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Substrates for soft X-ray microscopy based on Si_3N_4 membranes

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Silicon nitride membranes were experimentally obtained as substrates for biological samples, which are examined using a microscope with an operating wavelength of 13.8 nm. The free-hanging films obtained have a size of up to $1.5 \times 1.5\,mm^2$, which makes it possible to select an area of interest for investigation on the sample on the order of tens to hundreds of microns. The mechanical strength of the membranes satisfies that the samples do not tear the membranes and withstand transportation. The results obtained are an import-substituting technology for the manufacture of Si_3N_4 membranes. The resulting membranes have a transparency of more than 40% in the range of the „water transparency window" (2.3−4.4 nm) and EUV (13−15 nm). The developed technology will become the basis for creating cuvettes for living biological samples for soft X-ray microscopy studies.

Keywords: Si_3N_4 membranes, photolithography, soft X-ray microscopy, EUV microscopy, correlation microscopy.

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Introduction

One of the key roles is played by the sample holder in the soft X-ray microscopy. It should have the following parameters: be transparent in the range 2.3−4.4 nm (microscopy „of the water transparency window" [1]) and pass extreme ultraviolet (EUV) (microscopy in extreme ultraviolet [2,3]), and be resistant to ultraviolet radiation so that the holder does not collapse during operation on the microscope. Also, the holder should be mechanically strong to withstand the application of a sample and transportation [4]. At the moment, three types of substrates are used in the world for samples in soft X-ray microscopy:

1) polyvinyl-formaldehyde films with 20 nm carbon film, which is applied to a copper mesh with different cell sizes [3,5];

2) 300x4 glass capillaries (20) μm [1,6];

3) free-hanging membranes from Si_3N_4 [2,7].

Membranes from Si_3N_4 have several advantages. For example, they are more resistant to UV and EUV radiation. Their working surface is larger than that of a glass capillary. Membranes for soft X-ray microscopy can be made large, up to 1.5×1.5 mm, which allows selecting an area of interest for research on a sample of the order of tens — hundreds of microns.

Fig. 1 shows that a silicon nitride membrane with a thickness of 90 nm has a perfect transmittance in the range 2.3−4.4 nm and at a wavelength of 13.8 nm. The value of the transmittance coefficient can be varied. For example, a stronger membrane will be produced by increasing the thickness but with a lower transmittance.

The actual thicknesses of commercially available membranes range from 10 to 1000 nm, while the maximum membrane size is 15×23 mm with thicknesses from 30 nm [9].

To vary the size of the membrane, we selected a film with a thickness of 90 nm. Various biological samples with a thickness of 5−10 μm or more will be applied to the membrane, and the membrane should not burst under them. We will describe a technique for manufacturing free-hanging membranes Si_3N_4 of various sizes for their use as holders of biological samples for soft X-ray microscopy. This is an approbation of a well-known technology, but with the use of domestic equipment. It is also an import-substituting technology for the manufacture of such membranes, which are described in all details. The developed technology will

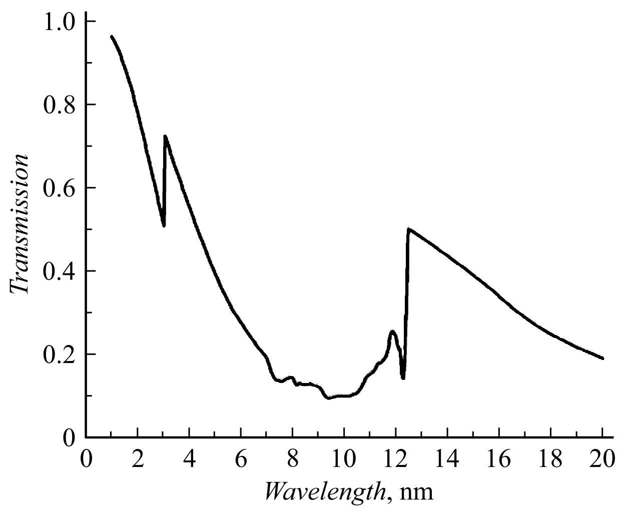

Figure 1. Theoretical dependence of the transmission coefficient of the film Si_3N_4 with a thickness of 90 nm in the range 1−20 nm [8].

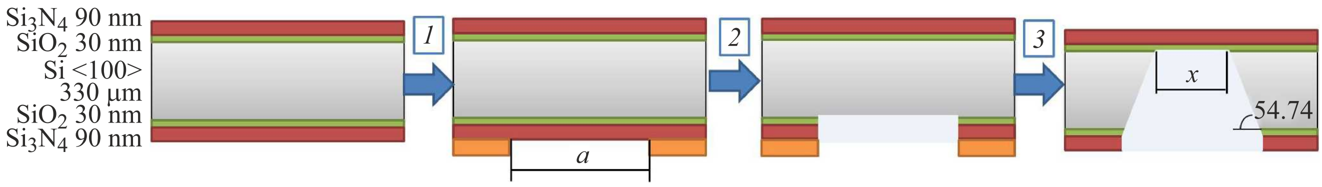

Figure 2. Membrane manufacturing scheme Si_3N_4. *1* — applying a photomask to a substrate and creating a technological drawing; *2* — removing layers Si_3N_4 and SiO_2 by ion etching for subsequent chemical etching; *3* — flushing of the photoresist and chemical etching of the remaining Si layer in two stages.

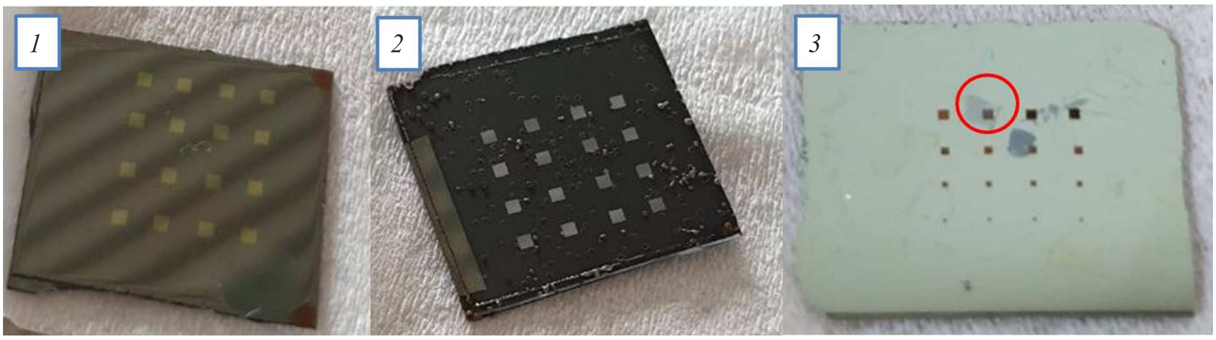

Figure 3. The results of some stages of manufacturing. *1* — photoresist with technological pattern; *2* — after ion etching; *3* — final membranes in the plate Si. A sample on the membrane is highlighted in a circle.

become the basis for creating cuvettes for living biological samples for soft X-ray microscopy studies.

1. Method of manufacturing a free-hanging membrane Si_3N_4

The process of manufacturing such membranes is discussed in the literature [10], but rather superficially. We will describe it in more detail. The process itself can be divided into three stages (Fig. 2, 3).

Initially, a commercial Si plate with a crystallographic orientation $\langle 100 \rangle$ thickness $330\,\mu$m is taken, on which thin films SiO_2 are applied on both sides (~ 30 nm) and Si_3N_4 (~ 90 nm), then „substrate“. A photoresist was applied to one of the sides. A commercially available positive resist FP-383 was used to obtain a photoresist mask layer.

The photoresist was applied to the substrate by centrifugation at a speed of 3000 rpm. The centrifugation time was 30−40 s. Next, the substrate was placed in an oven and kept 1 h at 85°C. The etching mask was manufactured using a laser scanning UV lithograph by Heidelberg instruments μpg 101 [11]. The mask was made in the form of a matrix of squares with the side „*a*“ (Fig. 2). After illumination the resist was developed in UPF-1B.

Next, ion etching of the layer Si_3N_4, SiO_2 and 100 nm Si was performed through the mask. The ion beam etching technology [12] was used to remove the layers. The technological source of accelerated ions KLAN-103M (NTK „Platar“ [13]) with an filament cathode and a flat ion-optical system was used. Aperture — an ellipse with half-axes 30 and 45 mm. Ar was chosen as the gas. The ion energy was 800 eV, and the current density was 0.7 mA/cm^2. Residual pressure in the chamber before the process — $2 \cdot 10^{-4}$ Pa, during the etching process — $1.3 \cdot 10^{-2}$ Pa. The etching depth was controlled using a sample−„witness“, which was used as a plate Si_3N_4, part of the surface of which was covered with a mask. As a result of etching, a step was formed, which was measured using white light microinterferometer TalySurf CCI 2000 [14].

Since part of the test sample is covered with a photoresist, which can be baked under the action of ion irradiation, the etching process was selected in such a way as not to overheat the substrate. The sample was irradiated for 5 s, after which it was closed with a shutter for cooling at 25 s. The number of such shutter opening and closing cycles was selected based on the etching rate Si_3N_4 and the required amount of material removal. After that, the resist was washed off in DMF.

Then the Si layer was chemically etched in 30% KOH solution in two stages. The first stage — etching in boiling solution to remove the base layer Si. The second stage — etching of the residual layer Si at room temperature. This is necessary so that the film Si_3N_4 does not break during etching in boiling solution. A huge number of bubbles (hydrogen) are released on the surface of the Si being etched, which can tear the membrane.

The result is a square membrane with a square side „*x*“ (Fig. 2). The size „*a*“ for the mask can be calculated from the geometry of the substrate section for obtaining the required membrane size „*x*“. The thickness of the plate

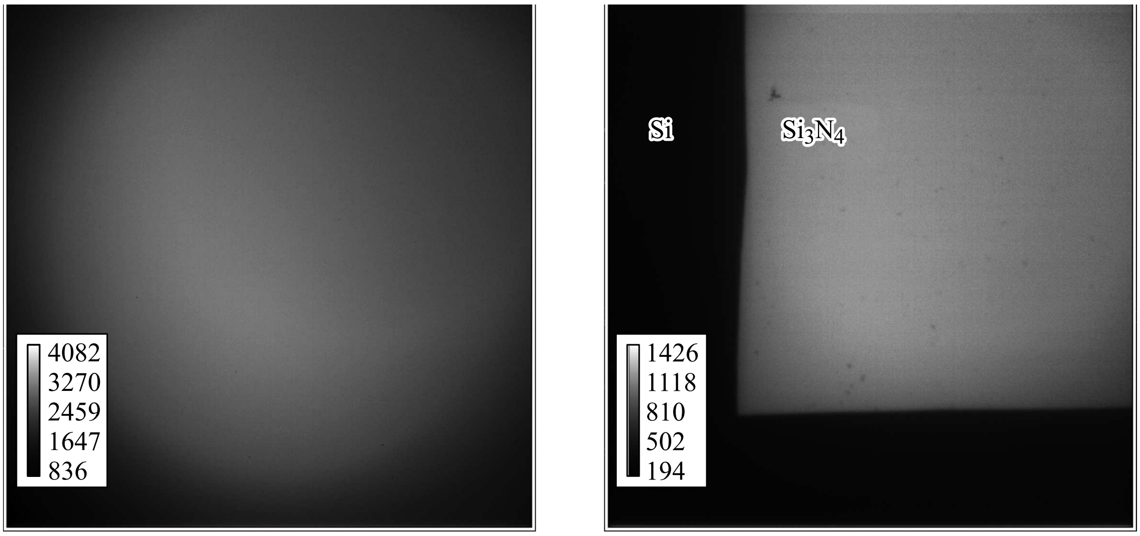

Figure 4. Illumination of the field of view of a microscope without a membrane (left) and with a membrane (right).

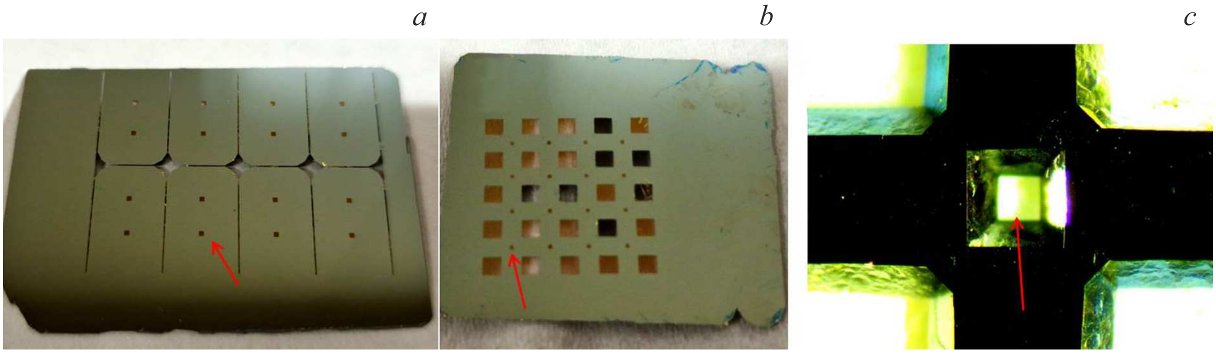

Figure 5. Finished membranes after all manufacturing processes using a photomask for contact lithography. The arrows indicate the membranes. *a* — membrane 0.5×0.5 mm; *b* — membrane 0.1×0.1 mm; *c* — the reverse side of the membrane 0.1×0.1 mm.

and the etching angle of this crystallographic direction are known. As a result, $a = x + 466\,[\mu m]$ is obtained.

Figure 4 shows two images obtained using a soft X-ray microscope (wavelength 13.84 nm) with an optical scheme for the lumen [15]. The left image shows the illumination of the field of view of a microscope without a membrane. The intensity without a membrane was 2700 conventional units and with a membrane of Si_3N_4 — 1100 units with the same exposure time in the same area. The intensity with a membrane was 41% of the intensity without a membrane, which is in good agreement with the theoretical transmission value of 90 nm Si_3N_4 at 44% (Fig. 1).

A photomask for contact photolithography was manufactured at the MVC design and production center in Nizhny Novgorod to accelerate the production of Si_3N_4-membranes of a given shape [16].

The photomask has a pattern for the manufacture of membranes for soft X-ray microscopy and optical microscopy (membranes with a square side of 500 and 1000 μm) and correlation microscopy (membranes with a square side of 100 and 200 μm).

The manufacturing procedure is fundamentally different in only one step: instead of laser scanning lithography, contact lithography is used. Namely, the positive photoresist FP-383 was illuminated by contact method through a photomask with the light of an ultraviolet lamp with a wavelength of ≈ 450 nm and the resulting mask was developed in an aqueous solution of the standard developer UPF-1B, diluted in a ratio of 1 to 4.5 parts of water. The hold time of the sample in the developer solution is 45 s. After that, ion etching and etching in KOH were carried out.

Fig. 5, *a* shows the final version of the plate with a membrane Si_3N_4, on which two windows can be punched through the technological slots of the plate. There are two variants of such plates in the photomask : with a window size of 1×1 mm or 0.5×0.5 mm. Fig. 5, *b* demonstrates another variant of substrates, where large squares represent technological holes for chipping small squares. There are two variants of small size windows in the photomask: 0.1×0.1 mm and 0.2×0.2 mm. Fig. 5, *c* shows the reverse side of the membrane, where the chamfers are visible, which

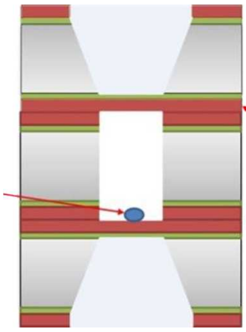

Figure 6. Cuvette for living biological samples. The object indicated by the arrow is located between two membranes Si_3N_4.

were designed in the photomask. An octahedral rectangle with a circumscribed circle with a diameter of 3 mm will be obtained if a window is punched, which is also perfect for transmission electron microscopy.

Various fixed biological objects can be applied to these membranes for their study using a EUV microscope. Also, on the basis of these membranes, it is possible to make cuvettes for live samples for X-ray microscopy „of the water window “. It is important to observe two critical parameters of the environment for cell life such as temperature and atmospheric pressure. Thin glass capillaries filled with water containing the studied items are used in X-ray microscopes all over the world. This is due to the fact that angular tomography is performed on X-ray microscopes [17,18]. The proposed cuvette will be used for z-tomography [15].

Several variants of composite Si_3N_4-cuvette are considered in the literature. For example, a cuvette for contact X-ray microscopy was proposed in the paper [19]. We propose another version of such a cuvette (Fig. 6), so that in the area where the living cell is located, the environment for vital activity remains. The sample will be located on one of the membranes Si_3N_4 in such a cuvette, above there is a plate with a window without a membrane and from above it is closed by a membrane Si_3N_4. The plates will be glued together. Thus, it is possible to study biological objects in their native state using an X-ray microscope and conduct their z tomography to restore their three-dimensional structure [15].

Conclusion

A technique was developed for manufacturing silicon nitride membranes with transparency in the region of 13.84 nm at the level of 41% and in the region of „of the water window“ (2.3–4.4 nm) at the level of 60–70%. Membranes are used as holders for fixed samples and can serve as the basis of composite cuvettes for living biological objects using soft X-ray microscopy. The produced membranes were tested as sample holders using a soft X-ray microscope.

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Conflict of interest

The authors declare that they have no conflict of interest.

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