Local elastic properties of biological materials studied by SFM

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The special methods of quantitative characterization of elastic properties of animal and human erythrocytes with atomic force microscopes (AFM) Solver P47 and Solver BIO were developed. The best AFM images in air were obtained using the semicontact resonance mode of measurements (Fig.1a). The highest resolution (10 nm) was achieved in air with a phase contrast mode, which allowed one to visualize the globular structure of the erythrocyte membrane (Fig.1b). Statistical analysis of more than 30 cells on the same AFM image showed that the relation between erythrocytes with different shapes (diskocyte, spherocyte, erythrocyte like as starfish) depends on the species of a test animal and changes from one to another test mammal of the same species.

Elaboration of immobilization methods for localization of the separate live erythrocytes on the glass substrates in liquid are being continued using poly-L-lysine. Poly-L-lysine provides the accurate localization of the erythrocytes on the glass in liquid due to electrostatic interaction between negative charged erythrocyte surface and the positively charged poly-L-lysine surface molecules. This method will be used for observation by AFM of erythrocytes and others cells in the buffer solution with a given pH level (in other words in the conditions close to natural). Solver BIO allows one to obtain the images of cells with atomic force and optical microscopy at the same time. It is important that it is possible to use the semicontact resonance mode of AFM measurements in air and liquid with Solver BIO. This makes possible more detailed observations of cells in air and liquid using atomic force microscopy and spectroscopy.

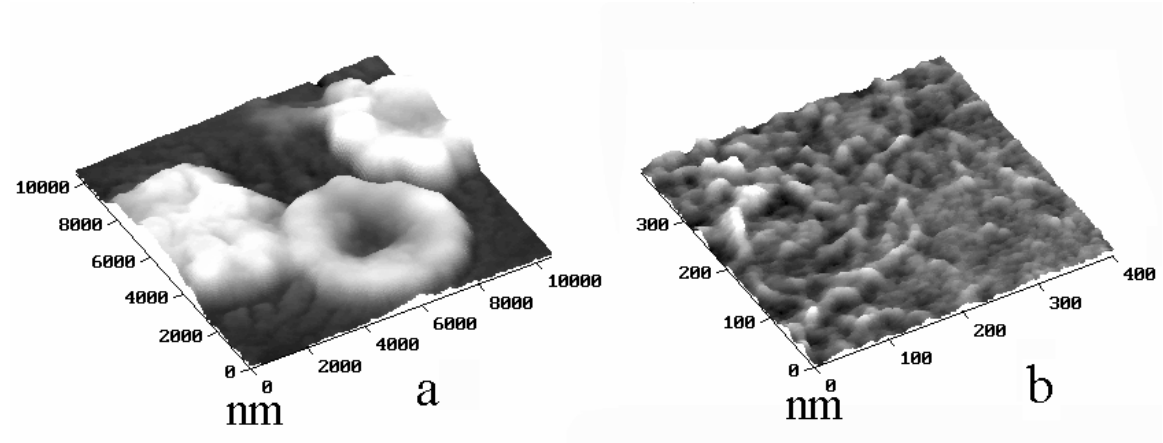

Fig.1. a – AFM images of human erythrocytes obtained in air,
b – AFM image of erythrocyte's membrane obtained with the highest resolution using a phase contrast mode.

When studying live erythrocytes in buffer solutions with a given pH by AFM, the high mobility of the erythrocyte capsule led to smearing of the obtained image (Fig. 2a). Therefore the maximal space resolution was about 200 nm.

The method for determining the quantitative characteristics of cells changing under the impact of external factors is being improved further on. Scanning force spectroscopy (SFS) proved to be the most promising method for studying biological micro-objects. It provides a unique possibility to directly measure the local elastic properties of the cytoplasmic membrane of live cells. The curve of deflection of the cantilever contacting a surface enables

one to measure the strength of the interaction of a tip with a surface and to determine the Young's modulus of the cell taking into account the elasticity constant of the cantilever. The details of obtaining the Young's modulus for the cell are described in our other report (*"Measuring local elastic properties of cell surfaces and soft materials in liquid by atomic force microscopy"*) presented at this symposium.

We have also obtained spectroscopic data characterizing the change in the amplitude of the vibration of the cantilever due to its interaction with the erythrocyte or the glass substrate. Analysis of these curves evidences that this procedure enables one to control elastic properties of erythrocytes and other soft material in liquid medium, but quantitative values of the corresponding Young's modulus are not yet obtained on the base of these data.

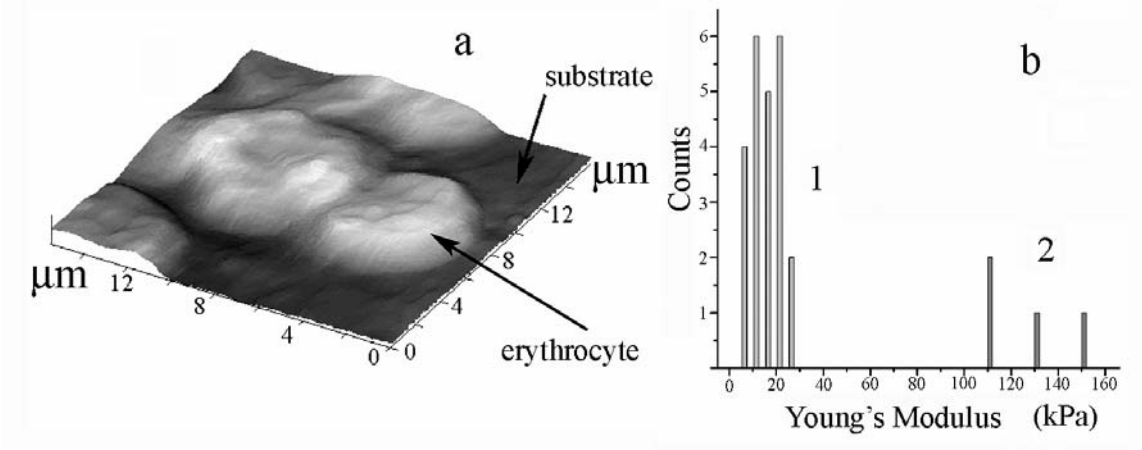

Fig.2. a – AFM image of human erythrocyte obtained in buffer solution,
б – histograms of the Young's modulus values for live erythrocytes (1) and those treated with formalin (2).

Literature survey we have undertaken showed that under the change of the external conditions the elasticity of cell membranes changes much stronger than the morphology of the cell [1]. We have carried out force spectroscopic studies of erythrocytes in a buffer solution on Solver BIO in the regime of the cantilever deflection. Experiments were carried out in a physiological solution (137mM NaCI, 2,7mM KCL, 1,47mM KH_2PO_4, 8,1mM $NaHPO_4$, pH=7,4) in order to make experimental conditions maximally close to the buffer solution of cells. In these experiments we took fresh rat erythrocytes obtained directly before the experiment. Glass treated with poly-L-lysine was placed in a solution with erythrocytes and was kept there for 1 hour. Then the unfastened cells were washed off, the sample was placed in a Petri dish with a buffer solution and SFS measurements were carried out. To study the change in the elasticity of erythrocytes glass was placed for 10 min into the 5% solution of formalin and then the SFS measurements in the buffer solution were carried out.

Fig. 2b shows a histogram obtained for the Young's modulus values for a "live" erythrocyte and an erythrocyte treated with formalin. According to our preliminary calculations, the Young's modulus for a "live" erythrocyte is 16,05±2,3 kPa, which agrees with the literature data on the elasticity of "live" cells. The Young's modulus for erythrocytes treated with formalin is ten times larger and makes 119,5±15 kPa.

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[1] Hong Xing You and Lei Yu. Methods in Cell Science **21**, 1-17. (1999).