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Specialized nanowhisker probes for high-precision investigation of native bio-objects in liquid by means of atomic force microscopy

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Abstract. Fabrication and investigation of specialized Pt-C single nanowhisker probes are carried out for high-precision study of objects with organic and inorganic nature in liquids by atomic force microscopy. The best modes and buffered media were revealed on calibration lattice TGQ1 choosing as reference inorganic structure. Preliminary results of the study of the structure of bacteria E.Coli in the native state were received in the PBS liquid. Fixation of the bacteria was performed on agar-agar with a mass fraction of 1.8%. The significant improvement of image contrast and resolution was found when using nanowhisker probes compare to standard Si tips.

1. Introduction

The studies devoted to investigation of inorganic and biological objects (cells, bacteria, viruses, proteins, DNA, etc.) in a liquid by atomic force microscopy (AFM) are of great interest nowadays [1, 2]. This is explained by the possibility to carry out precision «in situ» studies of biological objects in the native state with nanometer resolution. It's observable that reliable interpretation of the features of investigated native bio-structure greatly depends on the resolution ability of the method used.

The principal element in AFM is a probe (needle or tip) since the resulting image presents a convolution between geometric parameters of the probe and the surface of the sample [3]. Therefore, it is necessary to fabricate a new tool to ensure high-precision investigation of such objects with great accuracy. To improve the accuracy of AFM investigations the research works are carried out to develop the new types of probes based on nanowhisker structures [4, 5]. Such probes have a high...
aspect ratio, strength and stiffness with a small transverse dimension [6] that makes them valuable for probes application.

Therefore, the main aim of this article was a fabrication and approbation of the probes with nanowhiskers for high-precision research of native organic bio-objects in liquids by atomic force microscopy.

2. Experimental setup
The Pt-C nanowhisker structures (NW) were grown at the tip of the standard probes under the electron beam in a high vacuum and injection system of precursor gases [6]. Fabrication and geometries control of the NW were performed by scanning electron microscope (SEM) CrossBeam Neon 40 (Carl Zeiss, Germany) with integrated inlet gas system C₉H₁₆Pt.

The investigation of probes in selected liquids (PBS - phosphate-buffered saline, NaOH 0,1M/0,5M) was carried out in semi-contact mode. The investigation of the samples was performed by means of scanning probe microscope Ntegra Aura (NT-MDT, Russia). The studies were conducted on SNL-10 tips (Veeco, USA).

3. Results and Discussions
The NW probes with a length of about 400-600 nm and diameter of about 30-40 nm was fabricated with tip radius of curvature less than 10 nm. NW probes retain their configuration after 2-3 cycles of scanning, which is comparable with standard Si probes (Fig. 1). NW was produced by an electron beam in a vacuum in the presence of the precursor gas C₉H₁₆Pt [7]. Composition of NW is 30% of platinum Pt, 70% are residues of the precursor gas and carbon particles. NW were made at an angle of inclination relative to the tip axle about ~ 22-25 degrees to reduce possible vibrations when a double-tap contact with the surface (peculiarity of the tip holder).

The best solution for measurement was revealed as sodium phosphate buffer (PBS). The improvement of height differences visualization in 3-4 times was revealed on calibration grating TGQ1 using NW probes compare to standard probes (from 3.9 nm to 18.7 nm on the X axis, from 37.3 to 11.8 nm in Y axis). The surface roughness using NW probes was more than such at standard probes (Ra = 0,317 nm for the standard probes, Ra = 0,887 nm for NW probes), that indicating enlargement of penetrating ability of probes. The improvement of resolution using NW probes in NaOH buffers was found as well, but alkaline features of this media can defect upper layers of biological samples during the scanning process.

Figure 1. SEM images of the cantilevers with NW at the tips of standard Si probes obtained after scanning of the liquid medium (front and side view).
Figure 2. CLSM image (a) and AFM image (b) of E.Coli bacteria groups on a mica substrate with agar 1.8% in PBS buffer.

For immobilization of native bacteria E.Coli gelatin and agar-agar substances were used. Investigation of fixation results was carried out by confocal laser scanning microscopy (CLSM) by TCS SL (Leica, Germany). It was revealed that agar-agar with a mass fraction of 1.8% as an initial solution on a mica substrate provide best immobilization of the bacteria (Fig. 2).

Figure 3. AFM image of a single bacteria E.Coli and cross-section obtained by standard probe (a) and NW probe (b) in PBS buffer.

In the study of individual bacteria E.Coli the significant improvement of contrast and resolution was found using NW probes (Fig. 3). Thus, on the cross-sections derived from the specific single bacterium E.Coli in the same place, the increase of height differences (penetration ability) with NW probes (200 nm) and imaging of thin nanoscale structure along the bacterium (left peak on Fig. 3b) was observed. Presumably, the structure can introduce group of fibers around bacterium E.Coli. The standard probes cannot allow to reveal this particular nanoscale structure.

For detailed imaging and studying of fiber structure around E.Coli the single bacterium was visualized by NW probe (Fig. 4, a), and a longitudinal section was derived along the studied structure.
It was found that the size of such structures comprises a value of about 400-500 nm in diameter and 20-30 nm in height. However, it should be mentioned that the measurements were carried out in the liquid and such structures can rapidly adhere together in these environment. In addition, AFM image always represents the convolution of the probe and the surface being studied (fibers), which can be smaller compared to the dimensions of the probe.

![AFM image of single bacteria E.Coli (a) and the sectional structure along the fibers (b) obtained by NW probe in PBS buffer.](image)

The investigation shows that the application of specialized SNW probes has better results than standard silicon probes in the study of native organic and inorganic objects in liquids by atomic force microscopy.

4. Conclusions

Thus, application of probes with NW in liquid media improves the spatial resolution and contrast of the images in the study of native biological samples, in particular, the native bacteria E.Coli. The results obtained in this study can be used for a more detailed and accurate study of native objects with complex structure in liquid by means of atomic force microscopy.

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