Interferometric Technique For Investigation of Laser Thermal Retinal Damage

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We describe a new technique for investigating laser-tissue interactions based on the use of an interferometric laser exposure pattern. A Michelson interferometer is used to generate a sinusoidal fringe exposure pattern. The periodicity of the fringe pattern may be adjusted from macroscopic dimensions to a scale of microns without the need for an imaging plane. Since fringe pattern periodicity is more adjustable and directly measurable than laser spot size, this technique offers significant advantages for studying the effects of thermal damage and diffusion in the irradiated tissue. In addition, the comparison of tissue response with theoretical models is simplified since the sinusoidal fringe pattern is itself an eigenfunction of the thermal diffusion equation. This technique is demonstrated for argon laser photoacoagulation in the rabbit retina. Exposures at durations comparable to the thermal relaxation time produced spatially confined lesions, while those at much longer durations resulted in significant diffusion of the thermal damage beyond the primary targeted regions. The role of thermal diffusion can thus be assessed directly from the opthalmoscopic and histologic appearances of the lesions. This technique can be employed to study thermal diffusion and other transport phenomena occurring in laser-tissue interactions for a variety of laser sources and tissue targets. Invest Ophthalmol Vis Sci 28:1290-1297, 1987

Retinal photoacoagulation, the first established therapeutic laser procedure, is widely employed to treat such diseases as macular degeneration¹ and diabetic retinopathy.² The procedure has undergone intensive investigation with regard to mechanisms and extents of tissue damage. It is generally recognized that therapeutically desirable retinal lesions result from thermal effects in and around the irradiated regions.³ These effects include alteration of the genetic apparatus of cells, inactivation of enzymes, and denaturation of proteins and nucleic acids, which lead to necrosis, hemostasis and coagulation.⁴ A determination of the exposure conditions which produce highly localized rather than extended thermal damage is clinically relevant to the targeting of specific tissue sites. Sophisticated theoretical models have been developed to explain and predict laser-induced thermal damage in the retina,⁵-⁸ and these ultimately rely on laboratory measurements for verification or refutation. It is thus essential from both clinical and theoretical perspectives to have reliable experimental techniques with which to accurately assess thermal damage.

In addition to laser wavelength and exposure duration, spot size and beam profile have been identified as critical parameters affecting retinal tissue response.⁵,¹⁰ Until now, studies of laser damage and thermal diffusion in the retina have relied on the use of solid, circular spots to perform the exposures.¹¹-¹⁶ The correlation of tissue damage with exposure conditions is sometimes problematic, since these techniques rely on difficult and somewhat arbitrary measurements of laser spot size. In this paper we present a new interferometric exposure technique which allows highly precise determination and adjustability of exposure parameters.

Materials and Methods

Theory

The use of a sinusoidally varying interference pattern for laser exposure provides a convenient and powerful approach for the investigation of thermal diffusion in laser-tissue interactions. In contrast to standard exposure techniques, the periodicity of the interference fringe pattern provides an important exposure parameter which can in general be more easily...
and accurately measured than laser spot size and beam profile. In addition, the periodicity of the exposure pattern can be continuously varied from macroscopic dimensions down to a scale comparable to the wavelength of the incident light. Finally, the use of a sinusoidally varying exposure simplifies the theoretical modeling of thermal diffusion since the sinusoid is an eigenfunction of the thermal diffusion equation.

When two mutually coherent laser beams of intensity \( I_0 \) are directed onto a target, they will interfere to produce a sinusoidally varying interference fringe pattern of the form:

\[
I(x) = I_0(1 + \sin kx) \tag{1}
\]

where \( k = 2\pi/\Lambda \) (\( \Lambda = \) the periodicity of the fringe pattern). The periodicity of the interference pattern is determined by the optical path difference between the phase fronts of the two laser beams, and can be varied from macroscopic dimensions to those comparable to the laser wavelength by adjusting the two incident laser beams.

It should be stressed that the use of two coherently interfering beams to produce a sinusoidally varying exposure pattern differs appreciably from other exposure techniques such as projecting an image or producing a Moire pattern. These techniques construct the desired exposure at a specific image plane and thus require careful focusing onto the retina or the exposure pattern will be degraded. In contrast, the interference exposure pattern generates a sinusoidally varying exposure at all planes where the beams overlap without the need for an image plane. Thus, assuming that scattering is small, variations in focusing on the retina will produce only a scale change in the exposure without defocusing the fringes.

In order to simulate the conditions in the retina as accurately as possible, models of thermal diffusion in the retina have taken into account several factors. These include considerations of the exact locus of the optical absorption and thermal deposition during the exposure, as well as the role of thermal diffusion between retinal layers and in the transverse direction. In addition, the damage characteristic of the retina is itself nonlinear. While these theories can be used to model a wide variety of exposure conditions, the solutions to the associated differential equations are generally quite complex. The use of interference exposure patterns can provide a significant simplification of exposure conditions which facilitates the analysis and the correlation between those conditions and the resulting tissue response.

In order to illustrate these points, we consider a simple model for thermal diffusion. While this approach does not include many of the processes which occur in retinal photocoagulation, it nevertheless provides qualitatively correct predictions and demonstrates the potential applicability of the interference exposure technique to a wide variety of studies.

The transport of heat is governed by the thermal diffusion equation:

\[
C \frac{\partial T}{\partial t} = K \nabla^2 T + J(r, t) \tag{2}
\]

where \( C \) is the heat capacity, \( K \) is the thermal conductivity, and \( J \) is the source term representing the deposition of the heat via laser absorption. We begin by considering the diffusion associated with a temperature change produced by an exposure which is sinusoidally varying as in equation (1). For simplicity thermal diffusion between the retinal layers is neglected, and only the thermal transport transverse to the fringes is considered. The diffusion then reduces to a single spatial dimension. If it is assumed that the tissue is initially heated in a sinusoidally varying temperature distribution, it is possible to examine the effect of diffusion as a function of time. The solution of the thermal diffusion equation for a sinusoidally varying initial condition is:

\[
\delta T(x, t) \sim \delta T(1 + e^{-\kappa^2 t} \sin kx) \tag{3}
\]

where \( \kappa = K/C \) is the thermal diffusivity. As shown, the effect of thermal diffusion is to produce a homogenization of the sinusoidally varying temperature change. That change relaxes exponentially with a time constant:

\[
\tau = \frac{1}{k\kappa^2} = \frac{\Lambda^2}{(2\pi)^2} \tag{4}
\]

where \( \tau \) is termed the thermal relaxation time, and is a measure of the time required for thermal diffusion to produce a smearing out of the initial sinusoidal temperature distribution. It should be noted that \( \tau \) depends on the periodicity of the exposure pattern and increases as the square of \( \Lambda \).

The role of thermal diffusion in retinal damage is shown schematically in Figure 1. For exposure times which are short compared to the thermal relaxation time, the thermal diffusion away from the initially targeted areas will be minimal and the lesion will resemble the exposure pattern (in this case, the vertical lines of the fringe pattern). In contrast, as the exposure time is increased above \( \tau \), thermal diffusion can be seen directly in the structure of the lesion and is manifest as a fusing together of the fringe lines. Laser intensity, exposure duration, and fringe periodicity may be systematically varied to study thermal diffusion and measure the thermal relaxation times. The role of thermal diffusion for given exposure parameters may thus be assessed directly from the appearance of the lesion.
Lesion with negligible transverse diffusion  
Damage  
No Damage  
Dominant diffusion  

Fig. 1. Using a simplified model, the threshold for retinal damage is assumed to occur at a given radiant exposure. This is shown schematically as an inverse linear contour on a double logarithmic plot of laser intensity versus exposure duration. At the left of the graph, at times comparable to or less than the thermal diffusion constant, \( \tau \), there is negligible transverse diffusion, resulting in a lesion with a profile similar to that of the irradiating beam. Conversely, at the right of the graph, at much longer exposure times, there is significant lateral diffusion, resulting in tissue damage extending well beyond the sites of actual laser irradiation.

**Experimental Procedure**

Figure 2 shows a schematic diagram of the experimental apparatus. Continuous wave argon (Spectra Physics, model 171, Mountainview, CA) and helium-neon lasers were positioned so that their beams were collinear. An electronically controlled mechanical shutter was placed in the beam path to allow laser pulses of variable duration to be transmitted to the target. Two lenses and an aperture were used to enlarge and restrict the laser beams, which then passed into a Michelson interferometer. By appropriately adjusting the two mirrors of the interferometer, fringe patterns of various orientations and periodicities could be generated from the ordinarily Gaussian beam profiles. The resulting pattern was then directed onto a mirror attached to a slit lamp biomicroscope and focused into the target eye. Overall optical efficiency of the configuration was 25 percent.

A 3 kg chinchilla rabbit was used for the laser exposures. The rabbit was anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine (4 mg/kg) and the pupil of the eye to be treated was maximally dilated with 0.8% tropicamide and 5.0% phenylephrine hydrochloride. The rabbit was then placed in a specially designed holder and the cornea of the experimental eye was covered with a contact lens to permit focusing on the fundus. Illumination was provided by the slit lamp, and the low intensity helium-neon laser beam was employed for aiming purposes, while the path of the argon laser beam was temporarily blocked.

For this procedure, the lenses were adjusted to form a laser spot of approximately 1,200 \( \mu \)m diameter in the slit lamp's focal plane. With the 0.66 magnification factor of the contact lens, this corresponded to a diameter on the retina of \( \sim 800 \mu \)m. The interferometer's mirrors were set so that the laser pattern consisted of six vertical fringes (increasing in length from the periphery to the center of the spot) with a periodicity of \( \sim 160 \mu \)m. Laser intensity was not uniform over the entire beam area, but the various optical components were adjusted so that (with the exception of the spaces between the fringes) it approximated the original Gaussian profile of the argon laser emission.

Two rows of four exposures each were made with the argon laser, at 514.5 nm, directly below the medullary ray. Table 1 lists the parameters of the exposures, which consisted of four at a duration of 10 msec and four at a duration of 100 msec. Argon laser intensities, measured with a power meter (Coherent, model 210, Palo Alto, CA) immediately in front of the contact lens, were 2.0, 1.5, 1.25, and 1.0 W, and 0.5, 0.375, 0.3125, and 0.25 W, respectively. The minimum exposure duration necessary to produce threshold-level lesions was limited by the argon laser's maximum output of 8 W.

Fundus photographs were taken 1 hr following placement of the lesions. The eyes were then enucleated and the rabbit was sacrificed by intravenous...
injection of pentobarbitol. The treated eye was immediately fixed in a 2% glutaraldehyde, 4% paraformaldehyde, 0.1 M sodium cacodylate buffer solution. In preparation for light microscopy, the retina was embedded in JB-4 glycol methacrylate, cut at 2 to 3 μm, and stained with hematoxylin and eosin. All animal procedures were done in accordance with the ARVO Resolution on the Use of Animals in Research.

Results

A fundus photograph of the lesions is presented in Figure 3. With the exception of no. 4, all of the lesions are clearly visible ophthalmoscopically. The first three exposures, performed at 10 msec, resulted in lesions with distinct vertical segments corresponding to the fringe pattern produced by the interferometer. Some thermal diffusion occurred laterally, so that the clarity of the fringe pattern in the lesions is not as great as that in the irradiating argon laser beam, especially at the higher laser intensities. Lesion no. 3 consists of visible damage only in portions of the irradiated area, while no. 4 is below the ophthalmoscopically detectable threshold.

The bottom lesions, produced from exposures at 100 msec, are notable for the absence of easily discernible vertical segments. Although the irradiating beam consisted of discrete vertical fringes, this was obscured by the length of the exposure period, which was sufficient to allow significant lateral thermal diffusion. Lesion no. 5, produced at 0.5 W, is indistinguishable from an intense lesion produced by an ordinary beam of an ophthalmic argon laser. An inner white region is surrounded by two bands of different shades of grayish color. The central white portion of lesion no. 6 is not as circular as that in the previous lesion, and the overall lesion is smaller, due to the lower peak temperatures associated with irradiation at lower laser intensities. This trend is seen to continue in lesion no. 7, which, although only slightly above the ophthalmoscopically visible threshold, appears to have been produced by an essentially uniform beam. In contrast, lesion no. 3, the last supra-threshold lesion produced at 10 msec, has readily distinguishable segments corresponding to the separate fringes of the beam.

Histologic sections of lesions nos. 2 and 6 are shown in Figure 4A and B. The histologically observed damage correlates closely with the ophthalmoscopic appearance of the lesions. Lesion no. 2 (Fig. 4A) exhibits a distinct periodicity in retinal damage which corresponds closely to the ~160 μm periodicity of the exposure pattern. The observed morphology confirms the periodicity of the retinal damage observed ophthalmoscopically. The photoreceptors in the exposed areas are hyperstained, indicating a thermal necrosis from the spatially varying laser exposure. There are regions of compression within the photoreceptor layer. Multiple humps or ridges produced by mild edema are observed in the inner retinal layers. The lesions are near threshold, thus both the anterior limiting and Bruch's membrane are intact and there is only mild nuclear pyknosis.

In contrast, the histologic section of lesion no. 6 (Fig. 4B) gives no indication that the irradiating beam consisted of distinct fringes. This confirms the ophthalmoscopic observation which shows a large lesion without the presence of periodic structure. The histology indicates that the central white region of the le-

Table 1. Exposure parameters

<table>
<thead>
<tr>
<th>Lesion no.</th>
<th>Laser intensity* (W)</th>
<th>Pulse duration (ms)</th>
<th>Fluence (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>10</td>
<td>1.02</td>
</tr>
<tr>
<td>2</td>
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<td>4</td>
<td>1.0</td>
<td>10</td>
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</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>100</td>
<td>2.54</td>
</tr>
<tr>
<td>6</td>
<td>0.375</td>
<td>100</td>
<td>1.90</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>100</td>
<td>1.27</td>
</tr>
<tr>
<td>8</td>
<td>0.3125</td>
<td>100</td>
<td>1.59</td>
</tr>
</tbody>
</table>

* Measured in front of contact lens; values listed are averages—peak intensities are twice as great.

Fig. 3. Fundus photograph of the rabbit eye, taken 1 hr after eight exposures were made with the argon laser at 514.5 nm. Lesions nos. 1–4 (top row; no. 4 is below the ophthalmoscopically visible threshold) and 5–8 (bottom row) were made at 10 and 100 msec, respectively, at laser intensities given in Table 1. Note the distinct vertical segments visible in the top row, which correspond to the lines of the fringe pattern.
Fig. 4. Histologic sections of lesions 2 (A) and 6 (B). For a 10 msec, near-threshold laser exposure, retinal damage is highly localized on the sites of the irradiation (arrows in A). Conversely, for a 100 msec, near-threshold exposure, thermal diffusion causes delocalized retinal damage (B). There is a clear correlation between the extent and type of histologic damage and the ophthalmoscopic appearance of the respective lesions. Sections were stained with hematoxylin and eosin (original magnification ×172).

Discussion

Fringe patterns from low intensity helium-neon lasers have been used with some success to predict potential visual acuity in patients undergoing treatment for cataracts,18 amblyopia,19 corneal opacification20 and macular degeneration.21 This is done by projecting the fringe pattern onto the retina and determining the smallest fringe periodicity which the individual is able to resolve. Helium-neon laser inter-
ferometers for these and other applications are available commercially. To our knowledge, however, our technique represents the first reported use of interferometry for examining direct laser effects on the retina.

Retinal heating results from absorption of incident radiation by one or more of its three principal chromophores. These include melanin (located in the retinal pigment epithelium, with little variation in absorption across the visible portion of the spectrum), hemoglobin (in blood vessels, with highest absorption for yellow light), and xanthophyll (in the inner and outer plexiform layers of the macula, with highest absorption for blue light). Argon laser green (514.5 nm) radiation is absorbed primarily by randomly distributed melanin granules in the pigment epithelium, the monocellular layer which in the chinchilla rabbit is about 10 μm thick.\(^8\) Of argon laser intensity entering the cornea, only 18.5 percent is dissipated in the ocular media, while 51.5 percent is absorbed by the pigment epithelium.\(^22\) The physiological basis for retinal photocoagulation is the destruction of the photoreceptors by thermal action spreading from absorption (in the case of the argon laser) by the pigment epithelial melanin granules.\(^23\)

As laser treatments require increased precision, it is important to have a thorough understanding of the effects of various lasers on their intended targets. Of primary concern is the evaluation of current and potential clinical procedures and the testing of models for laser-tissue interactions. In addition, there is also a need to establish safety levels for inadvertent exposure to lasers in industry, the military and the laboratory.

The use of the interferometric fringe exposure provides a direct ophthalmoscopic and histologic indicator for the role of thermal diffusion in the localization of lesions. Experimental results are in qualitative agreement with our simplified model. According to equation (4), using a fringe pattern periodicity of 160 μm and a \(\kappa\) of \(1.3 \times 10^{-3} \text{ cm}^2/\text{s}\) (the value for liquid water), \(\tau\) for the retina is approximately 5 msec. The relatively large spot sizes used here made the results more readily discernible, but prevented us from achieving sufficiently high fluences at 5 msec. Even at 10 msec, however, it was apparent both ophthalmoscopically and histologically that the tissue had been irradiated by a beam with discrete vertical segments.

Ophthalmoscopic observations indicate a definite periodicity in the top row of lesions (Fig. 3), demonstrating that only minimal thermal diffusion occurred for the 10 msec exposures. Because of the threshold nature of retinal damage, the histologically observed retinal morphology does not exactly reflect gradual variations in damage corresponding to the sinusoidal exposure pattern. Rather, the retinal damage is present in areas where the induced temperature change exceeds the damage threshold. It should be stressed, however, that the histologically observed damage exhibits a distinct \(\sim 160 \mu\text{m}\) periodicity that corresponds to the laser exposure and thus confirms the ophthalmoscopic observation of distinct vertical damage stripes. It is interesting to note that the periodicity of the damage is less than the thickness of the retina itself, indicating that retinal damage may be highly localized in the transverse direction by appropriate choice of exposure parameters.

As expected, results from irradiation at 100 msec belied the fringe pattern of the argon laser beam. Exposure at some 20 times the thermal diffusion constant allowed sufficient time for heat to spread between the fringe-like areas that were actually irradiated. As intensity was lowered, overall thermal damage was less severe and approached threshold levels, yet always with damage covering essentially the entire circular area. The critical determinant of thermal diffusion is exposure duration, not intensity. These are the results predicted in Figure 1. For both exposure times, tissue damage was qualitatively similar to that observed for more conventional exposure methods.\(^24,25\)

Although the argon laser\(^26\) is the source most often used for retinal photocoagulation, for some pathologies and under certain conditions within the eye it is desirable to use other lasers, sometimes to selectively target specific areas or layers of the retina. The krypton laser,\(^27\) with emission at 568 and 647 nm, is frequently used for photocoagulation, and as new sources, such as metal vapor and dye lasers, become available, they may be tested to determine if they provide a particular advantage for clinical use. Overall retinal absorption varies considerably for the visible and near infrared wavelengths,\(^28\) and there is strong interest in customizing photocoagulation to specific retinal conditions.\(^29,30\) The interferometric exposure technique should facilitate the evaluation of, and distinction among, the thermal effects of different laser sources. It should be noted, however, that photocoagulation with the fringe pattern itself would probably not offer any improvement over existing therapeutic techniques. Rather, it is an investigative and diagnostic technique with which to more precisely study fundamental laser interactions with the retina. Our results graphically demonstrate characteristics of thermal diffusion in the retina that may be helpful in further refining existing clinical methods.

Retinal photocoagulation is not without its risks. Threshold levels for a given amount of tissue damage decrease along with exposure duration, and it has been suggested\(^31\) that procedures requiring less watt-
age are at lower risk for complications. Clinical photocoagulation is typically performed at exposure durations of at least 100 msec.\textsuperscript{32} In view of our findings, it would appear that some procedures might be more effectively performed with shorter times. In particular, treatment in the macular region and near blood vessels might be more accurately performed at times on the order of 1–10 msec, since tissue damage would be limited to the immediately targeted regions. Exposures with too short times would be inadvisable, however, since they run the risk of causing acoustic shock wave damage and hemorrhage.

While we have demonstrated the interferometric technique in the context of retinal photocoagulation, the majority of other biomedical laser applications also rely on thermal effects.\textsuperscript{4} This approach could easily be adapted to other tissues. Anderson and Parrish\textsuperscript{33} have proposed an intriguing method of laser surgery, “selective photothermolysis,” in which selective damage is determined not by precise aiming of the laser beam but by the unique absorption properties of the intended target. If the target has an absorption coefficient at least twice as great as that of the surrounding tissue at a given wavelength, preferential absorption will result in localized thermal damage if irradiation is performed at a duration similar to or less than the thermal diffusion constant. Shortness of the exposures is determined largely by the size of the target. Anderson and Parrish used this method to selectively damage blood vessels (3 × 10\textsuperscript{-7} s, 577 nm) and melanocytes (2 × 10\textsuperscript{-8} s, 351 nm), although selective photothermolysis is, in principal, applicable even at the subcellular level. Selective photothermolysis depends on the selective absorption properties of specific target chromophores for suitably brief pulses, while the interferometric exposure technique directly demonstrates thermal diffusion by employing selective irradiation with the fringe pattern. By appropriately adjusting the fringe pattern periodicity, the interferometric technique could be used to determine thermal damage and absorption characteristics of a wide range of potential targets of biomedical interest.

In principle, the interferometric technique can also be used to study other processes in laser-tissue interactions. The only factor limiting its applicability is the necessity of maintaining the coherence of the laser beams in order for the fringe pattern to be properly constructed. Procedures that involve significant scattering in turbid media would thus be difficult to study in this manner. However, one process that should be suitable for this method is excimer laser tissue ablation. This phenomenon has been considered in the eye principally for corneal surgery.\textsuperscript{34} Relative contributions of thermal and photochemical mechanisms to the ablation process are currently being debated, and are of considerable relevance to the procedure’s application to any potential tissue target. Histologic examination of tissue sections following irradiation with appropriately adjusted interferometric fringe patterns could be instrumental in elucidating the fundamental nature of this procedure.

**Key words:** fringe pattern, interferometer, laser, photocoagulation, retina, thermal diffusion

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## References


