

Effects of UV Radiation on Herring Sperm 's DNA in Aqueous Solution . A Raman Spectroscopic Study

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Abstract : The Raman spectra of herring sperm DNA in aqueous solution and after radiation of two UV regions (UVA 320 ~ 400 nm and UVA&UVB 280 ~ 400 nm) were investigated . The level of the UV radiation used in the experiment was similar to the normal solar irradiation in Nanjing region of China . The Raman spectral measurements indicated that the obvious changes of the molecular structure were not found in the DNA solution to the UVA radiation , the damage of the molecular structure in part was found in the DNA solution to the UVA&UVB radiation . The damage of the DNA molecular structure was increased with irradiation time . The damage was first observed in pyrimidine-dimer bases and deoxyribose .

Key words : Raman spectroscopy , DNA , ultraviolet radiation

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0 Introduction

The solar ultraviolet radiation is conveniently divided into 3 intervals on the basis of wavelength : UVA(320 ~ 400 nm) , UVB(280 ~ 320 nm) and UVC(200 ~ 280 nm) . Of these only UVA and UVB are of environmental significance , since UVC is effectively absorbed in the earth 's atmosphere by air , dust and steam and is strongly resisted by the ozonosphere^[1] .

Dramatic increases in the incidence of skin cancer have been recorded in recent years and are believed to be related in large part to increased elective exposure to sunlight^[2] . The research results of molecular biology indicated that the damage of DNA and gene mutation are important mechanism of causing cancer by ultraviolet radiation^[3] . UVB radiation(like UVC) is strongly absorbed by intracellular bio-molecules and induces DNA damage and mutation . Structural changes in DNA were considered to be

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one of the primary consequences of the deleterious effects of UVB on the cellular level^[4]. It has been shown that UVB radiation modulates the signal transduction response of skin cells by activating growth factors ,receptor phosphorylation and mitoge-activated protein kinases^[5]. Modulation of such pathways by UVB modifies cell growth and differentiation rates will lead to cancer development and progression. The research results recently show the radiation of a large quantities of UVA also caused skin cancer and UVA radiation may be help to bring about cancer forming which was induced by the UVB radiation^[6,7].

In the past ,nonsolar ultraviolet radiation (UVC) was frequently used as a tool to elucidate the intracellular signal transduction response to external stimuli. Studies on the response of herring sperm DNA to the damage effects of UV radiation have been reported^[8]. But the DNA in aqueous solution was exposed to UV radiation from germicidal lamp ,and probably most relavant lesions formed by UVC(253.7 nm) radiation. In this article we report that the sperm DNA in aqueous solution was exposed to the UVA radiation and the UVA&UVB radiation ,respectively ,to mimic the exposure to the solar ultraviolet region except the UVC radiation. The Raman spectra of sperm DNA in aqueous solution before and after the radiation of two UV regions were measured. The structural information and conformation change of the sample were obtained. The site ,mode and potential degree of UV radiation damages could also be understood. So the characteristic and reason of damage can be deeply investigated.

1 Material and methods

1.1 Material

The herring sperm DNA (highly pure fiber) from Germany Böhlinger-Mannheim Co. was dissolved in twice distilled water at pH 7.0. The final concentration of DNA was 5% (*w/w*). The DNA solution was put in a refrigerator for 60 h at a temperature of 4°C. Then the samples were put into a quartz tube to be irradiated with UV radiation and detected with Raman spectroscopy.

1.2 UV radiation

The herring sperm DNA solution in a quartz tube was irradiated with ultraviolet rays of a germicidal lamp. The quartz tube was put into a black vessel and was covered by special glasses ,which were produced by Shanghai Color Optical Glass Material Factory. When the sample solution was covered by a ZB1 glass and a ZWB2 glass ,whose size is :50mm long 50mm wide and 1mm high. The transmissivity of UVA (320 ~ 400 nm) radiation was determined by a PERKIN-ELMER LAMBDA 17 UV/VIS spectrophotometer and is shown in Fig. 1. The intensity of the ultraviolet radiation was measured with a digital laser powermeter (EG&G ,Model 460-1). The intensity of the ultraviolet radiation was 0.38 Wm^{-2} . The sample solution exposed to the UVA radiation for 0.5 h ,3 h and 15 h ,respectively. When the sample solution was covered by a ZWB2 glass ,whose size is :50mm long 50mm wide and 2mm high. The transmissivity of UVA&UVB (280 ~ 400 nm) radiation was also determined by the spectrophotometer and is shown in Fig. 2. The intensity of the ultraviolet radiation was 0.41 Wm^{-2} . The sample solution exposed to the UVA&UVB radiation for 0.5 h ,1 h and 5 h ,respectively. The intensity of the solar radiation in Nanjing region of China was 0.18 Wm^{-2} during our experiment times.

1.3 Spectroscopic method

Raman scattering was excited by using the output of a Coherent Series Innova 70 argon-ion laser. The

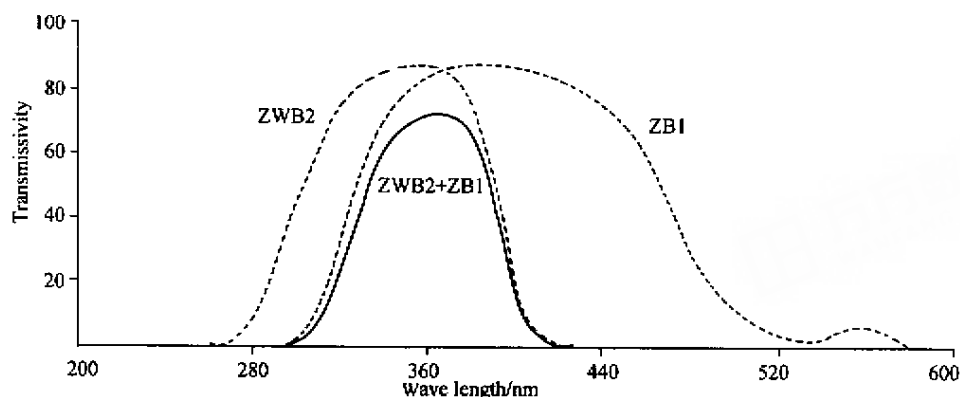


Fig.1 UV transmissivity of ZWB2 & ZB1 glasses

exciting line was 514.5 nm, and the power was 250 mW. Raman scattering was collected at 90° to the exciting laser beam and analyzed by a Jobin Yvon HRD-2 double monochromator fitted with a photomultiplier detector. This system was interfaced to an IBM 486 PC and controlled by Instruments S.A. Prism 3.1 software. Raman spectra were manipulated, including background subtraction, using the Prism 3.1 software running on the IBM 486 PC. The three mechanical slit widths of the spectrometer were set at 500, 500 and 500 μm . The scanning range was set at $600 \sim 1750 \text{ cm}^{-1}$, the scanning speed at $1 \text{ cm}^{-1} \text{ s}^{-1}$.

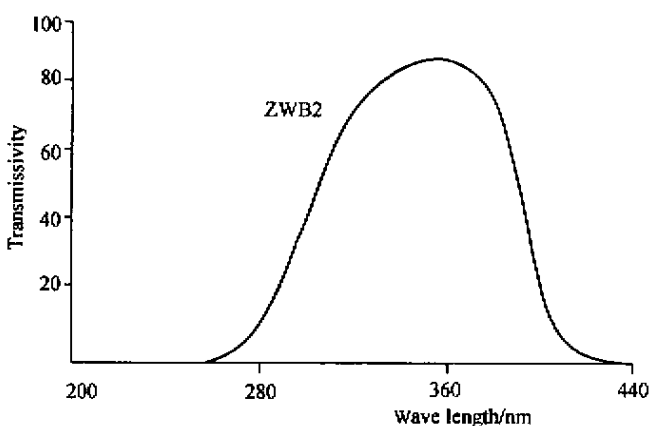


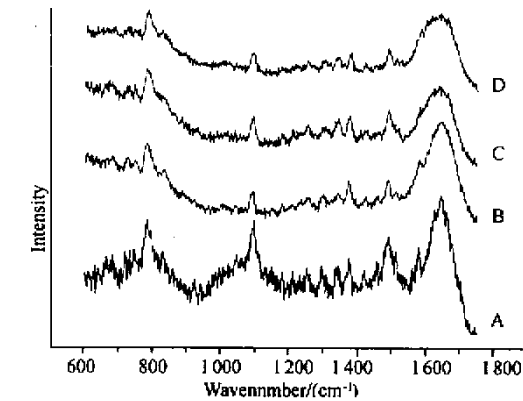
Fig.2 UV transmissivity of ZWB2 glass

Each Raman spectrum is the result of 5 or 6 measurements at random positions on DNA solution, which were averaged in order to minimize effects due to differences in local DNA composition^[9]. The averaged spectra thus obtained showed only minor variations, not of any consequence for the interpretations given below. The wave number calibration of the Raman spectra was made on the basis of an CCl_4 Raman spectrum recorded with the same instrument setting. Line positions of well-resolved lines are accurate within $\pm 2 \text{ cm}^{-1}$. The high-wave number band at about 3400 cm^{-1} of water was used as a reference for the intensity measurement. The experiment temperature was $(13 \pm 2)^\circ\text{C}$.

2 Results and discussion

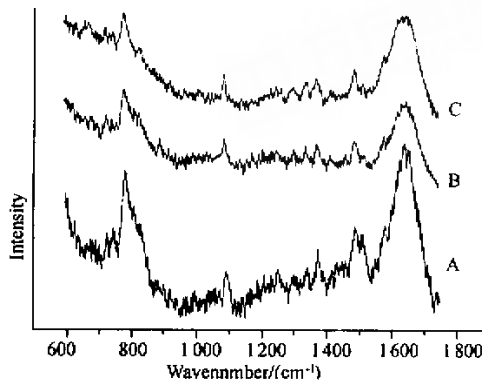
The Raman spectra of the herring sperm DNA in the aqueous solution and after the UVA radiation for the different times are shown in Fig.3. The Raman spectra of herring sperm DNA in the aqueous solution after UVA&UVB radiation for the different times are shown in Fig.4. The Raman shift and the tentative

assignment^[10~13] are shown in Table 1 and Table 2 ,respectively ,where A is the vibration characteristic of adenine ,G is guanine ,C is cytosine and T is thymine .They are listed in descending order according to their contributions to the Raman bands .



A : in aqueous solution ;B : after 0.5 h UVA radiation ;C : after 3 h UVA radiation ;D : after 15 h UVA radiation

Fig.3 Raman spectra of DNA in aqueous solution and after UVA radiation with different time



A 0.5 h ;B 1 h ;C 5 h

Fig.4 Raman spectra of DNA in aqueous solution after UVA&UVB radiation with different time

Table 1 Raman Spectra of Herring Sperm DNA with UVA Radiation

Raman shift/(cm ⁻¹)				Tentative assignment	Raman shift/(cm ⁻¹)				Tentative assignment
In aqueous solution	UVA radiation				In aqueous solution	UVA radiation			
	0.5 h	3 h	15 h			0.5 h	3 h	15 h	
641	643	643	637	C	1210	1210	1210		T
666	666	665	662	G ,T	1235	1235	1233		A
685	684	684	685	G	1253	1254	1253	1252	C ,A
733	728	727	725	A	1295	1299	1300	1298	A
747	749	748	746	T	1342	1343	1343	1341	A
784	784	783	785	T ,C ,O-P-O B-type	1377	1375	1375	1378	T ,A ,G
831	833	828	827	(O-P-O) B-type	1420	1422	1420	1421	A ,G
860	858	860	859	deoxyribose -phosphate	1458	1458	1458	1454	deoxyribose
924	923	925	926	deoxyribose	1490	1490	1491	1489	G ,A
942	945	943	944	deoxyribose	1515	1515	1520	1513	A
1046	1043	1041	1043	(C-O)	1582	1581	1581	1583	G ,A
1095	1095	1092	1092	(PO ₂ ⁻) B-type	1643	1643	1643	1641	C
1181	1179	1179	1176	T ,C	1662	1662	1662	1661	T ,C (C-O)

γ—stretching vibration ,A—adenine ,G—guanine ,C—cytosine ,T—thymine

Table 2 Raman Spectra of Herring Sperm DNA with UVA Radiation

Raman shift/(cm^{-1})					Raman shift/(cm^{-1})				
In aqueous solution	UVA&UVB radiation			Tentative assignment	In aqueous solution	UVA&UVB radiation			Tentative assignment
	0.5 h	1 h	5 h			0.5 h	1 h	5 h	
641	633	641	639	C	1210	1211	1210	1217	T
666	666	665	667	G ,T	1235	1235	1236	1235	A
685	685	686		G	1253	1253	1252	1253	C ,A
733	730	730	729	A	1295	1295	1304	1303	A
747	748	748	752	T	1342	1342	1339	1345	A
784	785	786	788	T ,C ,O-P-O B-type	1377	1375	1374	1374	T ,A ,G
831	831	832	833	(O-P-O) B-type	1420	1420	1416	1418	A ,G
860	860			deoxyribose -phosphate	1458	1459	1462	deoxyribose	
924	922	921	927	deoxyribose	1490	1491	1489	1491	G ,A
942	942	942	953	deoxyribose	1515	1516	1518	1517	A
1046	1045	1037		(C-O)	1582	1583	1583	1584	G ,A
1095	1095	1092	1092	(PO-2)	B-type	1643	1642	1635	1648
1181	1191	1178	1175	T ,C	1662	1661	1661	1662	T ,C (C-O)

γ —stretching vibration ,A—adenine ,G—guanine ,C—cytosine ,T—thymine

2.1 Influence to UVA radiation

The influence to UVA on the herring sperm DNA in the aqueous solution is slight in the Raman spectra(Fig.3). The main characteristic Raman peaks appeared at the same position as that of the native DNA solution. The Fig.3(B)(C) and (D) were similar almost with the Fig.3(A) in entire spectrum expect the region from 900 to 1200 cm^{-1} . The spectrum baseline of the region from 900 to 1200 cm^{-1} at Fig.3(A) was higher than others. This was the broad band of the fluorescent residual background of the DNA dissolved in aqueous solution when the DNA was illuminated by a visible laser. The trace amounts of fluorescing impurities were partial and slight. The fluorescence was completely eliminated and the baseline of the region from 900 to 1200 cm^{-1} was quickly come down after only o.5 h to UVA radiation. But the positions and intensities of the Raman peaks in this region ,for examples as at 1 095 ,1 046 and 1 181 cm^{-1} ,were almost the same as that of the native DNA.

The Raman spectrum of the herring sperm DNA in the aqueous solution after UVA radiation for 15 h is shown in Fig.3(D). It was also quite similar to the Raman spectrum of the native DNA solution(Fig. 3(A)) except that the bands at 725 827 and 1176 cm^{-1} were shifted to more than 4 cm^{-1} . Therefore ,it can be concluded that the influence to UVA radiation is weaker for herring sperm DNA in the aqueous solution ,even when the UVA radiation time had have lasted 15 h.

According to X-ray diffraction study ,double-stranded DNA has three forms : A form ,B form and C form. The three types of DNA can be differentiated by Raman spectroscopy. A peak at 805 ~ 818 cm^{-1} is always present when a fiber shows an A-type diffraction pattern. A peak at 825 ~ 838 cm^{-1} always appears in the B form^[14]. The Raman spectrum of the C form of DNA is characterized by a peak at 865 ~ 870

cm^{-1} [12]. These Raman peaks are sensitive to the spatial structure of nucleic acid. The peak position is shifted and the peak intensity is changed when the DNA conformation is changed. In Fig. 3(A) the characteristic band of the DNA backbone-chain phosphodiester bonds appeared at 831 cm^{-1} , which indicated that the native herring sperm DNA existed as the B form. The characteristic peak appeared at 833 cm^{-1} after 0.5 h UVA radiation in Fig. 3(B), at 828 cm^{-1} after 3 h UVA radiation in Fig. 3(C), at 827 cm^{-1} after 15 h UVA radiation in Fig. 3(D), respectively. The Raman characteristic bands at 688 , 784 , 1095 and 1420 cm^{-1} were also attributed to mainly the B form of DNA in Fig. 3(A) and they also appeared in Fig. 3(B), Fig. 3(C) and Fig. 3(D), respectively. No peak intensity showed obvious change , and the shifts of all peak position were observed at corresponding regions. These observations suggest that the influence of UVA radiation on the conformation of DNA solution was small.

2.2 Influence to UVA&UVB radiation

The results of the herring sperm DNA to the UVA&UVB radiation were completely different from that to the entire UV region radiation. Because the DNA are composed of pyrimine and purine bases ,which have the conjugate double bond. The UV radiation at about 260 nm was strongly absorbed by them. In previous experiment the DNA solution was exposed mainly to UV(253.7 nm) radiation from germicidal lamp with higher irradiance ,so that many base groups of the DNA were effected and the protonations of base pairs were found after 0.5 h to entire UV region radiation. It leaded to both AT and GC base pairs were disrupted and the DNA molecular conformation was damaged completely after 1 h to entire UV region radiation [8].

The influence of the herring sperm DNA to UVA&UVB radiation was not serious in this experiment comparing with the entire UV region radiation in the literature [8]. The herring sperm DNA mainly possessed the B-form structure in the aqueous solution in Fig. 3(A), contained typical characteristic Raman peaks of the B form. The Raman peaks at 784 , 831 , 1095 and 1420 cm^{-1} were main characteristic peaks of B-DNA nucleic acid backbone in the aqueous solution. The Raman peaks of the ring breathing vibrations of guanine at 685 cm^{-1} , adenine at 722 cm^{-1} , thymine at 748 cm^{-1} and thymine , cytosine at 784 cm^{-1} were markers for the C2 'endo-anti conformation of the nucleotides , typical structure of B-DNA. Apart from the band at 784 cm^{-1} , other Raman bands were almost found at original position after the UVA&UVB radiation. For example , the strong peak of the PO^{-2} group appeared at 1095 cm^{-1} , corresponding to the symmetric stretching vibration of the phosphonic peak , which was not relatively insensitive to DNA denaturation and protonation [10]. Its intensity and position will be changed when the DNA conformation completely changed. In Fig. 4(C), this peak appeared still at original site although after 5 h UVA&UVB radiation and its intensity had slight descent , which indicated the B form of the DNA solution remained basically unchanged. Further information was obtained from the peak centered around 831 cm^{-1} , which was ascribed to an antisymmetric O-P-O stretching mode of the B form DNA backbone , i.e. , with the furanose rings in a C2 'endo conformation [15]. This peak appeared at 831 , 832 and 833 cm^{-1} after 0.5 h , 1 h and 5 h UVA&UVB radiation in Fig. 4(A , B , C), respectively. These results also indicated that the spatial structures of the DNA main chain and phosphonic acid backbone were almost unchanged after UVA&UVB radiation.

The damage of the deoxyribose to the UVA&UVB radiation was found in herring sperm DNA

solution. For example, the bands at 924 and 942 cm^{-1} , assigned to the deoxyribose, remained at original position after 0.5 h and 1 h UVA&UVB radiation, but they removed at 927 and 953 cm^{-1} after 5 h UVA&UVB radiation, respectively. The band at 1458 cm^{-1} , also assigned to the deoxyribose, moved at 1459 and 1462 cm^{-1} in the former two cases of the UVA&UVB radiation, respectively, and disappeared after 5 h UVA&UVB radiation. The band at 860 cm^{-1} was quite similar to the former. It appeared at original position after 0.5 h UVA&UVB radiation, but its intensity largely decreased after 1 h UVA&UVB radiation and disappeared basically after 5 h UVA&UVB radiation. These results clearly illustrated that the deoxyribose in DNA solution to UVA&UVB radiation could be damaged.

The influence to UVA&UVB radiation on the four bases of the herring sperm DNA was different. The bands belonging to various groups of bases had respective changes. The bands at 1582 , 1490 , 1420 and 1377 cm^{-1} , these bands were due to be in-plane ring vibrations of the guanine and adenine, respectively appeared at almost the original sites after 5 h UVA&UVB radiation. The shift of the band peak was $< 3\text{ cm}^{-1}$. The bands at 1515 , 1342 , 1235 and 733 cm^{-1} , corresponding to the adenine, also respectively appeared at the original site after UVA&UVB radiation. These results indicated that the influence to the UVA&UVB radiation on the structure of adenine and guanine in the DNA solution was small.

The band belonging to cytosine ring at 641 cm^{-1} shifted to 633 , 641 and 639 cm^{-1} after 0.5 , 1 and 5 h UVA&UVB radiation, respectively. While the band belonging to cytosine at 1643 cm^{-1} appeared at the original site after 0.5 h UVA&UVB radiation, and it was broken to two peaks at 1635 and 1648 cm^{-1} after 1 h UVA&UVB radiation, and it was even more broken to two peaks at 1639 and 1650 cm^{-1} after 5 h UVA&UVB radiation. The bands of some base groups would shift and their intensities would decrease when a damaged base group was changed into another substance^[16]. The obvious change observed in these spectra indicated that the unstaking and damage of the cytosine ring had occurred.

The bands assigned to thymine showed a large shift after the UVA&UVB radiation, specially after 5 h UVA&UVB radiation. For example, the bands assigned to the thymine ring at 747 , 1181 and 1210 cm^{-1} shifted to 752 , 1175 and 1217 cm^{-1} after 5 h UVA&UVB radiation, respectively. Their respective change of band intensity was observed in Fig. 4(A, B, C). These observations suggested that more damages on the thymine had occurred after the UVA&UVB radiation. Consequently, especially the band assigned to PO_2 phosphate diester symmetric stretching mode of the B form DNA backbone and cytosine ring shifted from 784 cm^{-1} to 785 , 786 and 788 cm^{-1} after 0.5 , 1 and 5 h UVA&UVB radiation, respectively. We have known that the spatial structures of the DNA main chain and phosphonic acid backbone was almost unchanged after UVA&UVB radiation, and the pyrimidine bases could be damaged in DNA solution. Therefore, it could be inferred that the damage of the pyrimidine was the main source of a larger shift in the Raman band at 784 cm^{-1} after the UVA&UVB radiation.

The various DNA damages to UV radiation are included such as the damage of base pair, the destruction of glycane, the split of single, double strand, the cross-link of chain and the cross-link of DNA and protein. Among them the predominant UVA&UVB radiation-induced DNA lesions are the cyclobutane pyrimidine dimers and pyrimidine (6'-4') pyrimidone photoproducts, which are both implicated in the initiation of UVA&UVB radiation induced mutagenesis and oncogen activation, ultimately leading to skin cancer. It was likely proved in our experiment that the change of the Raman bands in the pyrimidine base

was found at first.

3 Conclusions

Raman spectroscopy was used to obtain qualitative information about the change of molecular composition and structure in the DNA solution to UV radiation. The results mentioned the above illustrate that valuable information about herring sperm DNA exposed to the solar ultraviolet region can be obtained in straightforward, noninvasive, and rapid manner. In this experiment, the influence to the UVA radiation on the conformation of the herring sperm DNA in aqueous solution was small. The obvious change of the DNA conformation was not found in the shorter time of the UVA&UVB radiation. The pyrimidine base and deoxyribose likely were damaged at first to the UVA&UVB radiation.

In this paper the results of the DNA structure change were obtained in the experiment position of mimic solar irradiation. The irradiance used was between 0.3 and 0.5 W/m², corresponding to the normal levels of the solar irradiance in Nanjing region of China. Brathen *et al* reported that the UVA irradiation was found to induce the formation of multinucleated cells in short time. Soriani *et al* reported that the transcription factor c-fos and c-jun is activated by UVA radiation in human skin fibroblasts in recent literatures. The UVA irradiation used was between 350 and 400 W/m² in their experiments, which was 1000 folds above of the irradiation level in our experiment. Therefore, the results proved in our experiment indicated that the influence on the DNA to UVA radiation was small upon the normal solar level in a limited time.

* Abbreviations : UV, ultraviolet ;UVA, ultraviolet-A(320 ~ 400 nm) ;UVB, ultraviolet-B(280 ~ 320 nm) ;UVA&UVB, ultraviolet-A and B(280 ~ 400 nm) ;UVC, ultraviolet-C.

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紫外辐射对鲱鱼精 DNA 溶液影响的拉曼光谱研究

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[摘要] 报道了鲱鱼精 DNA 水溶液的拉曼光谱及其分别被 UVA(320—400 nm)和 UVA&UVB(280—400 nm)两个区段紫外辐射后的拉曼光谱. 实验中所用的紫外辐射强度与中国南京地区冬季日光辐射强度相当. 实验表明 ,在仅用 UVA 区段紫外辐射时 ,短时间内未见 DNA 分子结构有明显的变化 ,而 UVA&UVB 区段的紫外辐射则对水溶液中的 DNA 分子造成一定的损害 ,照射时间愈长损害愈大 ,首先受到损害的是嘧啶碱基和脱氧核糖.

[关键词] 拉曼光谱 ;脱氧核糖核酸 ;紫外辐射

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Color-Image Generation by Use of Three-Color Fourier Computer-Generated Hologram

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Abstract :Color-image generation by use of three-color fourier computer-generated hologram is reported . It is illuminated by light bulb. The theory and experimental results are presented , corresponding questions are discussed also.

Key words :fourier computer-generated hologram ;color-image ;filter film

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