ISSN 0027-1349, Moscow University Physics Bulletin, 2023, Vol. 78, No. 2, pp. 214–220. © Allerton Press, Inc., 2023.
Russian Text © The Author(s), 2023, published in Vestnik Moskovskogo Universiteta, Seriya 3: Fizika, Astronomiya, 2023, Vol. 78, No. 2, 2320701.

BIOPHYSICS AND MEDICAL PHYSICS

Nanoscale Biosensor with Integrated Temperature Controller for DNA Diagnostics

I. I. Tsiniaikin[1]*, G. V. Presnova[2], I. V. Bozhev[1], A. A. Skorik[1], M. Yu. Rubtsova[2], A. S. Trifonov[1], O. V. Snigirev[1], V. A. Krupenin[1], and D. E. Presnov[1,3]**

[1] *Cryoelectronics Laboratory, Faculty of Physics, Moscow State University, Moscow, 119991 Russia*
[2] *Enzymology Department, Faculty of Chemistry, Moscow State University, Moscow, 119991 Russia*
[3] *Skobeltsyn Institute of Nuclear Physics, Moscow State University, Moscow, 119991 Russia*

Received August 2, 2022; revised September 15, 2022; accepted October 13, 2022

Abstract—A CMOS compatible technique for fabricating a sensor system based on field-effect transistors with a nanowire channel with an integrated thermoregulation elements is presented. The proposed system provides the necessary temperature regimes for many bioanalytical studies. Field-effect transistors with a nanowire channel were fabricated using of reactive-ion etching of the upper layer of a silicon on insulator wafer through a mask formed by electron beam lithography. Titanium thermoresistive strips for temperature control were located on the surface of the chip nearby to the nanowire transistors. Their fabrication is carried out simultaneously with the formation of contact pads to the transistor electrodes, which made it possible to avoid additional technological steps. A demonstration of a system with a built-in temperature controller for the determination of nucleic acids was carried out on model oligonucleotides. Increasing the operating temperature of the device to the ranges at which DNA hybridization allows increasing specificity and avoiding false positive results, as well as reducing the analysis time. The possibility of heating up to 85–90°C allows such devices to be reused.

Keywords: biosensor, nanowire, field-effect transistor (FET), microchip, silicon on an insulator, electron-beam lithography, resistance temperature detector, thermal management

DOI: 10.3103/S0027134923020157

INTRODUCTION

The development of new diagnostic methods for laboratory medicine that contribute to improving the efficiency of patient treatment is an urgent problem. In this work, we present one of the promising devices for diagnostics, a biosensor based on field-effect transistors (FETs), whose channel is made in the form of a nanowire. The principle of operation of such sensors is based on a change in the conductivity of a transistor nanowire channel due to a change in the electric field near its surface as a result of the selective binding of detected molecules with specific reagents immobilized on the nanowire surface. The binding of molecules to reagents is accompanied by a change in the charge of the resulting complex. Even a slight change in the electric field near the nanowire surface has a significant effect on its conductivity. One advantage of such devices is their high sensitivity, which is also due to the high ratio of the nanowire surface area to its volume. Due to this high sensitivity, the sensor makes it possible to measure very low analyte concentrations, which is important for solving many problems of modern diagnostic laboratories.

A significant advantage of sensors based on semiconductor nanowires is the absence of the necessity to introduce additional labels for registration biospecific interactions, which reduces the cost and simplifies the analysis. This provides the possibility for fast real-time measurements, as well as easily scale FET biosensor systems into complex diagnostic devices [1, 2].

A large number of studies have been aimed at solving technological problems and searching for materials for construction nanoscale biosensor devices [3–6]. The most convenient material for their fabrication is silicon on an insulator (SOI) material, which has long been produced industrially. The main advantages of using SOI are reliability, reproducibility of the

*E-mail: ii.tcinyaykin@physics.msu.ru
**E-mail: denis.presnov@physics.msu.ru

fabrication process of structures, and its compatibility with traditional semiconductor technology, which makes it possible to regime part of the measuring electronics in close proximity to the sensor. SOI is often used to fabricate nanowire structures used as biosensors [7–10], local electric field sensors [11, 12], and nanoelectromechanical sensors [13].

The development of fast, reliable, and sensitive DNA sensors that can identify infectious agents or diagnose various viral diseases has become increasingly important in recent years due to the emergence of epidemiologically dangerous situations that threaten global security. Such studies often require a certain temperature mode [14–19], so the introduction of a temperature controller into a biosensor system is an important objective.

For a DNA sensor, an increase in operating temperature of the device to the ranges at which DNA hybridizes most efficiently and selectively, makes it possible to increase specificity, avoid false positive results, and reduce the analysis time [15, 20, 21]. The implementation of a temperature control system and the possibility of heating up to 85–90°C allows the sensor to be reused many times. However, defining and maintaining the physical properties of liquids, such as thermal characteristics, in biosensor devices is a challenge, as the size of such systems becomes smaller: we are no longer talking about the micrometer scale, but about the nanometer scales. This property sets requirements and imposes restrictions on the operation of the device, since the accuracy of temperature control and the time of its setting play a significant role in the experiment.

In this work, we present a technique for fabricating biosensors based on field-effect transistors with a nanowire channel and an integrated heating and temperature control system. A wide range of bioanalytical tasks in the field of DNA diagnostics is solved using a biosensor with operating temperature control, and the reuse of such a biosensor is convenient and cost-effective. Creating a reproducible, accurate and stable system with a heating element is quite a challenge, and our work is aimed at solving this problem.

1. FABRICATING TECHNIQUE

Fabrication process of FET structures with a nanowire channel, which is a main sensitive element of the sensor, is described in detail elsewhere [2] and developed on the basis of previous studies [1, 11, 22]. The standard methods of modern microelectronics (optical and e-beam lithography, reactive ion etching (RIE), and vacuum RF sputtering and electron-beam evaporation) were used.

The commercially available UNIBOND Soitec SOI wafers were used for the sample fabrication.

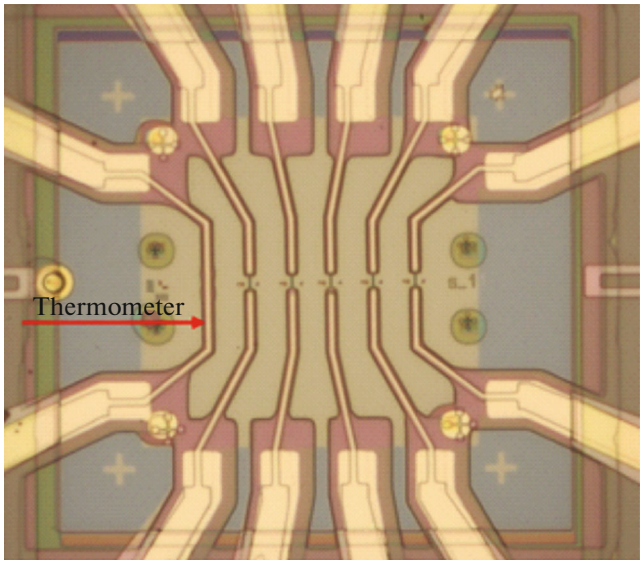

Fig. 1. An optical microscope image of the central part of the chip with a thermo-resistive sensor (thermometer).

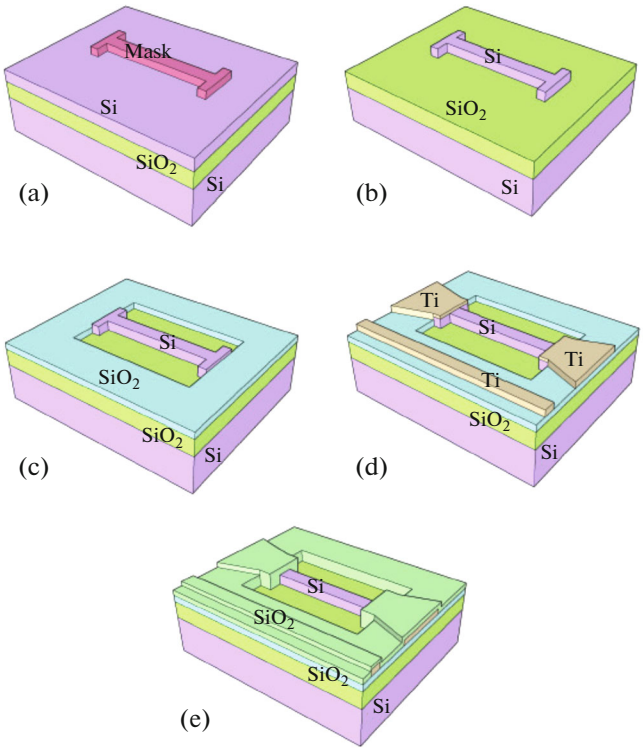

Fig. 2. The schematic sketch of the main fabrication steps of a field-effect transistors with a nanowire channel and a thermoresistive sensor. (a) Formation of a pattern mask using e-beam and optical lithography; (b) transferring the mask pattern to the upper SOI layer using RIE; (c) additional layers of SiO_2; (d) formation of metal contact pads; and (e) deposition of the upper insulating layers of the dielectric.

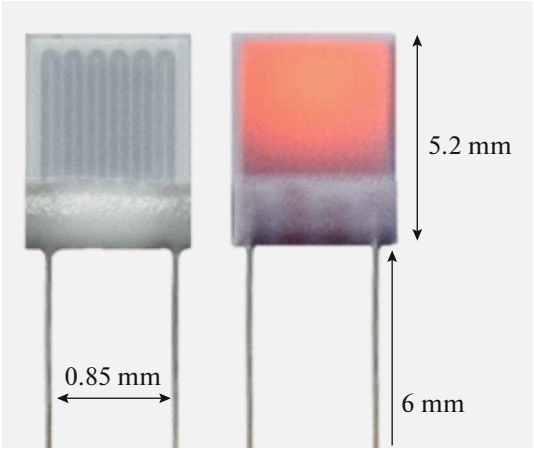

Fig. 3. A high-temperature thin-film heater H540 S.

Their parameters are as follows. The thickness of the upper silicon layer is 110 nm; orientation is <100>; doping is p-type with boron; resistivity is 8.5–11.5 Ω cm; thickness of the insulating intermediate layer of thermal oxide SiO_2 is 200 nm; and thickness of supporting silicon substrate is 750 μm.

Optical photolithography was used to quickly form a pattern of contact pads and insulating dielectric layers. The resist was exposed using a DRK-120 arc mercury quartz lamp in deep ultraviolet ($\lambda =$ 200 nm) for 2 min using a series of photomasks specially designed and fabricated on quartz glass.

E-beam lithography was used to form the nanostructure of the transistor and nearby parts inside the area of 100 × 100 μm.

The structure of the silicon nanowire was formed using anisotropic *reactive ion etching* (RIE) of upper silicon layer of SOI wafer in fluorine-containing plasma (SF_6, 2×10^{-3} mbar, 50 W, RDE-300, Alcatel). An aluminum mask (nanowire) with a thickness of 10 nm was prefabricated (Fig. 2a). The mask pattern was formed using *e-beam lithography* (300 μC cm^{-2}, 20 kV, Supra-40, Zeiss with Elphy Quantum, Raith) followed by vacuum deposition of aluminum (4×10^{-7} mbar, 0.3 nm s^{-1}, L-560, Leybold) by electron beam evaporation technique. As a result of RIE, the silicon regions, not covered by the aluminum mask were removed to the underlying layer, while the regions covered by the aluminum mask remained (Fig. 2b). The duration of the process (about 50 s) was controlled by laser reflecto-interferometry using the Multisem-440 diagnostic complex. After the etching step, the rest of the aluminum mask were removed in a weakly alkaline KOH solution.

When fabricating a big chips (7 × 7 mm^2) using SOI, the probability of a leakage current between the substrate and the input electrodes of the transistor increases due to the large area of contact pads (~2 mm^2) and local imperfection of SiO_2 dielectric layer of the SOI wafer. The fabrication of small chips is inconvenient for measurements in liquid. Considering these factors, there were necessary to increase the thickness of the dielectric on the entire surface of the sample, except for its central part (~150 μm^2) containing the structures of silicon nanowires. This allowed to additionally electrically isolate the metal contact pads and minimize the likelihood of the formation of conduction channels to the silicon substrate, which serves as a transistor gate. The thickness of the dielectric was increased by three successive RF-sputtered SiO_2 layers (O_2, 5×10^{-3} mbar, 200 W, 0.2 nm s^{-1}, Z-400 LeyBold). The thickness of each layer was 200 nm. The surface of the sample before each deposition was covered with a PMMA-MAA/PMMA two-layer resist mask with a total thickness of ~700 nm (Fig. 2c). The choice of a two-layer resist mask is due to the fact that the development rate for the copolymer is higher than for the PMMA, which made it possible to minimize the likelihood of the formation of vertical walls around the perimeter of the mask.

Metal contact pads were formed by *magnetron sputtering* of titanium (Ar, 1.2×10^{-2} mbar, 200 W, Z-400 LeyBold) onto a pattern formed by electronic and optical lithography (Fig. 2d). The thickness of the titanium layer was 80 nm. A titanium strip with a width of 10 μm was fabricated simultaneously in one cycle next to the nanowires. The titanium strip acts as a thermometer (a thermoresistive sensor) (Fig. 1).

After the formation of titanium contact pads, it is necessary to solve the problem of isolating thermoresistive sensors and conductive electrodes from contact with the liquid working medium. For this, the entire surface of the chip, with the exception of contact pads at the edges and nanowires in its central zones, was covered with SiO_2 dielectric layers by high-frequency magnetron sputtering (O_2, 5×10^{-3} mbar, 200 W, 0.2 nm s^{-1}, Z-400 LeyBold). For each layer, a separate resist mask was prepared; the pattern of the mask completely covered the pattern of contact pads, and each subsequent layer covered a large area. Such a multilayer coating is necessary for isolating metallic conductive wires and a heater from a liquid medium (Fig. 2e).

Samples with FET structures were mounted in a special ceramic holder, whose contacts were connected by ultrasonic bonding with a thin aluminum wire (~30 μm, a 7476 device, West Bond) to the areas of contact pads on the chip that were not covered by a dielectric. The aluminum wires and contact pads were isolated from the liquid using silicone sealant. At the same time, the working areas remained open.

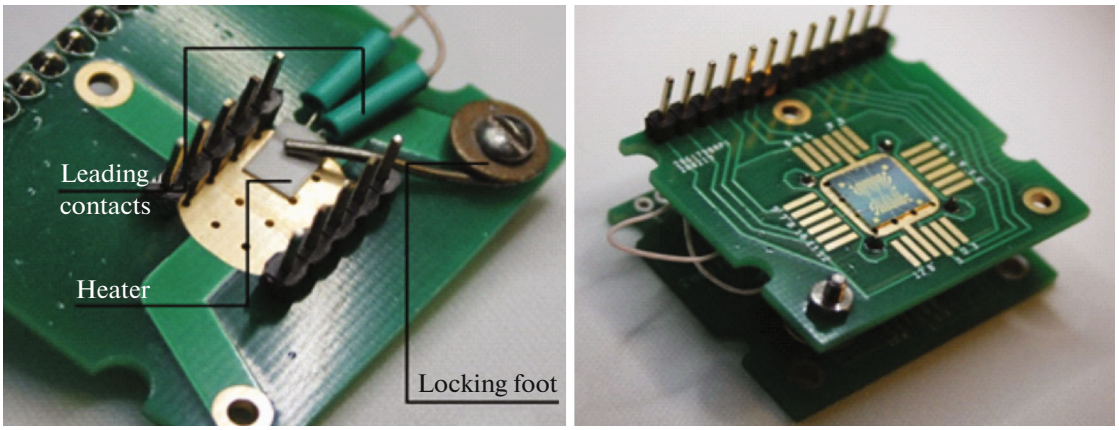

Fig. 4. A photograph of the heater located on the back of the holder is shown on the left; a photograph of the chip mounted on the holder is shown on the right.

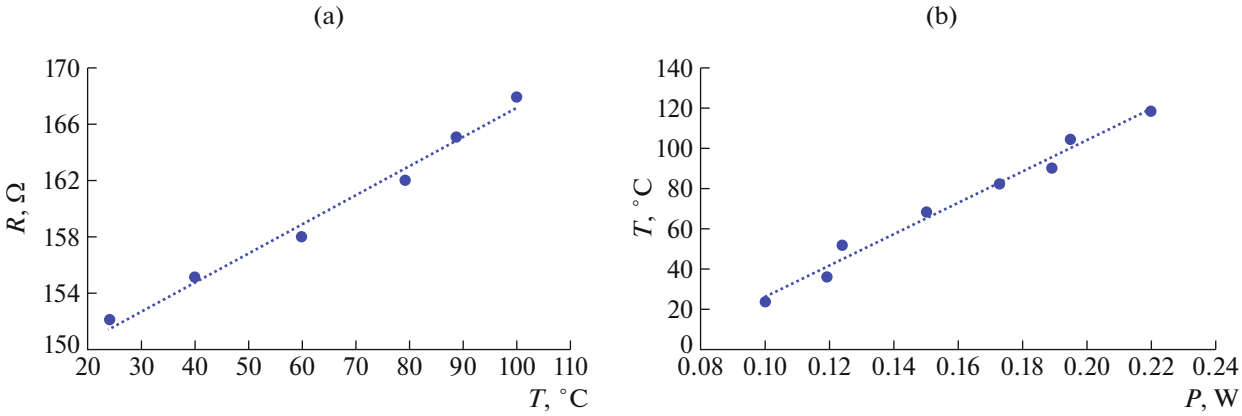

Fig. 5. Calibration of (a) the thermoresistive sensor $R(T)$ and (b) the thin-film heater $T(P)$.

2. A RESISTANCE TEMPERATURE DETECTOR AND A HEATER

2.1. Platinum Thin Film Heater

A commercially available high-temperature thin-film heater (H540 S) was used to set the sample temperature (Fig. 3). It consists of platinum wires on a ceramic substrate coated with a glass-ceramic layer. The heater have the following specifications: temperature range from -25 to $+800$°C, heating current of max 1000 mA, heating voltage of max 24 V, and deviation of $R_0 = \pm 0.5\ \Omega$).

The heater was tightly fixed on the rear side of the ceramic holder, which provided fast heating of the silicon sample. The system (Fig. 4), in which the working wires did not interfere with the experiment, was designed for the convenience of the experiment.

2.2. Thermometer Calibration

To calibrate the thermoresistive sensor, the samples were placed in a furnace. The samples were then heated from room temperature $T_{\text{room}} = 24$°C to $T = 100$°C. The resistance of the thermometer at the corresponding temperature was measured during heating after the thermal stabilization of the sample (about 2 min). Thus, the dependences of the resistances of titanium strips on the given furnace temperature $R(T)$ were obtained (Fig. 5a). A linear approximation (0.2 Ω per 1°C) was used, the temperature deviation was $\approx \pm 2$°C.

2.3. Heater Calibration

The heaters were calibrated at a fixed voltage of 5 V. The dependence of the temperature on the sample surface in the vicinity of the nanowires on the heater power $T(P)$ (Fig. 5b) was plotted using the calibration data of the thermometer $R(T)$ (Fig. 5a).

2.4. Measuring of Thermal Stabilization Time of a Sample

The time of thermal stabilization of the sample was experimentally determined. During the calibra-

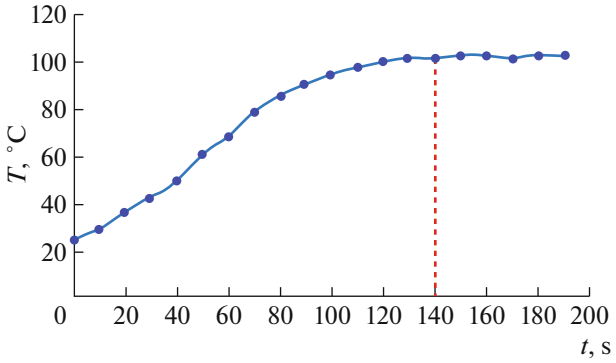

Fig. 6. Temperature dependence on heating time.

tion, the temperature was recorded at regular intervals (10 s). Based on the results of the data, the dependence of the sample temperature on the heating time was plotted (Fig. 6). The temperature of the experimental sample increased sharply in the range of 40–90°C. The sample was heated, the resistance of the thermometer changed. The temperature of the sample then gradually stabilized. The heater only maintains the desired set temperature (100°C). Thus, time t required for thermal stabilization of the sample is determined as 140 s.

3. USING A BIOSENSOR TO IDENTIFY MODEL DNA

The possibilities of using the biosensor with an integrated temperature controller for the determination of nucleic acids were demonstrated using a model system of short oligonucleotides (Fig. 7). The determination is based on the hybridization of a DNA target (target oligonucleotide to be determined) with a complementary in structure oligonucleotide probe immobilized on the surface of a modified silicon nanowire of a biosensor.

The covalent immobilization of an oligonucleotide probe with a thiol group at the 5′ end was carried out using the developed method for modifying the silicon surface with mercaptopropyl trimethoxysilane (MPTMS) and gold nanoparticles with a size of 4–6 nm [1]. Silicon nanowires were incubated in a 0.2 M solution of MPTMS in dry toluene overnight at 70°C, then washed twice with toluene, methanol, and water with stirring, and dried in air. Then, a suspension of GNPs of 4–6 nm was added, incubated overnight at room temperature, and washed twice with PBS.

The probe sequence

(5’-SH-dT)tt-ttt-ttt-ttt-ttt-ttt-ttt-ttt-
AGATTATCAATGATGAATTATATCTTGATG-3’)

included 30 bases, an SH group for immobilization on gold nanoparticles, and a spacer of 24 (2’ deoxythymidine)5’ triphosphates to increase the efficiency of hybridization on the surface. Oligonucleotide probes were spotted to the surface of modified silicon from solutions of 20 pmol · μL^{-1} in 0.25 M Na phosphate buffer containing 0.3 M Na_2SO_4. After immobilization, free oligonucleotide binding sites on the silicon surface in a solution containing 1% BSA and 1% casein in 10 mM K phosphate buffer pH 7.2 containing 0.15 M NaCl. The complementary target DNA sequence included 30 bases (CATCAAGATATAATTCATCATTGATAATCT).

The measurements were carried out as follows. A fixed concentration of the target DNA was passed through the biosensor for 40–50 min, then washed with a hot buffer solution (using a heater) or with a buffer solution at room temperature (without a heater, control). Standard hybridization buffer solution (0.3 M NaCl, 40 mM Na_2PO_4, 4 mM EDTA) pH 7.5 diluted 100-fold containing 0.5 mM $MgCl_2$ was used as a buffer.

When the conditions for complete molecular complementarity of two oligonucleotides are met, the duplexes formed on the surface of a nanowire with an immobilized probe are thermodynamically stable and affect the change in conductivity in a thin near-surface layer.

Figure 8 shows the responses of biosensor nanowires obtained during hybridization and washing with a heater (at an elevated temperature) and without heater (at room temperature, control). It can be seen from Fig. 8 that complexes of the target DNA with immobilized oligonucleotide probes are formed during hybridization. These complexes are stable and are not destroyed by washing with a buffer at room temperature (curve *1*). DNA duplexes are destroyed when washed with a hot buffer heated to 80°C using a built-in temperature controller, which can be seen from the decrease in the biosensor response to background values (curve *2*). This makes it possible to efficiently regenerate the biosensor surface for the next measurements.

Thus, the nanoscale biosensors with an integrated temperature controller (heater) are applicable for multiple measurements, which is promising for the molecular genetic analysis of nucleic acids. The use of an integrated temperature controller and a biosensor surface temperature control system significantly improves the analytical characteristics of nanowire biosensors (increases selectivity, reduces background signals, reduces analysis time, and allows multiple measurements).

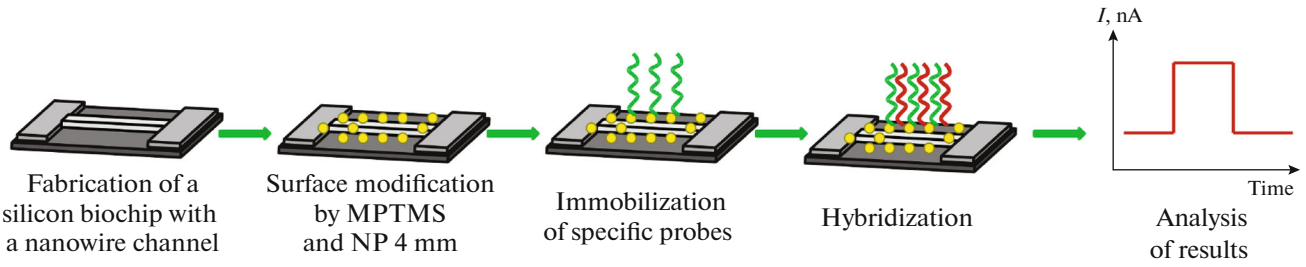

Fig. 7. The scheme of hybridization analysis of nucleic acids on a biosensor with an integrated temperature controller.

CONCLUSIONS

DNA analysis enables to identify pathology at an early stage in the diagnosis of various diseases. A buffer solution heated above the melting point of DNA complexes (up to 80°C) must be used to increase the specificity of the analysis. A sensor system based on a field-effect transistor with a nanowire channel with integrated thermoregulation has been developed and studied. The developed sensor system makes it possible to detect various biological objects with high sensitivity. The key feature of the system is integrated heating devices (thermal stabilization time is 140 s) and temperature control. This gives the possibility to measure at a stable temperature (40–90°C) and precisely control its change (≈ ±2°C). In DNA analysis, temperature stabilization results in the most efficient and specific determination of the target DNA and reduces analysis time. The possibility of stable heating up to 85–90°C (much higher than the melting point of duplexes) makes such a sensor promising for reuse.

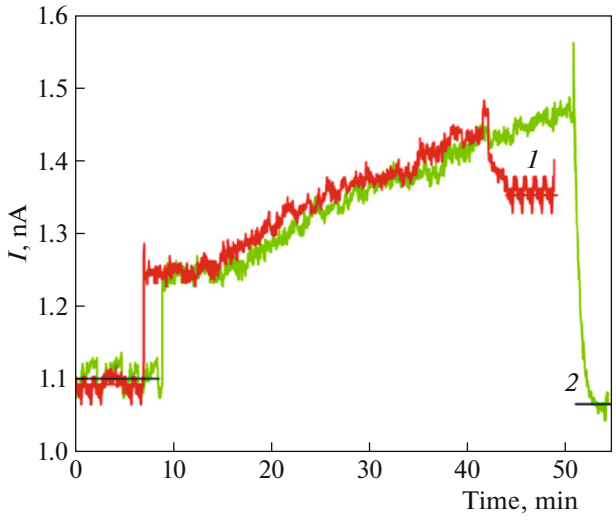

Fig. 8. The response of a nanowire with a built-in heater *2* and without a heater *1* when passing a model target DNA. Experimental conditions are as follows. Passing of buffer is 0–10 min; hybridization with 20 rM target DNA is ≈10–50 min; buffer washing with heating is ≈50 min (green, *2*); and passing wash buffer without heating is ≈40 min (red, *1*).

FUNDING

This work was supported by the Interdisciplinary Scientific and Educational School of Moscow State University "Photonic and Quantum Technologies: Digital Medicine."

ACKNOWLEDGMENTS

I. I. Tsiniaikin is especially grateful to the Foundation for the Theoretical Physics and Mathematics Advancement Foundation "BASIS." The equipment of the Educational and Methodological Center for Lithography and Microscopy of Moscow State University was used in the work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. G. Presnova, D. Presnov, V. Krupenin, V. Grigorenko, A. Trifonov, I. Andreeva, O. Ignatenko, A. Egorov, and M. Rubtsova, "Biosensor based on a silicon nanowire field-effect transistor functionalized by gold nanoparticles for the highly sensitive determination of prostate specific antigen," Biosens. Bioelectron. **88**, 283–289 (2017). https://doi.org/10.1016/j.bios.2016.08.054
2. I. I. Tsiniaikin, G. V. Presnova, I. V. Bozhev, A. A. Skorik, M. Yu. Rubtsova, A. A. Kamalov, S. T. Matskeplishvili, O. V. Snigirev, V. A. Krupenin, and D. E. Presnov, "A sensor system based on a field-effect transistor with a nanowire channel for the quantitative determination of thyroid-stimulating hormone," Moscow Univ. Phys. Bull. **75**, 645–656 (2020). https://doi.org/10.3103/s002713492006020x
3. A. Muller, X. T. Vu, V. Pachauri, L. A. Francis, D. Flandre, and S. Ingebrandt, "Wafer-scale nanoimprint lithography process towards complementary silicon nanowire field-effect transistors for biosensor applications," Phys. Status Solidi (a) **215**, 1800234 (2018). https://doi.org/10.1002/pssa.201800234

4. A. Gao, X. Yang, J. Tong, L. Zhou, Yu. Wang, J. Zhao, H. Mao, and T. Li, "Multiplexed detection of lung cancer biomarkers in patients serum with CMOS-compatible silicon nanowire arrays," Biosens. Bioelectron. **91**, 482–488 (2017).
https://doi.org/10.1016/j.bios.2016.12.072
5. M. H. Jakob, B. Dong, S. Gutsch, C. Chatelle, A. Krishnaraja, W. Weber, and M. Zacharias, "Label-free SnO_2 nanowire FET biosensor for protein detection," Nanotechnology **28**, 245503 (2017).
https://doi.org/10.1088/1361-6528/aa7015
6. V. P. Popov, M. A. Ilnitskii, E. D. Zhanaev, A. V. Myakon'kich, K. V. Rudenko, and A. V. Glukhov, "Biosensor properties of SOI nanowire transistors with a PEALD Al2O3 dielectric protective layer," Semiconductors **50**, 632–638 (2016).
https://doi.org/10.1134/S1063782616050195
7. Yu. D. Ivanov, T.O. Pleshakova, K.A. Malsagova, A.F. Kozlov, A.L. Kaysheva, I.D. Shumov, R.A. Galiullin, L.K. Kurbatov, V.P. Popov, O.V. Naumova, B.I. Fomin, D.A. Nasimov, A.L. Aseev, A.A. Alferov, N.E. Kushlinsky, A.V. Lisitsa, and A.I. Archakov, "Detection of marker miRNAs in plasma using SOI-NW biosensor," Sens. Actuators B: Chem. **261**, 566–571 (2018).
https://doi.org/10.1016/j.snb.2018.01.153
8. S. Zafar, C. D'Emic, A. Jagtiani, E. Kratschmer, X. Miao, Yu. Zhu, R. Mo, N. Sosa, H. Hamann, G. Shahidi, and H. Riel, "Silicon nanowire field effect transistor sensors with minimal sensor-to-sensor variations and enhanced sensing characteristics," ACS Nano **12**, 6577–6587 (2018).
https://doi.org/10.1021/acsnano.8b01339
9. F. N. Ishikawa, H.-K. Chang, M. Curreli, H. Liao, C. A. Olson, P.-Ch. Chen, R. Zhang, R. W. Roberts, R. Sun, R. J. Cote, M. E. Thompson, and C. Zhou, "Label-free, electrical detection of the SARS Virus N-protein with nanowire biosensors utilizing antibody mimics as capture probes," ACS Nano **3**, 1219–1224 (2009).
https://doi.org/10.1021/nn900086c
10. J. Choi, T. W. Seong, M. Jeun, and K. H. Lee, "Field-effect biosensors for on-site detection: Recent advances and promising targets," Adv. Healthcare Mater. **6**, 1700796 (2017).
https://doi.org/10.1002/adhm.201700796
11. A. S. Trifonov, D. E. Presnov, I. V. Bozhev, D. A. Evplov, V. Desmaris, and V. A. Krupenin, "Non-contact scanning probe technique for electric field measurements based on nanowire field-effect transistor," Ultramicroscopy **179**, 33–40 (2017).
https://doi.org/10.1016/j.ultramic.2017.03.030
12. D. E. Presnov, I. V. Bozhev, A. V. Miakonkikh, S. G. Simakin, A. S. Trifonov, and V. A. Krupenin, "Local sensor based on nanowire field effect transistor from inhomogeneously doped silicon on insulator," J. Appl. Phys. **123**, 054503 (2018).
https://doi.org/10.1063/1.5019250
13. D. E. Presnov, S. Kafanov, A. A. Dorofeev, I. V. Bozhev, A. S. Trifonov, Yu. A. Pashkin, and V. A. Krupenin, "High quality factor mechanical resonance in a silicon nanowire," JETP Lett. **108**, 492–497 (2018).
https://doi.org/10.1134/s0021364018190037
14. Yi. Cui, Z. Zhong, D. Wang, W. U. Wang, and Ch. M. Lieber, "High performance silicon nanowire field effect transistors," Nano Lett. **3**, 149–152 (2003).
https://doi.org/10.1021/nl025875l
15. S. Thomas, R. L. Orozco, and T. Ameel, "Microscale thermal gradient continuous-flow PCR: A guide to operation," Sens. Actuators B: Chem. **247**, 889–895 (2017).
https://doi.org/10.1016/j.snb.2017.03.005
16. J. S. Farrar and C. T. Wittwer, "High-resolution melting curve analysis for molecular diagnostics," in *Molecular Diagnostics*, Ed. by G. P. Patrinos (Academic, 2017), pp. 79–102.
https://doi.org/10.1016/b978-0-12-802971-8.00006-7
17. T. Menzen and W. Friess, "High-throughput melting-temperature analysis of a monoclonal antibody by differential scanning fluorimetry in the presence of surfactants," J. Pharm. Sci. **102**, 415–428 (2013).
https://doi.org/10.1002/jps.23405
18. J. J. Chen, Ch. M. Shen, and Yu. W. Ko, "Analytical study of a microfludic DNA amplification chip using water cooling effect," Biomed. Microdevices **15**, 261–278 (2013).
https://doi.org/10.1007/s10544-012-9728-6
19. H. Zhu, H. Li, H. Zhang, Z. Fohlerova, Sh. Ni, Ja. Klempa, I. Gablech, Ja. Hubalek, H. Chang, L. Yobas, and P. Neuzil, "Heat transfer time determination based on DNA melting curve analysis," Microfluidics Nanofluidics **24**, 7 (2020).
https://doi.org/10.1007/s10404-019-2308-9
20. H. A. Erlich, *PCR Technology: Principles and Applications for DNA Amplification* (Stockton Press, MacMillan Publishers, New York, 1990).
21. P.-Ch. Chen, D. E. Nikitopoulos, S. A. Soper, and M. C. Murphy, "Temperature distribution effects on micro-CFPCR performance," Biomed. Microdevices **10**, 141–152 (2008).
https://doi.org/10.1007/s10544-007-9119-6
22. G. V. Presnova, D. E. Presnov, V. G. Grigorenko, A. M. Egorov, and M. Yu. Rubtsova, "Oriented immobilization of antibodies and their fragments on modified silicon for the production of nanosensors," Moscow Univ. Chem. Bull. **71**, 110–115 (2016).
https://doi.org/10.3103/s0027131416020061

Translated by I. Obrezanova