The limit of mass determination with an AFM cantilever-based system

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Abstract. The application of an atomic force microscope (AFM) based microcantilever system for the determination of mass of gold nanoparticles (AuNPs) has been demonstrated. In this system, standard AFM microcantilevers for measurements in vacuum have been employed. The limit of mass determination with our AFM-based system has been determined to be of the order of $10^{-10}$ g. The prospects of employing AFM cantilever-based sensors for highly sensitive protein detection in proteomic studies and in diagnostics have been discussed.

1. Introduction
Nowadays, modern nanotechnology-based analytical systems employing molecular detectors, such as atomic force microscope (AFM), are being developed. Molecular detectors (AFM, nanowire sensors [1-3], etc.) are capable of detecting single target particles of nanometre size (such as nanoparticles [4], viral particles [2,5,6], and even individual biological [7,8] and synthetic [9] macromolecules). In molecular detectors, such a high sensitivity is achieved owing to the small size of the sensor element, which is comparable with that of the target molecule. Among nanotechnology-based systems, one can distinguish nanomechanical cantilever-based ones, which allow one to operate with condensed matter at the nanoscale, thus enabling determination of various characteristics of the investigated objects (mass [10], elasticity [8], etc.) at the level of single macromolecules or nanoparticles. In these systems, the target objects are adsorbed from the volume of analysed sample onto the cantilever, and the resonance frequency of the AFM cantilever (which decreases in a proportion to the added mass) is measured [11]. The adsorption of target objects increases the mass of the cantilever; this causes a shift in the resonance frequency of the cantilever, which is measured by AFM electronics. That is, in cantilever-based sensors, the mass of the particles adsorbed onto the cantilever is converted to the oscillation frequency signal. As target objects, nanoparticle-labelled single molecules of protein markers of diseases can be used [11]. The latter opens the prospects for employing AFM-based sensors as highly sensitive detectors in proteomic studies and in diagnostics [11]. In this connection, it is to be emphasized that the application of highly sensitive nanotechnology-based detectors in diagnostics is crucial, since the concentration of many clinically relevant marker proteins in the biological material is very low [12].

For the determination of mass at the nanoscale, several types of cantilever-based systems can be employed. In the paper by Naik et al. [13], the application of nanometer-sized cantilevers is discussed. Such systems exhibit an extremely high mass determination sensitivity of the order of $10^{-18}$ g. At the same time, the extremely small size of such cantilevers leads to long time required for the diffusion
transport of target objects to the sensor surface; this fact limits the use of nanometer-sized cantilevers. In the study by Kosaka et al. [11], standard AFM cantilevers were employed. The use of standard cantilevers is much more convenient, as it allows one to perform the detection of nanoparticle-labelled protein molecules in just one hour [11]. Measurements in air, however, require additional processing of the signal to reduce the influence of noise on the determination of resonance frequency [11]. In this regard, measurements in vacuum are preferable, since the influence of noise is reduced.

In our present study, the sensitivity of a microcantilever system employing standard AFM microcantilevers for measurements in vacuum, has been estimated. We have demonstrated that such a system allows one to register ~100 gold nanoparticles (AuNPs), what corresponds to 17 µL of 10^{-17} M solution of protein labelled with these nanoparticles. This approach is convenient, because no additional equipment (such as nanobalance [10,14]) is required to measure the mass of the adsorbed protein.

2. Methods
To measure the resonance frequency shift caused by adsorption of gold nanoparticles (AuNPs) onto the cantilever, the following technique was employed. AFM cantilever (PPP-CONTR-50, Nanosensors Inc., USA; resonance frequency 6 to 21 kHz; height 450±10 µm; width 50±7.5 µm; thickness 2±1 µm; force constant 0.02 to 0.77 N/m), which resonance frequency was measured prior to the experiment, was incubated in a ~1-µL drop of solution containing 20-nm-diameter AuNPs (synthesized using a citrate reduction method [15]). The incubation time was 5 and 15 min. After the incubation, the cantilever was dried and placed into the vacuum chamber of an NTEGRA Aura AFM (NT-MDT, Zelenograd, Russia), and its resonance frequency was measured and compared with that before the incubation. All measurements have been performed in vacuum at 0.1 Torr.

The mass of the AuNPs adsorbed onto the cantilever was estimated from the resonance frequency shift according to Kosaka et al. [11]:

$$\Delta m = \frac{2m(f_i - f)}{f},$$

where $f_i$ and $f$ are the resonance frequencies of the cantilever before and after the incubation, respectively, $m$ is the mass of the cantilever, and $\Delta m$ is the mass of the adsorbed AuNPs.

Therefore, given the mass of a single AuNP, the total number of the AuNPs adsorbed onto the cantilever can be determined. The size of AuNPs and the number of AuNPs adsorbed onto the cantilever were estimated by scanning electron microscopy (SEM) visualization of the cantilever with adsorbed AuNPs employing a Hitachi S5500 electron microscope (Hitachi, Japan).

Since the resonance frequency shift for a 20-kHz cantilever, registerable with the system employed in our study, is 20 Hz (which amounts to 0.1% from 20 kHz), the mass of the adsorbed AuNP causing this shift must be 1000 times smaller than that of the cantilever itself. Let us estimate the mass of the silicon (of 2.33 g/cm³ density) cantilever:

$$m = \rho V = 2.33 \text{ g/cm}^3 (450 \times 50 \times 1 \times 10^{-12}) \text{ cm}^3 = 5.24 \times 10^{-8} \text{ g}$$

For such a cantilever, 0.1% mass increase ($\Delta m/m=0.001$) corresponds to $\Delta m$ of the order of 10^{-10} g. Since the masses of 20-nm and 100-nm AuNPs (of 19.3 g/cm³ density) make up ~10^{-16} g and ~10^{-14} g, respectively, the number of these AuNPs causing $\Delta m=10^{-10}$ g corresponds to ~10⁶ (for 20-nm AuNPs) and ~10⁴ (for 100-nm AuNPs).

3. Results and discussion
Figure 1 displays the data obtained in our experiments. The resonance frequency curves shown in Figure 1a clearly indicate that adsorption of 10⁶ of 20-nm AuNPs causes a 20-Hz shift in the cantilever’s resonance frequency (which is in a good agreement with theoretical estimations) in contrast to the case with 10⁴ of 20-nm AuNPs, when no shift in resonance frequency was registered (Fig. 1b). The number of adsorbed AuNPs was altered by varying the cantilever incubation time in AuNP-containing solution.
Figure 1. The resonance frequency curves of the AFM cantilever obtained before (red curves) and after (blue curves) their incubation in AuNP-containing solution, and corresponding SEM images of single AuNPs adsorbed onto the cantilever surface, in the case of adsorption of $10^6$ (a, c) and $10^4$ (b, d) 20-nm AuNPs.

Typical SEM images of the cantilever surface with adsorbed AuNPs presented in Figure 1c,d confirm the data obtained in the resonance frequency measurements. Indeed, in Figure 1c (illustrating the case with the adsorption of $10^6$ 20-nm AuNPs onto the cantilever surface) one can clearly distinguish a number of AuNPs (seen as white spots in the SEM image), which tend to form multiparticle aggregates on the cantilever surface. On the contrary, only a few (<10 particles per ~25 μm$^2$ image) single AuNPs are distinguishable in Figure 1d corresponding to the adsorption of $10^4$ 20-nm AuNPs onto the cantilever surface. In the latter case, a small amount of AuNPs adsorbed on the cantilever surface is apparently insufficient to cause a registerable shift in the cantilever’s resonance frequency.

4. Conclusion
Due to high sensitivity of mass determination, the use of cantilever-based method for protein detection in biomedical applications is promising. To date, modern techniques allow labelling protein molecules with AuNPs in 1:1 ratio [12]. This fact gives an opportunity for the detection of such labelled protein molecules with high sensitivity. In this way, using 20-nm AuNPs for this purpose, the $10^{15}$ M protein detection limit can be attained upon analysis of 1 mL of sample. Moreover, if 100-nm AuNPs will be used instead of 20-nm ones, the detection limit of labelled protein molecules can be shifted down to $10^{17}$ M.

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