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BIOLOGICAL APPLICATIONS OF  
ULTRAVIOLET FREE-ELECTRON LASERS

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**ABSTRACT**

This review examines the possibilities for biological research using the three ultraviolet free-electron lasers that are nearing operational status in the United States. The projected operating characteristics of major interest in biological research of the free-electron lasers at Brookhaven National Laboratory, the Thomas Jefferson National Accelerator Facility, and Duke University are presented. Experimental applications in the areas of far- and vacuum ultraviolet photophysics and photochemistry, structural biology, environmental photobiology, and medical research are discussed and the prospects for advances in these areas, based upon the characteristics of the new ultraviolet free-electron lasers, are evaluated.

**INTRODUCTION**

**Background**

The idea for free-electron lasers stems from the work of John Madey,<sup>1</sup> who also was the first to observe stimulated emission<sup>2</sup> and laser operation (in the infrared).<sup>3</sup> Kim and Sessler reviewed the first 20 years of the development of free-electron lasers.<sup>4</sup> As with synchrotron radiation sources, free-electron lasers are usually too large and complex to be operated in individual laboratories, and several infrared free-electron laser user facilities are already operating in the United States, Japan, Russia and Europe.

There have been numerous proposals (and a few false starts) for development of free-electron lasers that will produce ultraviolet light and x-rays. In the United States we are fortunate to have three ultraviolet free-electron laser facilities teetering on the brink of routine availability. These free-electron lasers represent considerably different designs and are projected to have correspondingly disparate performance characteristics. All three UV FELs will require significant investments in experimental stations before they become productive user facilities.

Much of the rationale for development of ultraviolet free-electron lasers involves research in physics, chemistry and materials science, and, in the case of the Jefferson Laboratory FEL, possible applications in manufacturing. However, biological research, and related applied fields such as agriculture, medicine, and biotechnology already make extensive use of lasers and other sources of ultraviolet light, and can be expected to benefit from some of the remarkable properties of ultraviolet free-electron lasers.

I have joined with a small group of research scientists, most of whom are members of the American Society for Photobiology, (<http://www.kumc.edu/pol>) in organizing an *ad hoc* evaluation designed to identify some of the advances that ultraviolet free-electron lasers may stimulate in the biological sector and help lay a foundation for obtaining the user support facilities to make these resources available to the biological research community. Our plan is to complete the review early in 1998. This report is a preview of our combined effort based in part on my limited knowledge of the more extensive evaluations that my colleagues are preparing.

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## **DISCLAIMER**

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### Caveats

This report focuses on:

- Ultraviolet Radiation:  $\lambda \geq \approx 120$  nm ( $h\nu \leq \approx 10$  eV).
- Applications in the biological sciences (although many of the measurements could be used in chemistry, physics and materials science research).
- Research Applications (no manufacturing, routine analytical procedures or patient care).
- Linear (single photon) effects.
- The applications cited here represent only a sampling of the possible range of uses of FELs, not an exhaustive catalog.

### The Almost-Available American UV FELs

**The Duke University OK-4 Free-Electron Laser:** (Durham, NC) This laser is based on an undulator and resonator cavity mounted on a straight section of an  $\approx 1$  GeV synchrotron storage ring. The building and synchrotron storage ring are complete, and the FEL has operated, albeit not at the design specifications given in the table below. The laser can be operated either in "CW" mode (actually a continuous string of micropulses), or in two difference macro-pulse modes. Operation of a resonator cavity at  $\lambda = 200$  nm will require multiple, grazing incidence mirrors, and hence will require additional development. The Duke FEL facility also includes an infrared FEL and there are plans for extension into the x-ray region. The person to contact regarding performance characteristics is Dr. Vladimir Litvinenko, ([lv@fel.duke.edu](mailto:lv@fel.duke.edu)). The url for this facility is <http://fel.duke.edu/>.

**Brookhaven National Laboratory Ultraviolet Free-Electron Laser:** (Upton, Long Island, NY) This FEL is based on a linear accelerator (linac) as a source of relativistic electrons and a very long (10 m) single pass undulator. Single pass operation avoids resonator mirrors entirely and hence eliminates a major barrier to operation at shorter UV wavelengths. Compared to the other ultraviolet free-electron lasers considered here, the average power output of 10 mW sounds feeble, but compared to laboratory lasers, 10 mW of far UV is quite impressive. The low repetition rate and high peak power will likely be more important considerations for many spectroscopic experiments. The BNL design uses a conventional laser to generate a short pulse of electrons from a photocathode which are accelerated in the linac. Harmonics generated by a portion of the same pulse of conventional laser light are used to "seed" the laser amplifier. A clever aspect of this design is that the conventional laser pulse and its harmonics, which are rigidly synchronized with the FEL pulse, are available in the experimental area for "multi-color" experiments. The FEL is housed in a dedicated building, which has been completed. Most major components (seed laser, linac and undulator) are in place. A small space at the end of the undulator has been reserved for pilot experiments. Initial beam (nir/visible) is expected in 1998. Dr. Erik Johnson ([erik@bnl.gov](mailto:erik@bnl.gov)) is the project manager. The url is <http://www.nsls.bnl.gov/BeamRD/erik/sdl.html>.

**Jefferson Laboratory Ultraviolet Free-Electron Laser:** (Newport News, VA) This FEL facility is an outgrowth of the superconducting accelerator technology developed for the Continuous Electron Beam Accelerator Facility (CEBAF) at the Thomas Jefferson National Accelerator Facility. Like the BNL FEL, a beam of electrons is generated by a linac, but in this FEL the electron beam is recirculated to extract unused power. The building to house the FEL facility contains six large experimental laboratories located on the floor above the FEL. All of the components for a "demo" infrared FEL are in place and initial operation in the infrared is expected in 1998. Designs for additional components to provide a dedicated ultraviolet FEL are in place, but funding is not yet certain. However, higher harmonic output from the nominally infrared FEL may provide significant intensities of longer wavelength UV. The J-lab FELs are designed to demonstrate the feasibility of

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manufacturing applications and, as a result, the time-average output power levels projected for these lasers are simply astounding! However, the facility will also be available for research involving analytical applications. Dr. Steve Benson ([felman@cebaf.gov](mailto:felman@cebaf.gov)) is the source of the projected operating parameters. The url of their web site is <http://www.jlab.org/FEL/>.

A brief summary of the projected properties of these three FELs that are important to the biological research community is presented in Table 1.

**Table 1 Some Design Specifications of The North American FELs**

<b>PROPERTIES</b>	<b>Duke OK-4</b>	<b>BNL</b>	<b>Jefferson Lab UV FEL</b>
<b>Design type</b>			
accelerator	synchrotron storage ring	linac	linac with energy recovery
laser design	resonator	single pass amplifier	resonator
modes	"CW" = stream of $\mu$ pulses Giant pulse mode Super pulse mode see note below §	seeded beam amplifier or stimulated amplification of spontaneous emission (sase)	"CW" = stream of $\mu$ pulses Macropulse modes possible
<b>Spectral</b>			
range (fundamental)	50 nm to > 450 nm	70 nm to 1 $\mu$ m	190 nm to > 350 nm at full power plus rapidly decreasing intensity from 190 to $\approx$ 150 nm
resolution $\frac{\Delta\lambda}{\lambda}$	$10^{-4}$ ( $5 \cdot 10^{-7}$ to $3 \cdot 10^{-6}$ with "pulse line width narrowing")	$10^{-4}$ seeded beam mode  $10^{-2}$ sase mode	$\approx 10^{-3}$ @ 200 nm  $\approx 5 \times$ transform limit
<b>Temporal</b>			
duration (fwhm)	30 ps to 300 fs §	20 ps to 10 fs	2 ps
frequency	3 to 180 MHz	10 to 20 Hz	2.3 to 37.425 MHz
period	5.6 to 330 ns §	50 to 100 ms	26 to 440 ns
<b>Polarization (linear <math>\Delta I/I</math>)</b>	$\approx 1.0$	$\approx 1.0$	$\approx 1.0$
<b>Intensity</b>			
energy/pulse	1 to 10 $\mu$ J @ $\approx$ 50 MHz §	1 mJ	$\approx$ 30 $\mu$ J @ 37.425 MHz
average power	50 to 500 W @ $\approx$ 50 MHz §	10 mW	$\geq$ 1000 W!
peak power	30 to 300 kW §	100 MW	15 MW
pulse-to-pulse stability	1 to 2 %	50% sase mode 1 % seeded beam mode	20 % p-p /hr (depends on $\lambda$ ) few % per sec
beam quality	diffraction limited, i.e. TEM <sub>00</sub> gaussian mode	close to diffraction limited	close to diffraction limited, (annular transverse mode)

§ The Duke OK-4 FEL plans three pulse regimes: Regular, or "CW" mode consists of a continuous string of  $\mu$ pulses with the parameters given in the table above. Optional giant pulse mode provides bunches of  $\mu$ pulses lasting for  $\approx$  100  $\mu$ s with a repetition rate in the range from 50 to 200 Hz, and energies ranging from 0.1 to 3 J/macropulse. The peak power during a  $\mu$ pulse in giant pulse mode is  $\approx$  100 MW. Optional super-pulse mode (with resonant kick-off) will provide up to 20 or 30 mJ in macropulse consisting of a few ps  $\mu$ pulses at a repetition rate of 100 to 200 Hz. The peak power in super-pulse mode is  $\approx$  8 GW.

## FAR- AND VACUUM ULTRAVIOLET PHOTOPHYSICS AND PHOTOCHEMISTRY

Ultraviolet radiation can be used to probe the structure and dynamics of biomolecules, as discussed below, but it also produces chemical changes that impair biological function. The photochemistry and photophysics of biological materials produced by wavelengths less than 250 nm (photon energies greater than 5 eV) have been studied in far less detail than at longer wavelengths. Such wavelengths are totally absent from the solar spectrum reaching the surface of the earth, and hence have no significant environmental consequences, but are still of both fundamental interest, and may provide insights on the damages produced by higher energy ionizing radiation. Studies of the effects of far- and vacuum ultraviolet radiation have been hampered by the paucity of intense, tunable monochromatic sources.

### Ground State Absorption

Absorption of light by a ground state molecule is an obligatory precursor to the observation of photophysical and photochemical processes, as indicated schematically in Figure 1. The flux requirements necessary to measure a simple absorption spectrum are quite modest, certainly not requiring the capabilities of a free-electron laser, although certain demanding time-resolved experiments might benefit from the combination of short pulse width, high intensity and complete tunability that free-electron lasers provide. The ability to excite large numbers of molecules in a short time is also critical for some of the experiments mentioned below.

### Fluorescence and Phosphorescence

Most biological molecules that absorb strongly in the far- and vacuum ultraviolet are composed of light atoms: carbon, nitrogen, oxygen and hydrogen, hence minimizing spin-orbit coupling. Thus fluorescence (emission from the lowest electronic excited singlet state,  $S_1$ , to the singlet ground state,  $S_0$ ) and phosphorescence ( $T_1$  to  $S_0$ ) are easily distinguished by their excited state lifetimes: ns or ps for fluorescence and  $\mu$ s to s for phosphorescence. While the time structures of the Jefferson Laboratory and Duke free-electron lasers are nearly ideal for measuring fluorescence lifetimes by the method of single photon counting,<sup>5</sup> synchrotrons and certain pulsed lasers are also adequate for such experiments. Indeed, synchrotrons have the advantage of facile wavelength selection, and are thus the ideal source for measurement of excitation spectra. The single-photon counting method is limited to times greater than about 20 ps. Shorter lifetimes can be measured using streak cameras. The low-repetition rate of the BNL FEL is compatible with streak cameras for all temporal domains, but is inappropriate for single-photon counting measurements. While mechanical choppers can be used to study time-resolved phosphorescence into the ms time domain using conventional steady state sources, the time structure of the BNL FEL is well suited for time resolved phosphorescence in the  $\mu$ s domain, as are the macro-pulse modes of the Duke and Jefferson Laboratory FELs. The blazing intensities of the DUKE and Jefferson Lab FELs are overkill for the measurement of fluorescence and phosphorescence, but such measurements could be part of multi-parameter data acquisition protocols that also involved some of the more photon-hungry experiments described below.

### Internal Conversion and the Photoacoustic Effect

Energy deposited by absorbed photons that is not re-radiated as luminescence or sequestered in chemical reactions appears as vibrational excitations of both excited and ground electronic states. If such energy is delivered in repetitive pulses at a low (audio) frequency, either by mechanically chopping the incident beam or modulating the source, the periodic expansion and contraction of the sample generates sound waves that can be detected by a sensitive microphone. Such photoacoustic signals complement the information available from luminescence, but the detection process is far less

efficient. Thus photoacoustic spectroscopy is one of the experiments that may be made practical in the far- and vacuum ultraviolet by the high flux free-electron lasers.

### Absorption By Excited State Molecules

An alternate method of monitoring the transient population of the lowest energy excited singlet and triplet excited states is by observing the absorption of light from  $S_1$  or  $T_1$  to a higher energy state in the same spin manifold. The triplets and longer lived singlets can be monitored by fast electronic detectors and recording systems, but by using pulse-probe technology, in which times are computed by measuring the spatial offsets of probe pulses relative to the pump pulse that generates the excited state, temporal resolution has been extended into the realm of femtoseconds! The high intensity of some free-electron lasers is useful in such experiments because part of the energy from a pulse can be used to generate a broad spectrum of probe radiation perfectly synchronized with the pump. The BNL FEL has the additional feature that part of the conventional laser pulses that trigger the electron source and seed the laser amplifier are available in the target area, and are perfectly synchronized with the photons amplified in the FEL.

### Photochemistry

The photochemical products produced by the absorption of light are often determined by the electronically excited state from which they arise. Thus reactions that proceed *via* singlet states only may produce different products than reactions that arise *via* triplets, as shown very schematically in Figure 1. The photochemical reactions resulting from excitations to higher excited states may also be different due to mechanisms such as electron ejection, hydrogen abstraction and the population of  $\sigma^*$  orbitals that do not occur at lower photon energies. Determining the threshold energies of such reactions is often one of the most informative parameters to measure, and the tunability of free-electron lasers is well suited to this task. While sensitive modern chemical techniques can analyze very small quantities of a material,  $\mu\text{g}$  or  $\text{ng}$  quantities loom much larger when expressed in terms of the number of photons required to produce them in a low quantum yield reaction, *i.e.*, the photon requirement equals the number of moles of product required for analysis times Avogadro's number divided by the quantum yield of the reaction. In such experiments the tunability, monochromaticity and intensity of the source are the critical parameters. While synchrotron radiation has been used for such studies, especially in Japan,<sup>6</sup> the limited intensities available proscribe many interesting experiments.

## STRUCTURAL BIOLOGY

Remarkable progress has been made over the last two decades in elucidating the three dimensional structure of proteins, DNA and RNA. These achievements are due to advances in physical methods, particularly crystallography and nuclear magnetic resonance, and corresponding breakthroughs in molecular biology and biotechnology that have produced a cornucopia on materials for structural studies. Despite these developments, the links between structure and function remain elusive. One limitation of crystallography and NMR is that the duration of most experiments is very long compared to the times required for conformational changes in the macromolecules being studied.<sup>†</sup> Determination of the mechanisms that permit proteins to assume their functional conformation from among the ensemble of possible conformations of comparable energies has emerged as one of the most studied problems in biophysics and structural biology. The high flux rate and inherent time structures of free-electron laser radiation make them particularly attractive as sources for such studies.

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<sup>†</sup> Synchrotron radiation has made it possible to follow certain rapid changes in the crystal structure of proteins and other macromolecules using Laue methods. However the conformational change must be sufficiently small to preserve the integrity of the crystal.

### Time-Resolved Circular Dichroism

Circular dichroism, CD, is the difference in the absorption of left- and right-circularly polarized light. CD is only observed in systems lacking a high degree of symmetry,<sup>7</sup> and has been used extensively in studies of the conformation of proteins and nucleic acids.<sup>8</sup> In particular, the amide bonds of proteins give strong CD signals in the spectral region from 240 to 165 nm that have been used to estimate the net secondary structure of proteins. Perhaps more importantly, CD is exquisitely sensitive to changes in secondary structure, and can be used to follow the temporal course of conformational transitions. Conventional broad-spectrum sources as well as synchrotron radiation are adequate for measuring steady state CD spectra from the vacuum ultraviolet through the mid-infrared,<sup>8</sup> but temporal resolution is limited to  $\approx 1$  ms. Using laboratory laser sources, kinetic CD and magnetic CD experiments have been extended to nanosecond resolutions, albeit only in the visible and near UV.<sup>9-12</sup> The higher intensity far- and vacuum ultraviolet radiation provided by free-electron lasers may permit facile kinetic CD measurements in this more interesting spectral region, although pulse-to-pulse stability is a concern. The four-wave mixing method for the determination of CD recently demonstrated by Neyer *et al.* is noteworthy because the temporal resolution results from the duration of the pulse of laser light, and not from the ability to follow a continuous signal.<sup>12</sup> This is also one of the few biological applications of FEL radiation that exploits the coherence of the laser beam.

### Raman Scattering

Raman scattering is another spectroscopic method that has been demonstrated to be sensitive to the conformation of proteins, nucleic acids and other biopolymers.<sup>13</sup> The information content of a Raman experiment is usually increased substantially when the probing wavelength falls within the absorption band of some of the moieties of the biopolymer.<sup>14,15</sup> In the far UV where multiple moieties absorb, Raman spectra change significantly with wavelength, but the limitation inherent in the limited number of wavelengths available from laboratory lasers with appropriate properties for such experiments limits the interpretation of data. The continuous selection of wavelengths provided by free-electron lasers should significantly benefit far- and vacuum ultraviolet resonance Raman experiments. The high intensity provided by some free-electron lasers should also benefit the measurement of time resolved resonance Raman spectra<sup>16</sup> and Raman optical activity.<sup>17</sup> Two areas of concern in developing far- and vacuum ultraviolet Raman experiments using free-electron lasers are: (1) the high peak power of the pulsed FEL beam may generate non-linear signals that are difficult to interpret (S. Asher, personal communications) and (2) the spectral bandwidth of the laser beam must be of the order of  $\Delta\lambda/\lambda \leq 10^{-4}$ .

### Fluorescence and Phosphorescence: Time and Polarization Resolved

As noted above, the time structure of the Duke and Jefferson Lab free-electron lasers are nearly ideal for measurements of the time course of fluorescence using single-photon counting methods, but the intensities available from these lasers are several orders of magnitude more than required for luminescence spectroscopy. Moreover, most applications of fluorescence in structural biology probe the motion of fluorophores on a ns to ps time scale and hence involve determination of the time course of fluorescence anisotropy.<sup>7</sup> For the aromatic amino acids and the nucleic acid bases, the highest intrinsic anisotropies are achieved with excitation wavelengths near 300 nm, a region adequately served by laboratory lasers and synchrotron radiation.

## ENVIRONMENTAL ULTRAVIOLET PHOTOBIOLOGY

**The Cross Section for Damaging DNA for  $\lambda > 320$  nm is Small, But Finite**

DNA absorbs strongly for  $\lambda < 300$  nm, and both photochemical lesions and biological effects are easily detected using conventional laboratory sources of UV. Thanks to ozone in the stratosphere, very little solar UV with  $\lambda < 300$  nm reaches the surface of the earth. The absorption of unshielded DNA increases exponentially with decreasing wavelength for  $\lambda < \approx 320$  nm. This observation led to the sobering realization that a small change in the density of the ozone column might be amplified so as to result in serious consequences for life on earth.<sup>18</sup> However, several factors tend to limit the likelihood of catastrophic consequences from ozone depletion. The initial calculations of Setlow<sup>18</sup> explicitly excluded consideration of the local shielding of DNA in cells of complex organisms by the overlaying layers of scattering or pigmented tissues (*e.g.*, the stratum corneum of skin), hence overestimating the biological consequences of decreased stratospheric ozone.<sup>19</sup> Other studies ignored the damage produced by  $\lambda > 320$  nm,<sup>20</sup> and hence **overestimated** the increased damage percentage resulting from ozone depletion by **underestimating** the base-line values for ultraviolet-induced damage.<sup>21</sup> Although the cross section for damage by wavelengths greater than 320 nm is small, the intensity of terrestrial solar UV in this range is far higher than at shorter wavelengths, as shown in Figure 2. Biochemical and biological studies of the deleterious consequences of wavelengths greater than 320 nm are severely hindered by the present lack of tunable sources that can deliver high intensities of quasi monochromatic ultraviolet light at high intensities over large areas.

**Formation and Repair of DNA Lesions: Time-Dose Reciprocity Does NOT Hold.**

Biological organisms have been exposed to solar ultraviolet radiation during the entire time that life has existed on earth, and have developed multiple enzymatic repair mechanisms to mitigate the adverse effects of the damage UV produces in DNA.<sup>22</sup> Accurate determinations of the levels of DNA damage induced by exposure to UV and of the repair of such damage thus require that the entire dose be delivered in a time short compared to the time required for significant repair. For wavelengths greater than 320 nm, the low cross section for damage induction and consequent extended times of exposure complicates the interpretation of such experiments, particularly in higher organisms *e.g.*, human skin and intact plants. In addition, the Hg arc, the most frequently employed source for such studies has only a few emission lines above 320 nm, further limiting the available data.

**What Wavelengths Cause Malignant Melanoma?**

Several lines of evidence support the view that basal and squamous cell carcinomas, which constitute the overwhelming majority of most human skin cancers, result from exposure to the ultraviolet radiation in sunlight, and that the action spectrum for cancer induction more-or-less parallels the spectrum for DNA damage. Fortunately, such cancers are rarely life threatening. Melanomas, in contrast, occur with low incidence but are frequently malignant. Melanocytes are pigmented, and hence the possibility exists that longer ultraviolet and even visible wavelengths might contribute to their transformation. Indeed, data derived from a fish model suggests that the contribution of wavelengths greater than 320 nm are far more important in melanoma induction than in basal and squamous cell carcinomas.<sup>23</sup> While the incidence of malignant melanoma has increased drastically in recent decades, the significant involvement of longer wavelength UV would indicate that ozone depletion will not affect this disease. Knowledge of which, if any, wavelengths contribute the induction of melanoma is critical for devising strategies for reducing its incidence. The lack of high intensity, continuously tunable, monochromatic sources of near ultraviolet light are one of the several factors limiting progress in our understanding of melanoma induction.

## PHOTOIMMUNOLOGY

Exposure to ultraviolet radiation downregulates the immune system.<sup>24</sup> Both DNA in cells of the epidermis and urocanic acid, found in the stratum corneum, have been considered as the moieties whose absorption of UV triggers this response. As with DNA photoleasions, evaluating the relative contributions of the intense solar UV with  $\lambda > 320$ , which will not be affected by ozone depletion, and the less intense, shorter wavelengths which will be enhanced by ozone depletion is complicated by the small cross section for the immunological effects at the longer wavelengths and the limited ability of conventional UV sources to deliver intense, tunable, monochromatic radiation in this region. Using a conventional source, Drs. F. Noonan and E. DeFabo, Dermatology Department, George Washington University, the major proponents of the urocanic acid hypothesis, require more than twelve hours of exposure per mouse of 365 nm radiation to see immunological effects. Their apparatus can accommodate only three mice simultaneously, and a typical experiment may require from 20 to 200 animals to achieve acceptable statistics, hence rendering many experiments impractical (F. Noonan, personal communication). While the maximum power density that can be applied to a mouse is limited to  $\approx 100 \text{ mW/cm}^2$ , (D.Sliney, personal communications). If we assume that a mouse can be accommodated in an area of 5 by 10 cm, then up to  $\approx 200$  mice could be irradiated at one time. Presumably, the period of exposure would also decrease drastically.)

## PHOTOMEDICINE

Ultraviolet excimer lasers (usually 248 and 193 nm) are used in surgical procedures to ablate various tissues. These two wavelength clearly provide different properties, but the mechanisms underlying these differences are not well understood (R. R. Anderson, personal communication). An intense free-electron laser that could be tuned to any desired wavelength, perhaps coupled with a mass spectrometer to analyze the ablated tissue components, might provide critical insights into the ablation process and determine the optimum wavelength, time average and peak powers and time structure for various surgical applications.

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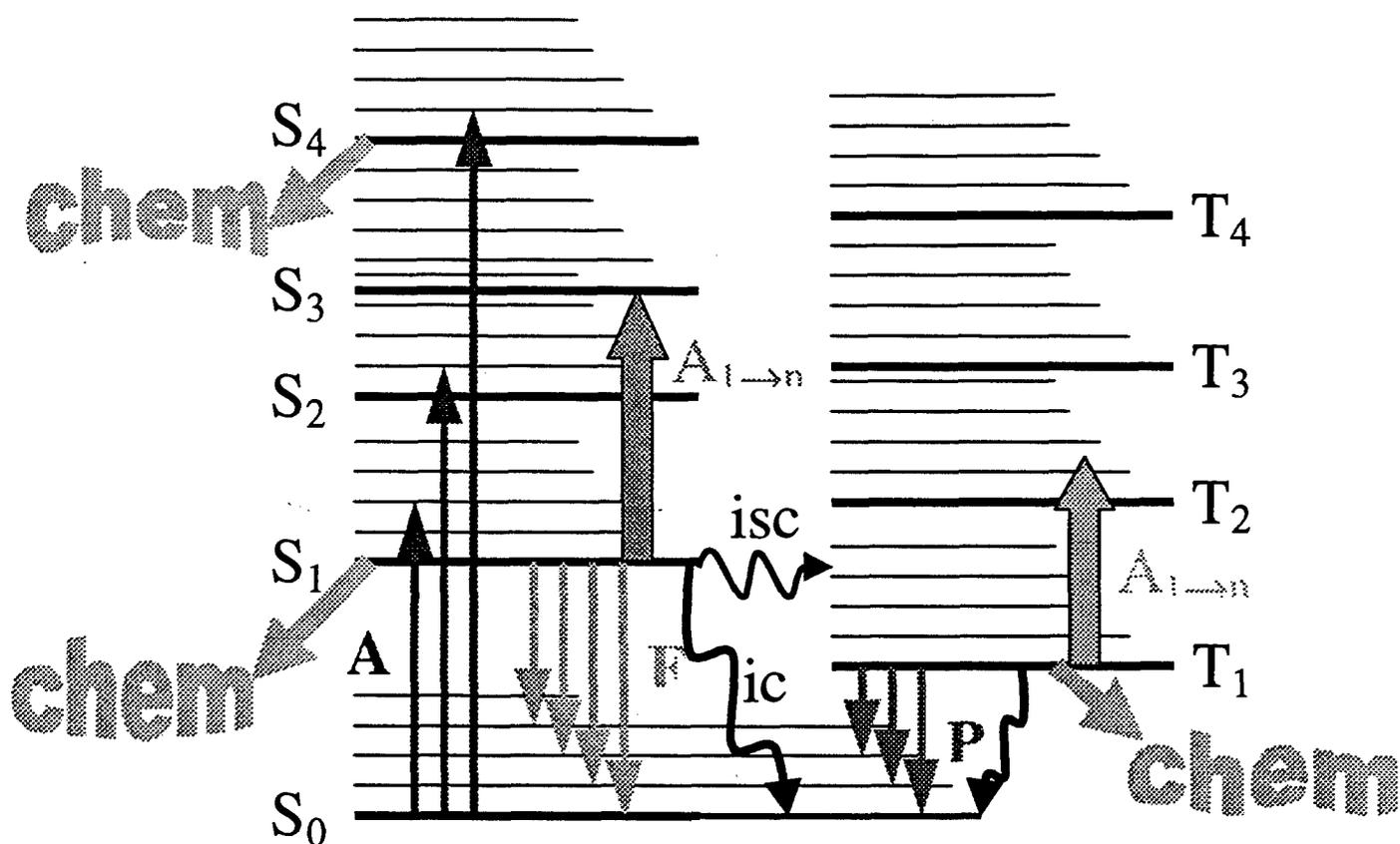


Figure 1 Energy level diagram of a typical organic or heterocyclic molecule found in biological systems indicating some of the photophysical and photochemical pathways resulting from absorption of a photon by a molecule in the ground state ( $\rightarrow$ ). Different photochemical reactions can occur from higher excited singlet states than from the lowest excited singlet or triplet states. Fluorescence ( $\rightarrow$ ) usually occurs from the lowest energy excited singlet state ( $S_1$ ) and phosphorescence from the lowest excited triplet state ( $T_1$ ) following rapid internal conversion (ic). The internal conversion process heats the sample slightly, a process that can be monitored by photoacoustic spectroscopy. Transient absorptions can be recorded during the time the molecule exists in the excited triplet or the excited singlet states. The excited triplet endures for times ranging from microseconds to tens of seconds, while excited singlets typically endure for tens of nanoseconds, at most.

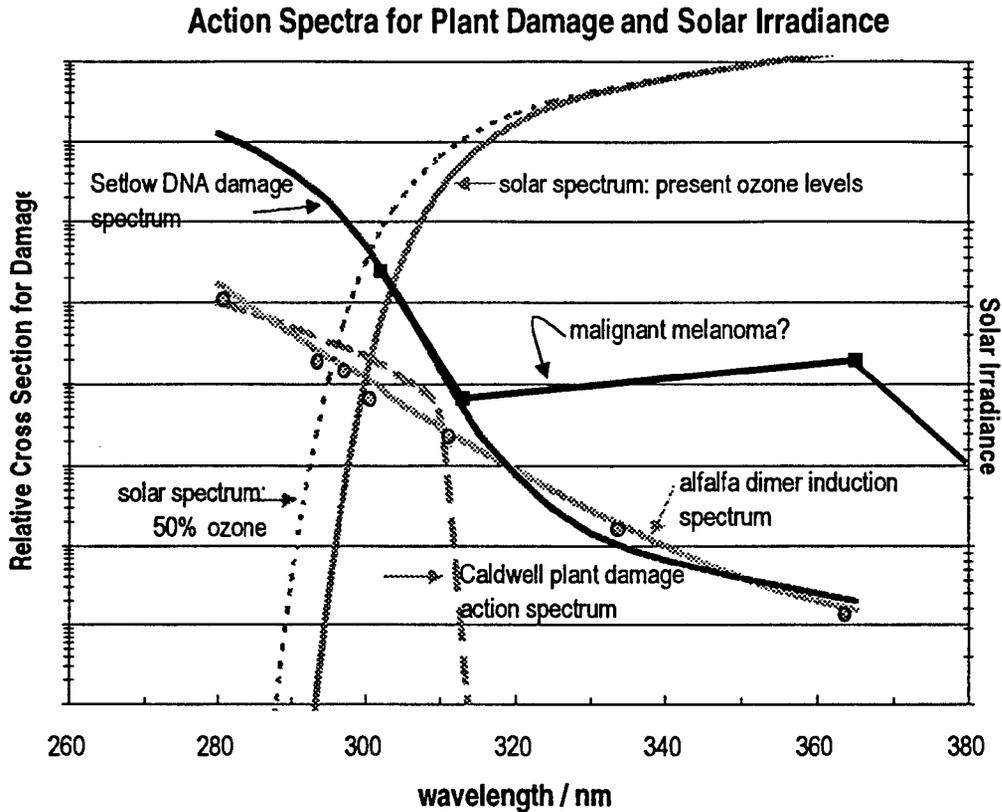


Figure 2 (left axis) Relative cross sections for biological damages plotted on a logarithmic scale as a function of wavelength: Composite action spectrum for damage to unshielded DNA<sup>18</sup> (—), absolute cross section for pyrimidine dimer induction in the DNA of alfalfa seedlings<sup>21</sup>(.....), generalized action spectrum for plant damage<sup>20</sup>(- - -), action spectrum for induction of melanomas in fish (*Xiphophorus*)<sup>23</sup>(—). (right axis) Solar irradiance at the surface of the earth for a solar elevation angle of 90° and total ozone columns of 0.32 cm (.....) and 0.16 cm (- - - -).<sup>25</sup>

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