Atomic Force Microscopy as a Quantitative Tool for Particle Characterization: From Microns to Angstrom Scale

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***Abstract:** Nanoparticles constitute a crucial and technology intensive area of research and development in the continuous expanding field of nanotechnology. They are becoming increasingly important in many areas, including data storage, plasmonic, photonic, microelectronic, energy, pharmaceutical, biomedical, and cosmetics etc. Using Atomic Force Microscope (AFM), individual particles of varying sizes ranging from µm to sub-nanometer level can be resolved and unlike other microscopy techniques, the AFM offers visualization and quantitative analysis in three dimensions. In this manuscript, AFM was effectively used to characterize different particles (SnO2, ZnO and TiO2) whose sizes varied between µm to angstrom level on a mica substrate. Further, the possibility of combining AFM and image post processing software Gwyddion, to extract quantitative data even for angstrom size particles are demonstrated.*

***Keywords:** AFM, Nanopartciles, Quantitative Analysis*

I. INTRODUCTION

Particle size is one of the most important parameters in materials science and technology as well as many other branches of science and technology, from medicine, pharmacology and biology to ecology, energy technology and the geosciences [1]. At the most basic level, we can define a particle as being a discrete sub-portion of a substance. The most common types of materials consisting of particles are: powders, granules, suspensions, emulsions, aerosols, sprays etc. Sizes of these particles can vary from few hundreds of nanometers to sub-nanometer level. For example, biodegradable polymeric nanoparticles used as carrier for controlled and sustained delivery of drugs have sizes between 10-1000 nm [2]. On the other hand, quantum dots (QDs) used in biomedical, material research field has their sizes vary between 2-10 nm [3].

Particles are generally characterized by their size, morphology and surface charge, using advanced microscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) [4]. Other techniques used for particle characterization are X-ray crystallography (for determining crystalline structure) [5], Dynamic light scattering (DLS, for particle size) [6], FTIR (for functional groups) [7], Vibrating sample magnetometer (VSM, for magnetic particles) [8], Zeta potential measurement (for surface charge) [9] etc. The unique advantages of an AFM.

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Over other surface characterization techniques. used for particle size and morphology characterization is its ability to image individual particles from angstrom level up to micron scale both in air and in liquid environment.

AFM measurements also require minimal sample preparation procedure compared to SEM and TEM and provide three dimensional (3-D) data. Further AFMs can also be used to determine beside sizes, the physical properties such as magnetic [10], electrical [11], thermal [12], and mechanical parameters [13] simultaneously. Post processing of AFM data by both free (e.g. Gwyddion) and commercial software (e.g. SPIP, Pico image etc.) can also provide quantitative data including particle counts, particle size distribution, surface area distribution and volume distribution of particles etc.

In this report, AFM was used as an effective tool to determine the surface morphologies of a number of particles (SnO_2, ZnO and TiO_2). Although AFM studies describing the morphologies of these particles is already reported in the literature [14-16] however, the main purpose of this study was to demonstrate the capability of AFM and post processing image software for extracting the meaningful quantitative parameters for particles having a wide range of dimensions e.g. from microns to even angstrom level.

II. EXPERIMENTAL

For single particle characterization, all the particles studied here were dispersed in an organic solvent like ethanol separately and sonicated for ~ 3 mins. In order to see individual particles, the solutions were further diluted with ethanol and AFM measurements were performed after depositing a 10 µl droplet on freshly cleaved mica substrate. Ethanol took ~1 min to evaporate from surface leaving the particles attached to the mica substrate. The dilution process and AFM imaging was repeated until individual particles appeared on the substrate. AFM measurements were carried out in tapping mode using an Agilent 5500 system under ambient condition. PPP-NCH cantilevers from Nanosensors with a nominal resonant frequency of ~ 330 kHz were used for imaging individual particles. Quantitative analysis of particles was carried out by using the powerful grain analysis option in Gwyddion image processing software.

III. RESULTS AND DISCUSSION

In this section the morphologies of particles varying from micron scale to angstrom level are presented. Further, grain analysis option in Gwyddion processing software was employed to determine the quantitative parameters of these

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Particles. Various steps involved in quantitative data extraction using Gwyddion post processing software are as follows.

- All the particles were first imaged on a mica substrate using tapping mode AFM.
- Raw image was levelled by using "Mean plane subtraction" option.
- To remove any tip effect on the particle morphology, "Model Tip" option (can be found under Data Process > Tip an Indentation > Model Tip in software) was used. Using manufacturer tip radius of 5nm for non-contact lever, the tip was remodeled as shown in fig. 1.
- To apply this remodeled tip in order to remove any tip effect (sometimes also called tip deconvolution effect) on image, the levelled image was first selected. "Tip dilation" option (Data Process > Tip and Indentation > Tip Dilation in Gwyddion) was applied to the selected image to remove tip effect on the image.
- The final image after applying "Tip Dilation" was used for quantitative particle analysis.
- The individual particles are separated the rest of the background in image by using "Mark by Threshold" (Data process > grain > Mark by Threshold in Gwyddion) option.
- Finally the quantitative parameters are obtained by "Statistics" option which can be found under Data process > Grains > Statistics.

A. Quantitative Estimation of SnO_2 Particles by AFM

SnO_2 powder particles being highly conductive, transparent and sensitive to gases have been studied intensively in the last decade. As a n-type semiconductor because of wide-energy-gap (3.6 eV), SnO2 has a wide variety of applications such as making transparent conducting films, catalytic materials, environmental monitoring, biochemical sensor, lithium rechargeable batteries, dye-sensitized solar cells and ultrasensitive gas sensors [17-20]. An AFM image of SnO2 particles (size 50 x 50 µm) on mica substrate after applying tip dilation correction is shown in figure 2a. The corresponding three dimensional image (3D) as well as a height profile along the red dotted line in figure 2a is shown is figure 2b and 2c respectively indicating the particle height variation from several hundred nanometers up to microns. These particles were separated from the rest of the image by applying Gwyddion "threshold" option as explained in the above in the result section and the threshold image is shown in figure 2d. The statistical parameters obtained for these selected particles are shown in figure 2e. A number of quantitative parameters such as total number of particles (78), total area they covered on surface (relative 5.19%) , their mean size (917.6 nm) are obtained by using the "statistics" option in grain analysis. For the correct estimation of these parameters, applying tip correction to the imaged surface is mandatory and failure to do so will result in incorrect evaluation of quantitative parameters. It is important to note that, Gwyddion being free software, the number of quantitative parameters obtained are limited. By using commercial software like scanning probe image processor (SPIP) or Pico image (PI), it is also possible to obtain many other quantitative parameters such as plotting histogram for particle distribution, selecting individual particles to learn their properties, surface area profiles etc.

B. Quantitative estimation ZnO Particles by AFM

ZnO NPs have attracted intensive research efforts for their unique properties and versatile applications in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, and spin electronics [21, 22]. In addition, they have the ability to boost the yield and growth of food crops [23]. Further applications include their use in sunscreen lotions for UV ray protection [24], as catalysts [25] etc. To get quantitative information on these medium sized particles, they were first imaged on a mica substrate. One of the image after applying the tip correction method is shown in figure 3a (image size 80 x 80 µm). The corresponding 3D structure and height profile along the red dotted line in figure 3a is shown in figure 3b and 3c respectively. The profile and 3D clearly demonstrates the particle sizes to be between 50-500 nm. The thresholding option was again applied to the image to separate particles from the rest of the surface as shown in figure 3d. Finally the quantitative parameters are obtained by using grain statistics option (figure 3e). As observed total numbers of particles are found to be 427 and they covered ~ 2.52 % of the entire surface. The mean grain size was found to be 480.5 nm. For finding other parameters readers are referred to have a look at figure 3e.

C. C. Quantitative Estimation TiO2 Particles by AFM

Titanium dioxide (TiO_2) is a multifaceted compound. It's the stuff that makes toothpaste white and paint opaque [26]. TiO_2 is also a potent photo-catalyst that can break down almost any organic compound when exposed to sunlight, and a number of companies are seeking to capitalize on titanium dioxide's reactivity by developing a wide range of environmentally beneficial products, including self-cleaning fabrics, auto body finishes [27]. Other applications include their use in sunscreen lotions as UV protector [24], in waste water treatment to remove pollutants [28], in pharmaceuticals to destroy cell membranes of viruses etc. [29]. Although TiO_2 particles are smaller in size compared to SnO_2 and ZnO, it is still possible to characterize them using an AFM and derive quantitative information of such particles. Figure 4a represents an AFM image of TiO_2 particles deposited on a mica substrate after tip correction (Image size 10 x 10 µm). The 3D view and height profile along the red line in figure 4a is shown in figure 4b and figure 4c. The height profile and 3D view demonstrates the particle sizes which varies from sub-nanometer (angstroms) to few 10s of nanometers. These particles were also analyzed in the similar fashion as done for other particles described above. The thresholding option was used to separate the particles (see figure 4d) followed by using grain statistics option to determine the quantitative data (figure 4e). Total number of grains was calculated to be 206 with a total covered area of ~ 2.21%. The mean grain size was found to be 87.50 nm. Other quantitative data obtained can be found in figure 4e.

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IV. CONCLUSION

Nanoparticles (NPs) being forefront of extensive research due to their potential applications in many field, there is a growing consensus for proper and accurate characterization of NPs in environmental media and biological systems to ensure their reliable and reproducible performance. Without such characterization, it is practically impossible to guarantee their optimal performance when used in various technical applications. In this context, AFM plays a vital role for both qualitative and quantitative analysis of particles. In this study by employing AFM, the possibility of getting both qualitative (particle image) and quantitative (particle size, distribution etc.) data for particles of various sizes (from micron scale to angstrom level) is successfully demonstrated. This study will work as a guide for non-experts in atomic force microscopy doing research in the field of nanoparticles to quantitatively analyze the particles of interest.

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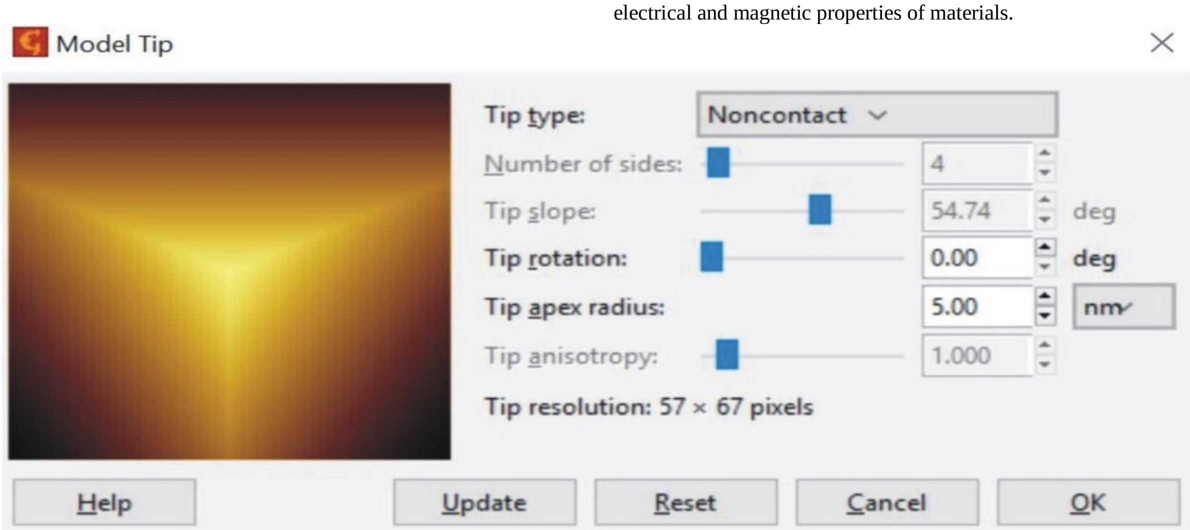

Figure 1: Modeled tip used for Removing Tip Effect From all the Images Before Carrying Out Quantitative Particles Analysis.

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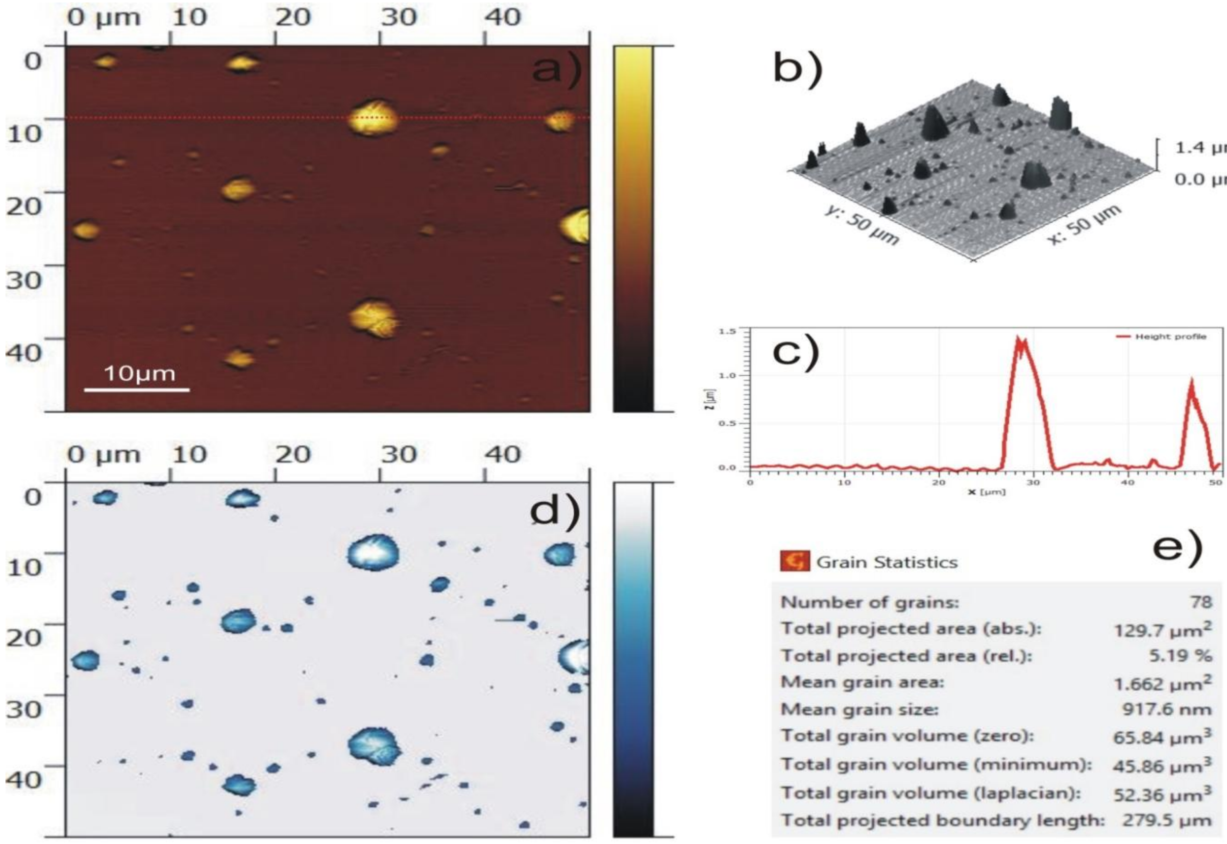

Figure 2: AFM image and quantitative analysis of SnO_2 particles deposited on mica substrate from water dispersion is represented in figure 2. Figure 2a, b demonstrate an AFM image (scan size 50 x 50 µm) and the 3D topography of the surface. Figure 2c represents the height profile along the red dotted line in fig. 2a. Separated individual particles from the rest of the surface using "grain analysis>mark by threshold" option of Gwyddion is shown in fig. 2d. Quantitative analysis of these particles using grain statistics option of Gwydion is represented in fig. 2e.

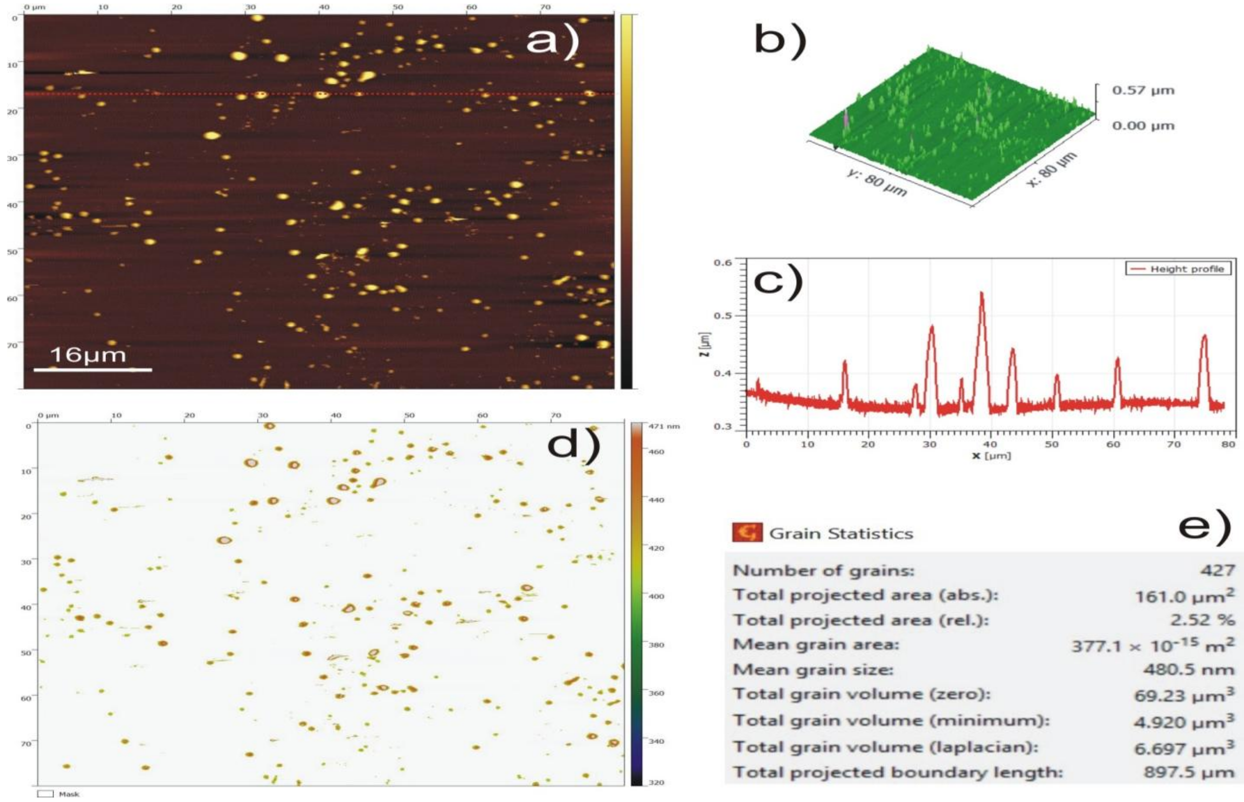

Figure 3: AFM image and quantitative analysis of ZnO particles deposited on mica substrate from water dispersion is represented in figure 3. Figure 3a, b demonstrate an AFM image (scan size 80 x 80 µm) and the 3D topography of the surface. Figure 3c represents the height profile along the red dotted line in fig. 3a. Separated individual particles from the rest of the surface using "grain analysis>mark by threshold" option of Gwyddion is shown in fig. 3d. Quantitative analysis of these particles using grain statistics option of Gwydion is represented in fig. 3e.

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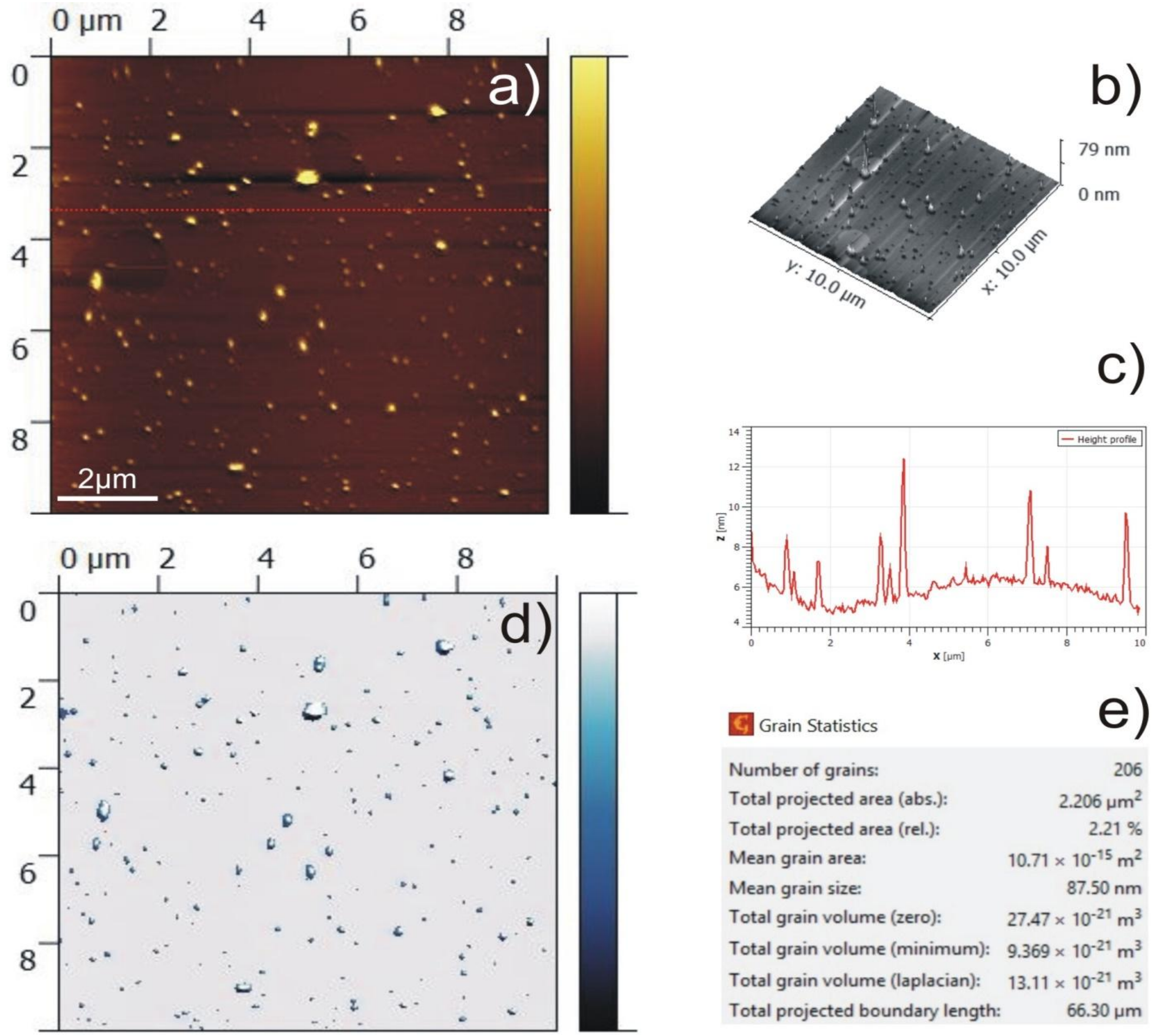

Figure 4: AFM image and quantitative analysis of TiO_2 particles deposited on mica substrate from water dispersion is represented in figure 4. Figure 4a, b demonstrate an AFM image (scan size 10 x 10 μm) and the 3D topography of the surface. Figure 4c represents the height profile along the red dotted line in fig. 4a. Separated individual particles from the rest of the surface using "grain analysis>mark by threshold" option of Gwyddion is shown in fig. 4d. Quantitative analysis of these particles using grain statistics option of Gwydion is represented in fig. 4e.

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