

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 15-01-2010		2. REPORT TYPE Journal Article		3. DATES COVERED (From - To) October 2009-January 2010	
4. TITLE AND SUBTITLE Ultrashort Laser Retinal Damage Threshold Mechanisms				5a. CONTRACT NUMBER FA8650-08-D-6930	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER 0602202F	
				5d. PROJECT NUMBER 7757	
6. AUTHOR(S) B.A. Rockwell, R.J. Thomas and A. Vogel				5e. TASK NUMBER B2	
				5f. WORK UNIT NUMBER 26	
				8. PERFORMING ORGANIZATION REPORT	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Air Force Research Laboratory 711 Human Performance Wing Human Effectiveness Directorate Directed Energy Bioeffects Optical Radiation Branch Brooks City-Base, TX 78235-5214				10. SPONSOR/MONITOR'S ACRONYM(S) 711 HPW/RHDO	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Research Laboratory 711 Human Performance Wing Human Effectiveness Directorate Directed Energy Bioeffects Optical Radiation Branch Brooks City-Base, TX 78235-5214				11. SPONSOR/MONITOR'S REPORT NUMBER(S) AFRL-RH-BR-JA-2010-0003	
12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution A: Approved for public release; distribution unlimited (approval given by local Public Affairs Office 10-019)					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Ultrashort lasers have been adapted for use in a variety of applications from micromachining of dielectrics to atmospheric spectro-chemistry. These lasers emit almost exclusively in the retinal hazard wavelength regime, making them potential sources for both accidental vision loss, but also candidates for biomedical applications where precise alteration of tissues is an objective. We review the mechanisms for damaging the retina at the threshold for the lowest energy where any change in tissue is barely perceptible. For laser pulses between several picoseconds and ten microseconds, the threshold retinal damage is produced by microbubble formation around melanosomes in the retinal pigmented epithelium. Below one nanosecond both stress-confinement in melanosomes and self-focusing reduce the threshold for damage as measured in corneal radiant exposure, although the mechanism for damage remains unchanged. Below several picoseconds, laser-induced breakdown produces intra-retinal damage, sparing the RPE at threshold levels. These mechanisms have been determined in the past decade and provide an understanding of trends in retinal damage with variation in laser parameters, but also elucidate potential techniques for producing precise alteration to tissues.					
15. SUBJECT TERMS irradiance, beam propagation, reflected beam, exposure time, Reflected Nominal Ocular Hazard Distance (RNOHD), laser safety, high-energy laser					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Samuel Y. O
U	U	U	SAR	10	19b. TELEPHONE NUMBER (include area code) NA



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Ultrashort laser pulse retinal damage mechanisms and their impact on thresholds

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Received 25 January 2010; accepted 10 February 2010

Abstract

Ultrashort laser pulses have been adapted for use in a variety of applications from micromachining of dielectrics to atmospheric spectrochemistry and multiphoton microscopy. These lasers emit almost exclusively in the retinal hazard wavelength regime, making them potential sources for accidental vision loss, but also candidates for biomedical applications where precise alteration of tissues is an objective. The present article reviews the mechanisms for damaging the retina at the threshold for the lowest energy, where any change in tissue is barely perceptible. For laser pulses between several picoseconds and 10 μ s, the threshold retinal damage is caused by microbubble formation around melanosomes in the retinal pigment epithelium (RPE). Below 1 ns, both stress confinement in melanosomes and self-focusing reduce the threshold for damage as measured in corneal radiant exposure, although the mechanism for damage is the same. Below several picoseconds, laser-induced breakdown produces intra-retinal damage, sparing the RPE at threshold levels. These mechanisms have been determined in the past decade and provide an understanding of trends in retinal damage with variation in laser parameters, but also elucidate potential techniques for producing precise alteration to tissues.

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Keywords: Ultrashort; Retina; Safety; Femtosecond; Melanin; Laser-induced breakdown; Ultrafast

Introduction

Since the production of the first pulses shorter than 1 ns in duration (here called ultrashort laser pulses), the potential for their application for dynamic spectroscopic measurements and nonlinear microscopy has been proven. The use of ultrashort laser pulses has also been shown to be irreplaceable in many other application areas. In 1985, Strickland and Mourou [1] introduced a technique called “chirped pulse amplification” to

produce ultrashort laser pulses with extraordinary peak powers. It was with the advent of these amplified laser pulses that ultrashort pulsed lasers became much more hazardous, and at the same time, useful for biomedical applications. As their application has increased, the international laser safety standards have adopted maximum permissible exposure (MPE) levels to allow for their safe use. These MPE levels are based on a large number of retinal damage thresholds measured *in vivo* [2–18] and a breadth of supporting research delineating the mechanisms of action for these unique laser pulses.

Fig. 1 shows a summary of retinal lesion threshold measurements as a function of pulse duration t for

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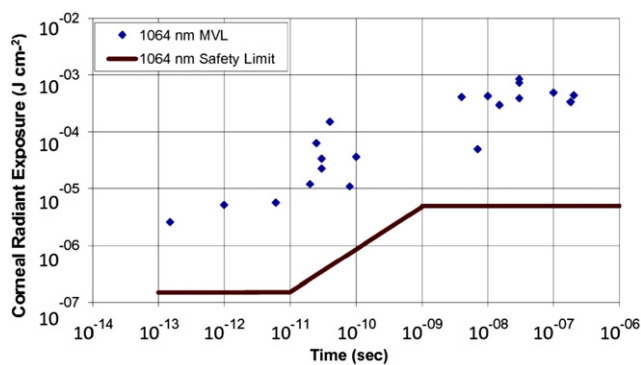


Fig. 1. Minimum visible lesion (MVL) thresholds (small diamonds) at 1064 nm and the 2007 maximum permissible exposure limits (ANSI and IEC) – solid line [19].

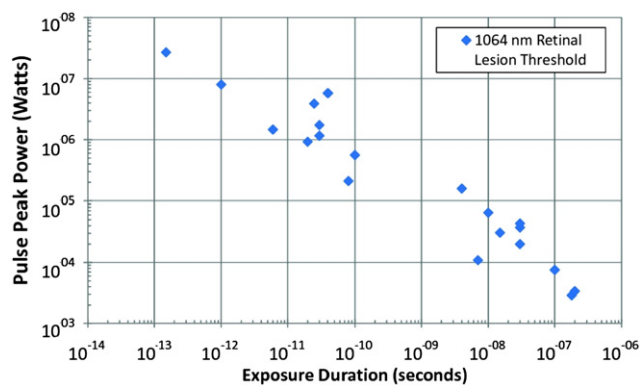


Fig. 2. Minimum visible lesion thresholds at 1064 nm plotted as a function of peak power in the pulse.

1064 nm laser exposure to the primate retina. Note that the vertical axis is the radiant exposure (measured at the cornea) needed to produce retinal damage, or in the case of the safety standard, to protect the retina. Several trends can be seen in this data set. First, for laser pulses of nanoseconds to microseconds in duration, there is a constant radiant exposure (i.e. energy into the eye) required to cause threshold damage to the retina. Second, for $t < 1$ ns there is a definite decrease in threshold energy at the cornea needed to produce retinal damage. Third, for $t < 10$ ps, the threshold remains approximately constant. Similar trends are seen in data from the visible (VIS) wavelengths. To understand the possibility of the impact of nonlinear optical phenomena affecting the threshold for retinal damage, it is instructive to examine the lesion threshold data plotted as a function of peak power. Fig. 2 shows the data of Fig. 1 plotted as a function of laser peak power (calculated by taking energy in the pulse divided by the pulse duration).

The eye is remarkably susceptible to accidental laser damage in the 400–1400-nm range. In this wavelength regime, collimated laser light is focused to a very small

spot on the retina. The retinal pigment epithelium (RPE) contains melanosomes, which are the primary absorber in this wavelength regime. For pulse durations > 10 μ s, the threshold retinal damage is produced by thermal denaturation of proteins or, for short wavelengths and exposures longer than several seconds, by photochemical effects [20]. These longer exposure durations are not the focus of this paper and will not be discussed further. In this article, the mechanisms for creating retinal damage for laser exposures between the minimum possible, which is about 10 fs, to exposures of 1 μ s in duration are reviewed.

Mechanisms for threshold-level retina damage

Melanin microcavitation (ps– μ s exposure)

Birngruber et al. [21] suggested that nanosecond laser lesions in the retina were produced by a mechanism other than thermal heating resulting in photocoagulation of the retinal layers as had been seen in longer exposure studies. Thompson et al. [22] examined in detail the thermal response of single melanosomes, demonstrating regions in which localized absorption would result in retinal damage thresholds lower than those produced by homogeneous absorption assumptions for the RPE. Roider et al. [23,24], along with related shorter-pulse works by Jacques and McAuliffe [25] and Pustovalov and Jean [26], were the first to show that there is a difference between trends in the retinal lesion threshold, if one considers purely thermal coagulation effects and trends observed in minimal lesion thresholds in the ns– μ s regime. They ascribed this difference to the lower threshold found analytically if one considers melanosomes as an initiation site for absorption and a resulting steam bubble producing photomechanical damage to the retina. This phenomenon is termed “microcavitation”. Schuele et al. [27] and Lee et al. [28] showed that the transition from thermal damage to bubble-induced damage occurs when the pulse duration is reduced below 5–10 μ s.

Microcavitation is described by considering the microscopic size and very large absorption characteristics of the melanosome in the retina. Fig. 3a shows a representation of melanosomes distributed in the RPE. These melanosomes are of the order of 1 μ m in diameter and absorb a majority of light that passes through the neural retinal layers. As the melanin particle absorbs the laser energy (Fig. 3b) their temperature quickly increases. For pulses < 1 μ s, the granules do not have time to conduct heat away and the particles become superheated. If the internal temperature increases above 150–170 $^{\circ}$ C [29] the granule acts as a nucleation site for a bubble to form (Fig. 3c).

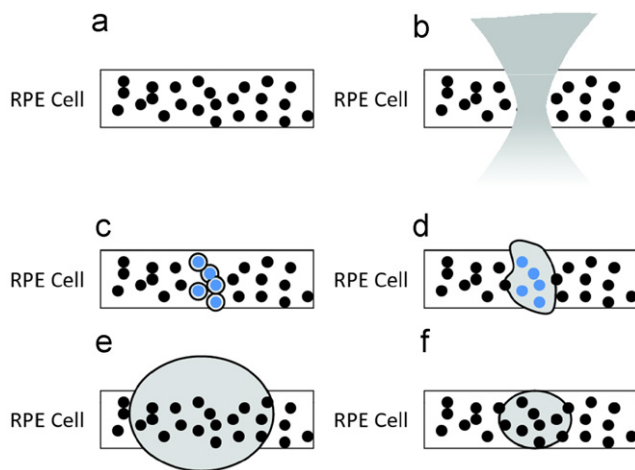


Fig. 3. Phenomenon of microcavitation in a retinal pigmented (RPE) cell is described (not to precise scale). Panel (a) pictorially shows an RPE cell. The rectangular box represents the cell walls approximately $20\mu\text{m}$ in diameter by $10\mu\text{m}$ in thickness. The small black circles represent highly absorbing melanosomes, approximately $1\mu\text{m}$ spheres. Panel (b) represents a ns-laser pulse exposing the eye and incident on this particular cell. Panel (c) shows that at approximately 100 ns after the peak of the laser exposure the granules have been superheated, which heats the surrounding layer of liquid above the nucleation temperature. This produces rapidly expanding microbubbles. Panel (d) shows that these microbubbles coalesce soon after their creation. For a 12-ns pulse and $H=0.37\text{ J/cm}^2$, the bubble has fully expanded, beyond the *in situ* cell wall diameter after approximately 250 ns from peak irradiance of the laser pulse, resulting in cell death. Panel (f) shows that the bubble collapses and eventually disappears.

Because the size of the laser beam usually exposes several granules, all these granules generate spatially separate bubbles, which coalesce and increase in size in the first 100 ns (Fig. 3d). Because of the kinetic energy of liquid acquired during the initial expansion phase, the bubble expansion continues until the kinetic energy is transformed into potential energy given by

$$E_B = \Delta p V. \quad (1)$$

Here V is the volume of the expanded bubble and Δp the difference between ambient pressure and vapor pressure inside the bubble. When the maximum bubble size is larger than the size of the RPE cell, its membrane is pushed to nearby cell structures. For 12 ns pulses and a radiant exposure of 0.37 J/cm^2 , the bubble reaches its maximum radius at approximately 250 ns [30]. The expanded bubble collapses because of the pressure difference Δp acting on the wall (Fig. 3f). Upon collapse, most of its energy is dissipated by shock wave emission, and it rebounds to a much smaller size than during the first oscillation [31]. This oscillating bubble quickly dies out, leaving a cell that looks microscopically similar to

the cell before laser exposure, but with a breached cell wall and a cell that is not metabolically active (i.e. dead). For μs pulses, the bubble dynamics around isolated absorbers differs from the picture described above: Now the first oscillation is shorter than the laser pulse, and once the bubble initially disappears, there is enough residual energy in the melanosomes to drive several subsequent oscillations with similar amplitude as that of the first oscillation [30].

Lin et al. [32] showed that microcavitation around small absorbing particles in cells results in cell death. This was elucidated using ns time-resolved stroboscopic illumination, which showed expansion of the microbubble resulted in the ability to stretch the cell walls at the bubble's maximum size, which is greater than the size of the static cell. They then assessed cell viability using fluorescent probes. Almost every cell that included microbubble expansion and implosion was selectively killed. They found that for exposure to thousands of pulses below the threshold to produce microcavitation around the microabsorbers, the cells remained viable. This is then understood to be the mechanism for retinal damage for pulse durations that cause microcavitation. As outlined in Fig. 3, the melanosomes in the RPE layer absorb laser energy from ps–ns laser pulses, and at a large enough retinal radiant exposure a microbubble is produced and results in a high probability of killing the RPE cell, which results in a retinal lesion.

There is an extensive body of literature that presents analysis of this phenomenon in RPE cells [22,23,27,28]. The medical application is to selectively affect the RPE without affecting the surrounding neural retinal layers [33]. The goal is to kill selected RPE cells and thereby stimulate RPE cell migration into the affected areas in order to improve the metabolism of the overlying retina. Many of these studies have considered primarily pulse durations in the μs regime. As can be seen from Fig. 1, there is a reduction in the energy necessary to cause a retinal lesion as the exposure duration decreases below 1 ns. Some have hypothesized that because of the nanostructure of melanosomes, stress confinement might explain these trends.

Melanosomes are spheroids with dimensions of approximately $1\text{--}2\mu\text{m}$ in diameter. These melanosomes are made of subparticles (melanin granules), which are approximately 30 nm in diameter and have a center spacing of approximately 40 nm [34]. Stress confinement is an increase in the internal pressure produced in a particle by laser-induced thermal expansion. The pressure does not have time to propagate away from the particle for short pulse durations. Stress confinement is produced when the laser pulse duration is less than the stress relaxation time. The stress relaxation time for $1\text{--}2\mu\text{m}$ melanosomes is of the order of 300 ps, and for the 30-nm melanin granules it is approximately 10 ps. This would indicate that stress confinement would

increase as the pulse duration is reduced from nanoseconds to picoseconds. If there is no change in the absorption characteristics of melanin, one would expect the threshold for microcavitation to be reduced with decreasing pulse duration in this pulse width regime.

Lin and Kelly [35] and Payne et al. [36] examined exposures producing microcavitation in RPE cells for fs–ns-exposure durations in an attempt to determine whether stress confinement effects can explain trends seen in the retinal lesion data (plotted in Fig. 1). Neither of these studies showed a significant difference between 10-ns exposures and 100-fs exposures. Alternately, Watanabe et al. [37] showed a slight decrease in ultrastructural disruption in skin melanosomes for pulse durations shorter than microsecond duration, but the most significant drop in this threshold was between ms exposures and μ s exposures. Birngruber et al. [2] evaluated the melanosomes in rabbit eyes after fs-retinal exposures, and found remarkably similar melanosome damage response for both ns and fs exposures.

Another consideration when evaluating microcavitation damage to the RPE is the absorption characteristics of melanin. The absorption of melanin falls off dramatically for wavelengths <420 and >900 nm [25]. Because the energy available for heating is proportional to the absorption coefficient, it is expected that the trends in microcavitation should follow the absorption coefficient of melanin. This has not been studied extensively and the exact wavelength where melanin absorption is overcome by absorption in water is not currently known. The international laser safety standards have adopted the same wavelength correction factors for purely thermal damage endpoints (ms–ks exposure) as with the ps–ms-melanin microcavitation retinal damage mechanism.

Self-focusing (fs–ns exposure)

Fig. 2 shows peak powers required to produce minimal threshold retinal damage with pulse durations between 100 fs to tens of microseconds. As can be seen, the peak powers in ultrashort laser pulses can approach megawatts for pulses <1 ns. At these sizeable powers, significant optical nonlinearities are expected to occur [38] in the focal area of the laser beam near the retina. An important nonlinearity is the dependence of the refractive index n on the laser irradiance I given by

$$n = n_0 + n_2 I, \quad (2)$$

which may lead to self-focusing of the laser beam. Weak self-focusing counteracts the diffraction-induced beam divergence while strong self-focusing results in a reduction of the beam diameter followed by an irradiance increase, even stronger self-focusing, and beam collapse. Beam collapse is the reduction of the beam diameter below the diffraction limit. Self-focusing is often linked

to optical breakdown, and for an understanding of retinal damage it is crucial to identify which phenomenon has a lower threshold and will thus occur first.

For self-focusing to occur, a critical power has to be surpassed, regardless of the spot size. For optical breakdown, however, a certain level of irradiance must be exceeded. With increasing spot size (i.e. decreasing focusing angle) the laser power must be increased to reach the irradiance threshold. Below a certain focusing angle, the power required for optical breakdown will thus be higher than the critical power for self-focusing. At even smaller angles, the power will exceed the critical value P_2 for beam collapse and filament formation [39]. A filament is a sub-diffraction-limited beam propagating with a nearly constant diameter for several centimeters in a focused system. Since small focusing angles are characteristic for ocular safety considerations, self-focusing in the vitreous and retina may occur at laser powers below the optical breakdown threshold.

When the peak power in a focused beam approaches the critical power, the irradiance at the focus is increased due to self-focusing [40] and this enhancement can be expressed as

$$I_{SF} \cong \frac{I_0}{1 - P/P_2}. \quad (3)$$

Here I_{SF} is the irradiance at the focus taking into account self-focusing, I_0 the linear irradiance with no self-focusing (i.e. long pulse), P the peak power in the laser pulse (pulse energy divided by pulse duration), and P_2 the second critical power for self-focusing [41] given by

$$P_2 = \frac{3.77 c \lambda^2}{32 \pi^2 n_2}, \quad (4)$$

where c is the speed of light in the material, λ the wavelength of light, and n_2 the nonlinear refractive index for the material, which, for vitreous humor was measured to be $(1.4 \pm 0.4) \cdot 10^{-13}$ esu (electrostatic units) [42]. Self-focusing irradiance enhancement has been shown to occur as peak power is increased until the P_2 power is reached. At that point, the beam spatially collapses and other nonlinear phenomena such as laser-induced breakdown (LIB) or supercontinuum generation result [39,41]. Supercontinuum is the generation of a very broad spectrum of light from an ultrashort laser pulse due to optical nonlinearities in the material of propagation.

In the eye, for the sake of evaluating the impact of self-focusing on retinal damage, it was firstly assumed that the radiant exposure at the retinal plane is constant to cause microcavitation for exposures from ps in duration to μ s in duration. This assumption has been studied by pulse-duration dependence of microcavitation around melanosomes [43,44]. It should be noted that this assumption neglects any variation in microcavitation threshold from either stress confinement or changes in heating rate due to

nonlinear absorption [45]. Because the vertical axes in Figs. 1 and 2 are radiant exposure at the cornea, one must consider the effect on the trends in these graphs from self-focusing. As the pulse duration is decreased, the irradiance at the retinal plane will increase due to self-focusing, but the radiant exposure to produce damage by microcavitation is constant. As a result, the radiant exposure at the cornea must be reduced to maintain the retinal radiant exposure. The result is a reduction in the amount of energy needed to enter the eye (i.e. corneal radiant exposure) as the pulse duration is reduced below 1 ns. If both sides of Eq. (3) are multiplied by the pulse duration, an expression for the corneal radiant exposure with peak power can be derived:

$$H_{SF} \cong \frac{H_0}{1-P/P_2}. \quad (5)$$

This provides a function to compare to the data of Fig. 1 and gives an idea of the reduction in corneal radiant exposure that can be expected due to self-focusing. It should be noted that this equation is valid only for peak powers below P_2 . The results of this calculation are shown in Fig. 4, where a reduction on the order of ten is shown up to P_2 , where the plot ends. Significant reduction of corneal radiant exposure required to cause retinal damage sets in for pulse durations below tens of picoseconds, slightly lower than the data indicate in Fig. 1. The results of Fig. 4 and Eq. (5) assume no aberrations, which is certainly not the case for the eye. The impact of aberrations on self-focusing has not been thoroughly examined. One would expect the trends of Fig. 4 to be changed with the aberrations of the eye fully considered.

As the peak power increases above P_2 , other nonlinear phenomena are produced. One such phenomenon is LIB [39]. As a result of plasma formation, the beam is defocused by the lower refractive index in the plasma. The interplay of self-focusing and plasma defocusing produces a filament when propagating in transparent materials [46].

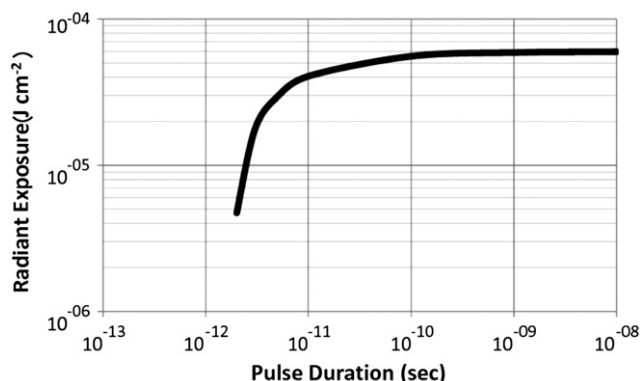


Fig. 4. Trend in reduction from self-focusing of corneal radiant exposure to cause retinal damage is shown.

Laser-induced breakdown (fs exposure)

Concurrent with the advent of the sub-ps laser research, efforts primarily by Kennedy et al. [47], Noack and Vogel [48], and Vogel et al. [49] led to development of theoretical descriptions of LIB in aqueous media. Laser-induced breakdown represents a rather violent phenomenon, in which the affected volume is ionized. The large irradiance in the laser focus causes nonlinear absorption, in turn resulting in plasma formation, large energy density, subsequent strong shock waves, and large bubbles [50]. These phenomena occur only at peak irradiance levels much larger than known photo-thermal and microcavitation mechanisms for damage to the retina for pulses of >100 fs duration [51].

For pulses of constant energy, the irradiance increases with decreasing pulse duration, and nonlinear absorption becomes even more important, even in the presence of strong linear absorbers such as melanosomes [51]. Finally, for pulse durations <200 fs, the energy threshold for optical breakdown in the transparent retina is lower than the threshold for bubble formation by linear absorption in the melanosomes.

As shorter-pulse lasers became available with sufficient pulse energies, research by Hammer et al. [52,53], Vogel et al. [54,55], and Noack et al. [56] systematically characterized the thresholds for the formation of the LIB and the efficiency of energy transfer from the laser to the plasma. The researchers were careful to include the specific geometry of the eye and to characterize the significant effects of optical aberrations, such that the lower bound of threshold could be identified. This research led to the hypothesis that LIB may be the threshold mechanism of damage to the retina for pulse durations of <200 fs. In addition, when peak powers are sufficient for the beam to collapse during propagation along its path through the eye (at known threshold energies seen below 10 ps), the peak power achieved by the beam is enhanced. This enhancement could in turn lead to LIB at measured thresholds in these 200-fs–10-ps ranges.

This hypothesis was tested through a number of experiments that characterized thresholds and morphology of effect to the retina. The works published by Cain et al. [7] and Toth et al. [5] confirmed that damage thresholds were within the measured realm of LIB. In addition, histology collected subsequent to exposures gave indication of full-thickness damage for LIB near the threshold for retinal damage as pulse duration decreases. This provides further evidence that LIB indicates the threshold for damage in 100–300-fs range (VIS/near infrared (NIR)).

Fig. 5 illustrates one of the most convincing pieces of evidence that nonlinear events are in play, and contrasts the physical response from two lasers at differing ends of the exposure duration realm. This figure demonstrates the damage distribution anticipated from a small

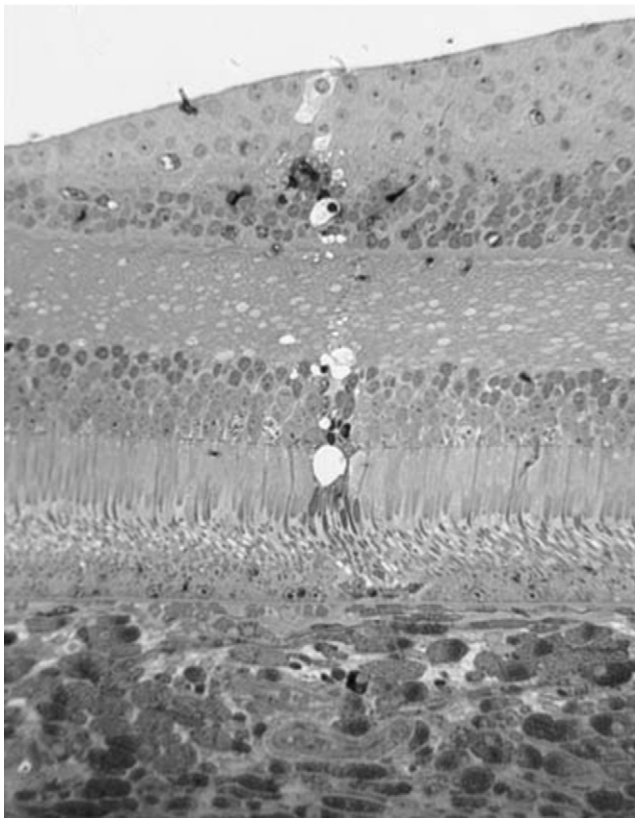


Fig. 5. Histopathology of fs-laser exposure to the retina. The picture shows a nonhuman primate retina from the inner limiting membrane (top) to the choroid (bottom). Damage extends throughout the inner retina, which is atypical for thermal damage or microcavitation (courtesy Cynthia Toth, M.D., Duke Eye Center).

numerical aperture focusing geometry where LIB occurs. The same distribution was reported more recently in experiments by Cain et al. [8]. The image was collected with energy just above the ophthalmoscopically measured damage threshold.

Group velocity dispersion (fs exposure)

Our final topic plays a role in damage threshold for the retina at sub-100-fs pulse durations. In these regions, the spectral content of the pulse can become sufficiently broad to observe a wavelength-dependent propagation speed in the pulse. In the VIS and NIR region of the spectrum, the longer wavelengths within the pulse propagate sufficiently faster than the shorter wavelengths to broaden the pulse. This effect, known as group velocity dispersion (GVD), is sufficient to decrease the peak irradiance in the pulse as it reaches the retina. The net result is a predicted increase in the threshold for damage to the retina.

The issue of GVD effect on damage threshold was described through theory in general focusing geometries

by Kempe et al. [57] and Kempe and Rudolph [58], and early on by Powell et al. [59], but were not characterized until a sub-100-fs study was conducted by Cain et al. [8]. The published findings indicated that a predicted and measured increase in damage threshold due to GVD effects was within a factor of two for a 40–50-fs pulse duration. An interesting aspect of the study was that a pulse could be “prepared” such that the GVD effects in the eye were compensated by the optics of the delivery system in front of the eye, and a lower bound of damage was characterized.

Conclusions

The radiant exposure threshold at the retina required to cause damage is the result of several new damage mechanisms for ultrashort laser exposures. Ophthalmoscopic evaluations of damage linked with histopathology, *ex vivo*, *in vitro*, and modeling efforts, have clearly delineated these mechanisms. For exposures between several picoseconds to microseconds in duration, microcavitation around melanosomes results in RPE cellular damage. Below 1 ns, this threshold is lowered by a combination of stress confinement in microgranules of melanin and self-focusing, resulting in a smaller beam on the retina. For fs–ps exposures, LIB is produced in the retinal layers. This is affected in the sub-50-fs regime by group velocity dispersion because the bandwidth of the laser pulse is very broad for these pulses. Proper understanding of these mechanisms opens the way for application of fs lasers to ocular surgery and treatment.

Acknowledgements

The work at the Air Force Research Laboratory was supported by the Air Force Office of Scientific Research. The authors would like to thank David Stolarski from TASC Inc. for his tireless editorial help with the manuscript.

Zusammenfassung

Schädigungsmechanismen der Retina durch ultrakurze Laserpulse und ihr Einfluss auf Grenzwerte

Ultrakurz gepulste Laser werden in einer Vielzahl von Anwendungen eingesetzt, von der Mikrobearbeitung dielektrischer Materialien, über die Atmosphärenspektroskopie bis zur Multiphotonen-Mikroskopie. Diese Laser strahlen fast ausschließlich im netzhautgefährdenden Wellenlängenbereich, wodurch sie zu potentiellen Quellen eines unfallbedingten Verlusts der Sehfähigkeit

werden, aber auch zu Kandidaten für biomedizinische Anwendungen, bei denen es auf präzise Gewebemodifikationen ankommt. Dieser Artikel gibt einen Überblick über die Schädigungsmechanismen der Netzhaut an den niedrigsten Energieschwellen, an denen eine gerade wahrnehmbare Veränderung des Gewebes eintritt. Für Laserpulsdauern zwischen einigen Pikosekunden und zehn Mikrosekunden wird die Schädigungsschwelle durch die Bildung von Mikrobläschen um die Melanosomen im retinalen Pigmentepithel (RPE) bestimmt. Unterhalb von einer Nanosekunde reduzieren sowohl das „stress confinement“ in den Melanosomen als auch die Selbstfokussierung die (als Bestrahlung der Hornhaut gemessene) Schädigungsschwelle, obwohl der Schädigungsmechanismus derselbe ist. Unterhalb einiger Pikosekunden erzeugen laserinduzierte optische Durchbrüche Schäden innerhalb der Netzhaut, wobei an der Schädigungsschwelle das RPE unbeeinflusst bleibt. Diese im Laufe des letzten Jahrzehnts entdeckten Mechanismen machen die Abhängigkeiten der Netzhautschäden von den Laserparametern verständlich, werfen aber auch ein Licht auf mögliche Techniken zur Erzeugung präziser Gewebeveränderungen.

Schlüsselwörter: Ultrakurz; Retina; Sicherheit; Femtosekunde; Melanin; Laserinduzierter Durchbruch; Ultraschnell

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