Scanning ion-conductance and atomic force microscope with specialized sphere-shaped nanopipettes

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Abstract. A scanning ion-conductance microscope was designed on the basis of scanning probe microscope NanoTutor. The optimal parameters of nanopipettes fabrication were found according to scanning electron microscopy diagnostics, current-distance I (Z) and current-voltage characteristics. A comparison of images of test objects, including biological samples, was carried out in the modes of optical microscopy, atomic force microscopy and scanning ion-conductance microscopy. Sphere-shaped nanopipettes probes were developed and tested to increase the stability of pipettes, reduce invasiveness and improve image quality of atomic force microscopy in tapping mode. The efficiency of sphere-shaped nanopipettes is shown.

1. Introduction

Atomic force microscopy (AFM) is one of the principal diagnostic methods to visualize the native biological objects (cells, bacteria, DNA, etc.) in a liquid with a high spatial resolution. However, AFM method leads to distortion of the soft biological objects while scanning process even in the less invasive tapping mode. Recently, in connection with the possibility of reducing the invasiveness of studying the native object in a fluid, a new method called scanning ion-conductance microscopy (SICM) was developed [1,2]. The interest to SICM is also explained by new mechanisms of image contrast that can give additional information of the object being studied, as well as the possibility of combining this method with "patch-clamp" technique [3,4].

Unlike tunneling and atomic-force microscopy, in SICM the ions transfer in electrolyte through narrow (D \sim 200 \text{nm}) and relatively long (L \sim 20 \text{mm}) pipette channel with charge exchange process at the electrodes. Emerging microfluidic flows are very sensitive to micro- and nanobubbles formed in the electrolyte, as well as to presence of microparticles and the quality of micropipette tip surface. Therefore, at the present there is an issue to find optimum schemes and methods for SICM [5,6]. A particular problem is the preparation of micropipette probe for SICM and fixation of biological objects while scanning. The ability to create a combined technique such as SICM, AFM and "patch-clamp" mode is of particular interest nowadays.
The aim of this work was to create a prototype of the scanning ion-conductance with integrated atomic force mode and develop technology for nanopipette probe fabrication for non-invasive studying objects of organic and inorganic nature.

2. Experimental setup
We have created a SICM based on scanning probe microscope NanoTutor (NT-SPb, Russia). Fabrication of nanopipettes probes (NP) was performed by means of PMP 107 Micropipette puller (WPI, USA). NP study was carried out by a scanning electron microscope SNE-4500M (SEC Co., Korea). Fabrication of NP was carried out on borate glass bar with a melting point of 820 °C. NaCl buffer was selected as electrolyte for SICM. A calibration grid TGZ (NT-MDT, Russia) was used as a test structure. Fabrication of the NP with spheres and control of their geometrical parameters were performed by means of scanning electron microscope (SEM) CrossBeam Neon 40 (Carl Zeiss, Germany).

3. Results and Discussions
SICM principle of operation is based on ionic conduction currents flowing between the two electrodes of Ag/AgCl, one of which is placed in bath with sample, while the second - in the probe itself (NP). Ag / AgCl electrode is characterized by a reversible redox reaction, which resulted that chloride atoms and electrons get into the solution, metalizing silver and vice versa. The current value depends on the diameter of the output MP and decreases when approaching MP tip to the object. At the same time the stability of MP, which depends on a number of factors, is effected on the stability of the servo system of SICM.

![Figure 1. General view of the NP and magnified areas of pipettes tips with hollows about 175 nm.](image)

During the study the loss of SICM image contrast was found with the small smoothness of MP tip front surface. Instability in the ion current also can be caused by small angles of verge convergence of the MP. To improve the stability of MP and contrast of SICM images the MP was fabricated by double exposure. In the process of heating the initial glass bar was gradually stretched and then abruptly torn. Moreover, in the stretch process MP twist along its axis was carried out. To smooth surface of the MP it was repeatedly short-term heat to reflow the tip (Figure 1).

Optimum contrast and image stability of SICM were found with MP convergence angle ~ (5-8)° and a diameter ~ (175-200) nm.

The dependence of the ion current from the distance to sample surface I (Z) and current-voltage characteristics I (V) was studied, the steepness of which is possible to diagnose the quality of the MP. Revealed offset potential in the process of SICM is indicating the necessity for periodic recharging of the electrode potential (electrochemical formation of AgCl layer on Ag). Topology visualization of polyethylene substrate by SICM and AFM indicates good correlation of results, which was obtained
by different methods. To determine the resolution of SICM on the surface of the polyethylene sample was created specifically periodic structure (replica from TGZ grid) with a period of about 3 µm and height of about 100 nm (Figure 2).

![Figure 2](image1.png)

**Figure 2.** SICM image of the calibration grid TGZ (a), magnified image of a single strip with cross-section line (b) and cross-section area (c).

The small breakdowns on SICM was noticed on the border of steps compared to the semi-contact AFM mode, but in general the AFM and SICM images demonstrated a good correlation. In addition, visualization of CHO cell culture (Chinese Hamster Ovary) was carried out by means of optical microscopy, AFM and SICM modes (Figure 3). The effectiveness of non-invasive diagnostics by means of SICM method was demonstrated.

![Figure 3](image2.png)

**Figure 3.** AFM image of the CHO cell in air (a), SICM image of native CHO cell in liquid with cross-section line (b) and cross-section area of the cell (c).

In SPM NanoTutor it is possible to perform a simultaneous study of objects both in SICM and AFM tapping mode using a pipette as a probe. However, when using a pipette in the AFM tapping mode leading to image noise and the gradual destruction of the pipette. To improve the stability of pipette, provide non-invasive interaction and improve the image quality, a calibrated Au sphere with a radius of about 200 nm was attached to the side of the NP (Figure 4). The attachment was carried out in two ways: with the help of Van-der-Waals forces between sphere and tip under the electron beam and by welding the sphere to the pipette by depositing the precursor gas (C). The second variant is preferable because of the greater stability of the NP and the dielectric characteristics of the C, which is important for the SICM studies.
Figure 4. SEM images of sphere-shaped NP fabrication: search for a sphere on a substrate (a), grabbing a sphere on a manipulator (b), image of standard NP (c), attachment of sphere by Van-der-Waals forces (d) and welding of sphere by depositing the precursor gas C (e).

Figure 5. AFM image of the calibration grid TGZ, measured by standard NP (a) and NP with sphere (b).
Figure 5 shows an improvement of the image quality using the NP with a sphere. The local soft contact of the sphere provides noise reduction and allows scanning of grid with greater accuracy (compliance in height and gap between grid lines). It should be noted that when using the sphere, the probe's resonance has decreased from about 9 kHz to about 5 kHz. The sphere does not affect the flow of ion current in the SICM mode, since it locates at a distance from the sample in SICM mode and does not block the hollow of the NP. In addition, the spheres reduce the invasiveness of the AFM method, which is important in the study of the biological objects.

4. Conclusions
Scanning ion-conductance microscope was presented based on «NanoTutor» microscope. It is shown that stable and contrasting SMIP-images obtained by nanopipette with the convergence angle ~ (5-8)°, diameter ~ (175-200) nm and smoothed tip obtained by local heat treatment. The dependence of the ion current from the distance to sample surface I (Z) and current-voltage characteristics were studied for nanopipettes, which is useful to obtain optimum MT fabrication regimes.

Revealed offset potential in the process of SICM was indicating the necessity for periodic recharging of the electrodes. A good correlation of SMIP images of test surfaces, including the biological samples, with the images obtained by means of AFM and optical microscopy was demonstrated.

Sphere-shaped nanopipettes probes were developed and tested. It is noticed that spheres increase the stability of pipettes, reduce invasiveness and improve image quality of atomic force microscopy in tapping mode. The efficiency of sphere-shaped nanopipettes is shown.

Acknowledgments
Work carried out with the support of the Russian Foundation for Basic Research (16-32-00806), the leading universities of the Russian Federation (grant 074-U01).

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