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Standard Guide for Size Measurement of Nanoparticles Using Atomic Force Microscopy[1]

This standard is issued under the fixed designation E2859; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The purpose of this document is to provide guidance on the quantitative application of atomic force microscopy (AFM) to determine the size of nanoparticles[2] deposited in dry form on flat substrates using height (z-displacement) measurement. Unlike electron microscopy, which provides a two-dimensional projection or a two-dimensional image of a sample, AFM provides a three-dimensional surface profile. While the lateral dimensions are influenced by the shape of the probe, displacement measurements can provide the height of nanoparticles with a high degree of accuracy and precision. If the particles are assumed to be spherical, the height measurement corresponds to the diameter of the particle. In this guide, procedures are described for dispersing gold nanoparticles on various surfaces such that they are suitable for imaging and height measurement via intermittent contact mode AFM. Generic procedures for AFM calibration and operation to make such measurements are then discussed. Finally, procedures for data analysis and reporting are addressed. The nanoparticles used to exemplify these procedures are National Institute of Standards and Technology (NIST) reference materials containing citrate-stabilized negatively charged gold nanoparticles in an aqueous solution.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1617 Practice for Reporting Particle Size Characterization Data

E2382 Guide to Scanner and Tip Related Artifacts in Scanning Tunneling Microscopy and Atomic Force Microscopy

E2456 Terminology Relating to Nanotechnology

E2530 Practice for Calibrating the *Z*-Magnification of an Atomic Force Microscope at Subnanometer Displacement Levels Using Si*(111)* Monatomic Steps (Withdrawn 2015)[4]

E2587 Practice for Use of Control Charts in Statistical Process Control

2.2 *ISO Standards:*[5]

ISO 18115–2 Surface Chemical Analysis—Vocabulary—Part 2: Terms Used in Scanning-Probe Microscopy

ISO/IEC Guide 98–3:2008 Uncertainty of Measurement—Part 3: Guide to the Expression of Uncertainty in Measurement (GUM:1995)

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions pertaining to nanotechnology terms, refer to Terminology E2456.

3.1.2 For definitions pertaining to terms associated with scanning-probe microscopy, including AFM, refer to ISO 18115–2.

[1] This guide is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.02 on Physical and Chemical Characterization.

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[2] Having two or three dimensions in the size scale from approximately 1 nm to 100 nm as in accordance with Terminology E2456; this definition does not consider functionality, which may impact regulatory aspects of nanotechnology, but which are beyond the scope of this guide.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] The last approved version of this historical standard is referenced on www.astm.org.

[5] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *agglomerate, n—in nanotechnology*, an assembly of particles held together by relatively weak forces (for example, Van der Waals or capillary), that may break apart into smaller particles upon processing, for example. E2456

3.2.1.1 *Discussion*—Using imaging based techniques, such as AFM, it is generally difficult to differentiate between agglomerates formed during the deposition process (that is, artifacts) and agglomerates or aggregates that pre-exist in the test sample.

3.2.2 *aggregate, n—in nanotechnology*, a discrete assemblage of particles in which the various individual components are not easily broken apart, such as in the case of primary particles that are strongly bonded together (for example, fused, sintered, or metallically bonded particles). E2456

3.2.2.1 *Discussion*—Using imaging based techniques, such as AFM, it is generally difficult to differentiate between aggregates and agglomerates.

3.3 *Acronyms:*

3.3.1 *AFM*—atomic force microscopy

3.3.2 *APDMES*—3-aminopropyldimethylethoxysilane

3.3.3 *DI*—deionized

3.3.4 *HEPA*—high efficiency particulate air

3.3.5 *NIST*—National Institute of Standards and Technology

3.3.6 *PLL*—poly-L-lysine

3.3.7 *RM*—reference material

4. Summary of Practice

4.1 This guide outlines the procedures for sample preparation and the determination of nanoparticle size using atomic force microscopy (AFM). An AFM utilizes a cantilever with a sharp probe to scan a specimen surface. The cantilever beam is attached at one end to a piezoelectric displacement actuator controlled by the AFM. At the other end of the cantilever is the probe tip that interacts with the surface. At close proximity to the surface, the probe experiences a force (attractive or repulsive) due to surface interactions, which imposes a bending moment on the cantilever. In response to this moment, the cantilever deflects, and this deflection is measured using a laser beam that is reflected from a mirrored surface on the back side of the cantilever onto a split photodiode. A schematic diagram of the system is shown in Fig. 1. The cantilever deflection is measured by the differential output (difference in responses of the upper and lower sections) of the split photodiode. The deflections are very small relative to the cantilever thickness and length. Thus, the probe displacement is linearly related to the deflection. The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nanometers. More detailed and comprehensive information on the AFM technique and its applications can be found in the published literature (**1**, **2**).[6]

4.2 Based on the nature of the probe-surface interaction (attractive or repulsive), an AFM can be selected to operate in various modes, namely contact mode, intermittent contact mode, or non-contact mode. In contact mode, the interaction between the tip and surface is repulsive, and the tip literally contacts the surface. At the opposite extreme, the tip interacts with the surface via long-range surface force interactions; this is called non-contact mode. In intermittent contact mode (also referred to as tapping mode), the cantilever is oscillated close to its resonance frequency perpendicular to the specimen surface, at separations closer to the sample than in non-contact mode. As the oscillating probe is brought into proximity with the surface, the probe-surface interactions vary from long range attraction to weak repulsion and, as a consequence, the amplitude (and phase) of the cantilever oscillation varies. During a typical imposed 100-nm amplitude oscillation, for a short duration of time, the tip extends into the repulsive region close to the surface, intermittently touching the surface and thereby reducing the amplitude. Intermittent contact mode has the advantage of being able to image soft surfaces or particles weakly adhered to a surface and is hence preferred for nanoparticle size measurements.

[6] The boldface numbers in parentheses refer to a list of references at the end of this standard.

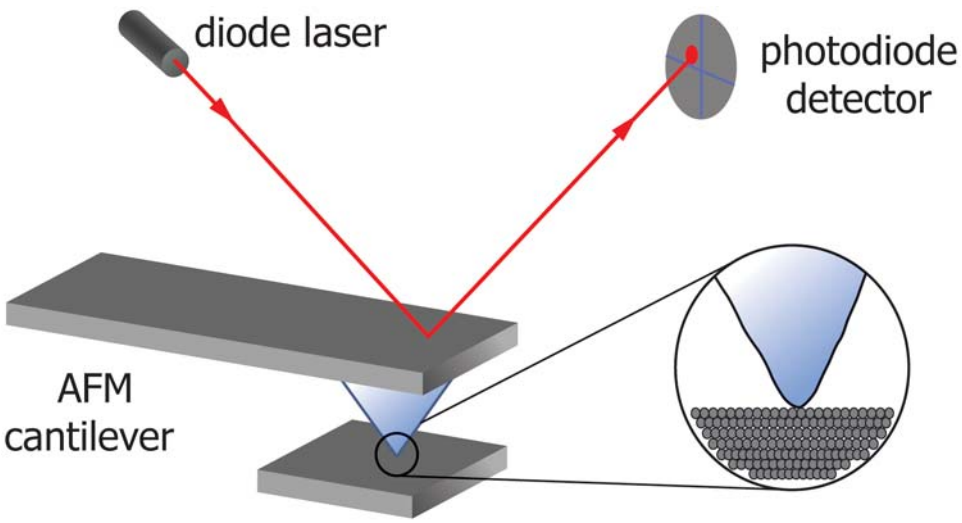

FIG. 1 Schematic Illustration of AFM Measurement Principle

4.3 A microscope feedback mechanism can be employed to maintain a user defined AFM set point amplitude, in the case of intermittent contact mode. When such feedback is operational, constant vibration amplitude can be maintained by displacing the built-in end of the cantilever up and down by means of the piezo-actuator.

NOTE 1—Operation of an AFM with feedback off enables the interactions to be measured and this is known as force spectroscopy.

This displacement directly corresponds to the height of the sample. A topographic image of the surface can be generated by rastering the probe over the specimen surface and recording the displacement of the piezo-actuator as a function of position. Although the lateral dimensions are influenced by the shape of the probe (see Guide E2382 for guidance on tip related artifacts), the height measurements can provide the height of nanoparticles deposited onto a substrate with a high degree of accuracy and precision. If the particles are assumed to be spherical, the height measurement corresponds to the diameter or "size" of the particle.

4.4 Procedures for dispersing nanoparticles on various surfaces such that they are suitable for imaging and height measurement via intermittent contact mode AFM are first described. The nanoparticles used to exemplify these procedures were National Institute of Standards and Technology (NIST) gold nanoparticle reference materials, RM 8011 (nominally 10 nm), RM 8012 (nominally 30 nm), and RM 8013 (nominally 60 nm), all of which contained citrate-stabilized negatively charged gold nanoparticles in an aqueous solution.

4.5 Generic procedures for AFM calibration and operation to perform size measurements in intermittent contact mode are discussed, and procedures for data analysis and reporting are outlined.

5. Significance and Use

5.1 As AFM measurement technology has matured and proliferated, the technique has been widely adopted by the nanotechnology research and development community to the extent that it is now considered an indispensible tool for visualizing and quantifying structures on the nanoscale. Whether used as a stand-alone method or to complement other dimensional measurement methods, AFM is now a firmly established component of the nanoparticle measurement tool box. International standards for AFM-based determination of nanoparticle size are nonexistent as of the drafting of this guide. Therefore, this standard aims to provide practical and metrological guidance for the application of AFM to measure the size of substrate-supported nanoparticles based on maximum displacement as the probe is rastered across the particle surface to create a line profile.

6. Reagents

6.1 Certain chemicals and materials may be necessary in order to perform one or more of the steps discussed in this guide, but the specific reagents used are at the discretion of the tester and may depend on which specific alternative procedures are chosen or relevant for a particular application.

6.2 *Adhesive tape,* if needed to cleave mica substrates.

6.3 *Atomically flat gold (111) on mica,* if needed as a substrate material.

6.4 *Colloidal gold, citrate-stabilized in aqueous solution,* if needed to test or validate sample preparation and measurement procedures.

6.5 *Deionized water, filtered to 0.1 μm,* as needed for sample preparation or to rinse substrates.

6.6 *Ethanol, reagent or chromatographic grade,* as needed to rinse substrates.

6.7 *HCl, concentrated (37 %),* if needed to clean silicon (Si) substrates.

6.8 H_2O_2*, 30 % solution,* if needed to clean Si substrates.

6.9 *Inert compressed gas source* (for example, nitrogen, argon, or air), filtered to remove particles.

6.10 *Mica disc,* if needed as a substrate material.

6.11 *Poly-l-lysine, solution (0.1 %),* if needed for preparation of functionalized substrates.

6.12 *Single crystal Si wafers, diced to appropriate size,* if needed as a substrate material.

7. Apparatus

7.1 *Atomic Force Microscope,* capable of making z-displacement measurements at sub-nanoscale dimensions.

7.2 *Bath Ultrasonicator,* as needed to clean substrates.

7.3 *Microcentrifuge ("Microfuge"),* as needed for sample preparation.

7.4 *RF Plasma Cleaner with* O_2*,* as needed to clean Si substrates.

8. Procedure

8.1 *Nanoparticle Deposition*—For AFM measurements, nanoparticle samples must be deposited on flat surfaces. The roughness of the surface should be much less than the nominal size of the nanoparticles (preferably less than 5 %) in order to provide a consistent baseline for height measurements. High-quality mica, atomically flat gold (111) (deposited on mica), or single crystal silicon can all be used as substrates to minimize the effect of surface roughness on nanoparticle measurements. Example procedures are provided for depositing nanoparticles on these three substrates. The sample deposition procedures outlined below were developed for use with negatively charged citrate-stabilized gold nanoparticles suspended in an aqueous solution at a mass concentration nominally 50 μg/g (as exemplified by NIST RMs 8011, 8012, and 8013). The procedures should work with other nanoparticles that carry a negative surface charge or zeta potential, including, but not limited to, commercially available citrate-stabilized colloidal gold. As suggested below, these procedures can also be applied to positively charged or neutral nanoparticles with some modification. Each procedure may require optimization by the user in order to obtain satisfactory deposition density and to minimize artifacts such as agglomerate formation on the substrate or build-up of organic films resulting from additives that might be present in the solution phase.