Safety of UV radiation for autofluorescence diagnosis of skin cancer

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It has been demonstrated that *ex-vivo* human skin autofluorescence is different for healthy and diseased tissue. In order to use these results for *in vivo* clinical applications, it is necessary to guarantee safe levels of UV radiation during skin scanning of patients and protect the eyes from scattered UV radiation coming from the skin surface. One of the goals of this work is to extend maximum limits of exposure to ultraviolet radiation in the spectral region between 315 and 400 nm. We also analyze irradiation levels of typical commercial ultraviolet LEDs and semiconductor lasers for application on skin cancer demarcation. Experimental measurements were not carried out in this paper.

Keywords: Ultraviolet; skin cancer; exposure; laser.

Se ha demostrado que la autofluorescencia en la piel humana *ex-vivo* es diferente para tejidos sanos y enfermos. Para usar estos resultados en aplicaciones clínicas *in vivo*, es necesario garantizar niveles seguros de radiación UV durante la exploración de la piel de pacientes y proteger los ojos de la dispersión de la radiación UV que proviene de la superficie de la piel. Uno de los objetivos de este trabajo es proveer de una guía de los límites máximos de exposición a la radiación ultravioleta en la región espectral entre 315 y 400 nm. También han sido analizados los niveles de irradiación de los LEDs ultravioleta comerciales típicos y de láseres semiconductores para la aplicación en la demarcación del cáncer de piel. No se realizaron mediciones experimentales en este trabajo.

Descriptores: Ultravioleta; cancer de piel; exposición; laser.

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1. Introduction

Exposure to natural and artificial ultraviolet radiations (UV) is the principal cause of skin cancer. Natural UV radiation levels are increasing due to ozone depletion, and also an increase in the incidence rates of skin cancer has been observed. Skin cancers of greatest incidence among the world population are squamous cell carcinoma and basal cell carcinoma, and these are more frequent on parts of the body that are commonly exposed to the sun. These types of skin cancer are rarely fatal.

In order to improve treatment prognosis and the cure rate of tumors, several optical techniques have been developed to detect cancer at an early stage. Among these optical techniques we can distinguish those based on fluorescence measurements either of native fluorophores or exogenous administered drugs [1,2]. These techniques are based on the absorption of UV, violet, or blue radiation by native fluorophores, and the emission of radiation at higher wavelengths [3,4,5].

In a previous study of autofluorescence measurements of *ex-vivo* human tissues, we found significant differences in autofluorescence intensity between the skin lesion and the corresponding control site. This study was based on a non-spectroscopic approach, exciting with 365 nm and detecting autofluorescence intensity at 465 nm in a narrow spectral band with 10nm of full width half maximum [6,7].

In order to extend these results to *in-vivo* human applications, we have reviewed some important aspects in regard to UV radiation for diagnosis purposes in medical applications. The goals of this paper are to provide exposure limits for UV radiation in the spectral region of 315 to 400 nm, and to analyze the irradiation levels of typical commercial semiconductor UV LEDs and laser diodes for this medical application.

2. Considerations on UV radiation

2.1. UV classification

The region of the UV radiation in the electromagnetic spectrum is from 10-400 nm (see Fig. 1). Three bands are relevant: UVA, also called near UV, long wave UV, or blacklight region; UVB, called mid-UV, erythema band or actinic UV; and UVC, also called far UV or germicidal radiation. According to the International Commission of Illumination (CIE), the spectral ranges of these bands are defined as: UVC from 100-280 nm, UVB from 280-315 nm, and UVA from 315-400 nm. According to the pigmentation effects, which

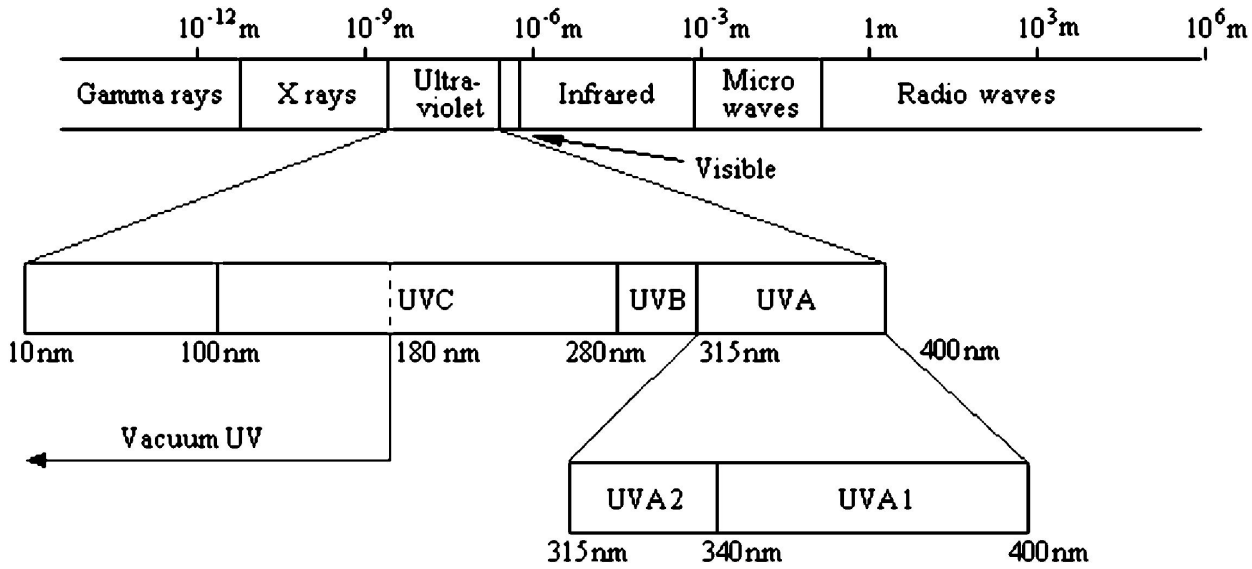

FIGURE 1. Schematic representation of UV radiation in the electromagnetic spectrum.

TABLE I. Typical values of depth in human skin for UV radiation intensity values at 37 and 1% (δ and d, respectively) of irradiance at the skin's surface.

Spectral region	wavelength (nm)	$\delta(\mu$m)	d (μm)
UVA	400	90	400
	350	60	280
UVB	300	6	28
	280	1.5	7
UVC	250	2	9.2

are wavelength dependent, UVA can be subdivided into two parts: UVA1 from 340-400, and UVA2 from 320-340 nm [8,9]. The spectral region below 180 nm is called vacuum UV.

3. UVA damage on skin and eye

3.1. Skin

Skin and eyes are the organs most exposed to UV radiation, and therefore the most injured. The skin consists of three main layers: the epidermis, dermis and subcutaneous tissues. A representation of human skin, its different layers, and their corresponding thickness are presented in Fig. 2.

The epidermis is mainly composed of squamous cells. The innermost layer of the epidermis is made up of a single layer of cuboidal cells, called basal cells. Placed at intervals between basal cells are melanocytes, specialized cells responsible for the production of melanin. The outermost layer of the epidermis, the stratum corneum, is composed of closely packed dead cells. The dermis and subcutaneous tissues are mainly connective tissue or fibers, and fatty tissue, respectively [10].

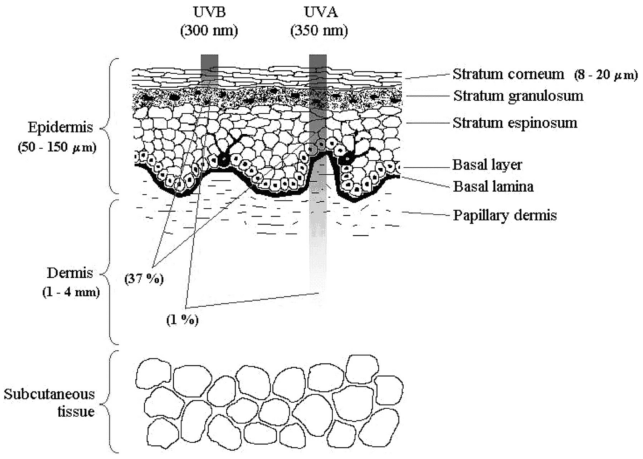

FIGURE 2. Schematic representation of human skin structure. Typical optical penetration depths for specific wavelengths in the UVA and UVB bands.

UVA is the most commonly encountered type of UV radiation. UVA penetrates more deeply in the skin than UVB. Typical values of optical penetration depths (δ) for specific wavelengths in UVA, UVB and UVC spectral regions are presented in Table I [11].

The stratum corneum is a native spectral filter for UV radiation, absorbing more UVB than UVA rays. On the other hand, the energy distribution of the sun, which is richer in UVA than in UVB radiation, makes it possible to reach of up to 70 photons of UVA for every photon of UVB on melanocytes [12].

Erythema is the most common photochemical response of the skin after exposure to wavelengths in the UVC and UVB regions. However, erythema can be produced by exposure to UVA alone at very high radiant exposures (> 10 J/cm^2) [13]. UVA exposure has an initial pigment-darkening effect (tanning) followed by erythema if the exposure is excessive.

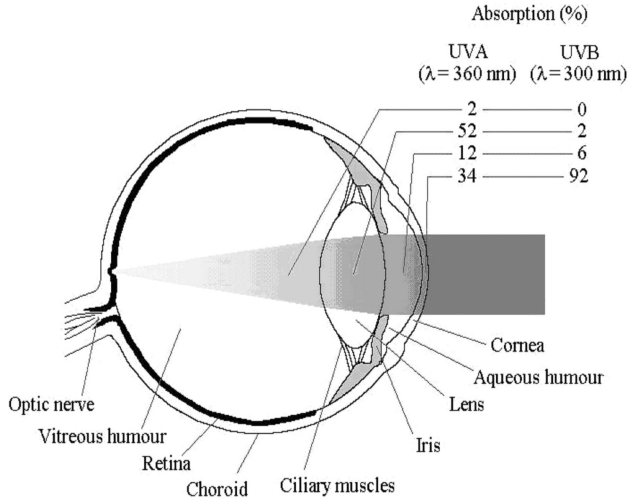

FIGURE 3. Schematic representation of human eye structure. Typical absorption levels for specific wavelengths in the UVA and UVB bands.

Overexposures to UVA also have been associated with toughening of the skin and suppression of the immune system. Repeated UVA overexposures also have been considered to be a cause of the later development of skin cancer.

There are recent studies that provide more information regarding UVA effects in normal human skin. D.W. Edström *et al.* have investigated the effects on human skin of repetitive UVA1 irradiation and visible light [8]. They found that repeated UVA1 (λ_{peak} = 365 nm, 64 mW/cm^2) irradiation of healthy human skin with suberythemal doses (20 J/cm^2) produces an increase of p53-positive keratinocytes and proliferation marker Ki67 and Cyclin A. These results indicate that applications with repeated suberythemal doses of UVA1 may cause DNA damage. Notwithstanding, the clinical implications of long-term exposure to UVA1 need to be considered more deeply.

The use of artificial UVA sources for medical and cosmetic purposes has been always limited because of the health risk to patients. In order to study how UVA and UVB exposures add up in the induction of squamous cell carcinoma, some authors have carried out different research work in animal models [14]. Their results support photoadditivity for effective UVA and UVB doses in risk analysis.

3.2. Eye

More than 99% of UV radiation is absorbed by the anterior structure of the eye, contributing to age-related changes in eyes and a number of serious ocular diseases including age-related macular degeneration, photokeratitis, cataract, cancer of the skin around the eye, corneal degenerative changes, pterygium (a fleshy growth on a normally clear cornea). As illustrated in Fig. 3, UV radiation is absorbed in different proportions by the structures of the eye. For UVA radiation, the main absorption is in the lens, that accounting for 52%, and cornea, for 34%, in the specific case of λ = 360 nm. Corneal injury from UVA radiation wavelength requires levels exceeding 10 J/cm^2 [13]. UV radiation damage to the eye may be cumulative and may increase the risk of developing an ocular disorder later in life. UVA has one nasty effect on the eye, known as nuclear cataract. This is a permanent tanning of the lens of the eye that occurs mainly in its center.

4. UV protection for skin and eye

UV radiation is capable of producing undesirable health effects. In order to minimize UV exposure to artificial sources it is important to take appropriate control measures such as the use of eyewear and protective clothing. In clinical application using UV sources, protection of both patients and staff must be considered. For instance, when a patient is being exposed to UV for phototherapy or diagnosis, sites not intended to be treated should be covered, and the eyes protected.

The areas of the body at major risk are the face, eyes, neck, forearms and the backs of the hands. The face can be protected by a shield, and this should also provide eye protection; the arms should be covered by clothing with a low UV transmission, and hands can be protected by wearing gloves. A variety of eye protection is available with various degrees of protection appropriate to their intended use. The appropriateness and selection of protective eyewear is dependent on the intensity and spectral emission of the UV sources, transmission properties of the protective eyewear material, design of the frame of the eyewear, and behavioral pattern of people near UV sources. Face shields, goggles, or safety spectacles which absorb UV should be worn where there is a potential eye hazard. [12]

Patient's eyes must always be protected from laser energy. For autofluorescence diagnosis of skin cancer in the immediate vicinity of the eye, we recommend the use of plastic shields for laser or non-coherent UVA radiation. This shield should be positioned on the surface of the orbit.

5. Safety exposure levels for UV radiation

For analyzing the safe levels of UV radiation for diagnostic applications, we have consulted the guides on UV radiation exposure limits (ELs) published by the international non-ionizing radiation committee (INIRC) of the international radiation protection association (IRPA) [13,15,16,17]. There are two different guidelines related to the level of coherence of the UV radiation, namely: Guidelines on limits of exposure to non-coherent UV radiation of wavelengths between 180 and 400 nm and Guidelines on limits of exposure to UVA radiation for lasers.

In general, as established in the purpose and scope of ICNIRP guidelines [17], diagnostic procedures should not be performed at exposure levels beyond the ELs. The ELs should be considered absolute limits for the eye, and "advisable" for the skin because of the wide range of susceptibility to skin injury depending on skin type [13]. The ELs

have been derived using physical and biological factors obtained from basic research work. This research include issues such as: UVA induced damage to DNA by indirect mechanisms, role of UVA in different events that could produce melanocytic and non-melanocytic skin cancer, etc. When dealing with ELs, one must be aware that irradiance values E (J/cm^2) are known and the exposure duration, Δt_{exp}, is controlled.

5.1. Guidelines on limits of exposure to incoherent UVA radiation

For the spectral region from 315 to 400 nm, the ELs for the unprotected skin and eye within 8 hours of exposure are respectively determined as follow: for the eye, it should not exceed 1 J/cm^2, and for the skin it should not exceed the values on the revised exposure limits of UVA radiation published in 1989 by the INIRCP committee of the IRPA [15].

For broadband sources, with effective irradiance (E_{eff}) weighted against the peak of the spectral effectiveness curve (270 nm), the following formula is applied:

$$E_{efff} = \Sigma E_\lambda S_\lambda \Delta_\lambda,$$

where E_{ff} is the effective irradiance normalized to a monochromatic source at 270 nm, E_λ is the spectral irradiance from measurements, S_λ is the relative spectral effectiveness (unitless) given in Ref. 15 and, Δ_λ is the bandwidth of the calculation or measurement intervals. The effective radiant exposure, spectrally weighted, incident on the unprotected eye or skin, should not exceed 30 J/m^2 [16].

5.2. Guidelines on limits of exposure to UVA laser radiation

The ELs for laser source exposure is divided in to two situations

a) single-pulse exposure: where Δt_{exp} is the pulse duration, defined at the half-peak power points of the pulse, and

b) continuous wave laser, where Δt_{exp} is the maximum anticipated time of direct exposure.

Note that for UV radiation, the aversion response time (0.25 s) cannot be used.

Skin and intrabeam laser ocular ELs in the 315-400 nm spectral region are established as follows: for Δt_{exp} from 1×10^{-9} to 10 s, the ELs (J/cm^2) are calculated as $0.56 \times \Delta t_{exp}^{1/4}$; and for Δt_{exp} from 10 to 30×10^3 s, the ELs are 1 J/cm^2.

The cumulative exposure duration (T_p) of a train of pulses is calculated as the sum of the individual pulse duration of each pulse in the train. The exposure dose for a train is calculated as theproduct of the average irradiance of the pulse train and the total duration of train (T_t), and this value should not exceed the value of the ELs defined for the T_p of the pulse train.

For repeated exposures to UV laser radiation, the exposure dose is considered additive over a 24-hour period. The cumulative exposure duration for repeated exposures, either to pulse trains or continuous wave lasers, is defined as the sum of T_p from each exposure, and we define it as T_p^*. The T_p for a continuous exposure coincide with the duration of the exposure. The additive exposure dose is calculated as the sum of the exposure dose of each exposure, and should not exceed the value of the ELs for the T_p^* calculated.

If on succeeding days exposures near the limit are expected, then the exposure limits for any 24-hour period should be reduced by a factor of 2.5 with respect to the single pulse limit.

6. Autofluorescence diagnosis of skin cancer

6.1. Autofluorescence diagnosis

Aufluorescence diagnosis of skin cancer is an optical technique that differentiates skin lesions from healthy tissue based on measurements of the fluorescence intensity emitted by native fluorophores present in different concentration in skin tissues. This autofluorescence is due to the absorption of the exciting radiation (usually UV, violet, or blue) by fluorophores that result in the emission of radiation at higher wavelengths. There are many native fluorophores such as: amino acid tryptophan, collagen fiber, elastin, reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinocleotide (FAD), and protoporphyrin IX, lipofusin, pyridoxal 5-phosphate, among others [3,4,5].

For skin tissues, the dominant fluorophores involved are keratin (concentrated mainly in the stratum corneum [18]), dermal collagen and NADH. These fluorophores have strong absorption in the UVA region of the spectrum.

We had developed a non-spectroscopic experimental array that excited flourophores at 365 nm using a filtered Hg arc lamp and detected autofluorescence intensity at 465 nm. Studies have been carried out in *ex-vivo* human tissues with the above mentioned array [7]. With this experimental set-up we have demonstrated that, for *ex-vivo* human tissues, there is a significant difference in the autofluorescence intensity of skin lesions and surrounding healthy skin.

There are several experimental system designs for autofluorescence diagnosis including both laser and noncoherent sources with wavelengths in either UV, violet, or blue spectral regions [19,20,21,22]. Commercial systems are still few in number, and they have been fundamentally based on bulky laser systems such as Ar^+, He-Cd and N_2 lasers. More compact systems and easier to handle, could be designed taking advantage of new UV semiconductor lasers.

TABLE II. Commercially available UV semiconductor lasers and LEDs.

Manufacturer	Device / Model	Beam Properties	Wave length (nm)	Output Power / Energy
Nichia	Laser diode NDHU200APAE2,	CW Divergence 9×24	375 ± 5	2 mW
B&W Tek Inc	Laser diode BWB-375	CW Divergence 2mrad	375 ± 5	3 mW
Power Technology Inc.	Laser diode IQ series modules	CW or analog modulated output.	375 ± 5	1,5 mW
	IQ1C1.5 (LD1435)	Circularization of beam.		
	$\emptyset = 38.1$mm, L= 157.5 mm			
Photonic	Laser diode Thermoelectrically cooled	CW Elliptical beam	375	1,5 mW
	TEC Laser diode modules			
Point Source	Laser diode (Laser diode	TEM_{00}	370	1 mW
	system iFlex-2000 fiber couple laser)			
IBH	Laser diode Nanoled 11	Pulsed (pulse length ¡ 0.1ns)	380	5 pJ
Marl Optosource	LED $\emptyset = 5$mm	CW Viewing angle $2\theta 1/2 = 10^\circ$	370	0.75 mW
		Spectrum halfwidth 12 nm		

7. Commercial semiconductor UV light sources

Semiconductor lasers and LEDs that emit in the UV region have been introduced recently on the market. Laser radiation in this spectral region has been fundamentally covered by excimer and solid state lasers. Because of the advantages of UV semiconductor lasers, special efforts are being made to produce and develop such devices at a commercial level. In Table II, some commercial semiconductor UV light sources are presented. Prices of semiconductor laser devices are still extremely high, ranging from 3000.00 to 6800.00 USD for the bare laser diode and modules versions respectively. UV LEDs have a much lower price than UV lasers. These LEDs can be purchased for 25.00 to 40.00 USD. A very important factor to consider in these new lasers is that their lifetime is still below 3000 hours.

Safety considerations concerning these UV semiconductor lasers include overall safety measures against user's modules, wearing appropriate safety glasses, and not looking at laser light directly or through any optical lens. Nichia laser diodes are classified in class 3B.

8. Applications of exposure limits to commercial UV laser diodes and UV LEDs

- UV laser diode NDHU200APE2 with λ = 375 nm, P = 2 mW, divergence $9^\circ \times 24^\circ$.

  For this laser diode (LD), we follow the criteria given for ELs in the guidelines for laser. We establish 10, 180, 30,000 s as practical values for exposure time. The exposure limits for skin regarding these exposure durations is 1 J/cm^2 and the corresponding allowed irradiance values are respectively: 100, 5.6 mW/cm^2 and 33.3 μW/cm^2 (This last one for 8 hours of continuous exposition).

  Visual autofluorescence inspection of some skin lesions can be performed during an exposure time of about 3 minutes (180 s), taking into account the beam divergence of this laser, it can be calculated that for distances of the LD to the patient's skin greater than 4.6 cm, the irradiance levels are safe.

- UV LED (Marl Optosource) with λ = 370 nm, P = 0.75 mW, viewing angle 10.

  Using an exposure limit of 32 J/cm^2 for 8 hours of continuous exposure [15], the irradiance levels are safe at distances between the LED and the patient's skin greater than 5.3 cm. For exposure times of about 180s the irradiance permitted turns out to be 177mW/cm^2. At such irradiance levels, the minimum distance from the LED to the patient's skin is as small as 4 mm.

9. Conclusions

1- Maximum limits of exposure to UVA radiation for skin and eyes has been given following the guidelines of the INIRC committee of the IRPA.

2- Technical characteristics of UV semiconductor lasers make them excellent candidates as an excitation source for skin cancer diagnosis systems based on autofluorescence properties of skin. But they are still expensive in comparison to semiconductor lasers in the red and infrared spectral region.

3- Geometric conditions for which irradiance levels of some semiconductor UV sources are safe have been found.

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