Counting grids for analysis of small biological objects

V. G. EVTUGIN[1,2], A. M. ROGOV[1,2], V. I. NUZHDIN[1], V. F. VALEEV[1], T. S. KAVETSKYY[3], A. L. STEPANOV[1,*]
[1]*Zavoisky Physical-Technical Institute, FRC Kazan Scientific Center of RAS, 420029, Kazan, Russia*
[2]*Kazan Federal University, 420008, Kazan, Russia*
[3]*The John Paul II Catholic University of Lublin, 20950, Lublin, Poland*

A new approach for fabrication of grid structures on substrates (devices) for visual characterization of ultra-small biological objects is presented. By Ar^+-ion mask implantation of silica substrates is applied to create grid structures with cells of 25 μm size and a depth of 100 nm. Demonstration of efficiency of such structures was carried out by scanning electron microscopy observation and energy dispersive spectrometry – EDX analysis using substrates with deposited *Staphylococcus* bacteria.

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1. Introduction

The proposed work is associated with certain stages of the implementation of the transition to personalized medicine. Nano- and microstructured materials could be used for standard countable statistical analysis, as well as for research in biology and medicine during sequencing, separation, detection, identification, quantitative and structural analysis of small biological molecules and micro-objects such as plant-cell populations (blood, cell-cultures), microorganisms, viruses, etc. [1].

One of the potential solutions such the problems of timely detection and subsequent localization, as well as the treatment of various types of infectious diseases, is the prompt diagnosis of various biological objects. Therefore, in recent years, various methods of microscopy techniques are successful applied such as fluorescent [2], confocal Raman [3], Fourier ptychographic [4], Mueller matrix microscopy [4], expansion [5], IR-absorption [6], scanning and transmission electron [7] and atom-force [8] types of microscopies. The rapid development of microscopic methods is due to the emergence of a new hardware base and the use of modern achievements in the development of new composite materials obtained on the basis of their chemical and physical properties. The creation of fundamentally new technological monitoring and analyzing systems, as well as the development of new methods based on them, could be suggested in the diagnosis and characterization of extremely small biological material (bacteria and viruses). Thus, there are opportunities that were not previously realized in practice for their calculation and statistical processing, form analysis, etc. All this allows everyone to significantly accelerate the diagnosis and analysis of pathogenic and conditionally pathogenic biomaterials, which will bring closer the transition and closer to personalized medicine and improve health care.

The aim of present work is the development, fabrication and testing of specific regular nanostructured substrates (devices) to ensure the calculation of ultra-small biological micro-objects using high-resolution electron and probe microscopy. To create such biological devices, it is proposed to use the technology of ion implantation modification and controlled surface structuring of dielectric materials. Ion implantation is currently one of the main techniques used in industrial semiconductor microelectronics for the formation of various types of nano- and microdevices [9]. The possibility of creating periodic surface structures suited for optical diffraction by ion-mask implantation with various ions on materials such as quartz glass [10, 11], and polymers [12] was previously realized. In this study, it is proposed to use implantation of silica glass with inert gas ions through a mask to form surface nanostructures (Fig. 1) suitable for use in the analysis of ultra-small biological objects such as *Staphylococcus* bacteria. First attempts for such approach was recently tested by visualization and analysis of *Bacillus subtilis* bacteria with scanning electron (SEM) and atomic-force (AFM) microscopy [8].

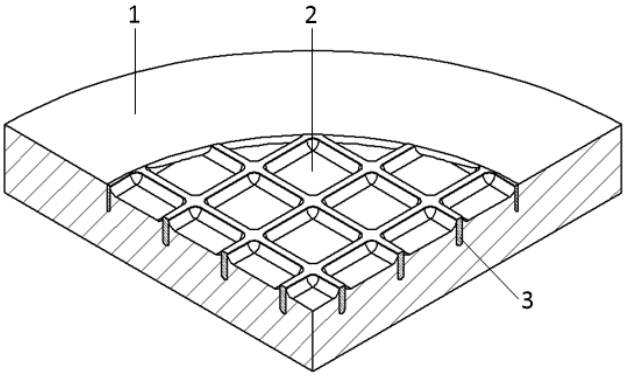

Fig. 1. Isometric drawing of a periodic microstructure (cross-sectional view) located in the surface layer of a glass substrate, comprising: a glass substrate (1); a network formed in the bulk of the glass substrate and consisting of periodic microstructured regions (2), delimited by sections of diffusion-impurity metal atoms (3)

2. Experimental

Implantation by singly charged Ar^+ ions with energy E = 40 keV, radiation dose $D = 5.0 \cdot 10^{17}$ ion/cm^2 and current density in the ion beam J = 20 μA/cm^2 was carried out with an ILU-3 ion-beam accelerator (Fig. 2) through a copper-nickel wire mask mesh (Fig. 3) into silicate glass coverslips (20 × 20 mm, thickness 170 μm). A mask in the form of a mesh was placed on the sample surface before implantation, at the end of which it was simply mechanically removed without leaving its parts.

The morphology of the structured surface of the implanted glass coated with microorganisms was studied by SEM, using a high-resolution microscope Merlin (Carl Zeiss) at a low accelerating voltage of 5 keV in the detection mode of secondary electrons. Elemental analysis was performed with an X-Max energy dispersive (EDX) spectrometer (Oxford Instruments) combined with a SEM at an accelerating voltage of 20 keV.

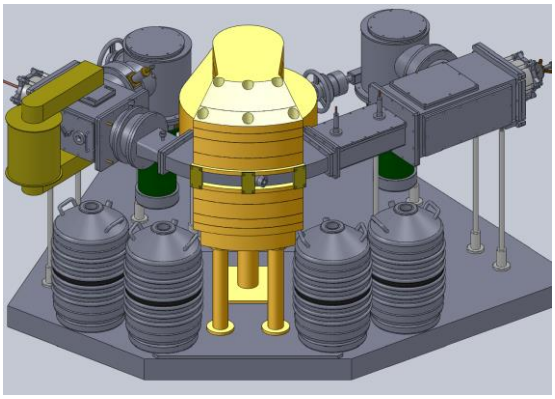

Fig. 2. Drawing of the ion accelerate construction – ILU-3 (color online)

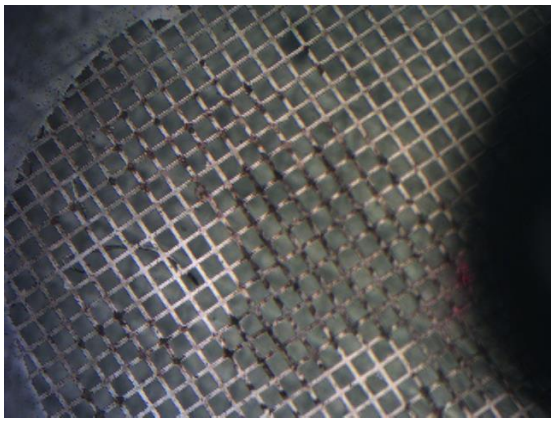

Fig. 3. Optical microscope image of a copper network before its irradiation and heating by an ion beam. The mesh is superimposed on the surface of the glass substrate

Staphylococcus bacteria from the collection of microorganisms of the laboratory of biosynthesis and bioengineering of the Institute of Fundamental Medicine and Biology in Kazan Federal University were used as biomaterial for deposition on the proposed substrates.

3. Results and discussion

Ion implantation is the process of introducing accelerated ions into the irradiated matrix to a certain depth depending on the energy [9]. The process of collision of implanted ions with the substrate atoms is accompanied by surface target sputtering. The nature of the sputtering depends on a number of factors such as acceleration energy of ions, ion mass, target substance density, etc. As it was demonstrated [10, 11], during the implantation of quartz glass by metal ions a sputtering and a decrease of the surface target level in the irradiated region relative to the surface of the initial substrate were observed.

In the present work, changes in the surface morphology of silicate glass subjected to high-dose implantation with Ar^+ ions through a mask were observed using the SEM method. Fig. 4 shows an SEM image of *Staphylococcus* bacteria on the flat glass substrate. The different scale SEM images of nanostructured glass surface with deposited *Staphylococcus* bacteria are presented in Fig. 5. The surface structure after ion implantation looks like as grid with size of separated cells same as in the mask (Fig. 5a). The cells fabricated by ion beam sputtering during implantation. The thickness of the sputtered glass layer (the depth of the lattice cells) for the selected radiation dose is about 80 nm [10, 11]. The width of the lattice cell is 500 μm. It is clearly seen that the dimensional parameters of the formed grid structures provide a convenient ratio for working with bacteria. As follows from Fig. 5b, some bacteria are deposited on the edges of the lattice, which could lead to some error in their statistical characterization. However, it is possible that the use of long-term implantation will allow the formation of deeper lattice cells, and this will isolate bacteria located on the edges of the lattice from the surface of the cells themselves and exclude them from the general analysis of microorganisms.

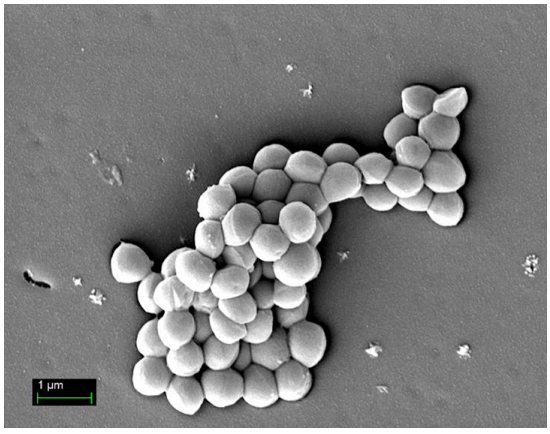

Fig. 4. SEM image of Staphylococcus bacteria on the flat surface of the glass

With the EDX image (Fig. 6), it is seen that when using mapping of structured surface containing bacteria as surface various chemical elements, it is possible to observe on the glass surface periodic irradiated and unirradiated regions in the form of a lattice corresponding to the size of the mesh mask. The applied bacteria are located on the background of a lattice determined by a specific chemical element. In particular, the distribution of copper in the form of a lattice, which is formed during the diffusion of copper into glass from a mask heated by ion current. Against the background of a lattice of copper atoms, it is possible to record and analyze the distribution of bacteria. When applying EDX maps of copper and carbon (Fig. 6), it becomes possible to analyze the distribution and packing density of bacterial populations even in the absence of a distinguishable visually profile geometric picture of the lattice structure of the substrate. This approach of EDX mapping could be allowed for manipulation not only with small concentrations of biological material, but also with their thick layers, since the electron beam penetrates through them prematurely easily.

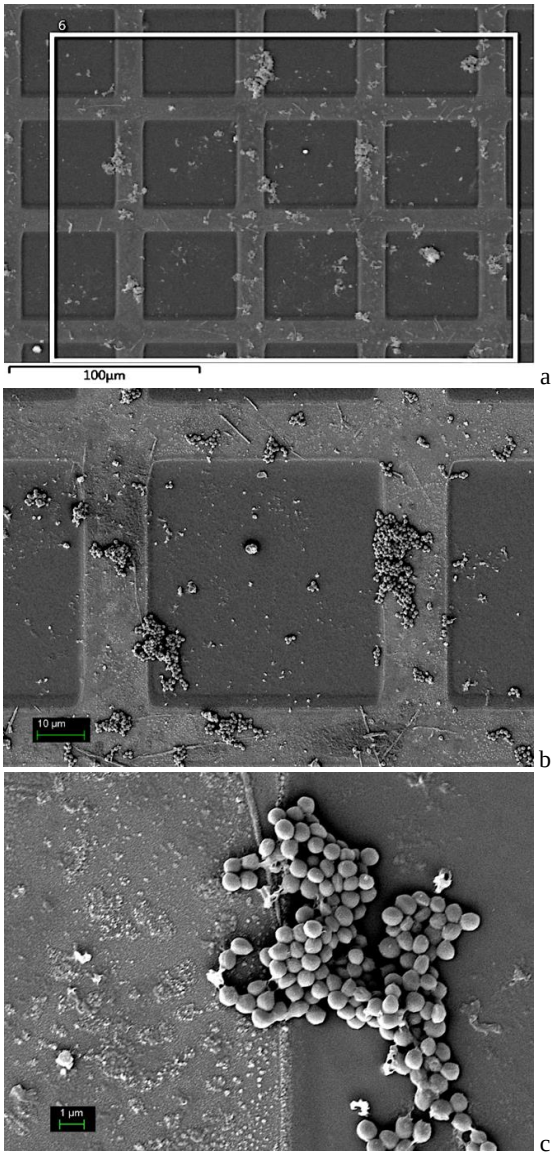

Fig. 5. Different scaler SEM images of the surface grid on the glass substrate with Staphylococcus bacteria

4. Conclusion

Thus, as opposed to electro-beam lithography or ion-synthesis of nanoparticles on glass [13, 14] the present work demonstrated the possibility of creating fundamentally new technological monitoring and analyzing systems (nanostructured glass substrates), as well as laying the foundation for the development of new methods for characterizing small cell material based on SEM and EDX analysis. The use of nanostructured substrates with a cellular structure formed by the method of ion implantation through a mask allows in principle to carry out calculations on complex biological microobjects. The large penetration depth of electrons in organic media during EDX mapping gives possibilities to obtain an image of the formed lattice even from under a dense layer of biological material, which significantly expands the possibilities of analysis of microbial biofilms.

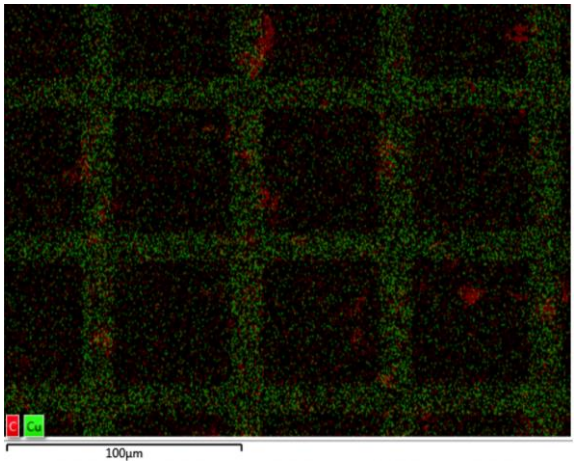

Fig. 6. EDX map for same area (Fig. 5a) for various chemical elements: carbon from bacteria and Cu from the grid (color online)

Acknowledgements

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*Corresponding author: aanstep@gmail.com