DOI 10.1007/s10812-023-01568-w
Journal of Applied Spectroscopy, Vol. 90, No. 3, July, 2023 (Russian Original Vol. 90, No. 3, May–June, 2023)

USE OF CONFOCAL MICROSCOPY TO MONITOR STRUCTURAL TRANSFORMATIONS IN NANOPILLARS BASED ON DNA AND CdSe/CdZnSe/ZnS QUANTUM DOTS

I. G. Motevich,[a] T. Erdem,[b] A. Akrema,[b] S. A. Maskevich,[c] and N. D. Strekal[a,*]

UDC 535.375.5;620.3

Chip system prototypes in the form of nanopillars were created from DNA complexes with CdSe/CdZnSe/ZnS quantum dots immobilized on a plasmonic gold film by the use of vacuum deposition technology and inorganic synthesis. The design and presence of terminal DNA labeled with Cy3 cyanine dyes makes it possible to carry out the hybridization reaction of this terminal strand with complementary DNA and to control the process by variation of the giant Raman scattering (GRS) and the fluorescence signal. The effect of molecular recognition of complementary DNA is accompanied by a change in the GRS spectrum, a 20-fold increase in the fluorescence intensity, and a decrease in the duration of fluorescence decay.

Keywords*: quantum dots, surface-enhanced Raman scattering, nanopillars.*

Introduction. The scientific school of spectroscopy, founded by B. I. Stepanov, A. N. Sevchenko, G. P. Gurinovich, A. M. Sarzhevskii, and other world-famous scientists, today opens up new horizons for international cooperation. This work was the result of scientific collaboration between scientists from Abdulakh Gül University (Kayseri, Turkey) and Yanka Kupala Grodno State University (Grodno, Belarus). Prototypes of chip systems were created in the form of an assembly of nanopillars of DNA molecules with quantum dots (QDs) on the surface of a plasmonic gold film connected by covalent addition of an α-SH DNA terminus and open to optically controlled specific binding of external DNA molecules labeled with organic chromophores. A unique technique of controlled self-assembly of DNA molecules labeled with quantum dots and cyanine dyes was used to form DNA nanopillars on the surface of plasmonic gold films obtained at Yanka Kupala Grodno State University. The binding of complementary DNA strands and the resulting change in the conformation of the nanopillars, leading to change in the energy of exciton–plasmon interactions, opens up the prospect of using the nanochips for the optically controlled molecular recognition of genetic fragments.

Earlier [1] we published results on the formation of fluorescent quasi-one-dimensional structures based on electrostatic complexes of cationic CdSe/ZnS QDs with DNA used as a molecular template as well as complexes of DNA with celandine alkaloids [2, 3], which are of practical importance in oncology. In the present work the formation of DNA–QD conjugates [4] using carbodiimide was studied.

The fundamental difference between a molecular beacon on nanopillars and molecular beacons operating in solutions based on the Förster mechanism of electronic excitation energy transfer is the possibility of registering three optically nonoverlapping responses in real time: shift of the plasmon resonance band, the QD fluorescence, and the Raman scattering of DNA and its molecular label. In this work we use confocal microspectrometry to show how the secondary luminescence signal of the nanopillars changes in relation to the conformation of the DNA and the distance between it and the plasmonic surface.

Experimental. Plasmonic gold films were obtained by vacuum deposition of gold onto quartz substrates using a VUP-5 vacuum unit as described in [5, 6]. CdSe/CdZnSe/ZnS QDs fluorescing in the red region of the spectrum were synthesized according to the protocol developed at Abdullah Gül University [7, 8]. According to measurements made with a

*To whom correspondence should be addressed.

[a]Yanka Kupala State University of Grodno, Grodno, Belarus; email: nat@grsu.by; [b]Abdullah Gül University, Kocasinan, Kayseri, Turkey; [c]Belarusian State University, A. D. Sakharov International State Ecological Institute, Minsk, Belarus. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 90, No. 3, pp. 456–461, May–June, 2023. Original article submitted March 15, 2023.

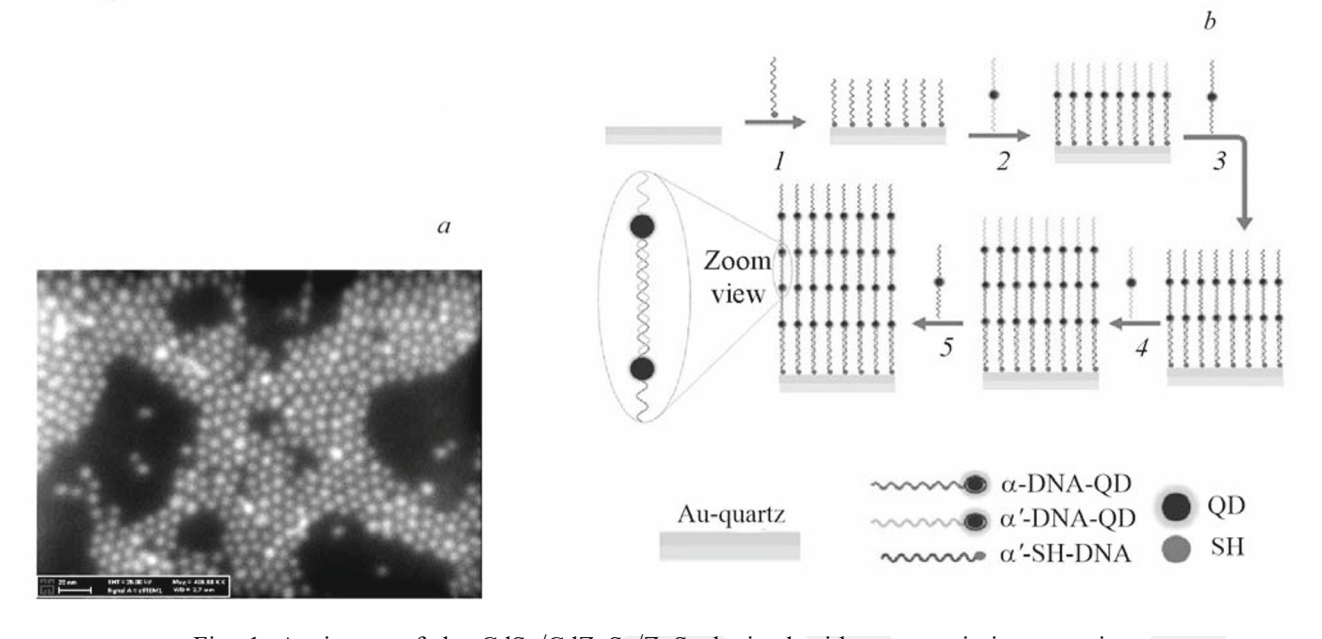

Fig. 1. An image of the CdSe/CdZnSe/ZnS obtained with a transmission scanning electron microscope (a) and a diagram of the formation of nanopillars based on DNA and QDs on the surface of plasmonic gold films (b).

transmission scanning electron microscope (Abdullah Gül University), the core/shell used in the QD work had dimensions of 8–10 nm (Fig. 1a). In order to remove unreacted reagents the obtained quantum dots were purified on a centrifuge at 3000 rpm for 5 min. The supernatant liquid was collected, and the QDs were precipitated with an excess of ethanol. The rotation rate for the QDs in the centrifuge was 5000 rpm for 5 min, and the precipitate was then redispersed in hexane. In order to obtain pure QDs the procedure was repeated twice.

Solubilization of the surface of the QD was realized by means of mercaptopropionic (MPA) and thioglycolic (TGA) acids. First 500 μL of the QD with a concentration of 10 mg/mL was flocculated with an excess of acetone and centrifuged at 10,000 rpm for 10 min. The precipitate was redispersed in 1 mL $CHCl_3$. Ethylenediamine and with vigorous agitation a 0.15 M solution of TGA or MPA were then added, and the mixture was stirred for 1 h. The organic and aqueous phases were separated, and the QDs in the aqueous phase were collected with a micropipette. The collected aqueous QTs were centrifuged with an excess of methanol in order to remove the remaining impurities.

The DNA modified with amino groups and cyanine dyes were acquired from the Oligomer company. The chemistry of carbodiimide cross-linking agents was used for conjugation of the amino-modified single-stranded DNA molecules (amine-ss-DNA) with the carboxyl groups present on the surface of the QDs coated with MPA or TGA. In a typical reaction a solution of 8 μM of the QD (25 μL) was diluted with a 50-mM borate buffer (200 μL, pH ~6.1) in a 4-μl glass vial. A 50-mM aqueous solution of N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 8 mL) and 50-mM aqueous solution of N-hydroxysuccinimide (NHS, 8 μL) was then mixed with the solution of the QDs. A solution of amine-ss-DNA with a 1:100 QD/DNA ratio was then added immediately, and the contents were stirred gently for 2 h at room temperature. During the conjugation reaction, the concentration of the salt in the solution was increased at a time interval of 30 min from [NaCl] = 0 M to [NaCl] = 1 M. The unconjugated DNA was then removed in an ultrafiltration unit with a molecular weight cut-off of 100,000, i.e., by centrifuging at 5000 rpm for 30 min and washing 3–4 times with 50-mM borate buffer solution (pH ~ 6.1). In a separate experiment the DNA–QD conjugates were tested for successful conjugation with complementary DNA labeled with Cy3 (not shown). The stoichiometric DNA/QD ratio amounted to 22 molecules of DNA to one QD. This ratio was estimated according to the method in [9].

In order to form the nanopillars of QDs with DNA it is necessary to screen the electrostatic repulsion, and NaCl was added to the dispersion of nanocrystals so that the concentration of the salt reached 100 mM. The alpha-DNA-coated nanocrystals (α-DNA–QD) (60 μL) were placed with a micropipette on the gold nanoislands coated with –SH–DNA, and the system was covered with a Petri dish for 30 min in order to limit evaporation. After 30 min the remaining solution was

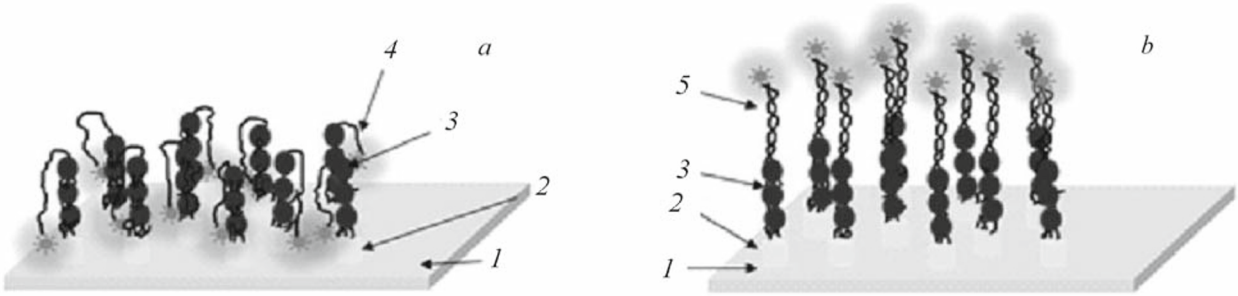

Fig. 2. The principle of "smart" self-assembly of the DNA on the plasmonic gold films: DNA nanopillars on the surface of a plasmonic gold film before (a) and after (b) attachment of complementary DNA molecules: 1) quartz substrate; 2) gold islands; 3) quantum dots labelled with DNA molecules; 4) single-strandard DNA molecules labelled with dye; 5) addition of complementary DNA molecules (the order of the bases is indicated in the text).

removed with a pipette, and the plasmonic film with the added nanocrystals was washed three times with the phosphate buffer solution by means of a micropipette in order to remove any uncoated nanocrystals. The first layer of DNA–QD complexes was thus formed. The second, third, and subsequent layers of DNA–QD were then deposited in a similar way with alternating α-DNA–QD and α′-DNA–QD. In this method the nanopillars are formed as a result of hybridization of the single-stranded α-DNA and complementary α′-DNA into double-stranded DNA rods located between the QDs in the resulting Zoom view of the layered structure (Fig. 1).

The two samples, each coated with α-SH and α′-SH DNA on gold nanoislands (Fig. 1b) amounting to five layers, had a length of ~205 nm (according to the number of bases in the nanopillars). Long-chain α-Cy3–DNA labeled with cyanine dye, where Cy3 is the cyanine dye, was then attached to the upper layer of this structure. In order to change the conformation of the nanopillars from pin (nonrigid, Fig. 2a) to rigid rod-like (Fig. 2b) long-chain (61 bases) complementary strands were attached to them. Thus, section 4 (Fig. 2a) is characterized by the following sequence of bases:

GCGTCGTGGCAACCTAGTTGCAGGCCAACGACAGCATTCGGTTAGGAGAGTGCGAGTCGCG

and finishes with the Cy3 dye. In Fig. 2b a single-stranded DNA binds complementary to this site with the base sequence:

CGCAGCACCGTTGGATCAACGTCCGGTTGCTGTCGTAAGCCAATCCTCTCACGCTCAGCGC.

The flexibility of the DNA molecules (Fig. 2) depends on whether the molecule is single-stranded or double-stranded. If the DNA is double-stranded it will be rigid whereas single-stranded DNA can have various conformations [10].

The secondary luminescence spectra of the DNA nanopillars labeled with the QDs and cyanine dye were measured on a 3D scanning confocal microscope with a Nanofinder S spectrometer (SOL Instruments, Belarus) directly from the substrates in the anhydrous phase. The excitation source was laser radiation with $\lambda = 532$ nm. The spectra were obtained with the Nanofinder S spectrometer provided with a 100× objective with a piezo scanner. The decay duration was measured on a picosecond pulsed spectrofluorimeter [11]. A PDL 800-B pulsed laser (Picoquant, Germany) with an LDH 400 laser head was used as radiation source with maximum spectral radiation density at $\lambda = 407$ nm, and the half-width of the spectral line was ~4 nm. The repetition frequency of the exciting laser pulses was 0.25 MHz.

Results and Discussion. Figure 3a shows the secondary emission spectra of the pin conformation DNA nanopillars containing QDs and Cy3 as fluorescent labels deposited on a gold plasmonic film. (The spectra in Fig. 3 are presented on two scales — absolute in nm and relative in cm^{-1}; on the inset the 0–2000-cm^{-1} range corresponds to the 530–600-nm range.)

As seen from Fig. 3a, in the Stokes region with respect to the laser excitation one of the spectra contains a fluorescence band for the Cy3 dye at $\lambda = 574$ nm. The other spectra contain QD fluorescence bands with maxima at $\lambda = 627$–639 nm. The shift of the QD fluorescence band and the predominant fluorescence quenching of the Cy3 dye indicate the absence of a

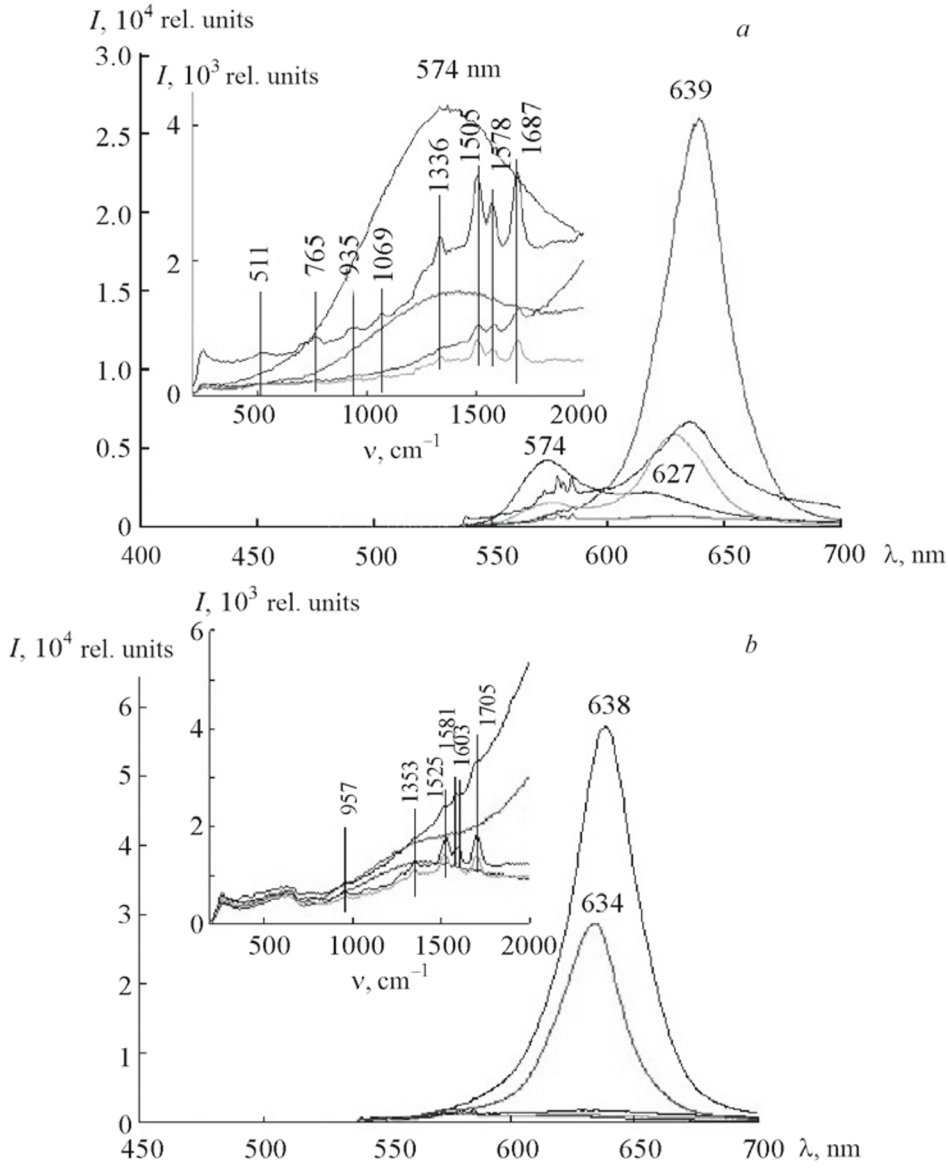

Fig. 3. Secondary luminescence spectra of a sample of the assembly of DNA–QD-Cy3 nanopillars in the nonrigid pin (a) and rod-like (b) conformations, recorded with spatial resolution at various points over the surface of the sample.

well-ordered structure for the assembly of nanopillars on the plasmonic gold film due to their low relative rigidity. Some of the terminal groups of the DNA with a terminal Cy3 are close to the gold surface, and the fluorescence of the dye is therefore quenched. Only one of the five spectra is characterized by noticeable fluorescence from Cy3 and here with even weaker fluorescence from the QDs. Thus, at the same points of the assembly of nanopillars, where the Cy3 fluorescence is strongly quenched, the QD fluorescence band is clearly pronounced, and the position of the maximum undergoes a hypsochromic shift with noticeable hypochromism.

The vibrational bands at 765, 1336, 1505, 1567, 1578, and 1584 cm^{-1} belong to the DNA vibrations enhanced by the surface of the plasmonic gold film [12]. These bands have highest relative intensity for one of the assembly points, where they are comparable with the intensity of the fluorescence band of the QD. This corresponds to a fragment of nanopillars that makes contact with the gold surface at the site where the DNA binds to the QDs.

The situation changes radically for the sample of DNA–QD–Cy nanopillars of rodlike (Fig. 2b) conformation (Fig. 3b). Thus, the relative fluorescence intensity of the QDs increases by a factor of 20 or more times this conformation

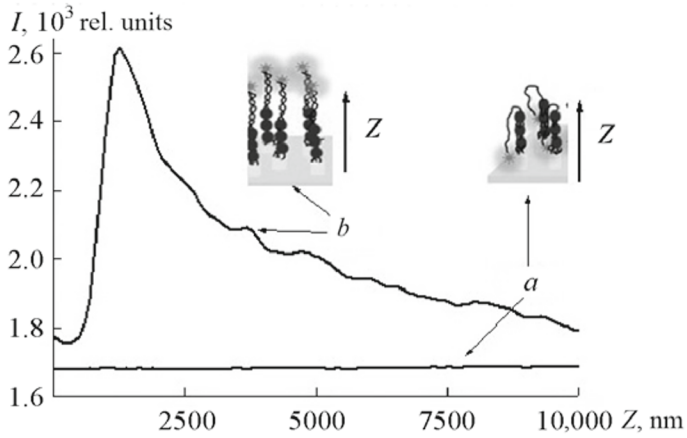

Fig. 4. Dependences of the fluorescence intensities at λ = 630 nm for the DNA–QD nanopillars in the pin (a) and rod (b) conformations on the distance to the substrate (*Z* coordinates along the normal from the samples).

compared with nanopillars in the nonrigid pin conformation (Fig. 2a), which may be due to at least two factors. First, the distances between the main donor–acceptor chromophores in the system (the Cy3 dye, QDs, and plasmon film) change. The QDs make a major contribution to the total fluorescence signal. Second, the assembly of nanopillars on the surface acquires an ordered structure, and the glow can be coherent [13]. The formation of individual domains with coherent luminescence can be confirmed by the decrease in the average QD fluorescence decay time from 0.168 to 0.156 nm compared with the nanopillars in the pin conformation. As for the previous sample, the position of the QD fluorescence maximum undergoes some shift. At points in the sample where QD fluorescence is quenched a weak (compared to the fluorescence intensity) GRS spectrum, consisting of bands at 957, 1353, 1336, 1525, 1581, and 1603 cm^{-1}, appears. In this case, according to the characteristic nature of its appearance, the band at 1580 cm^{-1} can be attributed to both Cy3 and DNA vibrations. The vibrations of the DNA cyanine label can appear at frequencies of 1336 and 1525 cm^{-1} [12].

Thus, the hybridization reaction and the change in the conformation of the terminal DNA in the assembly of DNA–QD–Cy3 nanopillars lead to a substantial increase of intensity and decrease of the duration of fluorescence quenching of the assembly. For practical application it is useful to study the dependence of the intensity of the individual bands in the secondary emission spectrum of the DNA nanopillars on the distance from the surface of the plasmon film, i.e., to scan along the *Z* coordinate (the plane of the sample here corresponds to the *XY* coordinate).

Figure 4 shows the dependences of the fluorescence intensity at λ = 630 nm, i.e., at the maximum of the fluorescence band of the QD for the samples in the pin and rodlike conformations. It is seen that with increase of the distance from the surface of the plasmon film, i.e., with increase of *Z* (the distance to the nanopillars), the fluorescence intensity in the DNA nanorods (Fig. 4b) changes little close to the surface, reaches its maximum at a distance of ~2λ from the sample, and then decreases monotonically (almost exponentially). On the decreasing part of the dependence maxima are observed at wavelengths of ~4λ, ~6λ, and ~8λ. The nonmonotonic character of the dependence and the presence of multiple 2λ oscillations (λ = 630 nm) indicates interesting optical characteristics in the created nanostructures. At the same time the fluorescence intensity of the QDs from the nanopillars of the pin conformation (Fig. 4a) remains practically unchanged at all distances from the sample and has low intensity. This fact indicates fluorescence quenching of the QD by the plasmon surface in the pin conformation.

Conclusions. From the created prototypes of chip nanostructures based on DNA, CdSe/CdZnSe/ZnS quantum dots, and an organic Cy3 label on the surface of plasmonic gold films it is possible to trace the molecular recognition reaction of single-stranded DNA strands as a result of changes in the giant Raman spectra and the intensity and duration of the fluorescence. It was found that the supramolecular structure of the obtained samples has new optical characteristics that show up in the nonmonotonic dependence of the fluorescence signal in reflected light.

Acknowledgments. The authors are grateful to S. V. Gaponenko for initiating this work.

The work was supported by the Belarusian Republican Foundation for Fundamental Research within the scope of the Tübitak-BRFFR international cooperation (Grant Nos. F21TYuB-021 and 220N155).

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