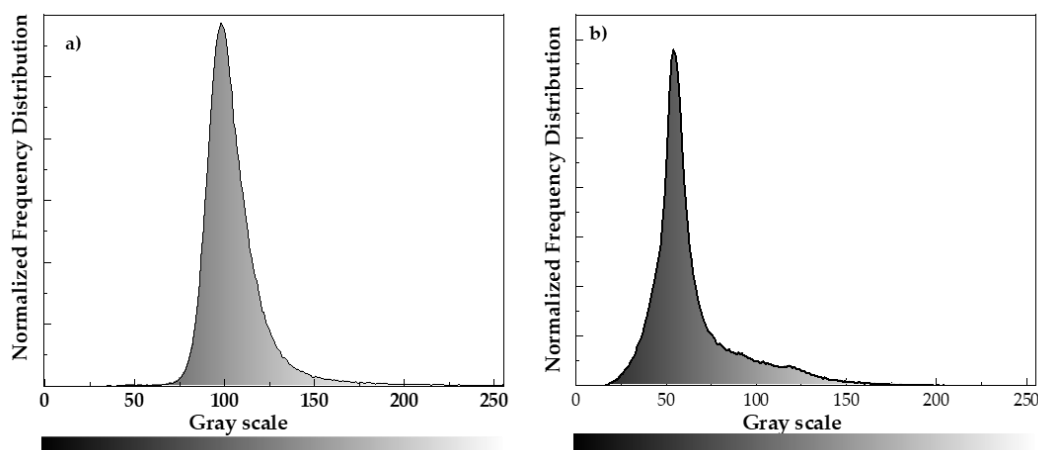


Figure 6. Analysis of the grey levels in the SEM micrographs shown in Figures 5. In particular, the two histograms refer to the following SEM micrographs: (a) Figure 5b; (b) Figure 5d.

This contrast enhancement obtained by applying a significant tilting to the 2D nanostructures (i.e., close to 90°) is due to the higher yield of SEs per PE, which results in a SE emission occurring in a region of the sample closer to the surface. In fact, SEs usually have low energy values, and therefore, they can escape the sample only in the case they are produced very close to the surface. An immediate sub-surface emission is achieved with the grazing incidence of the PE beam. In contrast, a vertical PE beam incidence causes SE emission from deep sample regions with consequent yield decrease.

4. Discussion

The results presented in this study demonstrate that SEM imaging performed under grazing-incidence conditions (~90°-tilting) significantly improves the morphological contrast of ultrathin MoS₂ nanosheets compared to conventional 0°-tilt imaging. This enhanced contrast enables the visualization of features such as sheet edges, folds, wrinkles, overlapping regions, thickness variations, pores, and fracture sites, which are largely undetectable under standard SEM geometry.

The grazing optical observation is a special visualization approach widely adopted in nature by the living creatures [19–24]; however, when visible photons are used to get optimized images, a working principle different from that involved in the SEM observation is active. Indeed, in the visible light oblique observation contrast is increased because of the shadowing, which is capable of better define the feature contours that are generated. In SEM observations an oblique positioning of the detector respect to the sample surface (that is, the ~90°-tilting of the sample) acts in a completely different manner; indeed, it allows to collect an extremely large number of SE coming out from the sample surface. Such a behavior can be easily understood on the basis of the following geometrical consideration. The SE path (x) in the sample subsurface region depends on the PE penetration depth (d) and on its incidence angle value (α), as $x = d \cdot \sin(\alpha)$.

The relative variation of the SE path ($\Delta x/x$) as a function of the incidence angle relative variation ($\Delta\alpha/\alpha$) is

$\Delta x/x = [\cos(\alpha)/\sin(\alpha)] \cdot \Delta\alpha/\alpha$. In the case of a very shallow angle of incidence ($\alpha \approx 0$), the $\cos(\alpha)$ value can be taken as approximately equal to 1 and the $\sin(\alpha)$ value can be approximated by α . Therefore,

$\Delta x/x \approx (1/\alpha) \cdot \Delta\alpha/\alpha$. Since α is quite close to zero, the $1/\alpha$ value is extremely large and therefore a significant relative variation of the SE path is obtained as a result of a small variation of the grazing angle. Under extreme grazing conditions (i.e., a sample surface almost vertically positioned), a little increase in the tilting angle (α decreases) determines an important decrease in the SE path. Since SE has a very low energy value (usually less than 30eV), they rapidly extinguish moving through the sample subsurface region and therefore a significant reduction of the path length by sample tilting from the vertical position may strongly increase the quantity of SE escaping the surface and therefore

the quality of the micrograph (contrast level). Indeed, in the hypothesis that the number of emitted secondary electrons, N_{SE} , is inversely proportional to the distance from the surface, x , it results: $\Delta N_{SE}/N_{SE} \approx - (1/\alpha) \cdot \Delta \alpha/\alpha$. The only limitation in this tilting operation is represented by the sharpness of the observation field that must be retained during the observation in tilting conditions. In particular, the tilting degree must be as high as possible, however it does not have to compromise the SEM image sharpness.

Compared to previous approaches that attempt to enhance SEM contrast through variations of accelerating voltage, detector positioning or surface metallization, the method proposed here is simpler, non-destructive and does not require instrument modification. Furthermore, it is conceptually related to natural optical strategies based on grazing illumination, adopted in biological systems to improve edge recognition and surface defect detection. In contrast to optical systems, however, the mechanism active in SEM is not shadow formation but maximization of SE collection from near-surface regions.

This methodology is particularly advantageous for two-dimensional materials and ultrathin coatings, whose functional properties strongly depend on nanoscale morphology. Although the technique may be less effective for thick, highly corrugated or insulating samples, where shadowing effects or charging may occur, it remains broadly applicable to other layered systems such as graphene, WS_2 , h-BN, and to nanostructured films and biomimetic surfaces.

5. Conclusions

In this work, a grazing-incidence SEM strategy based on $\sim 90^\circ$ -sample tilting was demonstrated to significantly enhance morphological contrast in two-dimensional MoS_2 nanosheets. Compared to conventional imaging with a tilt of 0° , this approach increases the number of detectable grey levels by more than 40% and reveals structural details such as sheet edges, folds, overlaps, pores and defects that are otherwise invisible.

The contrast enhancement originates from a higher secondary-electron yield, due to the reduced penetration depth of primary electrons and the more efficient escape of SEs from near-surface regions. This effect is achieved without loss of image sharpness, taking advantage of the intrinsic high depth-of-field of SEM.

The proposed method is simple, non-destructive and does not require instrumental modifications, making it suitable for routine SEM analysis of ultrathin 2D materials and nanostructured coatings. Although shadowing effects or reduced effectiveness may occur in thick or highly corrugated samples, the approach is widely applicable to other layered materials (e.g., graphene, h-BN, WS_2) and bio-inspired interfaces.

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