Vibrational spectra of DNA in the confined interglobular volume of photonic crystal

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Abstract The impact of confinement of DNA molecules in a limited volume of the cavity of photonic crystals (PC) on the vibrational properties of the DNA molecule and its conformation is studied. According to our preliminary study, the aqueous shell is removed when the DNA molecules are infiltrated into the PC cavities. Raman scattering (RS) DNA marker lines showed a dramatic conformational change of DNA in the PC cavities and the appearance of new unknown conformational states. We observed the enhancement of vibrational modes of DNA in the PC in comparison with free DNA of about tenfold and the absence of vibrational modes in DNA bases in a region of $1450\text{--}1700\text{ cm}^{-1}$. The observed features in the RS spectra of DNA are explained by the impact of confined interglobular volume and strong localization of the electromagnetic field. Namely, FDTD simulations in linear regime demonstrate the localization of light in cavities of PC with an approximately ninefold enhancement of the electric field within the photonic stop-band, which is the main reason for RS amplification.

Keywords DNA · DNA conformation · Vibrational modes · Raman spectra · Confined volume · Photonic crystal · FDTD simulations

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1 Introduction

DNA study is of great interest for biochemical and medical applications [1, 2]. Vibrational spectroscopy, namely, infrared (IR) absorption and Raman scattering (RS) [3] are used as analytical tools for the detection of DNA conformation and its interaction with ligands and nanoparticles as well as for DNA sequencing [2]. Biophysical experiments have to deal with an extremely small amount of substance therefore it is very important to develop high-sensitive methods of research. Suitable methods for DNA analysis are related to surface enhanced spectroscopy, namely, surface-enhanced infrared absorption spectroscopy (SEIRA), surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF). Typically, the local electric field enhancement due to the excitation of surface plasmon in systems with noble-metal (gold, silver) substrates or nanoparticles is used to enhance the optical response of DNA. Earlier in [4], we successfully used SEIRA to detect new peculiarities in the IR spectra of cancer DNA on rough gold surfaces and gold nanoparticles as well as SERS for small amounts of poly-A adsorbed on carbon nanotubes placed on Klarite substrate [5]. Currently, there are no platforms for SERS to detect large biomolecules, such as biopolymers and DNA. Spatial localization of the near field provides enhancement for small molecules (such as separate monomers of biopolymers). In the case of dielectric structures, the electric field enhancement can be obtained by excitation of cavity (defect) modes in the defects of periodicity in photonic crystals (PC) [6]. Recently, it has been demonstrated that non-linear optical generation can be enhanced in two- and three-dimensional (3D) globular PCs due to light focusing by globules pumped at wavelengths corresponding to different forbidden bands of PCs using the finite-difference time-domain method (FDTD) [7]. The enhancement of light harvesting in inverse TiO₂ opals due to the slow photon effect was theoretically demonstrated in [8], where the absorption of blue edge photons was observed. An enhanced optical signal (luminescence) has been shown for non-organic molecules such as dyes [9], quantum dots [10], and others after infiltration into pores of 3D PCs. At the same time, a two-dimensional photonic substrate was used for enhanced luminescence (label-free confocal images) from biomolecules and stem cells [11, 12]. We obtained the same results for DNA and cells (erythrocytes) on a 3D photonic substrate based on a synthetic opal [13, 14]. However, a change in luminescence (spectral shift and intensity increase) of DNA may indicate a destruction in the DNA chain (melting DNA) when it enters the PC cavity. Thus, the study of vibrational spectra can also identify conformational changes in the DNA structure in a confined volume.

In this work, the impact of a limited volume on the optical properties of macromolecules like DNA is determined and the enhancement of vibrational modes of the deoxyribose phosphate backbone of DNA molecules without presence of plasmonic nanostructures by using a 3D photonic opal-like crystal. In conventional Raman spectroscopy, it is possible to measure the signal from a drop of at least 30–100 μ l with a DNA concentration of 2–5 mg/ml. Usage of PC as a substrate allows reducing this amount, which is very important in various biological applications. It was also shown that after DNA infiltration into the PC, the signal from the DNA is severely distorted, which may indicate the formation of a new structure. Using numerical simulations of the linear response of 3D opal-like PCs in the framework of the FDTD, it was shown that this enhancement of the vibration modes is associated with a significant enhancement of the electric field in the region of contact of neighboring globules near the red edge of the photonic stop-band (PSB).

2 Materials and methods

Uniform SiO₂ nanoparticles were synthesized in a colloidal solution using the modified Stöber method [15]. A bulk synthetic opal was obtained by self-organization of globules during centrifugation and evaporation of the reaction mixture at a given rate. Finally, annealing of precipitated samples was performed in order to remove organic residues, extra- and intra-globular ethoxy groups and chemically bounded water at a temperature of 800 °C [16]. A $2 \times 3 \times 1 \text{ cm}^3$ crystal was used for the experimental study.

Ultra-pure calf thymus DNA fibers [17] were dissolved in distilled water (pH 5.5) in a cooling bath at room temperature to a concentration of 1 mg/ml. This DNA contains less than 0.2% of proteins, which is confirmed by UV-Vis spectroscopy in the region of 190–350 nm, resulting in the correct A260/A280 ratio [18].

The opal was impregnated with a 10- μl drop of aqueous DNA solution that penetrates deeply into the crystal under the action of capillary forces during 10–15 s. After infiltration, the sample was dried at room temperature for 30 min before Raman spectra measurements. The same drop of DNA solution was dried on Al foil and used for reference measurements. Note, the drop of DNA solution on Al foil or glass substrate dried within 50–60 min under normal conditions.

Raman scattering measurements were performed using a Schmidt–Czerny–Turner IsoPlane SCT-320 Imaging Spectrograph (Princeton Instruments), which has zero astigmatism, and a nitrogen cooled CCD PyLoN 400BR eXcelon (Princeton Instruments) [19]. In this work, excitation with a wavelength of 532 nm (Ti:sapphire laser) and power of no more than 10 mW/ μm^2 were used. The measurements were carried out with a grating of 600 l/mm, which provides a spectral resolution of 5 cm^{-1} in the range of 100–3800 cm^{-1} . Water immersion objective Zeiss W Plan-Apochromat 40 $\times$ /1.0 DIC VIS-IR in an inverted microscope configuration was applied.

Visible (400–700 nm) reflectance spectra were measured using a DMR-4 monochromator and variable-angle of incidence reflectance attachment in non-polarized light. The device was equipped with a silicone photodiode detector and an incandescent bulb lamp as a wide-band continuous spectrum light source.

Luminescence confocal images were obtained by a confocal laser scanning microscope Carl Zeiss LSM-510 META with Plan-Neofluar 10 $\times$ /0.3 Korr and 40 $\times$ /0.6 Korr objectives. The fluorescence images (512 $\times$ 512 pix) were registered by using a Zeiss AxioCam digital camera. PL excitation was carried out using an ultra-violet lamp HBO 100 and blue (FSet01 wf), green (Fset10 wf), or red (Fset20 wf) filters. Scanning electron microscopy (SEM) images of opals were obtained with an EPMA SEI JXA-8200 microscope.

3 Results and discussion

3.1 Photonic crystal based on synthetic opal

The electron micrograph of the PC surface presented in Fig. 1 indicates rather uniform close-packing of nanosized SiO₂ globules (at least on the scale of several hundred micrometers). The average sizes of SiO₂ globules (determined from the SEM data) are $223 \pm 20 \text{ nm}$ (Fig. 1b) and the sizes of the triangle cavities between the SiO₂ globules are 70–90 nm. As we can see from Fig. 1a, there are two types of point defects: the first type of defects is the absence of globules (mark A in Fig. 1a); the second type is formed as a result of failure of periodicity of the structure (mark B in Fig. 1a).

Fig. 1 SEM image of the surface of a synthetic opal (a) built from SiO₂ globules with diameter $D = 223 \pm 20$ nm and globule diameter distribution (b)

The ordered periodic structure of PC results in restriction conditions for light propagation in a narrow range of frequencies forming the PSB due to light interference and diffraction [3, 20]. The PSB position for a photonic crystal constructed from *fcc* packed spherical globules can be estimated by the modified Bragg equation:

$$\lambda_{\max} = 2a_{[111]} \sqrt{n_{\text{eff}}^2(\lambda) - \sin^2 \theta}, \quad (1)$$

where $a_{[111]} = D(2/3)^{1/2}$ is the distance between the planes of closely packed spherical globules in the [111] direction, D is the diameter of globules and θ is the angle of light incidence on PC. The effective refractive index, $n_{\text{eff}}(\lambda)$, for the synthetic opal depends on cavity filling and can be estimated in the effective media (linear) approximation for N components:

$$n_{\text{eff}}(\lambda) = \sum_{i=1}^N n_i(\lambda) f_i, \quad (2)$$

Fig. 2 Reflectance spectra (light incidence angle 10°) of the initial synthetic opal (black solid line) and infiltrated one with DNA water solution measured 20 and 80 min after infiltration (blue and red solid lines, respectively)

where n_i, f_i are the refractive index and the volume fraction of the i -component, respectively. For a dense *fcc* packing of identical globules, the globules volume fraction is:

$$f_1 = \frac{\pi}{3\sqrt{2}} \approx 0.74. \quad (3)$$

In the case of a 3D PC consisting of closely packed SiO_2 globules in some dielectric background, we use a two-component composite with $n_1 = n_{\text{SiO}_2} = 1.51$ and the volume fraction of Eq. (3) for the first component. For the second component ($f_2 = 1 - f_1$), we use air ($n_2 = n_{\text{air}} = 1.002$) or water ($n_2 = n_{\text{water}} = 1.334$) with/without DNA molecules because cavities of PC can be infiltrated.

Figure 2 shows the position of the PSB of PC measured at different stages of the experiment. The PSB of the initial sample occurs at 506 nm for a light incidence angle of 10° , which agrees well with the estimation using Eq. (1) for globules with $D = 223$ nm and $n_{\text{inf}} \approx n_{\text{air}} \approx 1.0$ ($n_{\text{eff}} = 1.366$). However, after filling the cavities with an aqueous DNA solution, the n_{inf} increases, which redshifts the position of the PSB maximum to 531 nm ($n_{\text{inf}} = 1.33$, $n_{\text{eff}} = 1.455$). During 80 min, while water is evaporated, the position of the PSB is returned consistently to its initial value and stops near 511–512 nm ($n_{\text{inf}} = 1.09$, $n_{\text{eff}} = 1.392$). Thus, after the evaporation of water at room temperature, the cavity pores of PC are infiltrated mainly by DNA and air, leading to a residual shift of 5–6 nm in the long-wave region towards the PSB of the initial PC.

Note, that the initial opal contains a small fraction of the absorbed water, which was observed by IR spectroscopy [21, 22]. When DNA penetrates into the cavities of PC, it displaces adsorbed water binding via hydrogen bonds with globules of the synthetic opal and resets its hydration shell [21].

3.2 Confocal luminescent imaging

The presence of DNA molecules dispersed on the surface (and in the subsurface region) of PC was confirmed by the luminescent confocal microscopy data. A detailed description of such an effect has been reported in [13]. After infiltration of the PC with DNA, the fluorescence

Fig. 3 Fluorescence images in the spectral range 470–520 nm ($\lambda_{\text{exc}} = 365$ nm) of the synthetic opal surface area near the edge of a drop (10 μl) of pure “DNA–water” solution (**a**) and the same one additionally sensitized by Hoechst 33342 dye (**b**) at room temperature. *Dark area* – initial PC surface, *luminous area* – DNA infiltrated into PC

intensity of the PC surface in this stain area is increased (Fig. 3a). Based on our visible spectroscopy data, we assume that the DNA molecules from an aqueous solution are sucked down with water into the cavities of the PC by capillary forces. To confirm that the observed enhanced emission in the range of 470–520 nm is caused by DNA, we additionally labeled them in aqueous solution by Hoechst 33342 dye (Fig. 3b). Comparing Fig. 3a and b, we can conclude that the photonic crystal acts as an enhancing substrate for the fluorescence of DNA *in vivo*, without dye labeling, even at room temperature.

3.3 RS spectroscopy

Figure 4 shows the Raman spectra of native DNA on Al foil and DNA infiltrated in the PC in the region of 400–1800 cm^{-1} and 2000–3500 cm^{-1} . The first insight into the spectra of the reference DNA and DNA infiltrated in the PC shows drastic differences between them: a change in the band frequency positions, redistribution of intensity, appearance of new bands and absent vibration bands in the region of 1400–1700 cm^{-1} , caused by missing vibrations of nucleoside *Ade* and *Gua* markers. The line frequencies and vibration assignments are presented in Table 1.

Fig. 4 Raman spectra of DNA on Al foil and into PC in the range 400–1800 cm^{-1} (a) and 2700–3100 cm^{-1} (b). Excitation with Ti:sapphire laser $\lambda_{\text{exc}} = 532 \text{ nm}$

The most drastic changes in DNA spectra were caused by conformation changes, manifested by the modification of vibrations for bases coupled with sugar and out-of-plane carbonyl bending vibrations, which are located in the region of 400–800 cm^{-1} . Here we divided the middle region into five parts and analyzed them according to Schrader [25]. Among them, the guanine ring mode coupled via the C1'-N9 to the sugar vibration is extremely sensitive to conformation. Namely, in this region, the most drastic changes in the RS spectrum of DNA in the PC were observed: appearance of strong lines at 441, 615, and 690 cm^{-1} in comparison with weak DNA lines on Al foil at 430 cm^{-1} (A-form), 622 cm^{-1} (A-form), 682 cm^{-1} (A-form) and 681 cm^{-1} (B-form). This means that DNA in a confined volume could change its conformation via the most flexible part of the DNA, namely, sugar with a drastic shift of the

Table 1 Raman frequencies (cm^{-1}) for canonical forms of DNA (A, B, and Z), melted DNA taken from literature, and DNA in PC and on Al foil

In current work		In references [3, 23]			Bands observed for melting [24]	Assignments
DNA in PC	DNA reference	A form	B form	Z form		
441		430				d [3]
615		622				Ade, Cyt, Thy [3]
				622		dG, dA syn [23]
690	680				-682	dG C2'-endo/anti
		682	681			dG [23], G [3]
		704				d [3]
	725	727	729			Ade [3]
	780	781	787			Cyt, Thy [3]
834			834			d (PO_2)Bk [3]
	883	881				d [3]
965	973	965	970			d [3]
1083	1065	1055	1054			d (νCO) [3]
	1096	1099	1094		-1094	d ($\nu_s\text{PO}_2$) [3]
1170	1176	1182	1182		+1183	dT, dC [23] (Thy, Cyt [3])
1260	1257	1255	1257		+1257	dC, dA [23] (Cyt, Ade [3])
1306	1293		1292		+1289	dC [23] (Cyt [3])
1314	1301	1301	1304		+1308	dA [23] (Ade [3])
1325	1325	1322	1319		+1320	dG [23] (Gua [3])
1335	1333	1336	1339		+1324	dA [23] (Ade [3])
1356	1368	1373	1376		+1365	dT, dA, dG [23] (Thy, Ade, Gua [3])
1430	1417		1422		+1381	d (CH_2 δ); Ade [3]
					+1412	d purine/syn [3]
	1481	1483	1489			Ade, Gua [3]
	1571	1574	1577			Gua, Ade [3]
	1668	1669	1669			Thy, Gua (C = Ov) [3]
2850						C-H [3]
2908	2892	2894	2896			d(CH_v) [3]
2934	2955	2950				d(CH_v) [3]
2968		2968	2964			d(CH_v) [3]

d – indicates the deoxyribose phosphate backbone; + or – sign indicates that the band has gained (or lost) appreciable intensity as a result of DNA melting

lines at about $7\text{--}9\text{ cm}^{-1}$. Along with this, the most intensive lines in the RS spectra, namely at 725 cm^{-1} (727 in A and 729 in B) as well as 781 cm^{-1} in A and 787 cm^{-1} in B are missing in DNA in PC. These lines belong to Ade (725 cm^{-1}) and Cyt (780 cm^{-1}) vibrations and DNA spectral features are recognized. It seems that these vibrations are drastically blocked in a limited volume.

RS lines between 800 and 1000 cm^{-1} are identified as the vibration modes of the phosphodiester backbone and sugar moieties, which characterize the transition between different helical DNA families. In this region, two weak bands at 834 cm^{-1} (B-form) and 965 cm^{-1} (A-form) with the missing of the band at 883 cm^{-1} (in reference, 881 cm^{-1} in A-form deoxyribose) are observed in DNA in the PC. It seems that the backbone and sugar vibrations are suppressed due to the interaction with the surface of PC globules. The absence of RS bands of DNA in the region of $1430\text{--}1700\text{ cm}^{-1}$ could be associated with PSB. However, in this case, the regular elements should be $1\text{--}5\text{ }\mu\text{m}$, which is difficult to explain.

In the $1000\text{--}1250\text{ cm}^{-1}$ region, phosphate groups with sugar units make the most contribution to the conformation of DNA with small changes in different forms. The vibration of

phosphate groups of DNA in the PC is observed at 1083 cm^{-1} in comparison with the reference DNA (at 1096 cm^{-1}) and A and B-forms (at 1099 and 1094 cm^{-1} , respectively).

The region between 1250 and 1450 cm^{-1} is called the 'DNA fingerprint' region where the vibrations of bases coupled via glycosidic C1'-N1 and C1'-N9 to vibration of sugar appear. There are a lot of bands of DNA in the PC, assigned to the bases- dC, dA: 1260 cm^{-1} (1257 in reference DNA), dA 1301 cm^{-1} (1306 in reference DNA), dG at 1314 and/or 1325 cm^{-1} (1322 -A and 1319 -B, 1325 in reference DNA), dA at 1335 cm^{-1} (practically coincides with A and B-form DNA) in A-form of DNA in PC, 1356 cm^{-1} - dA, dT, dG (does not coincide with 1368 cm^{-1} in reference to A and B-form DNA). The last band in the region observed at 1430 cm^{-1} for DNA in the PC could be assigned to C-H₂ bending. The changes in the spectra (deviation, deflection) could be connected with the bending of the DNA backbone and the exiting of molecular groups from their conventional position.

The vibrational bands of DNA in the region of 1450 – 1800 cm^{-1} are related to the double bond stretching of the residual bases. The intense Raman line near 1489 cm^{-1} (B-form) or 1483 cm^{-1} (A-form), 1481 cm^{-1} (our reference DNA) is connected partially with N7 of purine. This region is sensitive to base pairing and base stacking interaction. There is missing the DNA band in PC in this region. This can be caused by bounding of N7 of purine position with the surface of PC globules.

In the region of 2860 – 3000 cm^{-1} of CH₂ and CH₃ stretching vibrations, both redistribution of intensity and drastic enhancement of vibration intensities in comparison with RS spectra of DNA on Al and PC were observed.

In the DNA-PC spectra, we registered mostly vibrational modes of the sugar-phosphate backbone, namely, deoxyribose and phosphates and noticed drastic bending of the sugar-phosphate backbone. They are more intensive modes and their frequencies were shifted in comparison with those in conventional A and B forms of DNA. The vibrations of bases are suppressed in the entire IR region excluding vibrations of bases coupled with sugar. We observed missing the main DNA marker vibrations in the 600 – 800 cm^{-1} region and only weak base bands in the 1300 – 1380 cm^{-1} region certify the presence of DNA molecules.

From the comparison of DNA in the PC and melted DNA we found that the markers of melted DNA are closer to A-DNA form than to DNA in PC (see Table 1). Therefore, we support partial unzipping of DNA in PC as in our previous IR data [21]. However, it seems valid for a short circuit. In reality, we observed drastic decreasing of water molecules per nucleotide from 20 to 2–3 and decreasing of intensity of the high-frequency band shoulder at 1700 cm^{-1} responsible for DNA paring. The other idea about possible condensation of DNA in the cavities of the PC does not work if we compare marker bands of condensed DNA with those for DNA in PC cavities [26, 27]. The marker bands of DNA in PC do not fit the RS or IR marker band in condensed DNA [23, 28]. However, we noticed suppressed vibration of DNA in the region of 1430 – 1800 cm^{-1} in PC and enhanced vibrations in 400 – 1440 cm^{-1} and 2860 – 3000 cm^{-1} due to a specific PC feature, namely, enhancement via a localized electrical field in the PC cavity. When we registered Raman spectra from DNA in PC with excitation 633 nm , we did not register an enhanced signal. Probably, the enhancement of one of the vibrations and suppression of another depends both on DNA conformation and the map of electrical field redistribution in the confined volume of PC and the orientation of the field relative to the dipole moment of the DNA moieties. If the molecular group (fragment) is located in the maximum of intensity of the electrical field, the cross section of the RS process is increased [25].

DNA during infiltration in the PC cavities bends its backbone. It is proven by an increase of intensity and position shift, approved of marker bands of the backbone, namely in the region

400–500 cm^{-1} as well as in 700–1200 cm^{-1} in DNA infiltrated in the PC. Thus, DNA bends many times its backbone leading to a difference in DNA infiltrated in the PC in comparison with the reference DNA spectra.

The estimated averaged number of DNA molecules introduced into the cavities of PC is 1–2 molecules per cavity. We suppose that most of the DNA molecule is localized near the top of the triangular cavity or on the walls of the globules. The last event is less feasible. In the latter case, the structure of DNA becomes disordered and bonds between DNA and globules could appear.

Based on registered changes, it can be assumed that longer stretches of DNA are entropically elastic under tension. When the DNA is in solution, it undergoes continuous structural variations due to the energy available in the thermal bath of the solvent. This is due to the thermal vibration of the molecule in combination with continual collisions with water molecules. For entropic reasons, more compact relaxed states are thermally accessible than stretched out states and DNA molecules are found almost everywhere in tangled relaxed conformations. For this reason, one molecule of DNA will stretch under a force, straightening it out. The entropic stretching behavior of DNA from the point of view of the physics of polymers has been studied using optical tweezers [29, 30]. It was found that DNA behaves largely like the Kratky–Porod worm-like model under physiologically accessible energy scales. Thus, for DNAs in the form A or B in solution, it is better to use a SERS substrate with a rough surface, where the DNA molecule is located as single molecules.

With sufficient tension and positive torque, it is considered that the DNA undergoes a phase transition, with the bases spreading outward and the phosphates moving to the middle. This structure for overextended DNA was named P-form DNA [31] in honor of Linus Pauling. We suppose that DNA in the confined volume of the PC seems to be similar to the above-mentioned structures with altered positions of the vibration bands in comparison with conventional A and B forms. Meanwhile, a slightly changed vibrational frequency for adenine in the PC at 1335 cm^{-1} (1333 cm^{-1} in reference DNA) and an unchanged one for guanine at 1325 cm^{-1} (1325 cm^{-1}) can be used to detect a small amount of DNA.

3.4 Simulation of electric field enhancement in linear regime

The presence of a periodic structure in PC leads to observations of the PSB due to light interference on the periodically located scatters and electric field localization in the cavities of the PC (hotspots formation). The influence of strong light localization in a tiny volume of PC cavities on the enhancement of second-order non-linear optical effects, such as photoluminescence, is shown in [7, 11, 12] where the localization of the electric field is controlled by the parameters of the PC. In this section, we present the results of the calculation of the electric field in a 3D opal-like PC with parameters close to the experimental data to show the existence of areas with a local enhanced electric field in the linear regime that leads to increased non-linear optical response (the Raman scattering signal). The band structure for the *fcc* lattice consisting of 231 nm SiO_2 spherical particles in air is presented in Fig. 5. The calculations are performed for an opal-like PC consisting of SiO_2 spherical particles with a diameter of 231 nm in air by the FDTD method [32, 33] with a grid step of less than 1.5 nm in regions of rapidly changed fields. As can be seen from Fig. 5, there is no full photonic band structure, however there is the PSB near the *L* point (direction of the [111] plane).

The spatial distributions of the electric field intensity in a quasi-3D opal-like PC for normal incidence of light (along the *z*-axis coinciding with the [111] direction) for the wavelength

Fig. 5 Band structure for *fcc* lattice consisting of SiO_2 (231 nm) spherical particles in the air. In the inset, the Brillouin zone of the *fcc* lattice with labels at high-symmetry points is presented. The corresponding directions in the real space lattice are marked with arrows and Miller indices of respective lattice vectors. The shaded region corresponds to the scanning direction used for angle-resolved measurements

inside the PSB in three sectional planes (along the light propagation, $x0z$ and $y0z$ and on the frontal monolayer of dielectric spheres, $x0y$ – right side in the corresponding figure) are presented in Fig. 6b, c for two light polarizations along the x -axis (Fig. 6b, c) and along the y -axis (Fig. 6c). The quasi-3D opal-like PC under consideration consists of ten monolayers of dielectric spheres and its schematic illustration is presented in Fig. 6a. The orange parallelepiped shows a computation box with open-space conditions for the direction of wave propagation and periodic boundary conditions on the other sides. The violet arrow shows the direction of light propagation with the polarization of the electric field represented by blue arrows. The calculations are performed for incident light with a wavelength of 531 nm corresponding to experimental results. The intensity of the electric field is represented in the form of the electric field enhancement factor (EFEF) as the ratio between the electric field intensities inside of the PC, $(E_1)^2$ and those for the incoming field, $(E_0)^2$: $\text{EFEF} = (E_1/E_0)^2$.

As follows from Fig. 6, the electric field is attenuated from the surface to the depth of a quasi-3D PC at a distance of about ten globule monolayers for the incident wave with the photon energy near the PSB (531 nm). In parallel, we can observe an essential inhomogeneity in the electric field distribution with a local electric field enhancement. The highest values of EFEF are observed near the contact of two adjacent globules with the formation of so-called ‘hotspots’. In these regions, the EFEF reaches a factor of 8.6 in the case of electric field polarization along the line between the centers of two neighboring contacting globules (Fig. 6b, c) and 6.46 in the case of electric field polarization along the y -axis (Fig. 6d, e). This is due to the angle between the incoming electric field and the vector connecting two neighbor spheres, α , being 30° in the case of the electric field polarization along the y -axis. Thus, the EFEF is reduced by $\cos(\alpha)^2$ times.

The Raman spectra presented in this paper are measured using a $20\times$ ($\text{NA}=0.45$) objective in back scattering geometry. This means that the scattering signal was collected in a range of angles between -26.7° to $+26.7^\circ$. To analyze the impact of the angle of

Fig. 6 **a** Schematic illustration of the quasi-3D opal-like PC structure under consideration (electric field is polarized along the x -axis). Spatial distribution of EFEF in ten opal-like monolayers for various intersections by three planes xOz , yOz (**b**, **d**) and xOy (**c**, **e**) for two polarizations of normally incident light: (**b**, **c**) E_x ; (**d**, **e**) E_y . In the plane xOy , one periodically translated cell is presented at the right for both polarizations respectively. Light is propagated in a positive direction along the z -axis

incidence on the electric field enhancement factor, the dependencies of reflectance spectra and global and local EFEF maxima near the hotspot for various angles of incidence for a quasi-3D opal-like PC with a globule diameter of 231 nm are shown in Fig. 7. The global EFEF maxima were detected in the area of contact of two adjacent globules. The same is true for local EFEF maxima but it was made only for the outer surface ($z < 0$) of the quasi-3D opal-like PC structure under consideration. The spectra are blue shifted with an increase of the angle of incidence in accordance with the band diagram in Fig. 5. If the globule diameter is changed, the position of the spectra is also shifted with respect to the globule diameter and can be estimated by scaling the globule diameters due to a weak dispersion of SiO₂ permittivity. In addition, the PSB efficiency (maximum reflectance) decreases with the increasing angle of incidence. The global EFEF maxima (dashed lines in Fig. 7b) coincide with the maximum of the reflectance in Fig. 7a, whereas the local EFEF maxima (solid lines in Fig. 7b) are red-shifted relative to the position of the global maximum.

The spatial distribution of EFEF in the vicinity of the two contacting globules for the optimal diameter (231 nm) is shown in Fig. 8 for various angles of incidence. Here we can observe the moving of the EFEF maximum in the hotspot from the inner part to the outer part of the quasi-3D opal-like PC structure under consideration.

Fig. 7 Simulated spectra of reflectance (**a**) and maximum of the electric field enhancement factor (**b**) of the quasi-3D opal-like PC structure under consideration with a globule diameter of 231 nm for various angles of incidence. *Dashed lines* correspond to the global maxima of the electric field enhancement factor in the vicinity of a hotspot whereas *solid lines* correspond to the local maxima for the field enhancement factor in the vicinity of a hotspot for the outer surface ($z < 0$) of the quasi-3D opal-like PC structure under consideration

To summarize, the impact of the angle of incidence on the EFEF is presented in Fig. 9 for a fixed wavelength of an incident plane wave. The optimum value of the globule diameter at normal incidence is 231 nm, with a local maximum of the EFEF of 9.13 and the optimum is shifted to 233 nm when the angle of incidence is increased by 10° with a decrease of the local maximum of the EFEF up to 8.87. An additional increase in the angle of incidence results in a shift in the optimal diameter of the globule by more than 237 nm, with a decrease of the local maximum of the EFEF of less than 8.1. The intensity of the local EFEF maxima can be estimated as $\cos^2 \theta$, where θ is the angle of incidence. Thus, the electric field is still great,

Fig. 8 Spatial distributions of electric field enhancement in the vicinity of two contacting SiO_2 globules in the quasi-3D opal-like PC structure for incident light with a wavelength of 531 nm and various angles of incidence in the plane of incidence xOz

Fig. 9 Simulated dependencies of the maximum of the electric field enhancement factor for various globule diameters and angles of incidence. *Dashed lines* correspond to the maximum of the field enhancement factor in the vicinity of a hotspot whereas *solid lines* correspond to the maximum for the field enhancement factor in the vicinity of a hotspot for the outer surface ($z < 0$) of the quasi-3D opal-like PC structure under consideration

despite the negative effect of the angle of incidence. However, for a fixed globule diameter, the drop in the enhancement factor is more significant: 8.62 for 10 deg. and 6.02 for 20 deg. in the case of a globule with a diameter of 231 nm. The EFEF is 8.55 for a normal distribution of angles of incidence with $3\sigma = 27$ deg.

Hence, it was theoretically shown that electric field enhancement $(E_1/E_0)^2$ in the case of a quasi-3D opal-like PC structure reaches 9, where the Raman scattering is proportional to $(E_1/E_0)^4$ (EFEF²). Thus, the enhancement of the Raman signal can reach 81.

4 Conclusions

Our studies showed that DNA molecules are infiltrated from an aqueous solution into PC cavities. This leads to several new effects: a change in the DNA vibrational frequencies, a redistribution of the band intensity, the appearance of new bands and the disappearing of the vibration bands in the region of $1400\text{--}1700\text{ cm}^{-1}$. In particular, the missed marker of Raman scattering of the nucleoside Ade and Gua indicates drastic conformation changes of DNA in the PC cavity and the appearance of new unknown conformational states. In the case of a 3D photonic crystal, we lacked the spectral recognition of DNA, thus we can conclude that a B-DNA partial transition in stretching and torque forms is overstretched DNA or P-form.

It was numerically shown the formation of ‘hotspots’— areas in the interglobular space (PC cavities) using the FDTD calculation of the spatial distribution of the electric field intensity in the linear regime in a quasi-3D photonic opal-like crystal consisting of ten silicon dioxide globule monolayers for the incident light within the PSB. It was found that the optimal globule diameter is 231 nm to achieve the maximum of the electric field enhancement factor in the outer space of the structure under consideration in the case of a Raman scattering source with a wavelength of 531 nm. In addition, it was numerically demonstrated that the electric field

enhancement factor is suppressed by changing the angle of incidence and is reduced by about 34% for an angle of incidence of 20°.

Thus, the PC can be used to enhance certain vibrational modes of DNA (up to tens) in comparison with free DNA. DNA in the cavities of a photonic crystal has an unfolded form with unpredictable features induced by the environment of the PC and the electrical field redistribution.

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