Investigative Ophthalmology & Visual Science, Vol. 33, No. 2, February 1992

In Situ Measurements of Lens Fluorescence and Its Interference With Visual Function

Joseph A. Zuclich,* Randolph D. Glickman,*† and Arthur R. Menendez‡

Irradiation of the primate lens by near-ultraviolet wavelengths results in a blue fluorescence, which can be an intraocular source of veiling glare. This study quantitated the fluorescence intensity as a function of exciting intensity and wavelength. As the exciting wavelength was increased from 360 to 430 nm, the decreasing fluorescence intensity (for equal radiant exposures) was partially offset by a shift in the fluorescence spectrum to wavelengths of greater luminous efficiency so the luminance of the lens fluorescence remained approximately constant. The measured luminance of the lens fluorescence was high enough to imply degradation of visual function as a result of reduced contrast of the retinal image. To obtain an objective measure of visual deficit associated with the fluorescent glare, the visual evoked potential (VEP) elicited by counterphased sine-wave gratings was recorded while the subject eye was continuously exposed to the 413 nm emission from a krypton laser. The VEP amplitude was reduced in the presence of the exciting laser even at levels defined as "safe" (ie, where exposure levels are insufficient to induce an acute ocular lesion). Because the direct glare effect of the exciting radiation was negligible in this experiment, the VEP response loss is attributed to the effect of the lens fluorescent glare. Invest Ophthalmol Vis Sci 33:410–415, 1992

The ocular lens of the primate fluoresces in the blue when irradiated by near-ultraviolet (UV) wavelengths. This phenomenon, although usually unnoticed, is nevertheless present with exposure to the ambient solar environment. In the aging lens, the near-UV absorption band broadens and encroaches into the blue, while the fluorescence intensifies and migrates toward longer wavelengths.[1,2] Weale has estimated[3] that the reciprocal ratio between the luminance of a patch of sky and that of the fluorescence it induces is ~0.002 for the normal lens of a 30-year-old human (generally unnoticeable) but increases to 0.017 for a 60 year old (generally noticeable) and to 0.121 for an 80 year old. In the latter case, the fluorescence can be an intraocular source of "veiling glare," covering the entire field of view and intensity enough to impair visual function. With certain disease processes, including diabetes, lens optical aging may be accelerated.[4]

Our concern is with the fluorescence-associated glare induced by UV- and blue-wavelength lasers and conditions where otherwise "safe" laser exposures (insofar as inducing acute ocular tissue damage) might still result in a debilitating veiling glare. In this regard, we have conducted experiments to quantitate the in situ lens fluorescence as a function of exciting intensity and to get an objective measure of visual deficit associated with the fluorescence glare.

The expected wavelength dependence is summarized by the data in Table 1. When exciting near the lens absorption maximum at 360 nm,[5] the broad fluorescence is peaked at 440 nm but ranges from ~380 to beyond 500 nm.[6] With an excitation wavelength of 406.7 nm (krypton laser), a fluorescence peak of ~480 nm has been reported, while argon laser excitation at 457.9 nm has yielded a fluorescence peaked at ~520 nm.[7] Because the fluorescence spectrum shifts toward wavelengths of higher luminous efficiency as the exciting wavelength increases from 360 nm to blue wavelengths (Table 1), the apparent brightness (luminance) of the fluorescing lens would increase significantly if the same number of photons were emitted. In fact, the fluorescence yield decreases as the excitation wavelength is increased above 360 nm. Therefore, it was necessary to quantitate this fall-off in fluorescence yield vs the enhancement in luminous efficiency to identify the wavelength or wavelengths capable of inducing the most prominent veiling glare.

From *KRUG Life Sciences, Inc., San Antonio Division, San Antonio, Texas; the †Department of Ophthalmology, University of Texas Health Science Center at San Antonio, San Antonio; and the ‡Laser Branch, Directed Energy Division, Occupational and Environmental Health Directorate, U.S. Air Force Armstrong Laboratory, Brooks AFB, Texas.

Supported in part by Contract F33615-88-C-0631 (JAZ and RDG), let by the Laser Branch, Directed Energy Division, Directorate of Occupational and Environmental Health, United States Air Force Armstrong Laboratory, Brooks Air Force Base, Texas.

Submitted for publication: June 19, 1991; accepted September 26, 1991.

Reprint requests: Dr. Joseph A. Zuclich, KRUG Life Sciences Inc., San Antonio Division, P.O. Box 790644, San Antonio, TX 78279-0644.

Table 1. Lens fluorescence as a function of excitation wavelength

Exciting wavelength, λ_1 (nm)	*Fluorescence peak, λ_2 (nm)*	*Luminous efficiency at λ_2 (nm)*
360	440	0.023
406.7	489	0.208
457.9	528	0.83
488.0	538	0.93

Materials and Methods

Fluorescence spectra were recorded from isolated lenses suspended in saline in a cuvette and in situ from the lenses of anesthetized rhesus monkeys (*Macaca mulatta*). All animals used in this study were treated in accordance with the Animal Welfare Act; 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 unusual pain or distress.

The excitation source for wavelength-dependent studies was an Oriel (Stratford, CT) 1000-W xenon arc lamp directed through a grating monochromator to yield ~10 nm bandwidths. Additional studies were performed using the 413 nm output of a Coherent (Palo Alto, CA) krypton-ion laser (the laser optics actually yielded a triplet emission at 406.7, 413.1, and 415.4 nm with the 413.1 nm emission being the strongest). The lens fluorescence was recorded using a Photo Research (Burbank, CA) Pritchard spectroradiometer/photometer, Model 1980B, which yielded printouts of the integrated radiance (W/Ster · m^2) and luminance (photopic cd/m^2) of the fluorescence, as well as hard copies of the fluorescence spectra.

Because the Pritchard 1980B is a telephotometer, it allowed detection from selected spots on the irradiated lens. In general, the telephotometer (for in vitro or in vivo measurements) was targeted just behind the irradiated spot on the anterior lens surface and along an axis, making an angle of ~30° with the exciting light (which was directed along the visual axis). The telephotometer detector head typically was placed ~18" from the fluorescing lens with an aperture chosen to yield a 20′ arc field of view. This allowed the targeted area for the in vivo measurements to be fully within a portion of the lens seen through the dilated pupil while avoiding overlap with the sections of cornea and lens directly irradiated by the exciting light. The bandwidth of the monochromator in the telephotometer head was set at a nominal value of 10 nm.

Visual evoked potentials (VEPs) were recorded from anesthetized rhesus monkeys with scalp electrodes placed approximately at T_5 and T_6, which, in the rhesus monkey, lie over the foveal projection area of the visual cortex. Details of the instrumentation for data collection and data analysis techniques are published elsewhere.[8] A schematic diagram for the experiments reported here is shown in Figure 1. The anesthetized subject, with a dilated pupil, viewed the stimulus monitor as projected to the eye from a pellicle beamsplitter set in front of a fundus camera. The center of the projected stimulus field was prealigned along the optic axis of the fundus camera. The subject's fundus was viewed on a TV monitor using only the infrared component of the fundus camera viewing light (so the dark-adaptation state would not be disturbed). The multi-dimensional, translational-rotational stage on which the subject was mounted was adjusted as necessary to keep the fovea positioned on the optic axis of the fundus camera and, therefore, centered on the stimulus field.

During the VEP recording sessions, the 413 nm krypton laser radiation was introduced by directing the collimated beam through the pupil at an angle of 45° to the optic axis of the fundus camera (and the subject's visual axis). The small percentage of laser radiation transmitted through the ocular medium[9] was incident on the peripheral retina at a spot well off the field of view seen through the fundus video monitor. Laser power incident at the cornea varied from ~0.5 mW to 1.5 mW in a 5 mm diameter beam.

The visual stimuli were monochromatic sine-wave gratings presented over an 8° visual field. The sine-

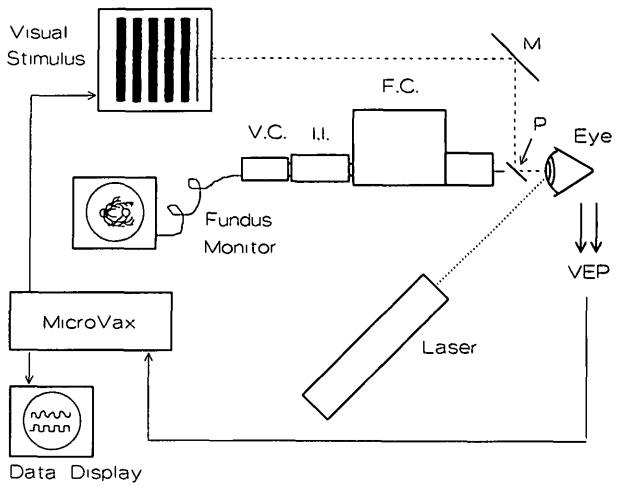

Fig. 1. Schematic diagram of lens fluorescence-VEP experiment. Abbreviations: F.C.: fundus camera; I.I.: Xybion IRO-01 image intensifier; Laser: Krypton-ion cw laser emitting at 413.1 nm, 5-mm beam diameter at eye; M: first surface mirror; P: pellicle beamsplitter; V.C.: Cohu 4815 video camera; VEP: visual evoked potential recorded from scalp of rhesus monkey.

wave gratings were presented at a spatial frequency of 4 cycles per degree, a space-averaged luminance of 60 cd/m^2, and counterphased at 6 Hz (12 reversals/s). VEP recordings were made for 60 s and averaged into a 1 s window. The amplitude of the VEP response was determined from the averaged records by Fourier analysis and was taken as the sum of the components occurring at the reversal frequency and the next two higher even harmonics. The grating contrast was varied from 20% to 100%, and the VEP amplitude was recorded at each of several contrast levels with and without the continuous-wave (cw) laser radiation.

Results

The fluorescence spectrum recorded from an excised rhesus monkey lens excited with arc-lamp radiation centered at 360 nm is shown in Figure 2. The trailing edge of the excitation envelope is seen at the left edge of the figure. Figure 3 illustrates the fluorescence from the in situ rhesus monkey lens when excited with the 413 nm radiation from a krypton-ion laser. Note that the fluorescence peak is shifted from ~440 nm when exciting at 360 nm to ~480 nm with 413 nm excitation.

Table 2 lists the fluorescence intensities (in terms of integrated radiance and luminance) recorded from the excised rhesus lens when exciting with arc-lamp radiation at several wavelengths. After equating for equal radiant exposures, little variation exists in either the lens radiance or luminance as the exciting wavelength is varied from 360 to 430 nm. As the exciting wavelength is increased above 430 nm, the fluorescence intensity drops precipitously, and the greater overlap between the excitation and emission bands (the exciting intensity now being relatively much

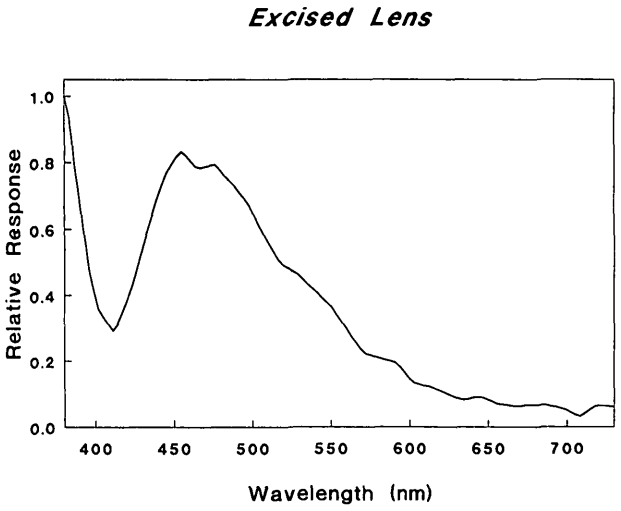

Fig. 2. Fluorescence of excised rhesus lens excited by 360-nm radiation from arc-lamp source.

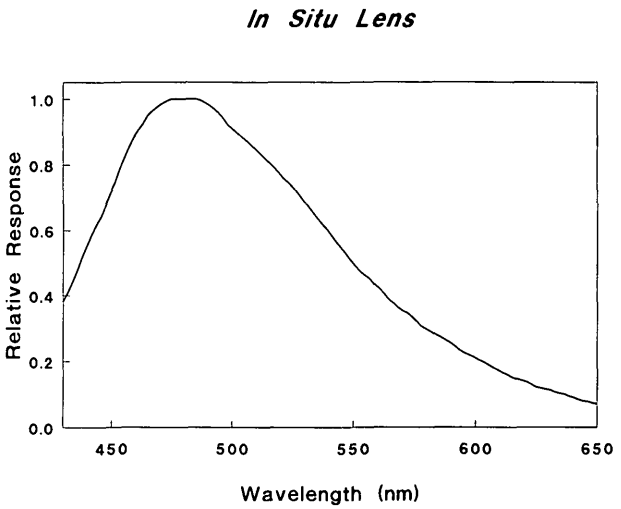

Fig. 3. In situ fluorescence of rhesus lens excited by 413-nm laser radiation.

greater) makes quantitative assessment of the fluorescence intensity problematic. Likewise, measurements of the in situ lens fluorescence indicate little variation in fluorescence luminance with exciting wavelengths between 360 and 430 nm but a rapid drop-off in luminance with longer exciting wavelengths.

For the in situ case with 413 nm laser excitation, we examined the scaling of fluorescence intensity with excitation intensity. Figure 4 shows, plotted as a function of cw-laser power, the peak spectral radiance (radiometric measure) and the luminance (photometric measure) of the fluorescence. Both curves exhibit a slope of 1.0 (dashed lines) with no sign of saturation of the fluorescence emitted. Higher exciting intensities were not used for the in situ case because of the potential for inducing thermal damage to the retina. However, with the excised lens, the cw-laser intensity was taken as high as 100 mW, which yielded a lens luminance of ~2000 cd/m^2, without sign of the fluorescent chromophore being saturated. At still higher cw-laser powers, we were unable to complete the fluorescence measurements because of thermal damage to the excised lens. The threshold observed for a thermally induced opacity in the lens is consistent with previously reported thermal damage thresholds.[10,11]

Table 2. Fluorescence intensities measured from excised lens

Excitation parameters		*Emission*	
Wavelength (nm)	*Radiant exposure (mW/cm^2)*	*Radiance (W/Sr/m^2)*	*Luminance (cd/m^2)*
350	0.019	4.5×10^{-4}	0.034
360	0.092	9.6×10^{-4}	0.16
400	0.054	5.3×10^{-4}	0.13
430	0.064	6.2×10^{-4}	0.18

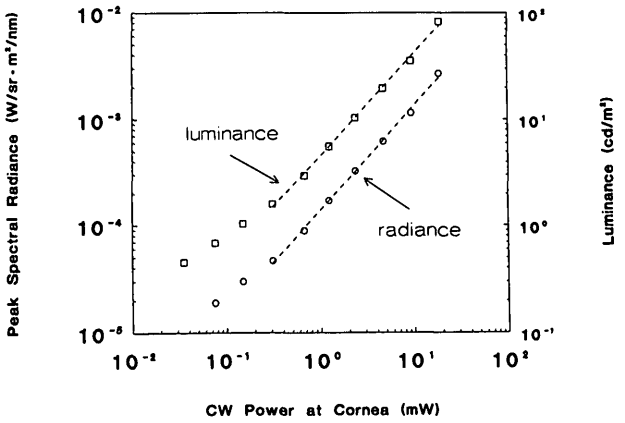

Fig. 4. Peak spectral radiance (circles) and luminance (squares) of lens fluorescence as a function of cw laser power. The slopes of both regression lines fit to the radiance and luminance data approximate 1 (actual: radiance = 0.984, r = 0.999; luminance = 0.956, r = 0.999).

Figure 5 depicts the VEP amplitude plotted as a function of stimulus contrast with and without cw-laser radiation (1.5 mW), as described in Materials and Methods. In general, the presence of the laser radiation resulted in a decreased VEP amplitude for all values of grating contrast. Similar results were generated in each of three VEP recording sessions with two rhesus subjects. The inset in the upper part of Figure 5 shows the predicted luminance of the direct veiling glare induced by a laser source (413 nm) at various angular separations from the grating stimulus, calculated from the Vos glare function.[12] At a separation of 45°, indicated by "θ" (at arrow in the inset), the predicted luminance of the veiling glare produced by this wavelength and incident power is less than 0.5 cd/m^2. The VEP responses, indicated by the label "+laser" in the lower two figures, have been plotted against contrast values adjusted for the effect of direct veiling glare. In the absence of any additional effect of lens fluorescence, the "normal" and "+laser" response curves should coincide. That they do not overlap is, therefore, attributed to the fluorescence emission.

Discussion

The fluorescence spectra from the excised lens (Fig. 2) and the in situ lens (Fig. 3) exhibit one broad, basi-

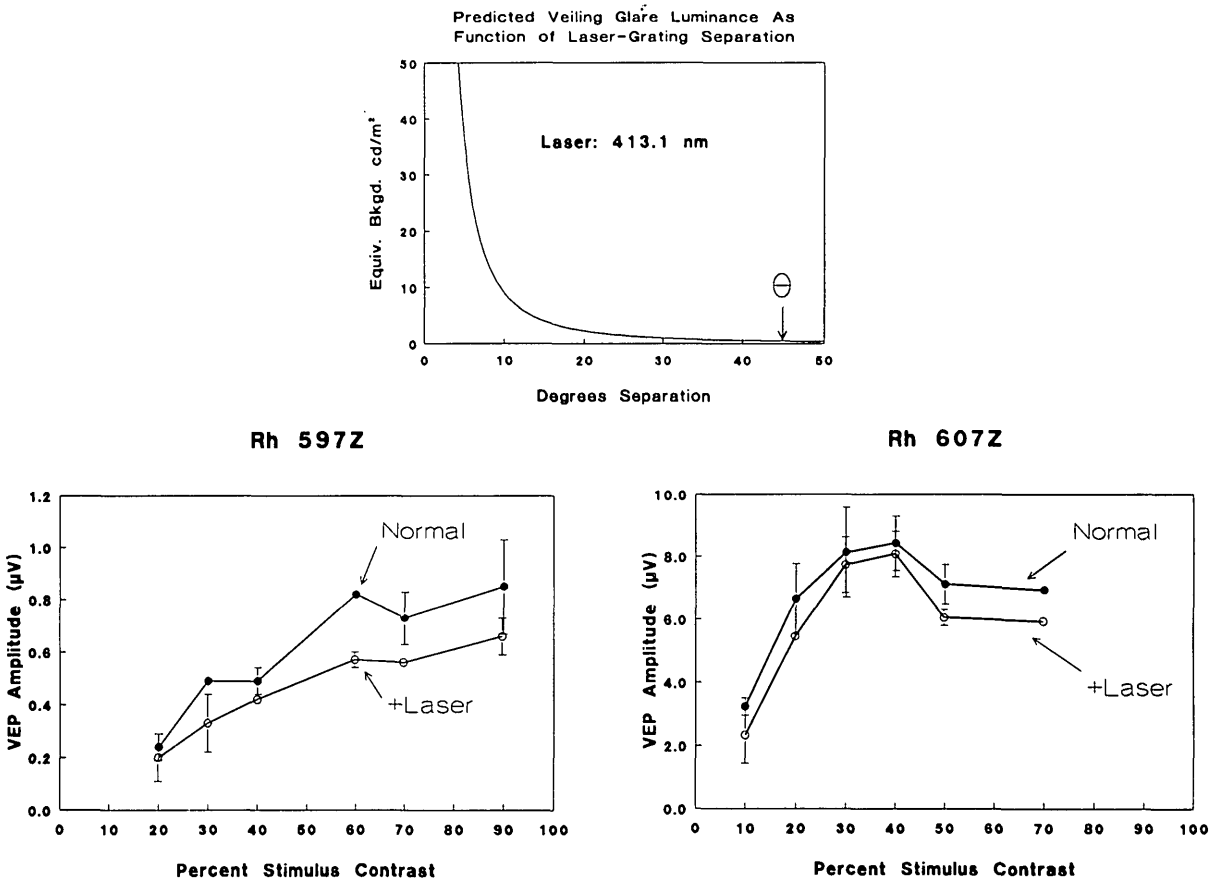

Fig. 5. Effect of laser-induced lens fluorescence on VEP. Top inset: predicted equivalent background luminance of a 413-nm, 1.5-mW incident power at cornea, laser source at various angular separations from a grating stimulus. Theta (at arrow) indicates the separation of 45° used in these experiments. Lower figures: VEP amplitudes with (+laser) and without (normal) presence of 413-nm laser. Results of recording sessions from two animals. Points indicate mean of two measurements; error bars indicate high and low values.

cally featureless peak. The in situ spectrum is somewhat broader even though it is excited with a laser source. This may reflect that it is recorded in an "optically noisy" environment—ie, in the presence of scattering of the exciting and the emitted radiation while passing through several media (tear layer, cornea, aqueous, and part of the lens) and reflections at the media interfaces. Also, a visible fluorescence (relatively weak) is noted at the corneal surface. All of these features may add to the veiling glare effect for the in situ case.

The data of Table 2 indicate the luminance of the fluorescing lens shows little change as the exciting wavelength is varied from 350 to 430 nm (for equal radiant exposures). In fact, the absorption of the primate lens, while strong, is relatively slowly varying throughout the near-UV and into the short-blue wavelengths but falls off very rapidly with further increase in wavelength.[13] Because the fluorescence emission becomes increasingly red-shifted as the exciting wavelength is increased, the higher luminous efficiency (Table 1) acts to offset the decreasing lens absorption from ~360 nm to ~420 nm, meaning that the lens luminance is a slowly varying function within that wavelength range. However, for longer wavelengths, the rapidly decreasing lens absorbance dominates the equation. Our conclusion from these data is that the potential for a veiling glare problem associated with lens fluorescence would be just as great for exposures to short-blue wavelengths (up to ~430 nm) as for UV exposures near the lens absorption peak at ~360 nm. As the primate lens ages and the lens absorption shifts further into the blue,[14] the lens veiling glare could become a problem for exposures to even longer wavelengths than indicated in this report. Indeed, several recent reports have indicated that visual performance may be improved by filters that block short, visible wavelengths, thereby reducing intraocular light scatter and lens fluorescence.[15,16]

The data shown in Figure 4 imply that even at modest exposure levels (of the order of 1 mW), the measured luminance of the lens fluorescence is high enough to degrade visual function by decreasing the contrast of the retinal image. The VEP experiments were undertaken to obtain an objective measure of this visual effect.

The consistent decrease in VEP amplitude with the presence of the cw-laser radiation demonstrates that, for the conditions chosen, the laser beam passing through the pupil results in a measurable visual deficit. The laser exposure conditions were chosen to approximate the case of a laboratory alignment laser (~1 mW) striking the eye at close range. This is generally considered an eye-safe exposure because the laser intensity is insufficient to result in an acute retinal lesion.[17,18] There could be, however, a very significant direct glare effect (ie, glare associated with the incident or exciting wavelength as opposed to glare associated with the induced fluorescence) from exposure to a 1 mW visible wavelength laser. For the case studied here, the transmission of the 413 nm krypton radiation through the ocular medium is low,[9,13] and the luminous efficiency of the incident radiation is ~0.002 compared to, for example, 0.235 for a red (HeNe) alignment laser or nearly 0.6 for the 514.5 green argon laser line. Therefore, the direct glare effect would be attenuated by about two orders of magnitude relative to that for the most common visible wavelength lasers. Moreover, we directed the krypton laser beam 45° off of the visual axis to minimize further the direct glare effect. Using the Vos glare function,[12] the predicted direct glare effect in our case (1.5 mW, 413 nm collimated beam, 45° off the visual axis) would be negligible, as shown in the inset at the top of Figure 5. Therefore, we attribute the decrease in VEP amplitude to the veiling glare associated with the lens fluorescence.

As the cw-laser intensity is increased above ~1.5 mW or as the beam is directed closer to the visual axis, the increased fluorescence glare and the direct glare effect would act to deny useful vision to the lased subject for the duration of the exposure. We further speculate, based on the absence of saturation of fluorescence intensity as the exciting intensity is increased, that intense short-pulsewidth exposures to UV and blue laser sources may, in a low-light environment, cause significant flashblindness, even for cases where vision is not otherwise compromised by an acute lesion or the direct flashblindness effect of the incident laser beam. In this case, the lens fluorescence-induced flashblindness could cause a visual deficit that persists for some time (seconds or longer) beyond the actual exposure duration.[19,20] An additional speculation is that lens fluorescence, because of its emission peak in the blue-green portion of the visible spectrum, may present a greater flashblinding stimulus to the scotopic system than to the photopic system. It would be difficult, however, to demonstrate this effect directly with the VEP, which is essentially a photopic response.

Key words: fluorescence, glare, laser, lens, visual evoked potential (VEP)

Acknowledgments

The authors thank Mr. William R. Elliott and Mr. Douglas J. Coffey for their excellent technical assistance. Excised primate lenses were provided by the Comparative Pathology Branch, Veterinary Sciences Division, USAF Armstrong Laboratory, Brooks AFB, Texas.

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