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Dielectric characterization of erythrocytes by electrostatic force microscopy

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The human cells have unique biophysical and biochemical properties that ensure interaction with the surrounding physiological environment to realize of specific functions. The study of mechanical, electrical and optical parameters of cells is important for clinical diagnostics.

The electrical properties of cells investigate generally by measuring a response of cells on external electric field [1]. The electric response depends on the shape, size, internal structure and conductivity of cell, and susceptibility of cellular components. It should be noted that the registration of response is usually performed on large cell populations that leads to average of measured parameters. In this case, the properties of individual cells are not taken into evaluation that lead to limited of measurement accuracy. The study of individual cells allows to determinate the heterogeneity of their characteristics within a cells population and to measure of samples containing cells of various types without the need for their separation [2]. Using of electrostatic force microscopy (EFM) to determine the dielectric response of individual bacterial cells with high precision and reproducibility was demonstrated in [3].

In this work, the object of study was red blood cells (RBC) that perform a fundamental physiological function of living organisms – transport of respiratory gasses. RBC were separated by centrifugation of whole blood, and then thrice washed with isotonic solution. In the next step, RBC were fixed of a 2%-paraformaldehyde solution for 2 h, then transferred to deionized water to achieve concentration equal to 10^8 cells/ml. Aqueous suspension of RBC (15 µl) was deposited on a freshly cleaved surface of highly oriented pyrolytic graphite (HOPG) and incubated for 30 min. Then the samples were washed in deionized water to remove non-adsorbed cells and air-dried at room temperature. The HOPG surface with immobilized RBC was scanned by atomic force microscope MFP-3D SA (Asylum Research) by EFM-method at various applied voltages. The conductive cantilevers of the ETALON HA_FM series (NT-MDT SI, Russia) with Pt coating were used, having the following parameters: k ~ 3.4 N/m, Q ~ 234, R ~ 35 nm.

Figure 1a shows an example of image and corresponding EFM-contrast (Fig. 1b) for an individual RBC on HOPG surface at + 3 V on probe. Figure 1c presents the phase shift ($\Delta\Phi$) between RBC and HOPG surface on EFM-profile.

Figure 2 demonstrates the EFM measurements for quantitative analysis of experiment data. RBC is modeled as disc shape with height H and a dielectric constant ε is located on the HOPG. The probe is a spherical shape with radius of curvature R that is at a fixed height h above the sample.

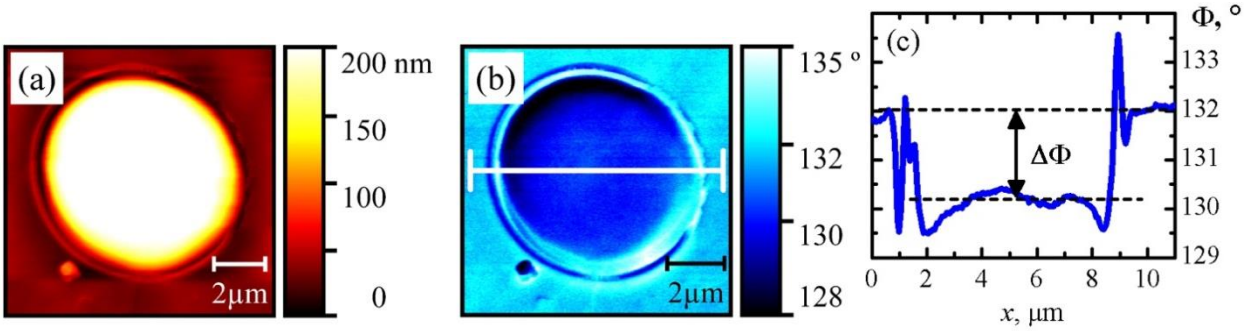

Figure 1. 2D-images of individual RBC on HOPG in first (a) and second (b) passes (h = 100 nm), and the corresponding profile of the EFM-image (c).

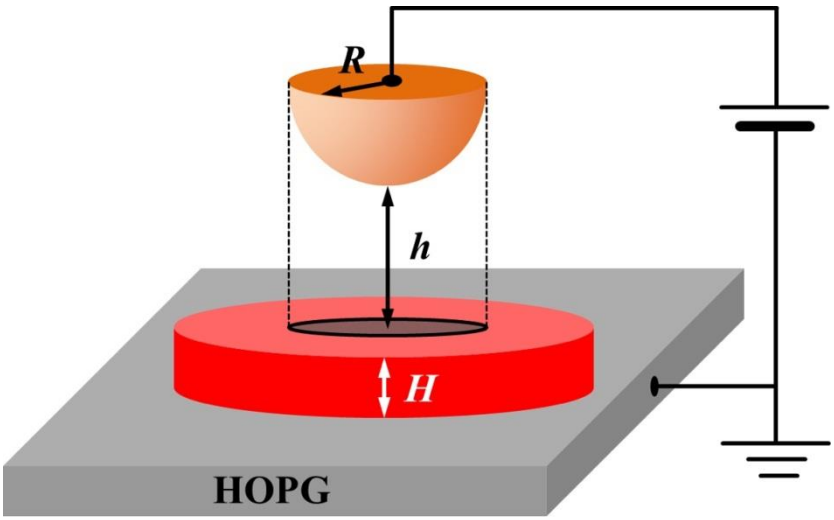

Figure 2. Scheme of EFM experiment (probe placed in the center under the RBC).

The dependence of phase shift tangent on square of the applied voltage for studied samples is linear. This indicates that the contrast of EFM-images for RBC is determined only by the capacitive coupling of the probe-sample.

Modeling of the EFM-profiles was performed according to the method described in [4]. To calculate the phase shift between RBC and the probe, the second derivative of the probe-RBC-HOPG capacitance was calculated using the equation:

$$\frac{\partial^2 C}{\partial z^2} = 4\varepsilon_0 \int_0^R \int_{y_1}^{y_2} \left[h + R - \sqrt{R^2 - x^2 - y^2} + \frac{H}{\varepsilon} \right]^{-3} dxdy ,$$

where ε_0 – vacuum permittivity, ε – dielectric permittivity of individual RBC, $y_1 = -\sqrt{R^2 - x^2}$, $y_2 = +\sqrt{R^2 - x^2}$ – the limits of integration along the *y*-axis.

By fitting the model phase shift to the experimental one, the mean value and range of the dielectric permittivity of individual RBC were determined, equal to 2.6 ± 0.8. Since the volume of the erythrocyte is filled with proteins, which, according to the literature, have dielectric permittivity values of 2-4 [3], the obtained value of ε is adequate.

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