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New Optical Properties of Synthetic Opals Infiltrated by DNA

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Optical properties of photonic crystals (synthetic opals) with DNA inserted inside the crystals have been registered and analyzed in the visible and IR ranges. A decrease in the intensity of vibration spectra of DNA infiltrated in opal is observed. Molecules of DNA placed in the cavities of a photonic crystal became invisible for the detection by visible and IR spectroscopies. Spectroscopic features of DNA in a photonic crystal indicate the formation of condensed DNA inside and at the surface of the photonic crystal. The emission of the "DNA – photonic crystal" system has been registered at room temperature. By confocal microscopy, we have visualized DNA at the surface of a photonic crystal. The experimental results are explained by assuming the formation of coupled photonic states of DNA and a photonic crystal.

Keywords Confocal microscopy; DNA; fluorescence; photon density of states; photonic crystal; stop-band; synthetic opal; vibrational modes

Introduction

Photonic crystals (PC) are promising due to their potentialities in the control over the propagation and the emission of light. When light travels in a periodic crystal structure consisting of elements with a spacing of the order of a light wavelength, it could be diffracted by the PC structure, which leads to the formation of stop-bands for the propagation of electromagnetic waves and a modification of the photon density of states in the visible range. A photonic band gap could be formed if the stop-bands are overlapped along all directions of the light propagation and for all polarizations. Globular PCs are of great interest for optics, optoelectronics, and sensor applications [1,2]. That is why the experimental study of their optical properties is of importance for to-day.

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The infiltration of biomolecules in PC results in modifications of the optical properties of both the PC and molecules [3]. As known [4], DNA possesses a low photoluminescence efficiency with quantum yields of fluorescence (FL) of 10^{-2} to 10^{-5} at room temperature. The FL from DNA can be observed at room temperature due to the signal enhancement with a special optical arrangement. Another possibility to do this is presented by local field enhancement effects, using, for example, the enhancement of a DNA optical signal by a metal surface [4] or PC. We are going to get enhancement of the luminescence spectra of DNA infiltrated in opal due to the possible formation of new bound photon-electron states. A special optic equipment has been applied to the investigation of the optical properties of synthetic opal infiltrated by DNA.

On the other hand, the introduction of biological molecules in PC leads to a change of the dielectric properties of a medium between the structural elements of PC and to the formation of stop-bands. The optical properties of biological molecules introduced in the confined volumes of the photonic crystal cavities should be changed as well. DNA being a unique molecule has a size that is close to the spacing between structural elements in synthetic opal. That is why the goal of our study is to register the IR modes of separated DNA molecules in a confined volume.

The DNA visualization is not a simple task. As usual, it could be done with AFM microscopy on a mica substrate charged by Mg^+, Zn^+, etc. or by STM microscopy at its staining by a metal [5]. DNA molecules, condensed DNA, or DNA films give no clear images in optical microscopy. We tried to overcome this problem and get an image of DNA on the PC surface by confocal microscopy.

Materials and Methods

Synthetic opals were produced in Zelenograd (Russia) and Dnipropetrovsk (Ukraine) [6,7]. Nanodispersive silica globules near 250 nm in diameter were synthesized by the method of Stober *et al.* [8] through the hydrolysis of tetraethoxysilane $Si(OC_2H_5)_4$ in a water-ethanol solution in the presence of ammonium hydroxide as a catalyst. The molar ratios of components in the reaction mixture were as follows: $NH_4OH:H_2O:C_2H_5OH:Si(OC_2H_5)_4 = 0.76:18:11:0.14$. We used a DNA–water solution (2 mg/ml) after the sonication during 0.5 h with a UZDN-A device (Sumy), denaturation at 100°C, and rapid cooling up to 0°C. After the DNA solution impregnates opal, the PC was dried, and this procedure was repeated for many times.

The structure and the optical properties of samples have been characterized by UV-visible optical and FTIR spectroscopies, fluorescence, confocal microscopy, and SEM analysis. We applied the conventional technique for optical transmittance and reflectance, as well as infrared (IR) reflectance and absorbance spectra. Spectra of reflectance in the absorbance mode [9] have been registered with the use of a gold support as the substrate for PC [10]. IR spectra have been registered with a Bruker IFS-66 FTIR instrument in the range 7000–400 cm^{-1}. Confocal microscopy images of opal and opal with DNA were obtained with an LCM 510 (Carl Zeiss, Germany) microscope, and optical microscopy images were got with a LOMO microscope. Spectra of FL have been registered with a DFS–12 (LOMO) and an LPI RAS (Russia) equipment. SEM images of opals were obtained with an EPMA SEI JXA-8200 microscope.

Results and Discussion

Structure of Opal

SEM images of the opal surface are presented in Figure 1. Opal globule sizes were found in the interval 230–280 nm.

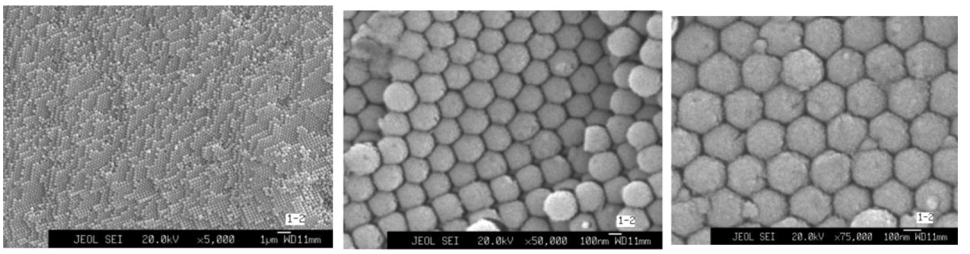
As seen from Figure 1b, the synthetic opal displays two types of short range ordering: one of them is hexagonal, and another one is of the octahedral type. Therefore, two different cavities sizes (27 nm for the hexagonal structure and 55 nm for the octahedral one) can be found in synthetic opal. In natural opals, the pores are filled by water. In synthetic opals, the pores are empty; however, sometimes small particles of silica are included in the pores (Fig. 1c).

Visible and IR Spectra

The transmittance and reflectance spectra of opals with different globule diameters ($d = 252$ nm and $d = 277$ nm) have been measured in the [111] direction in the 350–650 nm region (Fig. 2). The reflectance spectra were measured in the geometry of mirror reflection at an incidence angle of 7°. Unlike the reflection spectra, the spectral half-width of a transmission minimum depends more on the degree of disordering of the structure in bulk of a photon crystal (Fig. 2). The stop band for the 277-nm PC is wider than that for the 260-nm PC. The Bragg diffraction maximum occurs at 556 nm for $d = 252$ nm and at 613 nm for $d = 277$ nm globular PC in good accordance with transmittance spectra. All these data could be important for understanding the properties of PCs in the experiment with DNA visualization.

The near- and mid-IR transmittance spectrum of PC 0.85 mm in thickness are shown in Figure 3. In the IR reflection spectrum of opal (Fig. 4), we have registered the stretching (1122 cm^{-1}) and deformation (470 cm^{-1}) bands of O-Si-O, as well as a wide band of absorption of OH in the region of 3000–4000 cm^{-1}. The last fact is an evidence of water absorbed in opal.

Experiments with the DNA infiltration in opal have been done in the following way. First, we took an aliquot quantity of a DNA solution and dropped it on opal and CaF_2 simultaneously. Then we registered the IR spectra of DNA/opal and DNA/CaF_2 (see Fig. 5a). The minimal quantity of DNA that gave the IR signal on CaF_2 was equaled to 0.03 mg. In this case, the IR spectra of DNA/opal originated



(a) (b) (c)

Figure 1. Microstructure of synthetic opal (SEM images in the SEI mode): silica globules organized in terraces of a mosaic structure (a); domains with different types of packing (b); close view of ordered globules (c).

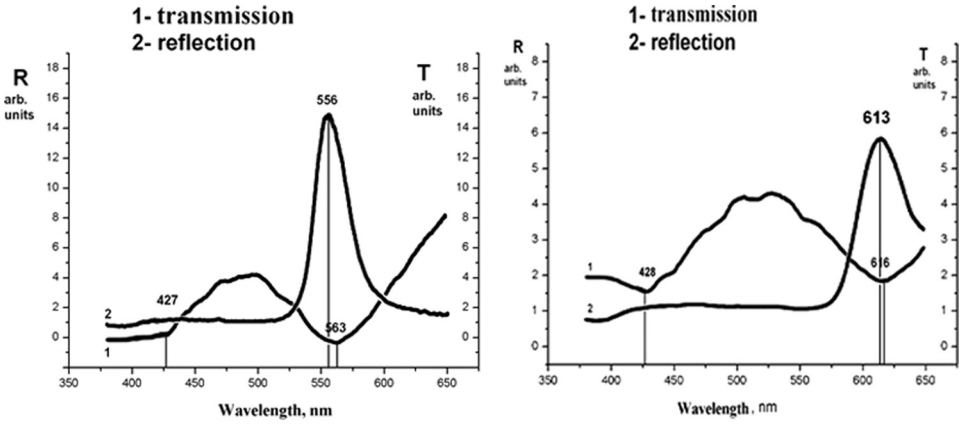


Figure 2. Bragg reflectance and transmittance spectra of synthetic opals with different diameters of silica globules: 252 nm (a); 277 nm (b).

from opal only. No bands were observed from DNA in DNA/opal (Fig. 5a, on the left). Then we add the DNA amount equaled to 0.6 mg (20 times more, as compared with the previous case) (Fig. 5b) onto both samples – opal and CaF_2. Again, we have registered the IR signal of DNA on CaF_2 (Fig. 5b, on the right) and not registered the spectra of DNA in DNA/opal. The visualization of the DNA/opal system did not show DNA at the opal surface. The last fact is not the enough evidence due to the difficulty to visualize DNA by optical methods. The increase in the DNA amount by 33 times in comparison with the initial experiment led us to successful results, and we managed to register DNA on the opal surface (Fig. 5c). Thus, increasing the total weight a drop of the DNA solution to 1 mg allowed us to visualize DNA with a confocal microscope. We suppose that PC was saturated by DNA, and our procedure

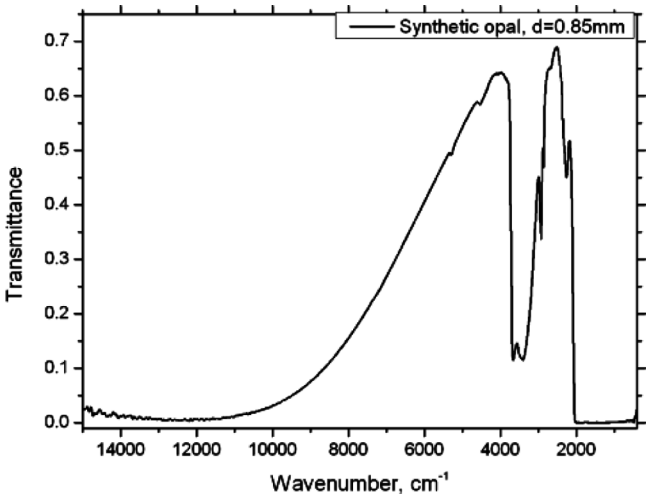


Figure 3. Near- and mid-IR transmittance spectrum of synthetic opal 0.85 mm in thickness.

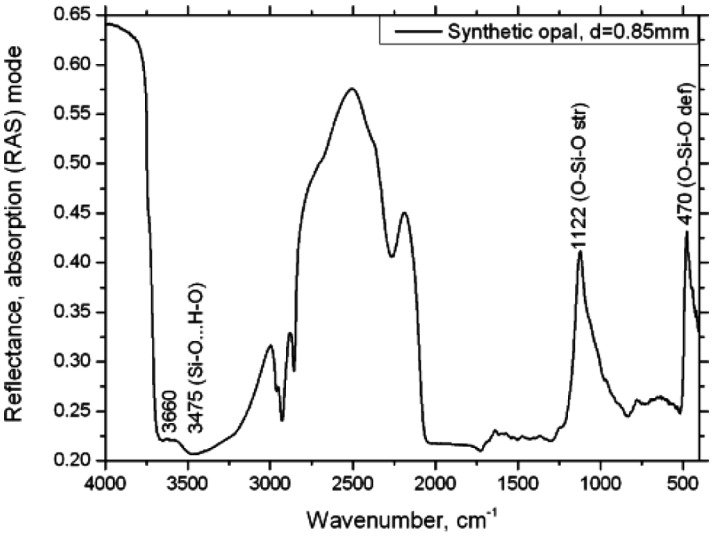


Figure 4. IR reflectance spectrum (in the absorbance mode) of synthetic opal 0.85 mm in thickness.

of application of additional drops of the DNA solution led to the formation of a DNA film on the PC surface. We note that this procedure of filling of PC was repeated many times with the use of different PCs, and we registered a decrease in the intensity of IR bands of DNA in opal in comparison with that for DNA adsorbed on the conventional IR support or glass. We suppose that we have started to reliably register the IR spectra of DNA in opal, when DNA saturated opal and appeared on its surface.

It is known that the FTIR spectroscopic method allows one to register the best spectra with a small amount of DNA [13], and increasing the number of DNA or other molecules leads to the saturation in some bands of absorption. Naturally, DNA aggregates scatter the light, however it became essential with increasing the amount of DNA and opposite our findings. In an IR spectrum, the scattering could be registered by the incline of the spectrum. However, we have not registered it in the IR spectra of DNA. By the way, changes in the IR spectrum of PC in the region of stretching OH vibrations near 3500 cm^{-1} were an additional evidence of the DNA insertion in opal (Fig. 5b, on the left). When the concentration of DNA molecules grows in opal, they replaced water molecules in opal pores from their sites of interaction with silica globules, and a rearrangement of the OH band near 3500 cm^{-1} occurs. The wide OH band in opal is replaced by a narrower band that is a characteristic band of DNA (Fig. 5c, on the left). So, the rearrangement of the band near 3500 is a marker of the DNA insertion in pores of PC (Fig. 5b, on the left). The additive effect of IR spectra from opal and DNA could not lead to the narrowing of the OH band.

Spectra of PC and the "PC–DNA" system have difference in the positions of vibration modes (1130 cm^{-1} and 480 cm^{-1}), and they are shifted to high frequencies due to increasing the dielectric permeability of the environment of PC globules, and DNA vibration modes do not yet appeared (Fig. 5b, on the left). Note that this amount of DNA is enough for the registration of IR spectra of DNA with any spectroscopic technique, including the traditional one.

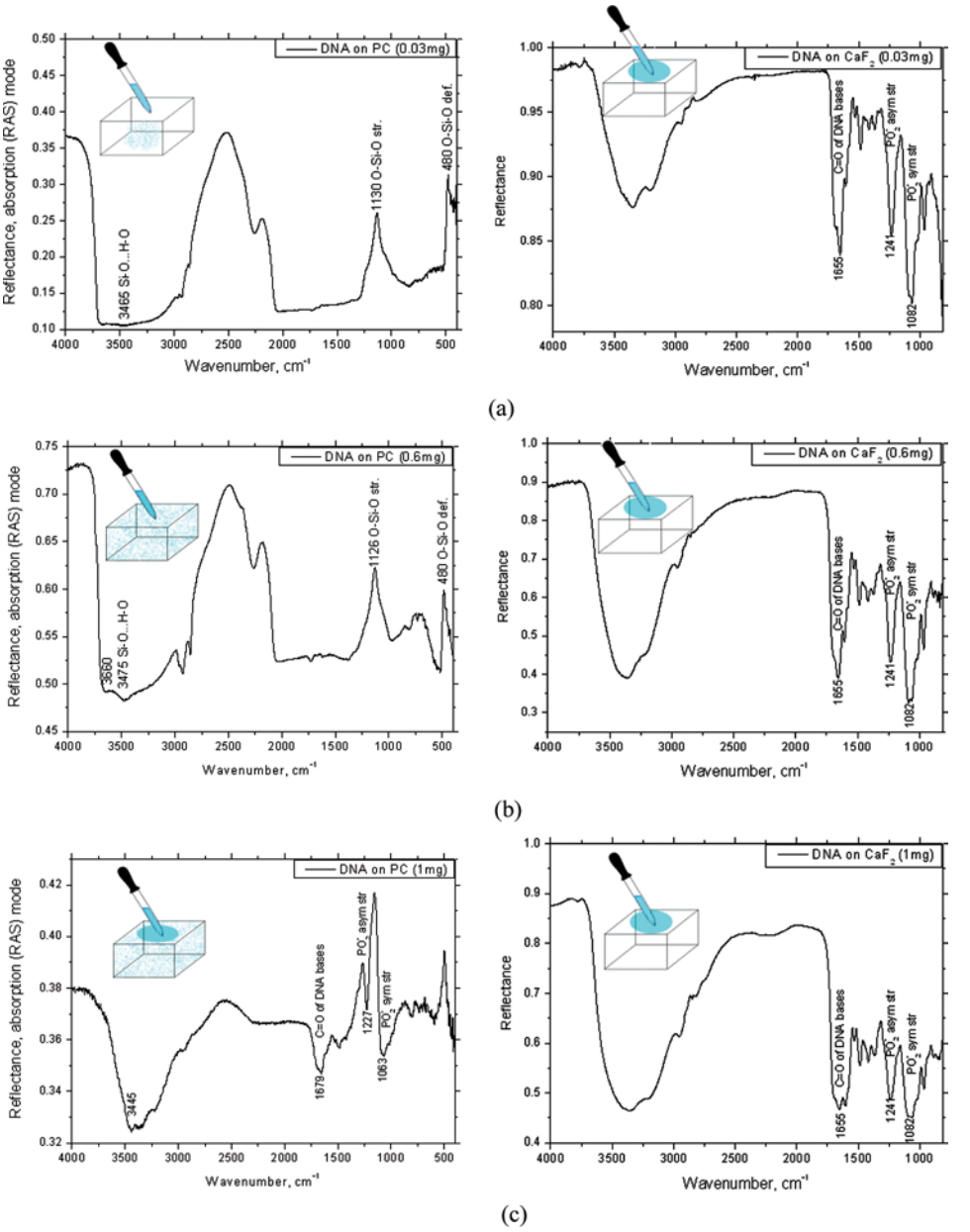


Figure 5. IR reflectance spectra (in the absorbance mode) of DNA infiltrated in opal (left) and absorbed on CaF_2 (right) in the same aliquot quantity: a) 0.03 mg, b) 0.6 mg, c) 1 mg.

The IR signal of DNA molecules in opal was decreased by 20–100 times for different bands in comparison with a signal from the same quantity of DNA molecules adsorbed on a CaF_2 substrate. Most changes in the DNA structure in a confined volume are connected with the formation of new H-bonds and the breaking of old H-bonds (Fig. 5b, on the left). New H-bonds appear in the region of OH and NH

Table 1. Vibration modes frequencies (in cm^{-1}) observed for denaturated DNA, PC, "PC-DNA" and their assignment

Samples			
DNA	Opal	Opal with DNA	Assignment
		3750–2850	Str. NH, OH, CH, H-bonded
	3660		Str. OH, H-bonded
	3475		
3352			Str. NH, OH, H-bonded
2950	2963	2963	Str. CH_3
2850	2930	2930	Str. CH_2
1238		1241	Asym. str. PO_2^-
	1122	1130	Str. O-Si-O
1094		1095	Sym. str. PO_2^-
		1082	
1069		1072	
1026			
	470	480	Def. O-Si-O

stretching vibrations (Table 1), and the increase (by twice) of the intensity of PO_2^- stretching vibrations is observed (Fig. 6). We suppose that such an increase of the intensity is related to the H-bonding of phosphate groups of DNA with the opal surface in a confined volume. From the positions of new bands in the sugar-phosphate backbone (Table 1) and their increased FWHM (up to 5–20 cm^{-1}), the formation of a new DNA conformation with elements of the B and Z forms [11–13] can be assumed, and this process is a result of the DNA condensation. Naturally, our supposition about changes in the DNA structure has relation to DNA inside PC and at the PC surface.

Emission from Opal

We used an experimental equipment (LPI RAS, Russia) [2,7] for the investigation of FL spectra, which gives us a possibility to enhance a quantum yield of DNA FL and to register the DNA FL in a solution at room temperature (Fig. 7). The fourth harmonic of a Nd:YAG laser (1.06 μm) was used for the excitation of FL in DNA and DNA in opal. DNA has a low yield of fluorescence at room temperature. However, we have got a spectrum of DNA due to a special equipment with high sensitivity and small contribution of scattered light (see [7]).

Opal has maxima of the secondary emission at 340 nm and 378 nm at room temperature. The maximum of FL in DNA has been registered at 350 nm with a shoulder at 378 nm at room temperature (Fig. 7). It is well known that FL of DNA takes place near 350 nm under the excitation at 260–265 nm [14,15].

We have obtained the FL spectra of PC and the "PC–DNA" system at the low temperature $T = 77$ K (Fig. 8). The position of the secondary emission of PC is practically unchangeable: 340 and 375 nm at the low temperature. The position of the DNA peak in opal at the low temperature is shifted to higher wavelengths by

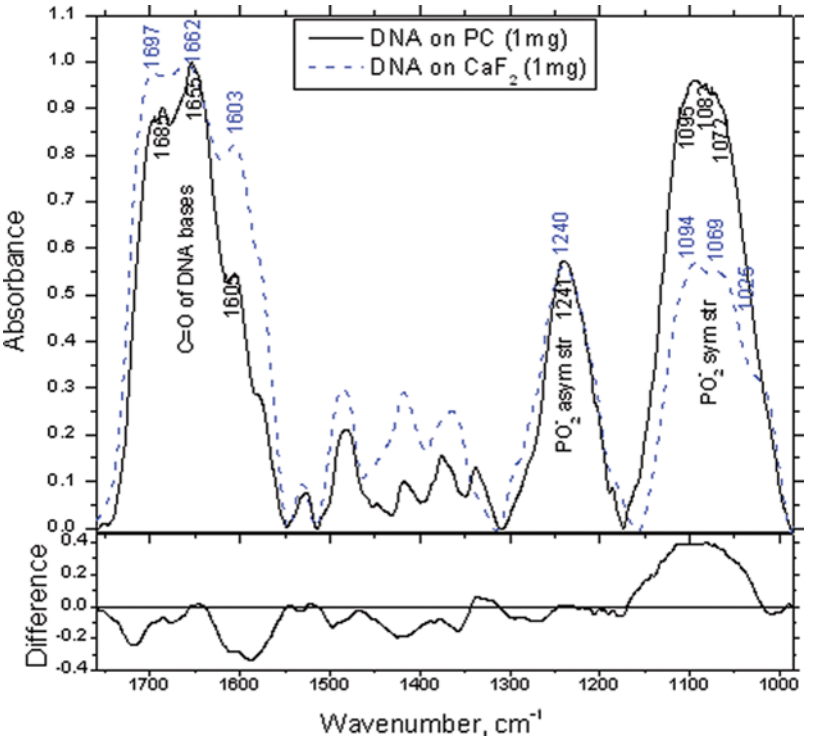


Figure 6. IR normalized absorbance spectra of denaturated DNA on CaF_2 (dashed line) and the "opal–DNA" complex (the mass of DNA in opal equals 1 mg) and their difference.

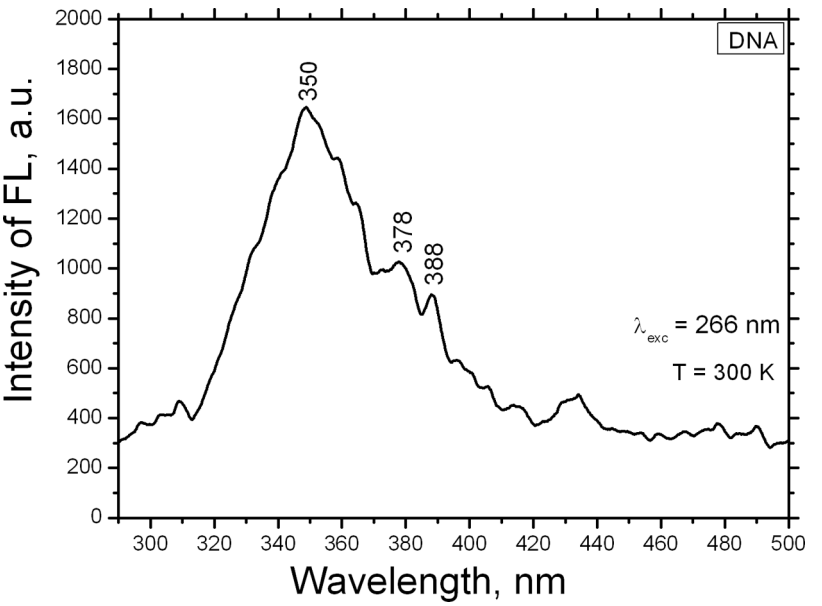


Figure 7. Fluorescence spectrum of DNA in solution ($\lambda_{exc} = 270$ nm) at room temperature.

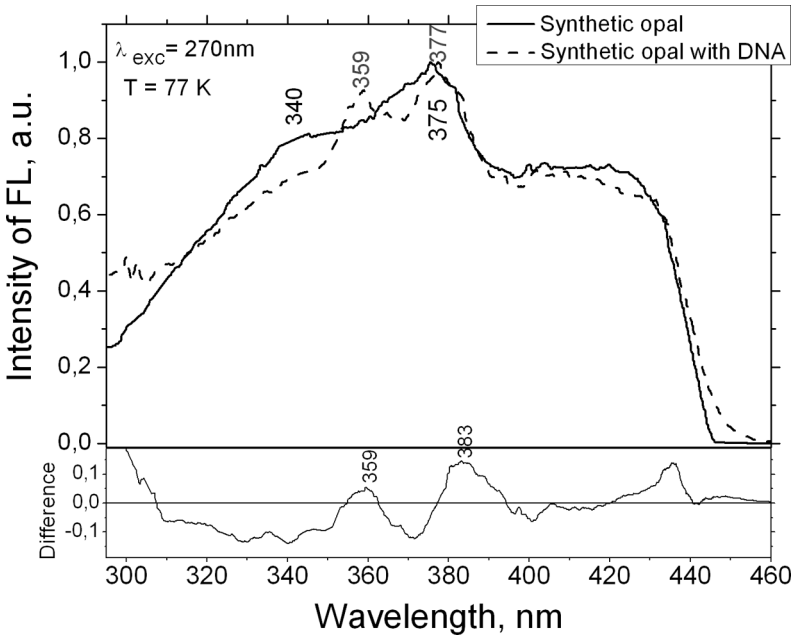


Figure 8. Fluorescence spectra of synthetic opal and synthetic opal with DNA at T = 77 K ($\lambda_{exc} = 270$ nm).

about 19 nm in comparison with that of free DNA (Fig. 7). The possible reason for this can be connected with the insertion of DNA in opal, which changes the DNA state and its conformation. At low temperatures, the FL of DNA and opal in the "DNA-opal" system has the same order of the intensity for both bands. We failed to resolve the emissions of DNA and opal in the "DNA-opal" system at room temperature (Fig. 9), and we can only conclude about the increase of the emission (Figs. 7–9). We suppose that the last fact is due to the essential increase of the radiation field caused by a slow diffuse motion of incident photons into the sample volume and a change of conditions inside the opal pores.

Confocal Microscopy

Confocal laser scanning microscopy (CLSM or LSCM) is a technique to get high-resolution optical images with depth selectivity [16,17]. The key feature of confocal microscopy is its ability to obtain in-focus images from selected depths. Images are acquired point-by-point and reconstructed with a computer, allowing three-dimensional reconstructions of topologically-complex objects. For opaque specimens, this is useful for the surface profiling; while, for non-opaque specimens, interior structures can be imaged. For the interior imaging, the quality of an image is greatly enhanced over that given by a simple microscopy, because the information from different depths of the specimen is not superimposed.

Confocal microscopes have the following benefits in biology relative to other microscopes: the refractive index of biological objects such as DNA is almost the same as a glass, so the optical microscope observation of these objects located on

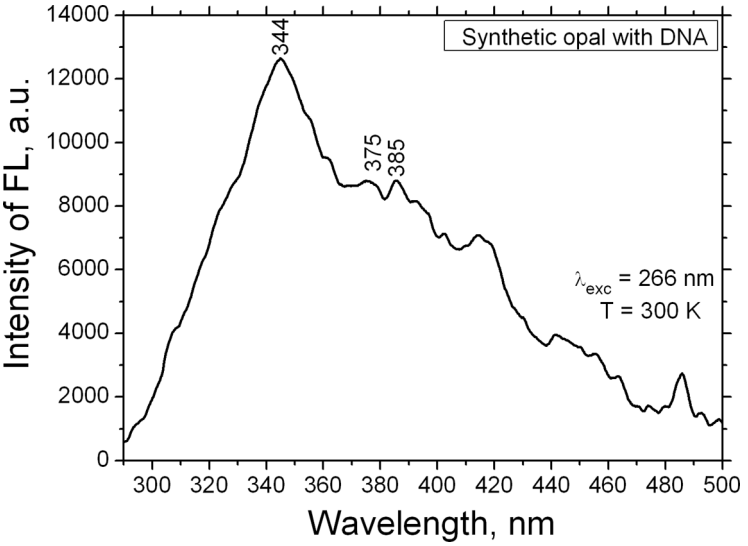


Figure 9. Fluorescence spectrum of synthetic opal with DNA ($\lambda_{exc} = 270$ nm) at room temperature.

the surface of a slide is usually very difficult. A confocal microscope which has high contrast and high depth resolution possesses two valuable features: it allows one to explore tissues at the cellular level in a state of physiological life, as well as to evaluate the results of the study (cellular activity) in four dimensions – height, width, depth, and time.

The PC surface has been visualized with conventional optical microscopy and confocal microscopy. Opal was illuminated with an UV lamp with a blue filter. In Figure 10, the regions of the ordering of a PC structure (domains) with sizes from 40 to 120 μm are shown. Optical microscopy has visualized the selected domains of the structure, namely, emitting domains. Confocal microscopy images of the synthetic opal surface and DNA localized at the PC surface are presented in Figure 11. Confocal microscope gives a possibility to visualize all domains near the PC surface

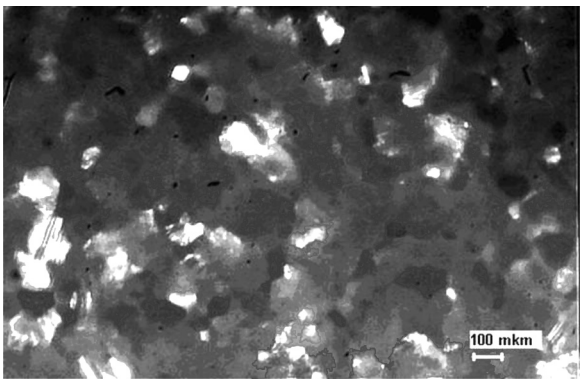
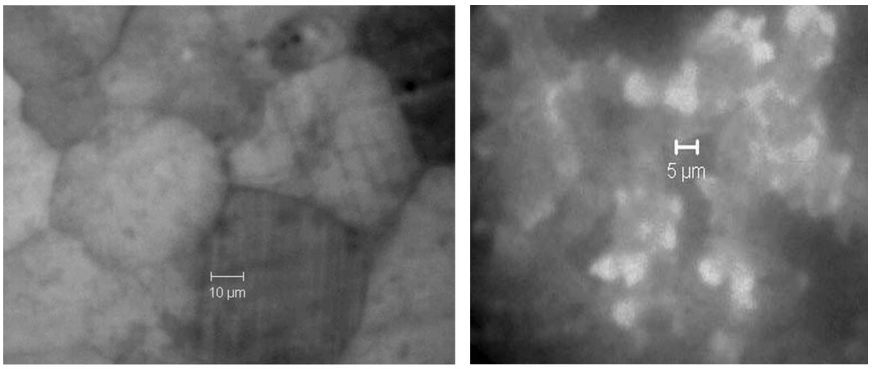


Figure 10. Image of synthetic opal after the infiltration with a DNA solution obtained with an optical microscope.



(a) (b)

Figure 11. Images of the synthetic opal surface (a) and DNA localized on this surface (b) obtained with a confocal microscope ($\lambda_{exc} = 365$ nm).

(Fig. 11a). After the cavities of PC being saturated by a DNA solution, DNA cannot penetrate deeply into PC more, by remaining on the PC surface, and we can visualized DNA. The reason for a possibility to visualize DNA is a property of the principal scheme of confocal microscopy which allows one to get an image from blocks of condensed DNA. The secondary radiation from PC is cut by the optical scheme of confocal microscopy and does not reach a detector, while PC shows the property of a bright light source for DNA molecules. Thus, in the case of a big amount of DNA (25 mg/cm^3 and more) infiltrated in PC, DNA molecules kept on the PC surface and can be visualized as emitting structures (Fig. 11b).

The FL of DNA cannot be excited by light waves 365 nm in length used by us in confocal microscopy. However, we have observed the emission of some molecular structured fragments of DNA (5 µm in size) at the opal surface (Fig. 11, b). We can suppose that this is connected with the formation of new emitting centres of the ''DNA–opal'' system. Our explanation came from our aim to enhance the optical signal from DNA inserted in opal, and the theoretical basis for it is available [18]. As we discussed above, the stop-band of PC starts near 400 nm and has slope with a big density of states that can arise from the coupling between DNA and opal photon states. In this case, the explanation of the visualization of DNA is related to the new above-mentioned property of PC to be a superlens [18].

Conclusions

We have registered a change of vibration band parameters and the drastic decrease of the intensity of vibration modes of molecules infiltrated in opal. DNA filled in the cavities of PC became invisible for the detection by the visible or IR spectroscopy. PC can be used as a hiding box for biological molecules.

DNA inserted into a confined space of cavities and at the surface of PC is folded in the unknown form with elements of the B and Z structures. Some spectroscopic features of DNA (markers of phosphodiester backbone) in PC denote the formation of condensed DNA.

The fluorescence of free DNA without labeling at room temperature and the emission of the ''DNA–opal'' complex with enhanced yield has been registered under

the excitation by the 265-nm light. We have visualized both the surface of PC and the DNA aggregates at the surface of PC due to the luminescent property of PC with confocal microscopy at $\lambda_{exc} = 365$ nm.

Acknowledgment

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