Surface Investigation, 2001, Vol. 16, pp. 1135–1141
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Quantitative Methods for Restoration of True Topographical Properties of Objects using the Measured AFM-Images.
2. The Effect of Broadening of the AFM-Profile

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Received 29 November, 1999

A method of the quantitative description of the broadening effect in atomic force microscopy (AFM) has been developed. The method allows one to restore real geometrical parameters of an object using two measured parameters of the AFM-profile (a height and a width at the half-height). An application of this method has allowed us to obtain the quantitative information about the molecular composition of the complex DNA–surface-active substance (SAS).

INTRODUCTION

The broadening effect is manifested in the fact that microobjects imaged by AFM have overstated lateral sizes. For example, this effect facilitates the molecule identification in AFM investigations of molecules of nucleic acids [1]: "broadened" molecules (a width of the profile of the DNA molecule is overstated 5–10 times) can be found easier at a shot with a large area that facilitates statistics collecting. By this fact, the broadening effect allows one to do without the additional contrast improvement (by uranylacetate etc.) of macromolecules in the investigation of nucleic acids.

The broadening effect is caused by the fact that the probe sharp point of a microscope has a finite radius of curvature. This instrumental error can hardly be overcome, since a decrease of the radius of curvature of the probe tip (the use of sharper probes) leads to an increase of the pressure in the contact region (at the same value of the contact forces). A greater pressure leads to greater contact deformations of the probe and the specimen, which increases both the lateral size of the contact area (limiting the reachable spatial resolution) and the radii of curvature of the contacting surfaces.

Contact deformations can be decreased when the specimen surface is observed in liquids, since in this case one can maintain the contact forces at the substantially lower level [2]. However, new problems can appear here, among which we mention the problem of fixation of the specimen at a solid substrate.

An additional mathematical analysis using model concepts about the geometry of the probe (a cone with a spherical tip, a paraboloid etc.) and *a priori* concepts about a shape of the investigation object are necessary to restore the real geometrical shape of the object with respect to its AFM-image.

An universal computer method of deconvolution of AFM-images has been proposed in the work [3]. The method includes two stages: the determination of the geometry of the used sharp point by test-objects and the convolution of the inverted geometry of the sharp point with the measured AFM-profile. This procedure allows one in many cases to restore the initial profile of the object with a high accuracy. This method was tested by solving the problem of restoration of the geometry of objects adsorbed on the surface of the plane substrate. The analysis has shown that lateral sizes of the object (a width at the half-height) restored according to the given method are substantially overstated under the condition that the radius of curvature of the object is less than that of the probe tip (the greater is the difference between the corresponding radii, the greater is the error). This circumstance complicates the applicability of the considered method for the investigation of biological objects (macromolecules, their complexes and others) because of small sizes of the latter as compared to the radius of curvature of the AFM probe tip.

A method of restoration of the volume of the investigated particle from the AFM-profile has been proposed in the work [4]. This method does not include the stage of the preliminary testing of the probe: both the geometry of the probe and the geometry of the investigated objects can be restored by the analysis of the same AFM-image. However, this method includes *a priori* assumption about a spherical shape of the investigated objects. This assumption is hardly justified when the problem of restoration of the geometry of biological objects characterized by low values of the elastic modulus is solved, because of the concepts about a substantial role of contact deformations in AFM investigations. The model allowing one to take into account the broadening effect, when the needle contacts with the deformed particle having an ellipsoidal cross-section, is more general. But, as far as we know, there are no works devoted to the application of this model for the analysis of experimental AFM-images, which is probably caused by algebraic difficulties appearing when the analytical solution of this problem is found.

STATEMENT AND SOLUTION OF THE PROBLEM OF RESTORATION OF A REAL WIDTH OF OBJECTS FROM THE MEASURED AFM-PROFILE

We applied the geometrical model to take into account the broadening effect (Fig. 1). This model takes into account the interaction of the object only with the probe tip (it is supposed that there is no contact with walls of the pyramid). This is justified in the case when a height of the investigated structures above the substrate does not exceed the radius of curvature of the needle tip.

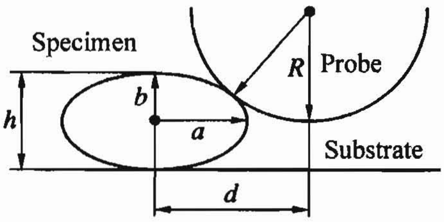

Figure 1. Geometry of the contact between the probe and the specimen. To the explanation of the broadening effect.

The probe tip is approximated either by a semi-sphere with a radius R or by a paraboloid of revolution (a cross-section of the needle-paraboloid is described by the relation $y = kx^2$, where k is the approximation coefficient). It was shown that the results of the application of both the methods are close to each other. A quantitative difference between them does not exceed 3–9%. It should be noted that the approximation of the needle by a semi-sphere is clearer and wider used in the literature. Below we present algorithms of both the approaches.

The investigated particle was described by the model of a oblate ellipsoid (with a, b and c semi-axes), i.e. we proceed from *a priori* concepts about the contact deformation of the specimen under the action of the probe. The problem was to find the value of a from the given values of b ($b = h/2$), R (or k for the parabolic approximation) and d (where d is the measured profile width of the AFM-image of the particle at its half-height).

Let us write the system of equations for an ellipse (a cross-section of the profile of the investigated particle) and circle (a cross-section of the profile of the needle):

$$\begin{cases} y_{\mathrm{ell}} = b\sqrt{1 - x^2/a^2} \\ y_{\mathrm{cir}} = R - \sqrt{R^2 - (x - d/2)^2}. \end{cases}$$

In the model of a parabolic needle, the second equation of the system is: $y_{\mathrm{par}} = k(x - d/2)^2$.

To solve the problem the following conditions for the contact point with the coordinates (x_0, y_0) were taken: lines tangent to an ellipse and to the circle (parabola) are equal and coordinates of the contact point satisfy the equations of an ellipse and circle (parabola):

$$\begin{cases} b\sqrt{1 - x_0^2/a^2} = R - \sqrt{R^2 - (x_0 - d/2)^2} \\ \mathrm{d}y_{\mathrm{ell}}/\mathrm{d}x|_{x_0, y_0} = \mathrm{d}y_{\mathrm{cir}}/\mathrm{d}x|_{x_0, y_0}. \end{cases} \tag{1}$$

In the model of a parabolic needle, the equation of a parabola is used in the system (1) rather than that of circle.

This system was analytically reduced to one equation with one unknown value x_0 for which an algorithm of a numeric solution was developed. Then the value a was determined from the found x_0. Write the equations for a spherical needle:

$$\left\{b^2 - [R - \sqrt{R^2 - (x_0 - d)^2}]^2\right\}\sqrt{R^2 - (x_0 - d)^2}$$
$$+ x_0(x_0 - d)[R - \sqrt{R^2 - (x_0 - d)^2}], \tag{2}$$

and for the determination of a from the found x_0:

$$a = \frac{x_0}{\sqrt{1 - [R - \sqrt{R^2 - (x_0 - d)^2}]^2 / b^2}}. \tag{3}$$

Analogously, for a parabolic needle, we have the equation relative to x_0 for the construction of the numeric solution:

$$k^2(x_0 - d)^3(x_0 + d) + b^2 = 0 \tag{4}$$

and the equation for the determination of a:

$$a = \sqrt{\frac{x_0(x_0 + d)}{2}}. \tag{5}$$

It was analytically shown that the equation (2) for a spherical needle has no a unique solution (at the corresponding range) only in the case when the following system of inequalities is fulfilled:

$$\begin{cases} R > d/2 \\ b > R - \sqrt{R^2 - d^2/4}. \end{cases} \tag{6}$$

Analogously, the equation for a parabolic needle (4) has no a unique solution (at the range we are interested in) in the case when the following inequality is fulfilled:

$$b > kd^2. \tag{7}$$

The meaning of these two limitations is obvious: if the AFM-profile is "sharp" enough, the needle by means of which it was registered should be so "sharp" as far as it is

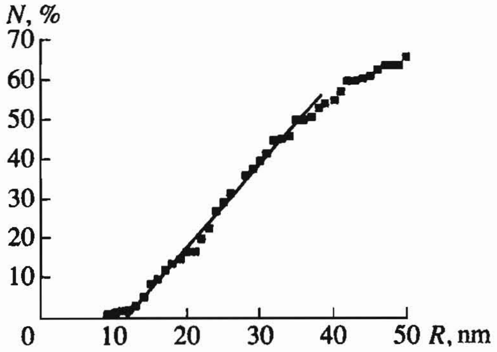

Figure 2. Dependence of the number N of the cases when the solution is absent on the radius of the needle approximation. Test of the satisfiability of the condition (6).

determined by relations (6) and (7).

Equations (2)–(5) were used for the numerical solution. Since the problem was solved numerically, it would be possible to try to use directly the system (1), reducing it, for example, to the system of linear equations. However, it turns out that the specific character of the system does not allow one to construct in this case the simple enough numerical solution, since the deviations from the precise solution, which are as small as one likes, lead to negative values in the radicands entering the system to be solved. By this fact, the proposed method is simple enough in spite of the necessity of preliminary analytical calculations. Besides, it has an important advantage: the test of the realization of the conditions (6) and (7) allows one to determined the cases of the absence of the solution in advance.

This test allows one to extract the additional and very important information about the properties of the probing sharp point: to determine the upper limit for the values of the radius of curvature R of the tip (the lower limit for the parabola coefficient k, respectively).

Determination of the precise value of the radius R (or k) for an individual probe requires its testing directly before the use (by means of test-objects, for example by virus particles [5]). However, in this case there is also the probability that in the scanning process the shape of the needle will be changed as a result of the interaction with the object. In this connection it is useful to obtain the information about the shape of the probe directly from AFM-images of the object under investigation.

The limiting value of R (or k), above (or below) which the number of cases of the absence of solution increases, can be found by statistics collecting of the height and width parameters of the AFM-image profile of the investigated objects and the further analysis of whether the relations (6) and (7) can be satisfied for the statistics collected. This will be the upper limit for the evaluated radius of curvature of the needle (Fig. 2). The lower limit for the radius of curvature of the probe is determined by contact deformations.

Concerning applicability of the developed method. It should be outlined that the developed method does not take into account the possible additional contribution into the broadening caused by the partial increase of the specimen by the probe during scanning.

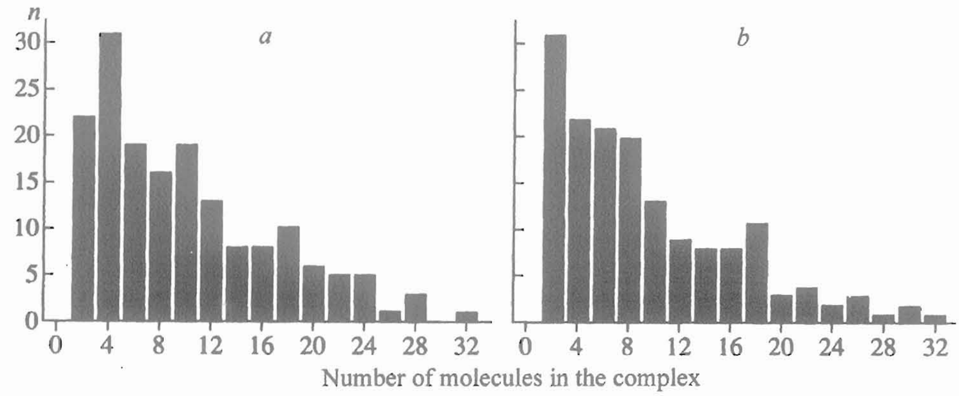

Figure 3. Histograms of the distribution of the number of DNA molecules entering the complexes with surface-active substances, with radii $R = 6$ (*a*) and 12 (*b*) nm; n is the number of the complexes.

This effect is caused by lateral forces of the interaction between the probe and the specimen which are characterized by the significant intensity when the investigations are carried out in the contact mode in the air even under minimization of normal forces. According to our evaluations, this effect can mainly be manifested in investigations of objects having a small value of the cross-section area (for example, single DNA molecules) and lead to 1.5–2.5-fold overstating of the value of the width d of the object and, as a consequence, of the restored value a.

The degree of the increase of the specimen by the probe can decrease if the intensity of the lateral force influence of the probe decreases. This is achieved by applying the mode of the discontinuous contact. The maximal effect of the decrease of lateral forces is achieved in measurements in liquid media.

APPLICATION OF THE DEVELOPED ALGORITHM FOR RESTORATION OF MORPHOLOGY OF DNA-SAS COMPLEXES

We applied the developed method for the restoration of the geometry of DNA complexes with surface-active substances (SAS) passed through the water/chloroform interface [6]. The tore diameter, the width of the profile at the half-height and the height above the substrate were measured for each toroidal particle. The mean values of these parameters were $D \sim 100$ nm, $d \sim 25$ nm and $h \sim 5$ nm. The two latter values were used in the restoration of the true width $2a$ of the particle profile according to the method described.

The graph of the dependence of the number of cases, when the solution is absent, on the radius of the approximated probe obtained by testing the fulfillment of the conditions (6) and (7) is presented in Fig. 2. One can conclude that the upper limit of the value R characterizing the probe used in visualization of the complexes is 12 nm (which corresponds to $k = 5.5 \times 10^{-2}$ nm^{-1}). The linear rise of the number of the cases, when the solution is absent, is observed above this value.

We counted the number of DNA molecules per each toroidal structure by using two values of R: 6 and 12 nm (which correspond to $k = 13.2\times10^{-2}$ and 5.5×10^{-2} nm^{-1} respectively). Mean values of the parameter a (found numerically) for these cases are 11 and 10 nm, respectively (a relative deviation is $\varepsilon = 0.5$). The results of the application of the parabolic model give, as a rule, the values greater by 3–9%. The corresponding numeric solutions in the considered case are 12 and 11 nm. Thus, we have shown that an oblate tore is the shape of the DNA-SAS complex. Restoration of the geometry of the complex allows one to analyze quantitatively its molecular composition (Fig. 3).

The authors are grateful to O. A. Pyshkina and A. S. Andreeva (Chemical Department, Moscow State University) for the help in the preparation of the specimens of the DNA-SAS complexes. The work was supported by the Russian Foundation for Basic Research, grant No 97-03-32778a.

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