Variation of laser-induced retinal injury thresholds with retinal irradiated area: 0.1-s duration, 514-nm exposures

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Variation of laser-induced retinal injury thresholds with retinal irradiated area: 0.1-s duration, 514-nm exposures

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Abstract. The retinal injury threshold dose for laser exposure varies as a function of the irradiated area on the retina. Zuclich reported thresholds for laser-induced retinal injury from 532 nm, nanosecond-duration laser exposures that varied as the square of the diameter of the irradiated area on the retina. We report data for 0.1-s-duration retinal exposures to 514-nm, argon laser irradiation. Thresholds for macular injury at 24 h are 1.05, 1.40, 1.77, 3.58, 8.60, and 18.6 mJ for retinal exposures at irradiance diameters of 20, 69, 136, 281, 562, and 1081 μm, respectively. These thresholds vary as the diameter of the irradiated retinal area. The relationship between the retinal injury threshold and retinal irradiance diameter is a function of the exposure duration. The 0.1-s-duration data of this experiment and the nanosecond-duration data of Zuclich show that the ED50 (50% effective dose) for exposure to a highly collimated beam does not decrease relative to the value obtained for a retinal irradiance diameter of 100 μm. These results can form the basis to improve current laser safety guidelines in the nanosecond-duration regime. These results are relevant for ophthalmic devices incorporating both wavefront correction and retinal exposure to a collimated laser. © 2007 Society of Photo-Optical Instrumentation Engineers.

Keywords: retina; laser injury thresholds; spot-size dependence; laser bioeffects; laser safety.

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1 Introduction

The eye is most susceptible to injury after exposure to a highly collimated laser beam because the laser energy is focused by the optics of the eye to a very small area on the retina of the eye. Most of the bioeffects database has been obtained in experiments designed to produce the smallest irradiance diameter at the retina in rhesus monkey eyes and these data are what primarily supports the provisions of laser safety guidance. The eye is also susceptible to injury following exposure to a laser beam that is diverged to irradiate a larger diameter on the retina. Current safety guidance reflects an understanding of the relationship between the quantity of laser energy that must be introduced into the eye to produce retinal injury and the diameter of the irradiated area developed in studies 30 yr ago. This relationship has become a subject of renewed interest because of the increasing use of instrumentation and devices that subject the retina to coherent or incoherent irradiation over a wide range of retinal irradiance diameters. The quest for high-resolution imaging of the human retina in vivo has led to the coupling of wavefront correction to defeat optical aberrations of the eye and highly collimated laser beams that are directed into the corrected eye resulting in near-diffraction-limited irradiance profiles at the retina. It is important to ascertain that these retinal exposures are safe.

Laser safety guidelines specify the maximum permissible exposure (MPE) as a function of exposure duration and laser wavelength for exposure to a collimated beam, identified in the guidelines as a small (point) source. By definition, a point source subtends a limiting visual angle αmin of 1.5 mrad. The MPE for a source subtending a larger visual angle α is obtained by multiplying the point-source MPE by a correction factor $C_E$ in American National Standards Institute (ANSI) and International Commission on Non-Ionizing Radiation Protection (ICNIRP), $C_E$ in International Electrotechnical Commission (IEC)] that is a function of α. While the angular or spatial distribution of the beam is the measurable parameter, it is the diameter of the irradiance profile D at the retina that determines the damaging potential of a given quantity of energy incident on the retina. The retinal irradiance diameter can be calculated from the source visual angle as $D = af_r$, where $f_r$ is the effective focal length of the eye in air. For a given value of α, D is smaller in the monkey eye ($f_r = 13.5$ mm), which is used in injury threshold studies, than in the human eye ($f_r = 17$ mm), for which the standards pertain. For comparison of the MPE to injury thresholds in the

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monkey eye it is useful to express $C_E$ as a function of $D$ (Table 1, Fig. 1). Note that $D_{min}$ is the retinal irradiance diameter in the human eye calculated for the limiting visual angle $\alpha_{min}$ and $D_{max}$ is the retinal irradiance diameter in the human eye calculated for $\alpha_{max}=100$ mrad. Also, $C_E$, $C_C$, and the MPE are directly proportional to $D$ between 25 $\mu$m and 1700 $\mu$m for the case of the human eye.

Zuclich et al.\textsuperscript{6,7} reported data showing that the $ED_{50}$ (50% effective dose) for 7-ns-duration exposures at 532 nm varied as $D^2$. The $ED_{50}$ is that quantity of energy entering the eye having a 50% probability of producing a minimum visible lesion (MVL) in the retina. Thermal models support the notion of a $D^2$ dependence of $ED_{50}$ for short-pulse exposures but suggest a different result for longer exposures and, in fact available $ED_{50}$ data for second-duration laser exposures are proportional to the irradiance diameter.\textsuperscript{8–10} These studies for continuous wave (cw) exposures were limited in the number of discrete retinal irradiance diameters evaluated and do not report data for exposures at all diameters necessary to completely establish the relationship between $D$ and $ED_{50}$. It was therefore the purpose of this study to provide new data relating the $ED_{50}$ for laser-induced retinal damage over a range of retinal irradiance diameters for cw exposures to an argon laser operating at $\lambda=514$ nm.

<table>
<thead>
<tr>
<th>$\alpha$ (mrad)</th>
<th>$C_E$</th>
<th>$D$</th>
<th>$C_E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha&lt;\alpha_{min}$</td>
<td>1</td>
<td>$D&lt;25\ \mu$m</td>
<td>1</td>
</tr>
<tr>
<td>$\alpha_{min}&lt;\alpha&lt;\alpha_{max}$</td>
<td>$\alpha/\alpha_{min}$</td>
<td>$25\ \mu$m $\leq D&lt;1700\ \mu$m</td>
<td>$D/25$</td>
</tr>
<tr>
<td>$\alpha&gt;\alpha_{max}$</td>
<td>$\alpha^2/(\alpha_{min}\alpha_{max})$</td>
<td>$D\geq1700\ \mu$m</td>
<td>$D^2/(25\times1700)$</td>
</tr>
</tbody>
</table>

$\alpha_{min}=1.5$ mrad; $\alpha_{max}=100$ mrad.

2 Materials and Methods

2.1 Apparatus

An optical system was assembled to expose the retina of a rhesus monkey eye to a laser beam having carefully controlled and measured divergence, duration, and power (Fig. 2). An argon laser provided cw laser irradiation at the wavelength of 514.5 nm. All exposures were of 0.1-s duration, controlled by the electronic shutter.

A beamsplitter deflected a constant proportion of the beam into a reference detector, while the remainder of the power passed through a lens and onto a mirror positioned to direct the laser beam into the eye to be exposed. A fundus camera enabled observation of the retina and selection of sites for exposure. The mirror was mounted on a translation stage so it could be moved to enable observation of the retina and then accurately repositioned for exposure. The fundus camera, mirror, and laser beam were aligned such that the laser energy reflected by the mirror passed through the center of the ocular pupil and struck the retina at the site corresponding to the crosshairs of the fundus camera viewing optics.

The direct output beam of the laser was spatially filtered and recollimated to produce a Gaussian beam having a measured divergence of 0.25 mrad for the collimated beam exposures. This arrangement produced the smallest retinal irradiance diameter exposures. To produce larger retinal irradiance diameter exposures, a beam-expanding telescope enlarged the beam to overfill an aperture that limited the beam diameter at lens L1 in Fig. 2. Lens L1 was positioned to produce a diverging beam that was then directed into the eye. A tophat irradiance profile was produced at the retina. The beam divergence $\theta$ was fixed by the aperture diameter and the focal length of the lens, and was measured at the eye position via a CCD-camera-based laser beam profiler system. The lens-to-cornea distance was adjusted such that the laser beam at the

![Fig. 1](image1.png)  
**Fig. 1** Dependence of the source-size correction factor $C_E$ on the retinal irradiance diameter.

![Fig. 2](image2.png)  
**Fig. 2** System for exposure of monkey retina *in vivo* to a laser beam having selectable irradiance diameters at the retina.
cornea was 3 mm in diameter. The retinal irradiance diameter was calculated using the relationship \( D = f_e \theta \), where \( \theta \) is the beam divergence in radians, and \( f_e \) is the focal length of the rhesus monkey eye, assumed to be 13.5 mm. The beam divergence and the corresponding values of \( D \) were recorded at the points where the beam irradiance fell to \( 1/e \) times the peak value. Before the rhesus monkey was positioned, a calibrated detector was placed to receive directly the power that would normally enter the eye. The ratio of the power at this position to the power at the reference detector was obtained with the attenuator removed. Subsequently, when the eye was exposed, the power entering the eye for each exposure was determined by multiplying the power at the reference detector by this ratio and by the transmission of the attenuating filter chosen to give the desired exposure.

### 2.2 Experimental Subjects

Rhesus monkeys (Macaca mulatta) were used in this study. Each animal was sedated via an intramuscular injection of ketamine hydrochloride. Anesthesia was induced via an initial induction dose of propofol and maintained using propofol via syringe pump for exposure. Proparacaine hydrochloride, phenylephrine hydrochloride, and tropicamide induced cycloplegia and full pupil dilation in the eye to be exposed. A retrobulbar injection of lidocaine temporarily paralyzed the eye muscles to preclude eye movement during exposure. A lid speculum held the eye open for exposure. The cornea was periodically irrigated with physiological saline solution to maintain clarity. The animal was wrapped in a heating blanket to maintain core temperature and vital signs were continuously monitored.

### 2.3 Procedure

Dose response data were obtained for exposure to the collimated beam and for exposure to beams having nominal divergences of 0.25, 5, 10, 20, 40, and 80 mrad. The measured values and corresponding retinal irradiance diameters are given in Table 2. For each test, an animal was positioned and exposures were placed in an array in the macular and extra-macular retina. Suprathreshold laser-induced marker burns were placed as a guide to placement and subsequent identification of the exposures. The exposure sites were examined by ophthalmoscope and digital fundus photography 1 and 24 h after exposure and the presence or absence of a lesion noted for each site. The response at each site was correlated to the dose at that site. The data relating the probability of damage to the exposure energy was processed by the statistical technique of probit analysis to determine the ED\(_{50}\). For each exposure condition, the data allowed determination of the ED\(_{50}\) for macular injury detected at 1 h postexposure, macular injury detected at 24 h postexposure, extramacular injury detected at 1 h postexposure, and extramacular injury detected at 24 h postexposure: The probit analysis also provided the 95% confidence limits about the ED\(_{50}\) and the slope of the probit curve, defined as the ED\(_{50}\)/ED\(_{90}\). The measured quantity for the dose was the total intraocular energy (TIE), defined as that energy incident on the cornea within the area of the ocular pupil.

### 3 Results

The data of this study are summarized in Table 2 and Fig. 3. The values for \( \theta \) and \( D \) are given at the points where the irradiance falls to \( e^{-1} \) of the peak value. All irradiance profiles except the collimated beam case had a uniform, tophat distribution. The retinal irradiance distribution for the collimated beam arrangement was Gaussian. The value of \( D \) given in Table 2 for this case assumes an optically perfect eye. In Fig. 2, the collimated beam data are plotted at a diameter of 20 \( \mu \)m, which is the diameter subtended by \( \theta_{\text{min}} \) in the monkey eye.

### 4 Discussion

Over the range of retinal irradiance diameters from 130 to 1000 \( \mu \)m, the ED\(_{50}\) for 0.1-s, 514-nm laser-induced retinal injury is proportional to the diameter of the irradiated retinal area. This stands in distinct contrast to the ED\(_{50}\) data for 7-ns, 532-nm laser retinal exposures wherein the ED\(_{50}\) for laser-induced retinal injury is proportional to the area (therefore \( D^2 \)) of the irradiated retinal area over the same range of diameters (Fig. 4). Figure 4 also includes the MPE computed for 7-ns exposures and for 0.1-s exposures. The MPE is provided by the laser safety guidelines in units of corneal radiant exposure (in joules per square centimeter) averaged over a 7-mm aperture. When the MPE (in joules per square centimeter) is multiplied by the area of a 7-mm pupil (in square centimeters), the total intraocular energy (in joules) introduced into the eye for an exposure at the MPE is obtained. The total intraocular values of the MPE are used in Fig. 4. Lund et al.\(^{14}\) and Schulmeister et al.\(^{15}\) concluded that the form of \( C_E \) should vary as a function of the exposure duration. Based on an analysis of the existing database and an exhaustive exploration of the effects of exposure duration variation and retinal irradiance diameter variation on the injury thresholds as predicted by thermal models, those authors proposed that the value of \( D_{\text{max}} \) separating the regime wherein the ED\(_{50}\) is proportional to \( D \) and the regime wherein the ED\(_{50}\) is proportional to \( D^2 \) should vary with exposure duration.

For both exposure durations, the ED\(_{50}\) expressed in terms of TIE no longer decreases proportionally to the irradiance as the irradiance diameter decreases below about 100 \( \mu \)m, but remains essentially constant for all smaller irradiance diameters. A number of investigations that included data for an irradiance diameter of 80 to 100 \( \mu \)m as well as data for greater and smaller irradiance diameters show that the incident energy required to produce retinal injury in an intact monkey eye does not decrease for retinal irradiance diameters smaller than about 100 \( \mu \)m, but reaches a minimum at that diameter and remains relatively constant for all smaller diameters.\(^{6,7,9,16}\) These investigations included a number of wavelengths and a broad range of exposure durations (Fig. 5).

The underlying cause for this behavior is a subject of debate.\(^{17-19}\) Possible explanations include (1) small-angle forward scatter of the laser beam within the eye, which distributes the energy over a larger diameter than expected at the retina; (2) larger than predicted uncompensated aberrations of the eye of the anesthetized monkey; (3) limited capability of the investigator to detect retinal alteration contained within
diameters less than 100 μm unless the additional energy is introduced to produce a more severe and therefore more visible alteration; and (4) intraretinal scatter which leads to an irradiance profile at the retinal pigment epithelium (RPE) layer that is larger than the irradiance profile that is incident on the uppermost layers of the sensory retina. Computer models designed to compute the incident laser energy required to produce thermal retinal injury predict that the required energy will decrease with decreasing retinal irradiance area until the diameter of the irradiated area at the retina is less than 100 μm. Experiments with bovine eye explants, wherein the anterior portions of the eye including the neural retina are removed so that the RPE layer can be directly irradiated, show that indeed the energy required to damage the RPE does decrease with the diameter of the irradiated area for irradiance diameters down to 20 μm. These experiments enable precise control of the diameter and energy of the incident beam at the RPE and provide positive identification of injury via a fluorescent-dye-based assay of cell viability. The model predictions and explant results produce a relationship between the incident energy and the retinal irradiance diameter that is not in agreement with in vivo experiments, wherein the incident energy is determined for a MVL in the retina of anesthetized monkeys.

Table 2 The ED\textsubscript{50} for laser-induced retinal damage in the monkey eye as a function of retinal irradiance diameter, observation time, and retinal location after 100-ms exposure to a 514.5-nm argon laser.

<table>
<thead>
<tr>
<th>θ (mrad)</th>
<th>D (μm)</th>
<th>Retinal Location</th>
<th>Observation Time (h)</th>
<th>ED\textsubscript{50} (mJ) (TIE)</th>
<th>95% Confidence Limits</th>
<th>Slope (ED\textsubscript{84}/ED\textsubscript{50})</th>
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<td>0.25</td>
<td>3.4</td>
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<tr>
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<td>1.77</td>
<td>1.53–2.03</td>
<td>1.27</td>
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<td>14.9–28.2</td>
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of focusing a collimated incident beam down to 4 to 5 μm at the retina. Measured visual acuity in the rhesus is consistent with a focus that fine.21–23 Yet the in vivo threshold data indicate that the effective minimum irradiance diameter on the retina is about 100 μm. Guidance for the safe levels of exposure of the eye to laser radiation has been derived based on damage threshold studies in the laboratory using anesthetized nonhuman primates. Focus is a dynamic process that is purposely defeated in these nonhuman primates while performing measurement of the retinal injury level. The actively focusing eye of an aware human might more accurately focus incident laser radiation and achieve a smaller irradiance diameter at the retina. There have been no reported instances of retinal injury following laser exposure at or below the MPE. Still, biomedical optics researchers are employing wavefront correction methods to achieve even better focus in the human eye for high-resolution imaging. Retinal injury threshold data from studies using anesthetized nonhuman primates might not accurately reflect the hazard in such an application.

Figure 6 shows that the current guidelines provide a margin of safety for 100-ms exposures, even given the possibility that the point source ED50 might be lower than that obtained through in vivo experiments. There is no compelling reason to change the form of C_E for cw exposures. On the other hand, the current guidelines do not provide a safety margin when compared to the data of Zuclich et al. for 532-nm Q-switched exposures.6,7 That experiment employed the same experimental procedures as described for this study. The ED50 determined in that study for retinal damage when D=85 μm is essentially identical to the MPE with no margin of safety. It is evident that the provisions of the guidelines must be adjusted for short-pulse exposures. As noted, an indicated adjustment would change the form of C_E such that the MPE for short-pulse exposures varied as D^2. Such a change would result in...
MPEs which are a better fit to the data for $D > 100 \text{ \mu m}$, but it creates an interesting problem when considering point-source exposures. Providing a margin of safety for large $D$ while at the same time maintaining the point-source MPE at the current level would require that $D_{\text{min}}$ be increased to 80 to 100 $\text{\mu m}$ (Fig. 7). This formulation is supported by the in vivo threshold data but not by thermal models and explant data\textsuperscript{15,17} and is difficult to reconcile with the known visual acuity. Conversely, if the value of $D_{\text{min}}$ remains at 25 $\text{\mu m}$, then the value of the point-source MPE must decrease substantially. This choice is supported by thermal models and the in vitro data but runs counter to a large base of point-source in vivo threshold data. Neither of these formulations is of itself satisfactory, and the final formulation will consider other factors, including the exposure-duration dependence of $\text{ED}_{50}$ values for point-source exposures. A smaller safety factor may be acceptable for the larger diameter condition, assuming that the $\text{ED}_{50}$ values for large $D$ have less uncertainty than the $\text{ED}_{50}$ values for small $D$.

Recently, optical wavefront correction systems have become available that have the capability to compensate for ocular aberrations.\textsuperscript{25} In essence, the wavefront correction system predistorts the wavefront of the beam incident on the eye in a manner to compensate for the aberrations of the eye. The beam is therefore focused on the retina to a diameter very close to the diffraction limit imposed by the diameter of the ocular pupil. Such a system, incorporated into the exposure system for determination of retinal injury thresholds, would enable the investigator to ascertain the true refractive state of the subject animal eye and to ensure that the retinal irradiance diameter is not limited by uncompensated aberrations. This would provide data required to resolve in part the disagreement between monkey and explant injury thresholds for retinal irradiance diameters smaller than 100 $\text{\mu m}$. The authors propose to conduct such an experiment.

5 Conclusions and Recommendations

This study has examined the dependence of the threshold for laser-induced retinal injury in the monkey eye on the diameter of the irradiated area on the retina. For retinal irradiance diameter $D > 100 \text{ \mu m}$, $\text{ED}_{50}$ is proportional to $D$ for 0.1-s exposures, whereas $\text{ED}_{50}$ has been shown to be proportional to $D^2$ for nanosecond-duration exposures. This is in agreement with the existing laser bioeffects database and with models of thermal retinal injury. For $D < 100 \text{ \mu m}$, the $\text{ED}_{50}$ remains essentially constant, independent of $D$. This finding is contradicted by injury threshold studies in retinal explants. There is more than one possible explanation for the diameter dependence for $D < 100 \text{ \mu m}$, and more data are required to elaborate on this issue. The incorporation of wavefront correction into future experimental designs will provide essential data. These data point to a need for adjustment of the current guidelines for the safe use of lasers for pulses in the nanosecond-pulse-duration regime.

The developers and users of ophthalmic devices incorporating adaptive optics should view the findings of this paper as a caution. Perhaps the current guidelines do not provide full
subjects did not experience pain or distress.

Note that all animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and the ARVO Resolution on the Use of Animals in Research. All experiments involving animals used appropriate levels of anesthesia so the subjects did not experience pain or distress.

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