

# Evaluation of Tools for Nanoparticles-modulated Hyperthermia by Atomic Force Microscopy

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## ABSTRACT

In 2004-2005 authors carried out the experiments [1] which make it possible to expect increase in the effectiveness of the tumor treatment by the method of percutaneous radiofrequency ablation (RFA): the tissue-damaging effect of RFA-induced hyperthermia does increase in the presence of nanoparticles.

This article told how Atomic-Force Microscopy (AFM) can be used for evaluation and characterization of nanoparticles supposed to be the modulators of hyperthermia and for studying of dynamics of an electrodes surface change.

**Keywords:** AFM, hyperthermia, percutaneous radiofrequency ablation, nanoparticles

## 1 AIM

Any acting nanoparticles, including ones, supposed to be used as hyperthermia modulators, must be introduced into an organism in the form of medicinal formulation, which should ensure:

- 1./ Stable concentration to guarantee the dosage control.
- 2./ Protection from seizure and neutralization by the natural shielding forces of organism.
- 3./ For the nanoparticles the special importance acquires the need of averting the formation of conglomerations.

Besides, it is necessary to evaluate the dynamics of a change in the expensive electrodes, used for the hyperthermia induction, surface properties.

Some of the enumerated properties can be evaluated by measurements with the aid of the AFM: sizes and concentration of nanoparticles, their capability to form the conglomerations, the dynamics of a change in the electrode surface properties.

The aims of the work were: the development of the methods of this estimation and their approbation based on several configurations of hyperthermia-modulating tools.

## 2 MATERIALS AND METHODS

AFM-system NTEGRA Prima, NT-MDT Co., Zelenograd, Russia.

Particles of rhenium sulfide and particles of silver with sizes of 80 and 120 nanometers, dispersed in different gels. Electrodes and temperature-sensitive elements for the universal complex for the RFA called "Metatom-2" manufactured by "Technosvet", Russia.

*Development or selection of the specimen preparation methods*

The suspension of nanoparticles in viscous fluid or gel was placed by thin layer to the microscope slide and was investigated by the AFM in the semicontact regime. It turned out that preparations on the basis of dextran and glue formed the steady structures, which could be repeatedly scanned with the reproducible results.

*The estimation of the regular flat structures sizes*

This was accomplished by repeated measurements of their linear dimensions (special function of regular NTEGRA-system software). The second method of evaluating the sizes of flat formations consisted in the measurement of the linear dimensions "of the disagreement zones" or, in other words, the zones of the artifacts appearance. The fact is that before scanning the special semiautomatic procedure selecting the parameters of the Cantilever fluctuations is carried out. With a substantial change of the surface properties during scanning parameters selected did not ensure shaping of the correct relief of surface (appearance of the artifacts in the form of pyramids, figure 1).

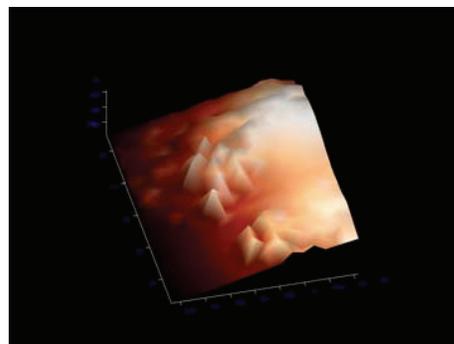


Figure 1: Example of "disagreement zone". Such sections treated as the zones of the protrusion of the solid nanoparticles above the surface of viscous phase.

Obviously, it is impossible for such sections to determine the height of the peaks, since the mentioned pyramids reflect not the components of surface, but the internal processes of the scanning system auto-oscillations.

*Estimation of the vertical sizes of formations*

The real height of formations is not equal to the height  $h1$  of the highest point of formation above the level of colloid. For its estimation it is necessary to determine the thickness of colloid  $h2$  and to add it to  $h1$ . We have found the areas with destroyed colloid (figure 2),

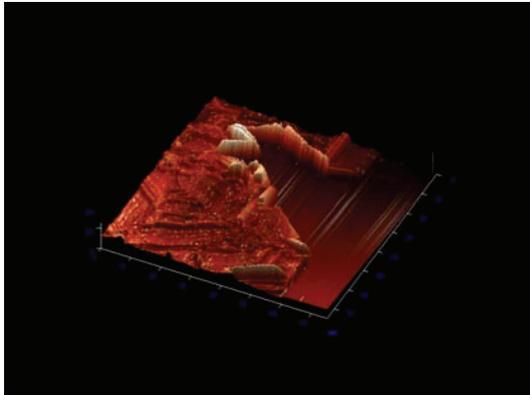


Figure 2: Area of destroyed colloid suitable for determination of colloid level.

where  $h2$ -estimation is possible in the sections (figure 3).

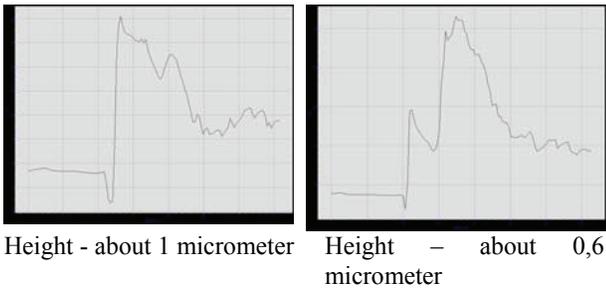


Figure 3: Determination of colloid thickness.

*Estimation of the internal structure of formation*

Characteristic vertical and horizontal dimensions must be close to each other for the solid structures, while for the conglomerations the horizontal sizes are expected to predominate above the vertical (figure 4).

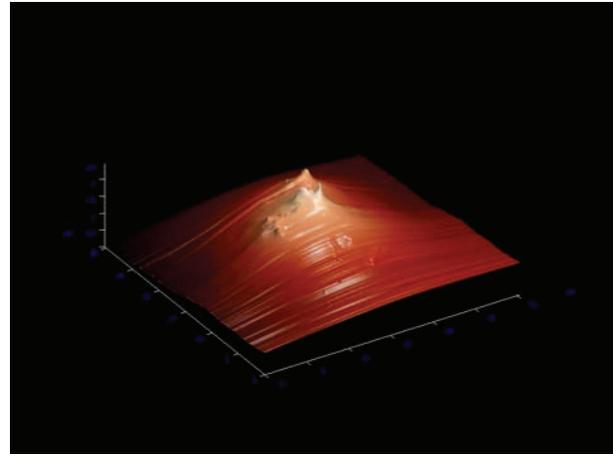


Figure 4: Example of conglomeration. Horizontal sizes are about 20 micrometers, vertical sizes – about 2 micrometers.

*Estimation of the distances between the formations.*

The minimum distance between the formations correlates with their sizes and can be determined by regular software of the NTEGRA system during image processing.

**3 ESTIMATION OF ELECTRODE SURFACE DYNAMICS**

The presence of two sets of the parameters, which characterize its form, is the special feature of object of study (the electrode).

The first one defines the long tube with diameter 1-2 millimeters, being seen by the naked eye its surface appears to be smooth.

The NTEGRA system does not have equipment for fastening of this type of specimens (and this should be considered as a deficiency in the context of the conducted study). This is the reason why during each scanning the larger or smaller (always random) inclination of the subject of studies relative to the plane in which cantilever moves has been demonstrated. The circumstances named lead to the appearance of the regular artifacts (illustrated in figure 5) during each scanning.

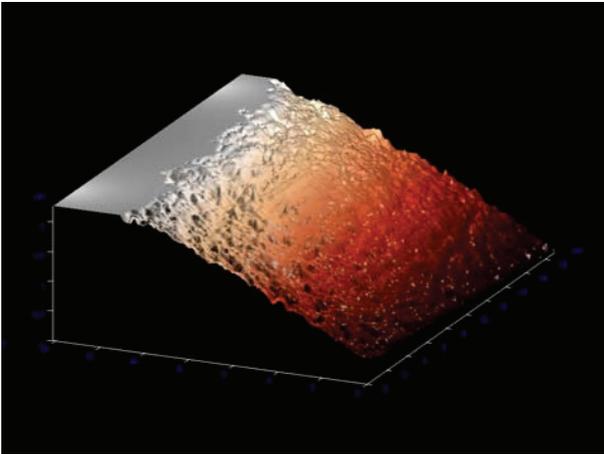


Figure 5: Regular artifacts: 1. Plane above illustrates the exit of the scanning system beyond the limits of linear interval because of too large difference in the heights of the different points of specimen. 2. The decrease of flat surface width occurs because of nonstandard fastening of model (inclination in the direction of the surface expansion).

The second set of the parameters characterizes the fine structure of the electrode surface, whose change under the influence of repeated cycles of application - sterilization is the object of study. The image of inclined fragment in figure 4 is apparently realistic taking into account the difference of vertical and horizontal scales. However, it is easy to note that the thin details, supposed to be the object of study, are located not on the plane, but on the complex surface, which is the part of the tube of electrode.

Software tools of NTEGRA system can convert complex surface into the plane. In particular, figure 6 shows the surface from figure 5, scanned in the regime “Subtraction of 4th order curve”.

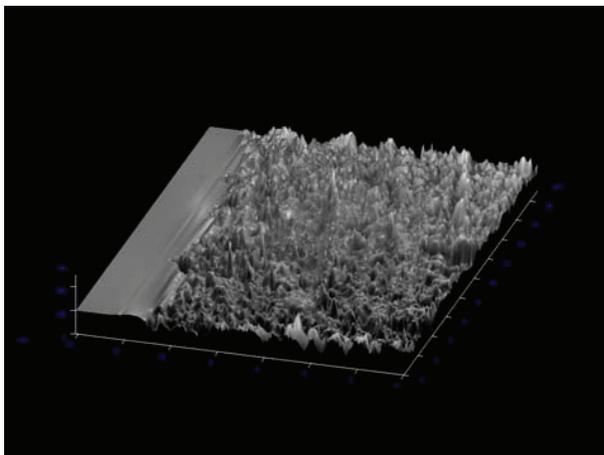


Figure 6: The surface from figure 4, scanned in the regime “Subtraction of 4th order curve”.

After scanning specimens in the regime mentioned the following parameters which characterize the fine structure of the surface were calculated:

- Rq - Root mean square roughness, ISO 4287/1, nm;
- Ra - Average roughness, ISO 4287/1, nm;
- Rz ten point height, ISO 4287/1, nm. The parameter expresses surface roughness by the selected five maximal heights and hollows, nm.

Tables 1 and 2 present the obtained characteristics of two examples of electrodes, obtained in three stages. First stage - immediately after removing from factory package (columns 1 in both tables), second stage - immediately after the first use for the RFA - induction (columns 2 in both tables), third stage - after the first sterilization of electrodes (columns 3 in both tables).

	Stages of lifecycle of electrode		
	1	2	3
Rq	66,99	123,91	67,29
Ra	51,3	91,81	46,80
Rz	948,25	2057,79	599,05

Table 1: Surface dynamics of the first electrode.

	Stages of lifecycle of electrode		
	1	2	3
Rq	28,79	444,40	48,47
Ra	21,0	346,01	35,37
Rz	164,61	2237,32	412,50

Table 2: Surface dynamics of the second electrode.

The substantial increase in all the roughness parameters was noted after use of electrodes.

- All parameters, which characterize the roughness of the surface of electrode, increased after its use for RFA-induction.
- After electrode sterilization the parameters of roughness of the first electrode surface practically returned to the initial values (prior to the beginning of use). The roughness parameters of the second electrode also substantially decreased but remain above initial.
- It is possible to guess that the elements, which determined the high roughness of the surface of electrode, appeared after the first use, disappeared as a result of sterilization (fragments of the carbonized tissues?).
- If the previous thesis is correct, then the roughness of the surface of electrode after sterilization can characterize the quality of the sterilization

## 4 CONCLUSIONS

In the work are proposed the methods of evaluating the sizes of the nanoparticles, dispersed in the viscous carriers, and also the estimations of the internal structure of larger formations.

The electrode surface dynamics discovered in our studies has obvious interpretation and corresponds to the expectations.

Methods are approbated based on several configurations of hyperthermia-modulating tools.

## REFERENCES

- [1] V.I. Chissoy, I.V. Reshetov, P.N. Luskinovich, A.A. Shepelev, G.D. Efremov, O.V.Matorin, S.S. Sukharev. The Evolution of Views of in vivo Use of Nanoparticles for Tumor Treatment. Technical Proceedings of the 2005 Nanotechnology Conference and Trade Show, Nanotech2005, vol. 1, Pages 31-34