Colloid Probe Characterization: Radius and Roughness Determination

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In the last 10 years the atomic force microscope (AFM) has routinely been applied to the direct measurement of surface forces. A particle is often attached to the tip of the force-sensing cantilever in order to control the chemistry and the geometry of the interaction. Usually a spherical particle (colloid probe) is employed, and the measured interaction force is normalized by the radius, enabling comparisons to be made with both theory and other techniques. Here we report a simple method for characterizing the radius, surface roughness, and regularity of colloid probe particles. The colloid probe is "reverse imaged" by employing a surface with very sharp features. A standard calibration grating is suitable. No further equipment is required. The method is straightforward and will be of value to researchers employing the AFM for force measurement.

Introduction

The idea of attaching a microsphere at the end of an atomic force microscope (AFM) cantilever as a probe to sense forces between an individual colloid particle and a planar surface was introduced some years ago.[1] Since then the potential of an AFM as a force measuring apparatus has been widely exploited, to study many types of interactions.[2−12] Quantitative force measurements with the AFM require a strictly controlled interaction geometry, so a spherical geometry was suggested and is nowadays normally chosen for ease of comparison with predicted forces. In fact the measured forces are normalized by the radius and compared with theoretical curves (which are normally derived for the interaction of two flat surfaces[13]), using the Derjaguin approximation.[14] Generally the microsphere radius is determined by scanning electron microscopy (SEM) to an accuracy of ~0.1 μm (relative error 0.5−1%) assuming that the particle is perfectly spherical and therefore the appropriate radius of interaction is equal to the global radius of the probe. This characterization must be done after the force experiments as the sample must be coated with a conducting material for SEM imaging. Some laboratories either do not have access to SEM facilities or choose not to use them for simplicity and therefore determine the radius of the colloid probe by optical microscopy.[15,16] This introduces a relative error of 10% or more.

Here we describe a method to characterize the colloid probe that makes use of reverse AFM imaging. Reverse AFM imaging is a well-known phenomenon to AFM users and often a problem when imaging a surface formed by structures sharper than the AFM tip.[17−19] As a result the sharp surface features image the AFM tip, and "tip artifacts" appear in the image. Our method extracts information from the "artifacts" of reverse imaging to determine the colloid particle radius and surface roughness. For this purpose a commercial grating consisting of an array of very sharp spikes is imaged with the colloid probe. The resulting AFM image consists of a dense array of images of the part of the colloid particle that contacts the spike during the scan. This is the region of the colloid probe that approaches the flat surface during force measurements. Therefore it is precisely the radius of curvature and the surface roughness in this region that are appropriate for evaluating force data. Since the array of spikes is fairly dense, the actual area of the particle that is reverse imaged by each spike (the apex of the microsphere, the "cap") is small but sufficient to derive information on the contact area. During each scan the particle is imaged by all the spikes in the scanning area, resulting in a large number of images of the particle. The radius of the sphere is simply calculated from the width

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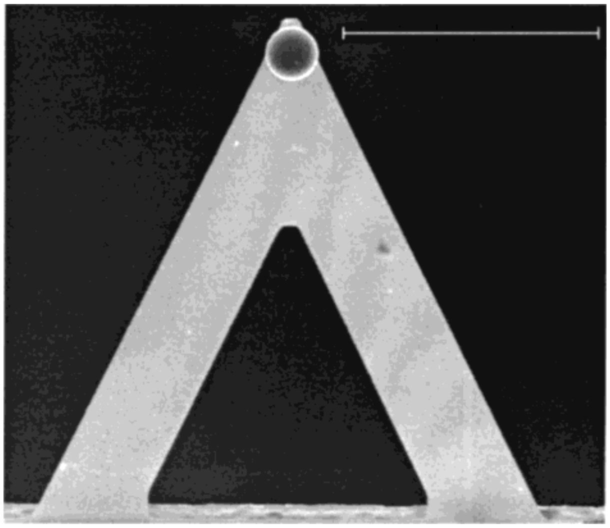

Figure 1. A scanning electron microscope image of a colloid probe of the type used in our experiments. The colloid probe is made by attaching a spherical silica particle (~20 μm diameter) to the tip of a commercial AFM cantilever. The bar is 100 μm.

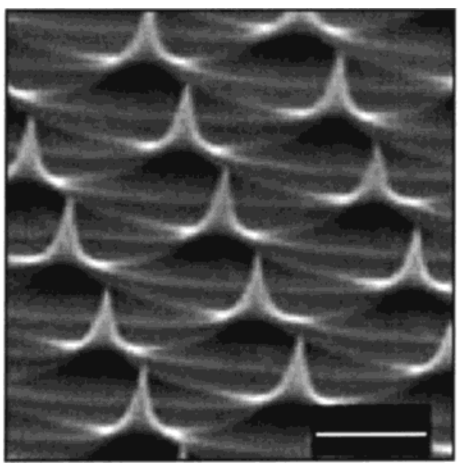

Figure 2. A scanning electron microscope image of the Silicon Grating TGT01 (NT-MDT, Moscow). The separation of the spikes is precisely known (pitch 2.12 μm, diagonal pitch 3 μm). The bar is 1 μm.

(w) and the height (h) of the imaged object with the formula

$$R = \frac{h^2 + (w/2)^2}{2h} \quad (1)$$

These values are easily determined using a section analysis (see parts b and c of Figure 3). The image of the cap not only provides the dimensions of the particle but also further reveals the presence of debris on its surface or deformations in its shape. This enables a faulty colloid probe to be identified prior to force measurements.

Experimental Section

Silica microspheres (Duke Scientific Corp., Palo Alto, CA) of radius ~10 μm and surface roughness 0.11 nm (root mean square) were used. The roughness was determined by AFM over 100 × 100 nm^2. The microsphere is attached with the minimal quantity of glue at the terminal point of a standard silicon nitride V-shaped cantilever of length 200 μm and leg width 40 μm (Digital Instruments, Santa Barbara, CA) (see Figure 1). The silicon calibration grating TGT01 (NT-MDT, Moscow), as shown in Figure 2, was imaged with a cantilever bearing a colloid probe, thereby obtaining an image that consists of an array of spherical caps (Figure 3a). These spherical caps are each images of the region of the colloid probe that will interact with a flat surface during a force measurement. A few repeated scans of the array, typically 20 μm × 20 μm in area, are performed in contact mode with each colloid probe at moderate speed (1−2 Hz) and applied pressure. After acquisition the images are flattened. It was found that for scan sizes significantly smaller than 10 μm, this process can introduce significant errors in the height data. Hence larger scan sizes are advised. These images are analyzed using section analysis and eq 1 to determine the radius of curvature of the cap.

The TGT01 grating is designed to characterize AFM tip shape, tip angle, and tip curvature angle. The grating is made of a 2 × 2 mm^2 array of very sharp spikes with tip curvature radius less than 10 nm and tip angle less than 20°. Convolution of the spike radius with the colloid sphere will introduce negligibly small errors. The spikes are approximately 0.6−0.8 μm tall. The pitch between closest spikes is exactly 2.12 μm, and the diagonal pitch is 3 μm. A Nanoscope III Multimode AFM (Digital Instruments, Santa Barbara, CA) was used for imaging. After AFM investigation the colloid probes were gold coated and imaged with a Cambridge S360 scanning electron microscope.

Results

An example of an image taken with a colloid probe is shown in Figure 3a. The cross sections of several spheres in each image are analyzed, and the width and the height of each cap are measured in order to calculate the radius with eq 1. The fact that the sizes of the caps imaged are not exactly the same in Figure 3a is due to very slight differences in the height of the spikes on the calibration grating. This does not adversely effect the results as the lateral dimensions increase accordingly with the vertical ones to provide the same average radius for the sphere.

Table 1 presents average values for the radii of nine microspheres (named in alphabetical order) investigated with this method. The radii obtained with our method are in good agreement with those obtained from SEM images. The difference in the radii measured with the two techniques is approximately 4%. This is certainly a more accurate estimate of the radius than that obtainable with an optical microscope, even though it is inferior in precision to that obtainable by SEM. The difference between the two methods for sphere C is considerably larger than those for the other spheres. This may be due to an aspherical colloid probe, where the radius of curvature of the cap is different for the global radius. This will not be apparent in the SEM image when the deviation from sphericity is small.

There are three advantages in using this method. First, if the fragile colloid probe is damaged following a force experiment or the sphere is lost, the data collected can still be analyzed and compared to the theoretical predictions. This would not be possible if the radius is not known. Second, for aspherical particles it is important that the radius of curvature of the probe in the region that approaches the flat is determined. This is achieved automatically when reverse imaging is used. Third, this method will also reveal the presence of debris on the surface of the sphere. This is shown clearly in Figure 4, where the image reveals a defective sphere. This sphere would obviously be discarded avoiding the risk of collecting false force data. Some commercial microspheres also have "baby" spheres on their surface, as result of a nonperfect synthetic process, which cannot be detected by optical microscopy. If these protuberances happen to be in the area of the microsphere that contacts the planar surface in force experiments, this will adversely influence force data. Again these protuberances can be evidenced with the AFM reverse imaging of the grating and they appear as small dots located at the same point of the sphere.

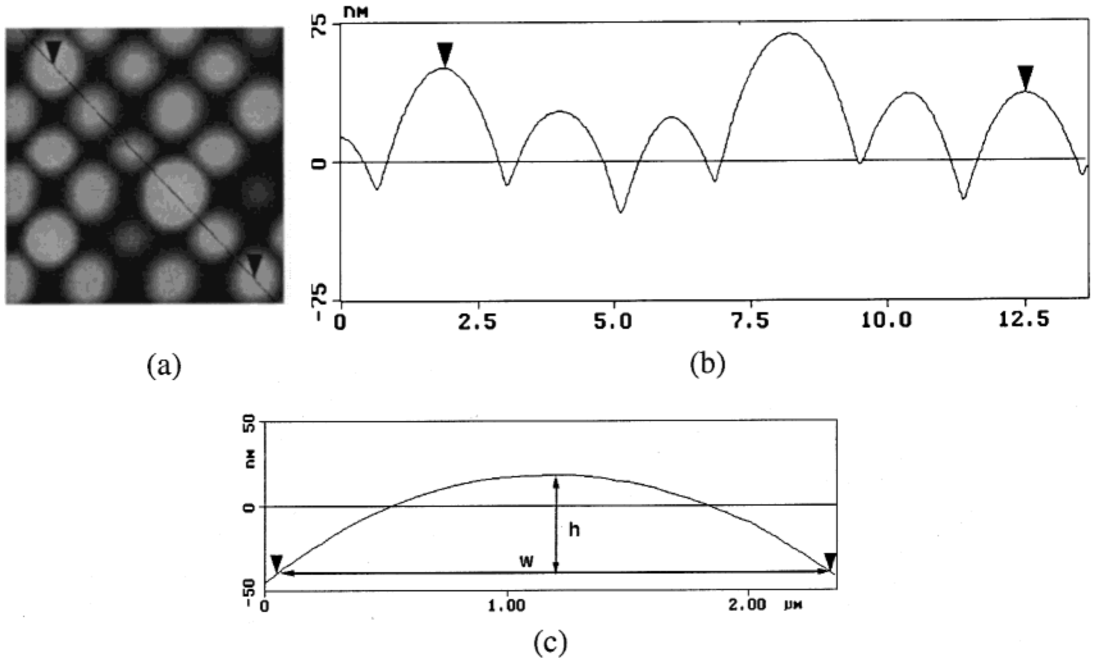

Figure 3. (a) AFM image of TGT01 grating obtained with a colloid probe (D, see Table 1). Image dimensions 13.7 × 13.7 μm². (b) Cross section through the indicated line in (a). The distance between the cursors in (a) and (b) is 10.63 μm, in good agreement with the expected length of five pitches for the TGT01 grating (5 × 2.12 μm). (c) Cross section of a single sphere from which the width (w) and the height (h) of the cap are derived.

Table 1[a]

microsphere	av radius obtained from AFM (μm)	av radius obtained from SEM[b] (μm)	rel % difference in the averages
A	10.0 (±0.4)	9.98 (±0.03)	0.2
B	10.2 (±0.5)	9.97 (±0.04)	2.3
C	9.5 (±0.4)	10.41 (±0.02)	8.7
D	10.4 (±0.2)	10.59 (±0.03)	1.8
E	9.4 (±0.1)	9.3 (±0.1)	1.1
F	9.4 (±0.2)	9.77 (±0.04)	3.8
G	9.6 (±0.2)	9.91 (±0.05)	3.1
H	9.6 (±0.1)	9.82 (±0.05)	2.2
I	10.5 (±0.4)	10.25 (±0.05)	2.4

[a] This table shows that the reverse AFM imaging method gives a value for the colloid particle radius in good agreement with the SEM value. In brackets are reported the uncertainties in the determination of the mean ($\sigma/N^{1/2}$, where σ is the standard deviation and N is the number of measurements). The average radius obtained with the reversed AFM imaging is found by measuring the size (height and width) of about 30−40 spherical caps in about three to five different AFM images. Note that each image of the spherical cap is an independent measurement because it results from the imaging from a separate spike in the array. The precision can be improved by increasing the number of measurements. [b] Note that the instrument used for SEM images can achieve an accuracy up to 0.1 μm on each distance determination.

It is essential that the piezo tube is accurately calibrated. The vertical calibration (z direction) should be determined using an appropriate standard such as the gratings commercially available. Indeed, this is essential for accurate force measurements. It is possible and advisable to determine the calibration in the x and y directions from the reverse images of the colloid probe as the distance between spikes is known for the calibration array TGT01. As we have found some variation in these values even for recently calibrated scanners, it is advisable to do an internal calibration on each image applying a correction factor to the lateral measurements in each direction. On correction of the horizontal measurements with this internal calibration, the measurement of the width of the cap can be considered reliable.

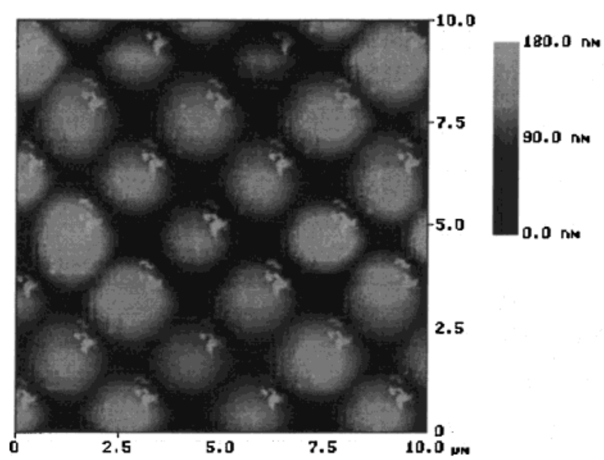

Figure 4. AFM image of TGT01 grating obtained with a nonspherical colloid probe. The repeated presence in the image of a blurry object on the same part of the cap clearly provides evidence of the existence of debris on the surface of the microsphere. Such a colloid probe would be unsuitable for use in force measurement.

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