

Scanning Probe Study of Human Blood Mitochondria Immersed in Sucrose Buffer Solution on Mica Substrate

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Introduction

More than twenty years have passed since invention of the scanning tunneling microscope (STM) in 1981 [1]. During this time STMs and other probing tools developed and improved to great extent. In 1986, an atomic force microscope (AFM) has been constructed, gave rise to yet one a very important breakthrough in this field of science and technology [2].

Starting from the second part of 90-th, new multifunctional microscopes have been brought into practice, being capable to operate not only as STMs or AFMs, but also using a lot of quite new physical principles, such as probing of electric, magnetic and van der Waals forces, inelastic tunneling, etc. To date, science has in disposal the third generation of the scanning probe microscopes (SPMs) having immeasurably more operation regimes and facilities comparing with the first generation of STMs.

A leader in manufacturing of this type microscopes in Russia is NT-MDT Corporation (Molecular Devices and Tools for Nano Technology), whose collaboration with this work has been done.

Despite that of paramount importance in applications of SPMs traditionally are solid state physics, microelectronics and micromachining technology, much efforts have been done to employ these devices in biology and medicine since the very beginning [3].

An attractiveness of the involved methods is due to high resolution and small destructive influence on a sample, being of main advantage in comparison with conventional electron microscopy.

Besides, the electron microscopy needs a special preparation of a sample, whereas an object is being studied practically in its native state if use is made of SPMs. A difficulty in using SPMs to biology and medicine problems is that the corresponding samples have much less conductivity as compared with metals and semiconductors, when a resolution of the order of 0.1 nm (atomic-scale resolution) is a matter of fact.

Also, biological objects have lower order of symmetry and prove to be chemically inhomogenous. However, despite these difficulties, a lot of interesting biological objects

have been imaged to date, using SPMs: DNA, enzyme, fragments of plasmatic membranes, eucariotic and procariotic cells, viruses, etc. [3,5,6].

In this work, for the first time, topography- and phase-contrast images of mitochondria have been obtained, being extracted from mononuclears of fresh human blood.

Experimental details

Using fresh human blood, a fraction of mononuclears has been extracted by centrifugation in a density gradient of ficoll-urographin [7]. Further, a mitochondria suspension has been removed from the cell deposition using a standard sucrose buffer solution (0.25 M tris-HCl and 1mM EDTA, pH 7.4) and method of differential centrifugation [7]. Noncompletely destroyed cells and nuclei were removed by centrifugation of 1000g, 10 min, 4⁰ C. To precipitate the mitochondria, a supernatant was subjected to centrifugation of 10000 g, 15 min, 4⁰ C.

Furthermore, the mitochondria deposition was resuspended in 100 μ L 0.25 M sucrose solution. All the stages have been done on ice. The mitochondria availability has been controlled by succinatdehydrogenase reaction [7]. The transportation has been done in ice during 2.5 hours.

A serial scanning probe microscope P-47-N (NT-MDT Co.) has been used to study the mitochondria. The suspension was placed on a mica substrate and adsorbed mitochondria were studied in a semicontact SPM regime (in liquid) at normal atmosphere and room temperature. The semicontact mode in liquid is more preferable as it does not practically exert any destructive influence on such objects.

The triangle cantilevers were used with the frequency of 10.5 kHz (in liquid) and the force constant of about 0.1 N/m. As a result, we have obtained steady topographic images and phase contrast images (Fig.1) which can be attributed to structure of internal membrane of mitochondria. We see that the images look like “beans” having dimensions 0.5x0.5 microns, and having a folded intrinsic membrane, characterized by the reiteration period of 0.2 micron. These images are in close agreement with current conceptions about mitochondria structure.

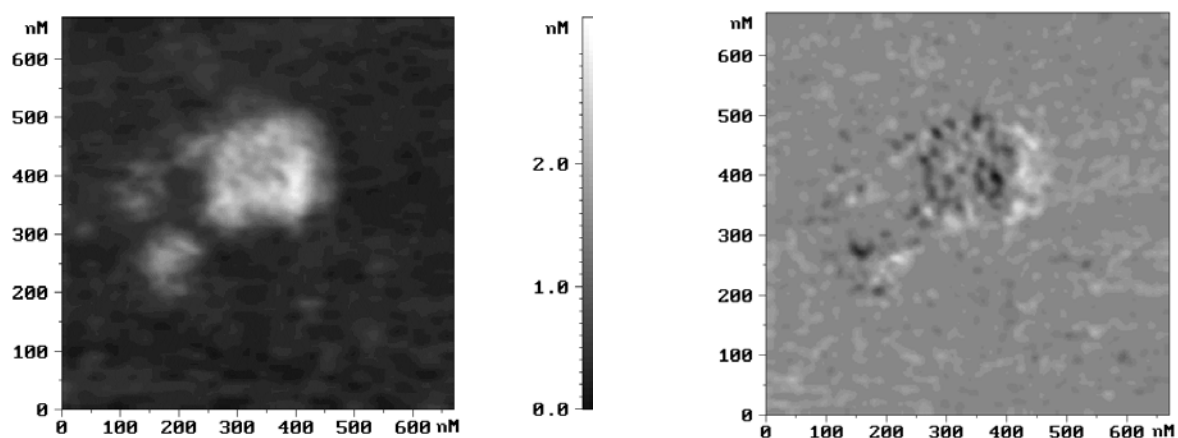


Fig.1 Topography (left) and phase (right) AFM images of human mitochondria in liquid.

Topographic (left) and phase (right) contracts of good quality were observed of mitochondria, which look like «beans» having dimensions 0.5x0.5 microns, and having a folded intrinsic membrane, characterized by reiteration period of 0.2micron.

Conclusion

As far as we know, we were probably the first succeeded in obtaining the human mitochondria images in liquid. The method of the sample preparation has been developed and optimized for the SPM study. In future, it allows us to continue investigation of changes relevant to the mitochondria evolution during different physiological and pathological processes both inside the cells and inside an organism as well.

Acknowledgements

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References

- [1] G. Binnig , H. Rohrer, Surface Sci. 126(1/3)(1983)236.
- [2] G.Binnig, C.F.Quate, Ch.Gerber, Phys. Rev. Lett. 56/9(1986)930.
- [3] P.K.Hansma, V.B.Elings, O.Marti, C.E. Bracker, Science, 242(1988)209.
- [4] M.S.Haikin, A.P.Volodin, A.M.Troyanovskii, V.S.Edelman, Priory i tehnika eksperimenta, 4(1987)231.
- [5] M.Radmacher, M.Fritz, H.G. Hansma, and P.K.Hansma P.K.,Science 265(1994)1577 .
- [6] R.Lal , B. Drake , D. Blumberg , D.R. Saner , P.K. Hansma , S.C. Feinstein, Special Communication (1995) 275.
- [7] Metody biologicheskikh issledovaniy, Ed. Prokhorova M.I., Publ. House of Leningrad University, 1982, pp.29-33; 207-212.