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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, health, and environmental 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.

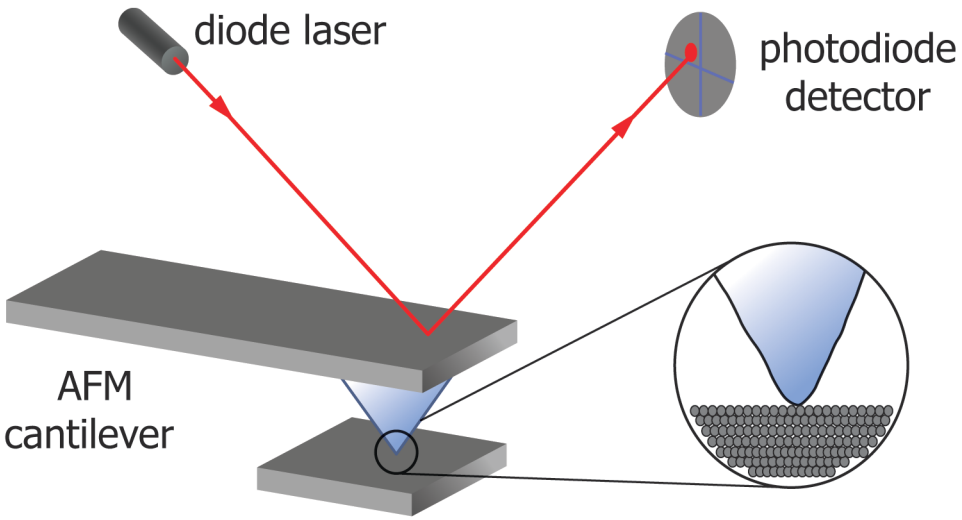


FIG. 1 Schematic Illustration of AFM Measurement Principle

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.

NOTE 2—Substrate preparation and sample deposition should be conducted in a manner that minimizes the potential for contamination and artifacts. For instance, to the extent possible, these operations should be conducted in a HEPA filtered clean bench or work area. Similarly, prepared samples should be stored in a manner that maintains their integrity and precludes contamination.

8.1.1 *Mica Substrate*—Mica is a layered mineral that can be readily cleaved along alkali-rich basal planes to form clean, atomically flat surfaces extending over large areas. To prepare the substrate, a mica disc must be cleaved to produce a clean surface. Place the disc on a clean, lint-free cloth or directly on an AFM puck. Press a piece of adhesive tape against the surface of the disc and then smoothly remove the tape from the mica. The top layer of the mica should appear on the tape. Continue to cleave the mica until a full layer is removed and the exposed surface is visually smooth. Typically, this step needs to be repeated several times, and requires visual inspection of the cleaved surface.

8.1.1.1 After cleaving, the mica disc is ready to be activated so as to promote adhesion between the substrate and the gold nanoparticles. The NIST gold nanoparticle RMs are dispersed in solution and stabilized by adsorbed citrate ions that give the particles a negative charge. The mica substrate can be activated to have a positive charge that readily binds negatively charged particles to the surface. The substrate is activated using diluted 0.1 % poly-l-lysine (PLL) solution to provide a positively charged surface. To create the solution, dilute 0.1 % PLL solution 1:10 with filtered deionized (DI) water (for example, add 0.5 mL PLL to 4.5 mL DI water). Use clean glassware for dilution and coating. Store the diluted PLL solution in a refrigerator between 2 °C and 8 °C until needed. Fully immerse the mica disc in the diluted PLL solution for 30 min at room temperature. To minimize evaporation, cover the solution with a glass dish. After the time has elapsed, remove the mica from the solution and blow dry with a filtered inert gas stream (for example, air, nitrogen, argon).

8.1.1.2 After drying, apply ≈25 µL of undiluted gold nanoparticle solution onto the PLL-modified mica substrate using a micropipette. The gold solution should spread evenly across the surface. Incubate at room temperature using the following schedule as a guide:

(1) 60 nm particles: 10 min.
(2) 30 nm particles: 5 min.
(3) 10 nm particles: 30 sec.

The incubation times are appropriate for 50 µg/g colloidal gold suspensions, but can be varied to modify the particle density on the surface as required for particles of different size, composition or concentration; incubation times should be verified or optimized for each application. Rinse the substrate with filtered DI water and gently dry with a filtered inert gas stream. The sample is now ready to image.

8.1.2 *Silicon Substrate*—An electrostatic deposition procedure such as that described for negatively charged nanoparticles on mica can also be conducted using silicon as the substrate material. Dice a small sample (for example, 5 mm × 5 mm) from a silicon wafer or obtain pre-diced silicon substrates from a commercial source. Clean the sample using the following procedure: treat for 5 min in a wet oxygen plasma cleaner,[7] treat for 10 min in a clean glass beaker with acetone placed in a low intensity ultrasonic bath followed by 10 min sonication in a clean glass beaker with ethanol. Blow substrate dry with inert gas stream.

8.1.2.1 If a plasma cleaner is not available, the following alternative cleaning procedure can be used. Place silicon substrate in a solution containing a 6:1:1 volumetric ratio of DI water: concentrated HCl (37 %): 30 % H_2O_2 solution, and treat in a low intensity ultrasonic bath for 2 min to 10 min. Solution is a strong oxidizer and very acidic, and thus should be prepared and handled with due caution; always dilute acid into water. Follow treatment with a DI water rinse to remove any residual acid or peroxide.

NOTE 3—Pre-made cleaning solutions for silicon wafers are commercially available. Other cleaning procedures can be found in the literature. If using an alternative procedure, avoid treatments that tend to remove the native oxide layer (for example, basic solutions, such as those containing ammonium hydroxide). Be advised that some commonly used cleaning solutions for removing organics from glass surfaces (for example, acidified peroxide or piranha) are extremely aggressive and appropriate care should be taken if using such solutions.

8.1.2.2 The cleaned wafer supports a thin, native oxide layer. The substrate can then be treated to produce a positive surface using an amino-silane coupling agent, such as 3-aminopropyldimethylethoxysilane (APDMES). Place a drop of APDMES on the Si surface. Allow the APDMES to react with the underlying substrate for 2 h inside a sealed glass vial. Remove the excess APDMES by rinsing with ethanol followed by DI water.

8.1.2.3 After drying, apply ≈25 µL of undiluted gold nanoparticle solution onto the APDMES-modified silicon substrate using a micropipette. The gold solution should spread evenly across the surface. Incubate at room temperature using the following schedule as a guide:

(1) 60 nm particles: 60 min.
(2) 30 nm particles: 30 min.
(3) 10 nm particles: 15 min.

The incubation times are approximate and should be verified or optimized for each application. To prevent evaporation, the substrate with gold solution droplet should be sealed inside a humidified chamber (for example, under an inverted glass beaker with DI water reservoir). Following incubation, rinse the sample first with ethanol, followed by DI water, and gently dry with a filtered inert gas stream prior to analysis.

8.1.3 *Gold Substrate*—An atomically flat gold (111) surface (deposited on mica) can be obtained commercially and used as a substrate for nanoparticle sizing. If necessary, clean the gold surface using ethanol and dry using a filtered stream of inert gas. It is recommended that ultrasonic cleaning not be used, as this may delaminate the gold layer from the underlying mica.

8.1.3.1 The gold substrate can be functionalized in a manner similar to that described for mica and silicon above, but using thiolated compounds that react chemically with the gold surface. For instance, an amino-thiol compound could be used to impart a positive surface charge to deposit negatively

[7] Plasma cleaners vary widely, and therefore specific settings and treatment times may vary and should be verified for each device. As a guide, typical settings would be RF power ≈40 W and pressure ≈0.2 mbar (20 Pa).

charged nanoparticles. In this case, one should follow the procedure described for APDMES above, but instead use an appropriately selected functionalized thiol compound (a variety of thiolated functional compounds are available commercially).

8.1.3.2 The nanoparticles can also be deposited on a native (that is, not functionalized) atomically flat gold surface using the drop-cast method, in which a drop of the test suspension is allowed to evaporate on the substrate surface. However, nanoparticles, including colloidal gold, are frequently stabilized with a surfactant or other capping agent, some of which may also be dissolved in the solution phase. As a result, AFM imaging may show a residual organic layer on the substrate and nanoparticle surface, which can potentially influence the accuracy of the nanoparticle measurements. If this is the case, the user may wish to adopt the following alternative preparation procedure for drop-casting, which utilizes a centrifuge to remove any excess surfactants or capping agents (for example, citrate ions) from the solution phase.

(1) Place an approximately 1 mL aliquot of gold suspension into a 1.5 mL microcentrifuge tube ("microtube") and use the rotation speed and spin times listed below as a guide. Remove and discard a portion of the supernatant from the microtube (according to the dilution ratio given below), then replace with DI water to obtain the proper dilution of the native suspension. No change in the stability of the suspension should be observed during this process. The following guidelines are appropriate for the gold nanoparticles using a typical bench-top microcentrifuge ("microfuge") capable of holding standard microfuge tubes (for example, up to 2 mL nominal volume); it may be necessary to vary these parameters in order to optimize deposition and minimize artifacts.

(a) 60 nm particles: dilution ratio 1:3; speed 5000 rpm (83.3 Hz) equivalent to 2040 g; time 5 min; volume of the suspension between 0.8 mL and 1 mL.

(b) 30 nm particles: dilution ratio 1:5; speed 8000 rpm (133.3 Hz) equivalent to 5220 g; time 6 min; volume of the suspension between 0.8 mL and 1 mL.

(c) 10 nm particles: dilution ratio 1:8; speed 14 000 rpm (233.3 Hz) equivalent to 16 000 g; time 20 min; volume of the suspension between 0.8 mL and 1 mL.

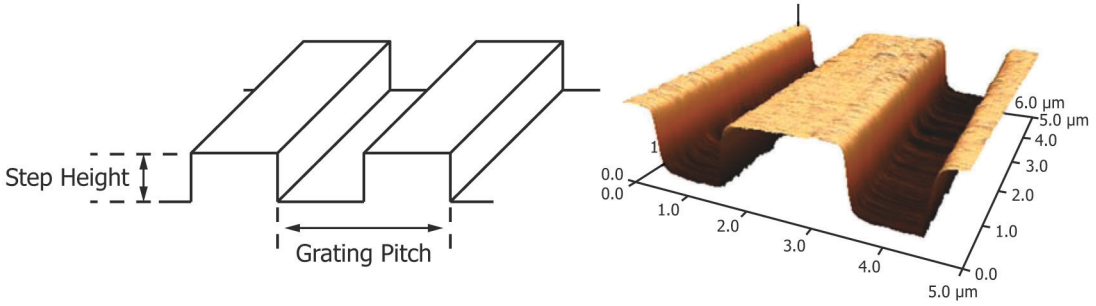
After the dilution and centrifuge process, a droplet ($\approx$ 0.05 mL) of the suspension can be placed on the substrate using a micropipette and dried in air. To ensure removal of moisture, the deposited film can be additionally dried at an elevated temperature that is compatible with the nanoparticles and substrate (for example, $\approx$70°C for gold nanoparticles on gold/mica substrate). The sample is now ready to image.

8.2 *Optical Microscope Inspection*—Inspect each sample using an optical microscope before AFM imaging to confirm that an appropriate level of deposition has occurred (that is, particles are well separated and not clumping) or to locate areas of the substrate where one can expect a reasonably good dispersion of the particles, or both. In the case of drop-cast test samples, the exterior of the dried droplet includes excess stabilizing agents (for example, citrate), while the interior is free of these agents with suitable particle distributions. Optical inspection may be hindered if the target particles do not absorb or scatter sufficient light, and the procedure is most useful for determining if over-deposition has occurred (that is, particle density is too high).

8.3 *AFM Imaging and Size Measurement:*

8.3.1 *Accuracy (Height Calibration)*—In order to obtain accurate measurements, the axial (z)-displacement of the piezoelectric stage needs to be calibrated using available traceable standards. In Fig. 2, we show a schematic diagram and AFM image of a calibration grating, which consists of a one-dimensional array of rectangular SiO_2 steps on a Si wafer. For this particular grating, the step height was certified to be 19.5 nm $\pm$ 0.8 nm. After choosing a suitable grating (the step height of the grating should be similar to the characteristic height of the nanoparticles), measure the calibration grating in at least three locations using a sharp AFM tip and compare the average measured value to the certified step height. If the values are markedly different (for example, exceeds the uncertainty associated with the certified artifact), consult the AFM manufacturer on how to re-calibrate the z-displacement of the piezoelectric stage. Additionally, one may consult Practice E2530 regarding the calibration of z-scale using a Si(111) monatomic step artifact for nanometer and sub-nanometer displacements.

8.3.2 *Imaging Mode*—Nanoparticles are fixed to the substrate via weak physical forces (for example, electrostatic and van der Waals forces). As a result, intermittent contact mode is a suitable imaging mode in which the cantilever is driven to oscillate up and down near its resonance frequency by a small piezoelectric element mounted in the AFM tip holder. The



NOTE 1—For this particular grating, the step height was certified to be 19.5 nm $\pm$ 0.8 nm.

FIG. 2 Schematic Diagram and AFM Image of a Calibration Grating Consisting of an Array of Rectangular SiO_2 Steps on a Si Wafer