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Applications of the Jefferson Lab free electron laser for photobiology†

H. F. Dylla, S. V. Benson, G. R. Neil and M. Shinn

Thomas Jefferson National Accelerator Facility, Newport News, VA 23606

R. H. Austin

Princeton University, Princeton, NJ

and

John Sutherland

Brookhaven National Laboratory, Upton, NY 11973

ABSTRACT

A versatile free electron laser (FEL) user facility has recently come on line at the Thomas Jefferson National Accelerator Facility (Jefferson Lab) providing high average (kilowatt-level) power laser light in the infrared. A planned upgrade of the FEL in this facility will extend the wavelength range through the visible to the deep UV and provide the photobiology community with a unique light source for a variety of studies. Planned and potential applications of this FEL include: IR studies of energy flow in biomolecules, IR and visible imaging of biomedical systems, IR and visible studies of photodynamic effects and UV and near visible studies of DNA photodamage.

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1. INTRODUCTION

The first FEL in the Jefferson Lab FEL user facility became operational in 1998 and in 1999 this device, called the IR Demo, exceeded its full power specifications by generating over 1.7 kW of average power at 3.1 microns.[1] The facility has been designed to exploit the unique characteristics of an FEL driven by a superconducting, recirculated linac: it efficiently produces high-average powers with a continuous pulse train of sub-picosecond laser pulses at high repetition rates (18.7-75 MHz). The initial FEL can produce kilowatt class light over the mid-infrared (2-8 microns). Minor additions to the hardware can extend the operation through the visible to the UV at low powers (10-100 watts). A planned upgrade of the facility, which is currently being designed and will be installed in late kilowatt range[2]. The combination of broad tunability, high quality optical output, high-average power and sub-picosecond pulse lengths make these FELs useful for a wide variety of basic science, applied science, industrial and defense applications. Planned experiments involving materials processing, material analysis, atomic and molecular physics, and chemical dynamics have been described in an overview of the initial FEL user program previously published in these proceedings[3]. The output characteristics of the operating FEL and its planned improvements and upgrades also make the device useful for a range of applications in biophysics, photobiology and biomedical sciences. These activities build on the excellent body of work that has been established by the Medical FEL Program funded by the Office of Naval Research at the FEL User Facilities at Stanford University, Vanderbilt University, Duke University and the Universityof California at Santa Barbara.

In this paper we first briefly describe the operating characteristics of the IR Demo FEL and its planned evolution. We then describe several biological applications of the device in the IR, visible and UV wavelength ranges. These applications involve: (1) the study of energy transport in biomolecules; (2) investigation of photodynamic effects and the related clinical application termed "photodynamic therapy"; (3) imaging techniques useful for biological and biomedical systems that involve the detection of ballistic and diffusely scattered light, particularly in the medically useful transmission window in the red/near infrared (700-900 nm); (4) investigation of DNA photodamage effects in the near UV and far visible (300-400 nm); and (5) IR photdesorption spectroscopy of molecules.

2. DESCRIPTION OF THE IR DEMO FREE-ELECTRON LASER

The IR Demo FEL is shown schematically in Fig. 1 and a listing of its current operating characteristics is shown in Table 1. The device uses a photoemission electron gun which produces a high brightness short-pulse electron pulse train at 320-350 kV and repetition rates of 18.7-75 MHz. This initial beam is pre-accelerated to 10 MeV in a pair of superconducting accelerating cavities operated at 1.5 GHz. After injection into a main acceleration module that contains eight superconducting cavities, the beam is accelerated to 40-50 MeV prior to injection into the wiggler/optical cavity region which comprises the FEL portion of the device. The wiggler is a 40 period permanent magnet wiggler located equidistant between two mirrors that make-up an 8m near-concentric optical cavity. The upstream optical cavity mirror is a 90% reflector which emits the useful portion of the FEL output which is subsequently transported to six user laboratories located above the FEL hardware. The waste electron beam transported from the wiggler region is recirculated back through the linac section in reverse phase for recovering the linac energy which was not converted to laser output. This design element significantly increases the operating efficiency and lowers the capital cost of the device. Details on the design, construction, and commissioning of the IR Demo can be found in Refs. 4—6. The IR Demo generated its first light in June of 1998 and in July of 1999 commissioning activities were completed after the device exceeded its design specifications by generating more than 1.7 kW at 3.1 microns. Shortly thereafter, the first user experiments were begun on a trial basis as the user labs were being commissioned. The results of our initial characterization of the laser output are given in Ref. 1 and a description of the 600 m^2 user lab facilities is given in Ref. 8.

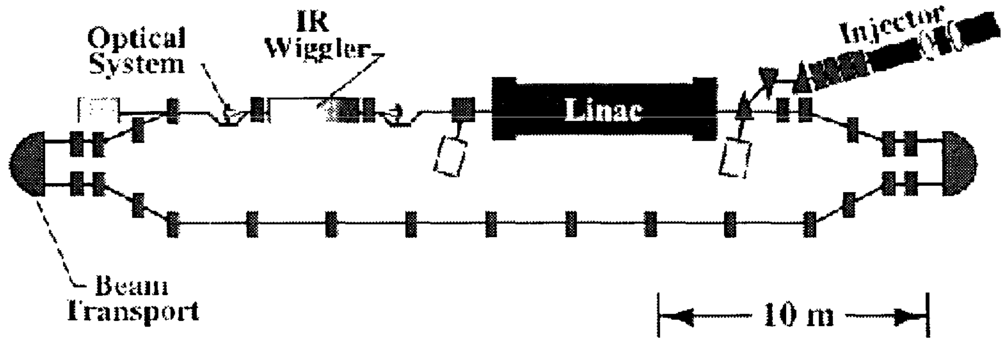

Figure 1. IR Demo Layout

Table 1. IR Demo Performance

	Specification	Achieved
Average Power	600–1000 W	1720 W
Wavelength range	6.5–3 μm	6.2-3 μm
Micropulse energy	~25 μJ	23 μJ
Pulse length	~2 ps FWHM nominal	0.5-1.7 ps
PRF 37.425, 18.7 MHz	74.85, 37.425, 18.7 MHz	
Bandwidth	~ 0.2–0.5%	~0.3
Timing jitter	< 0.2 ps	not yet measured
Amplitude jitter	< 20% p-p	<10% p-p
Wavelength jitter	0.02% RMS	not yet measured
Polarization	linear, > 100:1	>6000:1
Transverse mode quality	< 2x diffraction limit	≤2x
Beam diameter at lab	2 - 4 cm	1.5-3.5 cm

As shown in Table 1, the output of the IR Demo produces high quality laser light in a very useful format for a variety of laser-material interaction experiments. The high repetition rate and high average power output make the source very useful for photon-starved applications where the average power can be used without damaging the sample: this is often the case for gas phase samples or for condensed matter where large area samples can be used, or where the host material has a low absorbance in the wavelength range of interest. The pulse length of the optical output is typically sub-picosecond, although it can be varied by factors of two above or below the nominal value. This short pulse is particularly useful for enhancing the coupling efficiency of the incident laser light to the target material. The energy per pulse of the individual pulses is modest, ~20 μJ, although this is more than sufficient for exciting most optical transitions in a variety of physical systems. A design is underway for a pulse processing device that will be installed in the user lab. Following a scheme that was demonstrated at the Stanford FEL Center, a pulse stacking arrangement based on a resonant cavity will be used with a laser-triggered extraction mirror that can extract higher energy pulses at lower repetition rates than the FEL drive laser input.[9]

Tuning curves for the IR Demo FEL are shown in Fig 2 along with demonstrated high average power outputs obtained with low loss, dielectric cavity mirrors. At these highest powers the FEL can be tuned within the nominal bandwidth of the dielectric mirrors (+/- 5%) by varying the driver accelerator energy. For applications requiring modulation of the energy, this can be done at modulation rates up to 1 kHz. For lower power applications, broadband mirrors can be installed and the FEL can be tuned over most of the range indicated in Fig. 3. By sacrificing the recirculation aspect of the device, electron energies down to 20 MeV can be transported straight through the wiggler to a straight-ahead dump, and if proper mirrors and output windows are installed, the operating range can be increased to approx. 14 microns. On the short wavelength end of the spectrum, the IR Demo was recently demonstrated to lase on the fifth harmonic (1 micron) when the fundamental wavelength was 5 microns.[10] This opens up the possibility of extending the short wavelength limit using both 3rd and 5th harmonic operation to the visible range and near infrared (~600 –1000nm) by only investing in different mirror sets.

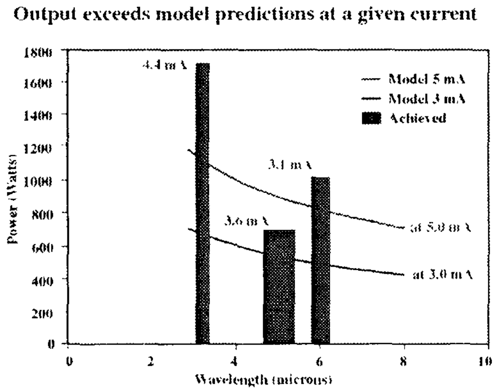

Figure 2. Tuning curves for the IR Demo FEL

A group of FEL users in the atomic and molecular physics community has proposed a novel means of using the IR Demo for spectroscopic work. By using IR Demo output at 1 micron, multiple Optical Parametric Oscillators (OPO) can be constructed at a fraction of the cost of a complete OPO system and the ensemble would retain the advantage of being time-synched at the ps level by virtue of the common FEL driver.[11] This "OPO Farm" concept would provide broadband 300nm –3 micron light at modest powers (watt level) for a variety of spectroscopic applications. By applying the high power FEL output at 1-2 microns to standard non-linear harmonic conversion techniques, output at the 10 watt level, which mimics the FEL time structure could probably be produced at fairly modest costs.

A full extension of the advantage of the IR Demo design to broader bandwidth will occur when the present system is upgraded in the 2002 timeframe. An upgrade to the IR Demo is currently under design which will increase the accelerator energy by a factor of at least 3, double the drive current and add at least two additional wiggler/optical cavity arrays. This upgrade is capable increasing the high average power output in the mid infrared to 10 kilowatts and extending the kilowatt level of operation through the visible to the deep UV (~250 nm). A summary of the wavelength extension options for the IR Demo involving both the incremental improvements mentioned above and the major 10 kW upgrade are given in Table 2. Readers who are interested in the current status of the operation of the IR Demo and planned user experiments can refer to the FEL web site at www.jlab.org/FEL/.

Table 2. Summary of Wavelength Extensions

Near term using IR Demo Lase at the third harmonic and triple or quadruple using crystals This gives >0.5 μJ at 18.7 MHz in the 300-400 nm range. (>10 W)
Use the IR Upgrade (higher energy but longer wiggler wavelength) Lase at third or fifth harmonic and triple or quadruple to 300–400 nm Yields >2 μJ at 4.68, 9.36, and 18.71 MHz (>40 W)
The UV Demo FEL using 160 MeV beam Lase at wavelengths as short as 250 nm (fundamental lasing) Yields 10–30 μJ at 2.34, 4.68, 9.36, 18.71, and 37.43 MHz (>500 W)

3. APPLICATIONS OF THE JEFFERSON LAB FEL TO BIOPHYSICS, PHOTOBIOLOGY AND BIOMEDICAL SCIENCES

As noted in the previous section, the Jefferson Lab currently provides a source of high-average power laser light across the mid-infrared spectrum that has particular advantages for laser applications: broad tunability, an ultrashort pulse length (sub picosecond), high repetition rate (18.7-75Mz) time structure, stability and high optical quality. A number of the members of the FEL user community have proposed applications of this FEL for problems of interest to the biophysics, photobiology and biomedical community. Several of these applications are direct extrapolations of previous studies that have been done at other FEL user facilities and several are unique and take advantage of the particular traits of the Jefferson Lab FEL. We discuss first several topics that can exploit the present IR capabilities of the IR Demo FEL and then discuss applications that can take advantage of the extension of the FEL's operating range to the visible and UV.

3.1 Energy transfer in biomolecules

IR spectroscopic techniques are important tools for determining the energy residence times and energy flow mechanisms in complicated condensed matter systems such as biomolecules. Detailed studies of such energy transport problems in both real and model biomolecular systems have taken advantage of the development use of both table top IR laser systems (based on solid state laser pumped OPO systems) and IR FELs over the last few years.[12] The tunability allows a particular resonance to be excited and the short pulse structure allows dynamical studies on a time scale that is interesting for energy flow in these systems. Energy transport in biological sytems is poorly understood because of the complexity of biological structures. Nonetheless, an understanding of energy deposition, storage and transport in such systems is an extremely important complement to understanding the structure and function of the living cell, organs and organisms. There appear to be a variety of circumstances under which vibrational energy in a biomolecule can travel in a coherent manner over large distances without being subject to the usual dissipation by equipartition of the energy into a thermal distribution of vibrational modes of the structure.[13] This energy flow may indeed be mediated by solitons as first speculated by Davydov.[14]

High time resolution pump-probe studies of several biomolecules were performed using the Stanford FEL by Schwettman and his co-workers.[15] These studies have continued and have recently been expanded by Austin and his colleagues using the FELIX FEL to study the dynamics of the amide-I absorption band in myoglobin.[13] These most recent studies have shown energy decay times that are indeed much longer (5-12ps) than would be expected if energy relaxation of the amide-I feature relaxed by the normal route of vibrational energy transport which has characteristic times of a ps or less. These experiments are very difficult to do because of the relatively low absorption coefficient of the biomolecule in solution, the relatively high absorption coefficient of water in the neighborhood of the amide-I band (5.8-6.5 microns), the ease at which the biomolecule can be denatured by overheating, and the temporal stability needed in the light source to do sub-ps dynamics, and the wavelength stability needed because of the relatively narrow bandwidth (~1%) of the amide--I absorption. The experiments by Austin et al.,[13] were extremely demanding both on the source (the FELIX FEL) and the experimenters because of the extremely small relative transmission rates that were measured in this thermally sensitive system and the need to have excellent amplitude and timing stability in the source.

If these experiments are repeated using the JLab FEL, signal to noise improvements should be evident because of the much higher repetition rate and timing stability afforded by the quaisi-cw nature of the JLab FEL. A condensed matter system such as aqueous solutions of biomolecules can only take partial advantage of the high average power capabilities of the IR Demo because of sample power limits. However, the samples in question can usually be prepared with 2-D symmetry and if the optical quality of the laser source allows a larger sample area to be irradiated- a straight-forward use of larger projection/collection optics for directing the incident beam and collecting the transmitted beam from the sample will allow the S/N ratio to scale with sample area at the same thermal power flux limits.

The present wavelength capabilities of the IR Demo (2-8 microns) with extensions to a wider band (1-11 microns) with no significant changes in FEL hardware[3] allow most of significant vibrational absorption bands in biomolecules to be excited.

3.2 Applications to Photodynamic Action and Photodynamic Therapy

Photodynamic Action (PDA) is a phenomena in photochemistry that has been studied for the last half century. The effect uses a dye molecule to efficiently couple the energy of an absorbed photon to a subsequent chemical reaction in the host system of the dye. The initial photoexcitation leads to radicals that are excited by energy transfer from the excited dye molecule. The effect has become a basis of an important means of cancer therapy based on the use of dyes which are designed to be preferentially absorbed by the fast growing cells in cancers.[16]

The approved clinical applications involve the use of visible light that be transmitted to accessible tumor sites such as skin cancers, lung cancers and, more recently, exposed brain cancers.

The first studies were done using the first available laser systems, ruby lasers, which emit light at 694 nm which overlaps the absorption of a commonly used biological dye molecule (methylene blue).[17] The clinical procedures that been developed over the last three decades involve the use of porphyrin compounds such as sodium porimer (Photofrin), aminolevulinic acid (ALA), lutetium texaphyrin (Lutrin) and tin ethyl etiopurpurin (Purlytin). These dyes are usually administered to a patient 24 hrs before the light treatment and then a laser source that matches an absorption band in the dye is applied to the tumor area. Clinically significant cancer destruction and enhanced patient survival rates are documented using this technique which is called photodynamic therapy (PDT). The perceived mechanism for PDT's effectiveness is believed to be the production of singlet oxygen within the cell walls. The absorbed light energy in the dye decays to a long lived triplet state which overlaps the energy bands of singlet oxygen. This reactive form of oxygen is highly toxic to living cells. Therefore, the effect requires the use of dye that has preferential absorption to tumor sites, strong absorptivity at wavelength where there is a suitable light source, little or no toxicity to the patient in the absence of light exposure, and high efficiency for production for singlet oxygen.

To date, there are no ideal dyes that meet all of the above criteria. The most commonly used dye (photophrin) instills significant photosensitivity in the patient to ambient light sources for many weeks after the initial dye treatment. Secondly, this dye and the other dyes which have met the required FDA approval for clinical use are most effectively excited by short wavelength (green) light which overlaps the primary ("Soret") absorption band in the dye molecules. However, it is the longer red to mid-infrared 700-900 nm wavelengths that are most effectively transmitted by living tissue. For clinical treatments, target fluences are in the range of 50-200 J/cm^2 and delivery rates with existing sources are typically 50-100 mW/cm^2. Therefore, reasonably high average power sources (1-10 watts) are needed to expose typical tumor areas in clinically acceptable times.

The search for PDT dyes that meet all of the above criteria have necessarily been limited by pre-selecting dye compounds that could be excited by existing solid state laser systems. Because of their simplicity and cost such lasers have the most practical appeal for clinical applications.

This rapidly expanding and promising field of cancer therapy could take advantage of an FEL such as the IR Demo for continuing development of optimal PDT dye chemistries and appropriate mechanisms for exciting the dyes. Harmonic operation of the IR Demo brings its operating range (800- 1000nm) and power delivery (~100 watts) in the ideal range for doing these experiments.

A recent experiment was done by Konig et al.,[18] using a commercially available short pulse (200 fs) Ti-sapphire laser operating at 780 nm and 2 mW average power. By focussing the laser output on photosensitized cells, they were able to demonstrate PDT effects (oxygen toxicity) using non-linear, two photon absorption of the 780 nm laser output. Thus, they were able to excite the dye at the more energetically favorable Soret band using incident radiation (780 nm) that is more readily transmitted in tissue. Given the average power constraints of such fs class lasers, the effect is highly localized to the focal region where the power densities allowed non-resonant 2-photon absorption. The high-average power and short pulse structure of the IR Demo allows this and other non linear absorption effects to be investigated for possible utility for PDT.

Another research area for PDT involves the possibility of direct excitation of singlet oxygen in-vitro by incident near-IR light at 1-2 microns. Again there are presently no reasonable power laser sources at this wavelength. If direct excitation appears to be a more efficient means of singlet oxygen production, it could lead to a clinically useful procedure where the laser light can be directed to accessible tumors such as skin lesions and organ lesions that can be irradiated with directable light guides (which is a well established imaging technique for optical tomography).[19]

3.3 Ballistic and Diffusive Light Imaging in Biomedical Systems.

Light travels through biological systems in a highly diffusive manner since the constituent materials and structures are efficient scatters of visible and infrared light. Pioneering studies have been done by B. Chance and co-workers[20] and R. Alfano and co-workers[21] on both the understanding of the diffusive transmission of light through biological systems and the exploitation of this effect for novel imaging and spectroscopic instrumentation. Figure 3 illustrates an imaging technique used by Alfano et al., that takes advantage of both short pulse laser illumination (typically mode-locked solid state lasers) and fast-gated detectors to resolve the extremely small component of light that reflects directly off of an interface in a turbid medium from the predominant component which diffuses through the medium. The technique is particularly useful for biological systems if the imaging is done with light in the transmissivity window shown in Fig. 4 that lies between 700 and 900 nm- above the absorption of hemoglobin and below the near-IR absorption bands of water.[22] Prototype imaging systems have been developed on this principle that have been applied to model biological systems. Recently, spectroscopic systems have been developed that use the higher flux diffusive component. With clinically useful light sources tissue depths of cm lengths have been explored with spatial resolutions approaching a mm.

The broad tunabiliy of the IR Demo, the ps pulse structure and the high repetition rate make this an ideal source for extending these developmental studies in biological systems and other complex turbid media.

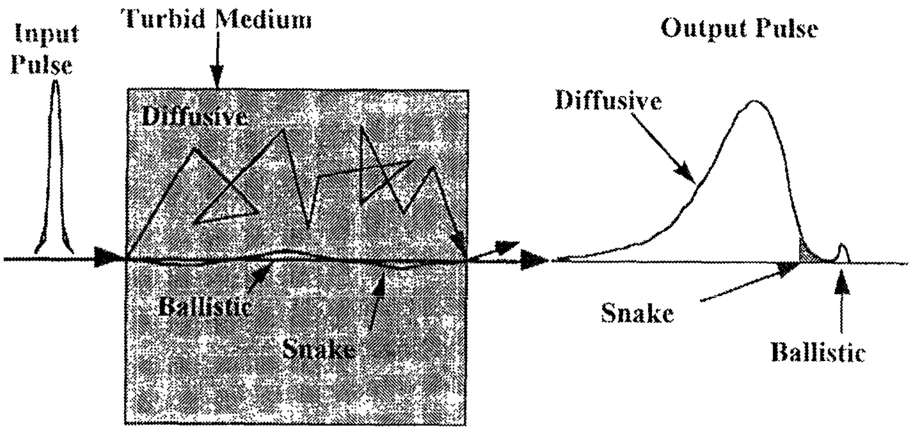

Figure 3. A schematic diagram of light scattering by a turbid medium.
(Adapted from R. R. Alfano, et al., Ref. 21)

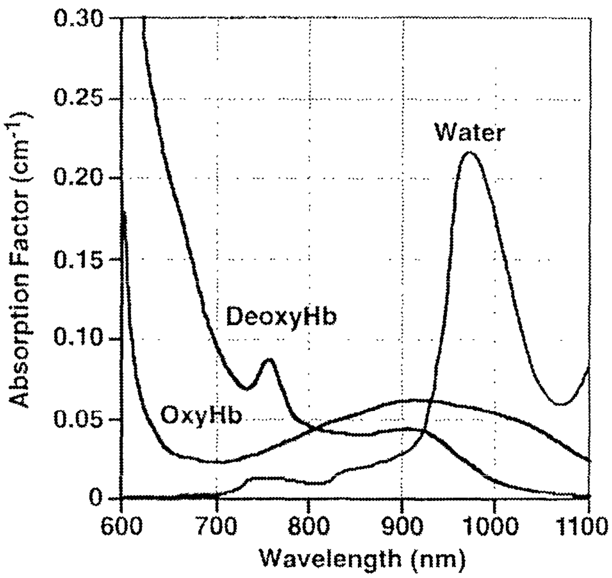

Figure 4. The transmission window in the near IR in tissue between the absorbance of Hemoglobin and water (Adapted from B. Chance, Ref. 22)

4. APPLICATIONS TO PHOTODAMAGE STUDIES IN THE UV AND NEAR UV.

In a companion paper in this conference proceedings, J. Sutherland describes the wide range of photobiology problems that exist in the UV and near UV related to DNA damage.[23] UV photodamage effects are a very timely area of research because of the uncertainties in extrapolating terrestrial UV exposure variations due to atmospheric ozone depletion and the extrapolation of DNA damage effects as a function of wavelength from biochemical through cellular to animal systems. For example, the light-induced DNA damage vs. wavelength in the region of interest (Fig. 5), shows that there is significant uncertainty in the near UV and far visible due the small value of the damage cross sections in this range. However, it is also in this range that solar radiation is rapidly rising in intensity as the wavelength increases to the visible. Predicted effects of ozone depletion on the solar radiation curve convoluted with the uncertainties in the low cross-section damage effects leads to significant uncertainties in the net reduction of ozone induced effects on the biosphere.

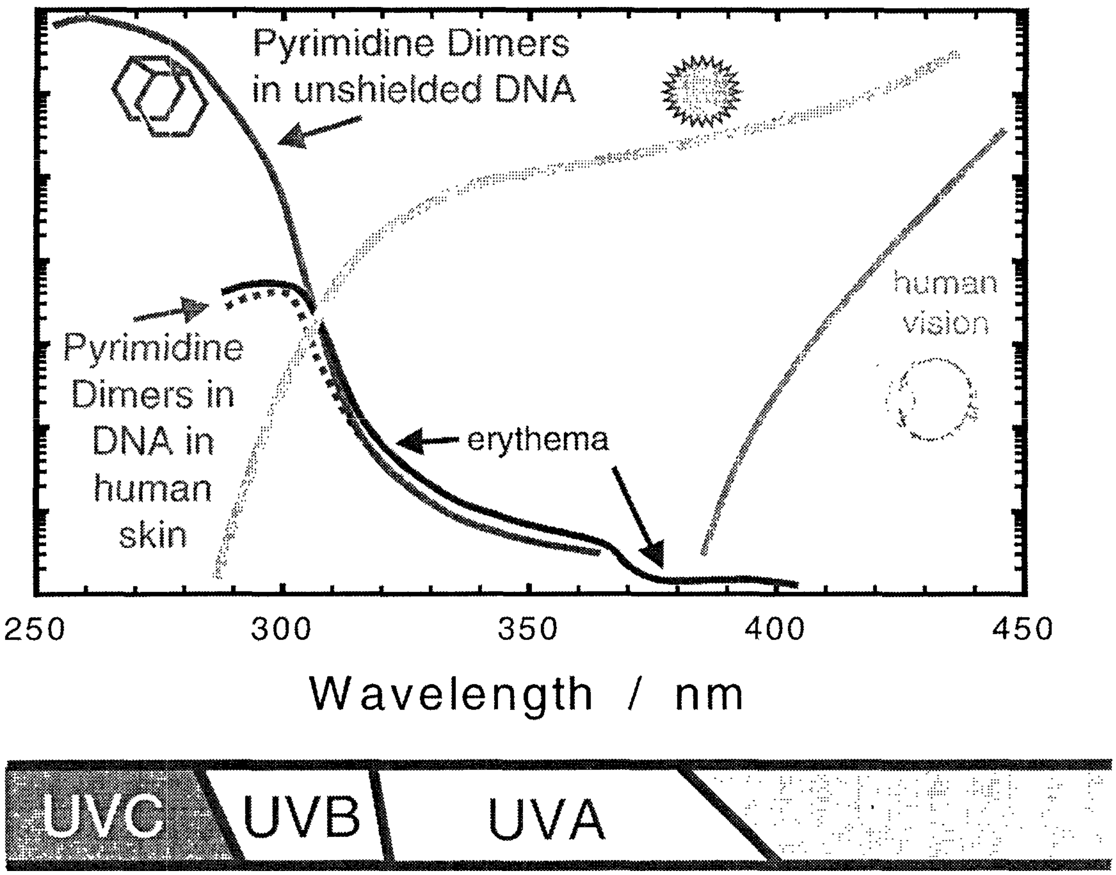

Figure 5 Schematic representations of the action spectra for the induction of DNA damage in unshielded DNA and in DNA in human skin, erythema, human vision and a typical terrestrial mid-day mid-latitude solar spectrum.

(Figure courtesy J. Sutherland, Ref. 23)

On the basis of a survey of the photobiology community, Sutherland concludes that a light source that has the following attributes would be most useful for studying the above problem: tunability in the near UV to visible (300-400 nm) to overlap the most uncertain range in the action spectra, reasonable monochromaticity (1 nm) so that discrete points in the action spectra can be measured and compared; high average power (~100 watts) so that large sample (100's) of small animal systems (e.g., mice) can be irradiated in clinically useful times. The near-term plans to operate at the IR Demo directly at harmonics or as source for conventional non-linear harmonic generation (as described in Sect. 2 above) would allow these studies to progress at the 10 watt level which would be ideal for exploring molecular studies. The planned upgrade of the IR Demo FEL for 10 kW operation in the IR and 1 kW operation in the UV would provide all the desired capability. For details the reader is referred to the companion paper by Sutherland.[23]

5. UV/IR PHOTODESORPTION ANALYSIS OF BIOMOLECULES

Multi-photon absorption at IR wavelengths has been shown to be a useful means of desorbing thermally labile and complex structures such as biomolecules[24] which then can be subsequently analyzed by laser induced fluorecence or mass spectrometry. Having a high peak intensity and tunable IR source allows energy to be introduced into selective vibrational transitions of the biomolecule or the target molecule-surface complex, thus enabling resonantly enhanced desorption effects.

Two additional characteristics of the IR Demo can be exploited for further optimizing biomolecule desorption spectroscopy. A large body of recent work[25] has noted the efficacy of ps sources for maximizing the coupling of laser energy into condensed matter systems. The simplistic reason for the optimization is the approximate overlap of the laser pulse length with the characteristic vibration time of simple diatomic adatoms and the characteristic time for electron-ion coupling in the solid substrate. If laser energy is incident with pulse lengths longer than approximately a ps then energy is fed into the bulk system; moreover if the laser energy is supplied above the atomic ablation threshold, which would be required for the desired desorption effect, than the longer pulse would allow the formation of laser-induced plasmas which can rob energy from the incident laser light and post-ionize or fragment the desorbed molecule. Again, the full high average power available from the IR Demo is probably not useful ; however, for samples that can be prepared with 2-D symmetry, significantly more average power can be usefully applied for signal-to-noise gains than is available from conventional systems. The IR light that is available with the present capabilities of the IR Demo are immediately useful for these type of laser desorption experiments. Conventional time-of flight mass spectrometers or existing OPO systems can be used for desorbed fragment analysis systems. With the availability of tunable UV at the 10 watt level with harmonic generation or higher (~ kW) with the IR/UV upgrade to the Demo, then additional sensitivity for these types of experiments are gained. Such a system will be a unique resource for protein sequencing and biomolecule structure analysis when combined with existing protein chemical analytical techniques.

6. SUMMARY

The Jefferson Lab FEL has recently come into operation in the mid infrared range. The unique combination of output characteristics: high average power, broad tunability, high repetition rate, and short pulse (ps) time structure makes the device very useful for a wide variety of laser material interaction studies and development projects. Potential applications of the FEL to photobiology and biomedical sciences have been highlighted involving: energy transport studies in the IR, DNA photodamage studies in the UV and far visible, ballistic photon medical imaging in the red and near infrared, and IR photodesorption studies.

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