

Threshold Determinations for Selective Retinal Pigment Epithelium Damage With Repetitive Pulsed Microsecond Laser Systems in Rabbits

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■ **BACKGROUND AND OBJECTIVE:** In both clinical and animal studies, it has been shown that repetitive short laser pulses can cause selective retinal pigment epithelium damage (RPE) with sparing of photoreceptors. Our purpose was to determine the ophthalmoscopic and angiographic damage thresholds as a function of pulse durations by using different pulsed laser systems to optimize treatment modalities.

■ **MATERIALS AND METHODS:** Chinchilla-breed rabbits were narcotized and placed in a special holding system. Laser lesions were applied using a commercial laser slit lamp, contact lens, and irradiation with a frequency-doubled Nd:YLF laser (wavelength: 527 nm; repetition rate: 500 Hz; number of pulses: 100; pulse duration: 5 μ s, 1.7 μ s, 200 ns) and an argon-ion laser (514 nm, 500 Hz, 100 pulses, 5 μ s and 200 ms). In all eyes, spots with different energies were placed into the regio macularis with a diameter of 102 μ m (tophat profile). After treatment, fundus photography and fluorescein angiography were performed and radiant exposure for ED₅₀ damage determined. Speckle measurements at the fiber tips were performed to determine intensity peaks in the beam profile.

■ **RESULTS:** Using the Nd:YLF laser system, the ophthalmoscopic ED₅₀ threshold energies were 25.4

μ J (5 μ s), 32 μ J (1.7 μ s), and 30 μ J (200 ns). The angiographic ED₅₀ thresholds were 13.4 μ J (5 μ s), 9.2 μ J (1.7 μ s), and 6.7 μ J (200 ns). With the argon laser, the angiographic threshold for 5 μ s pulses was 5.5 μ J. The ophthalmoscopic threshold could not be determined due to a lack of power; however, it was >12 μ J. For 200 ms, the ED50 radiant exposures were 20.4 mW ophthalmoscopically and 19.2 mW angiographically. Speckle factors were found to be 1.225 for the Nd:YLF and 3.180 for the argon laser. Thus, the maximal ED₅₀-threshold radiant exposures for the Nd:YLF were calculated to be 362 mJ/cm² (5 μ s), 478 mJ/cm² (1.7 μ s), and 438 mJ/cm² (200 ns) ophthalmoscopically. Angiographically, the thresholds were 189 mJ/cm² (5 μ s), 143 mJ/cm² (1.7 μ s), and 97 mJ/cm² (200 ns). For the argon laser, the maximal ED₅₀ radiant exposure threshold was 170 mJ/cm² angiographically.

■ **CONCLUSION:** The gap between the angiographic and the ophthalmoscopic thresholds for the 200 ns regime (4.5 times above angiographic ED₅₀) was wider than for the 1.7 μ s regime (3.3 times above the angiographic ED₅₀). This would suggest the appropriate treatment would be 200 ns pulses. However, histologies have yet to prove that nonvisible mechanical effects increase with shorter pulse durations and could reduce the "therapeutic window." When comparing the thresholds with 5 μ s pulses from the argon and Nd:YLF laser, it demonstrates that intensity modulations in the beam profile must be considered.

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INTRODUCTION

Retinal photocoagulation is considered one of the most important treatments in the field of ophthalmology. It was first investigated by Meyer-Schwickerath in 1949 using sunlight for irradiation of the retina¹ and further developed by Maiman in 1960, who presented the first Ruby-laser.² Today, conventional retinal laser treatment is performed using the argon laser (514 nm) with exposure times that are typically 100 to 200 ms. After applying laser energy to the retina, usually an ophthalmoscopically visible greyish-white lesion results. Following the thermal destruction of the retinal pigment epithelium (RPE) that is the primary absorption site, an irreversible coagulation of the outer and inner segments of the neuroretina caused by heat conduction will occur.³⁻⁶ In some diseases, such as proliferating diabetic retinopathy, this thermal denaturation is responsible for the therapeutical success.

It was also shown that a variety of macular diseases, including diabetic macular edema, diabetic retinopathy, age-related macular degeneration, or central serous retinopathy, are treated successfully with conventional laser irradiation. In spite of this, the benefit for the patient has to be considered carefully in macular irradiation because of the resulting scotomas that could lead to a severe loss of visual acuity.

Many macular diseases are thought to be associated only with a reduced function of the retinal pigment epithelial cells. Therefore, a method for the selective destruction of the RPE cells without causing adverse effects to the choroid and neuroretina, especially to the photoreceptors, seems to be an appropriate treatment.⁷ The selective effect on RPE cells, which can absorb about 50% of the incident light because of their high content of melanosomes,⁸ was first demonstrated by Roider on rabbits using 5 μ s Argon laser pulses at 514 nm with a repetition rate of 500 Hz.⁷ By irradiating the fundus with a train of μ s laser pulses, it is possible to achieve high peak temperatures around the melanosomes. This leads to a destroyed RPE, while only a low sublethal temperature increase in the adjacent tissue structures is obtained.⁹ These effects are ophthalmoscopically invisible and can only be visualized by angiography. The selective destruction of the RPE cells sparing the photoreceptors was proven by histologic examinations at different times post treatment. The selectivity of 1.7 μ s pulses could also be proven by micropertimetry in clinical trials.^{10,11}

The aim of this work was to investigate the thresh-

olds for ophthalmoscopic and angiographic visibility as a function of pulse duration in the μ s-time regime to optimize present treatment modalities. In the regularity and homogeneity of the irradiance, the radiant exposure across the laser spot was also measured. Using short-pulsed laser exposures, intensity modulations caused by interference might influence the thresholds. If a highly irregular speckle formation occurs at the fiber tip, the maximum radiant exposure is more significant for threshold determination than the mean radiant exposure usually used for ED₅₀-calculations.

MATERIALS AND METHODS

Lasers

Laser lesions were created with 1) a frequency doubled Nd:YLF laser, and 2) a continuous wave argon laser:

- 1) An arc-lamp excited, intracavity frequency doubled Nd:YLF laser (Quatronix Inc., model 527DP-H) was modified with an active feedback electro-optical Q-switched system to generate pulse durations up to several μ s at a wavelength of 527 nm.¹² For the experiments, pulse durations of 5 μ s, 1.7 μ s, and 200 ns (100 pulses, 500 Hz) were used. The energy was transmitted by a 105 μ m core diameter fiber (Ceram Optec GmbH, Optran UV-A 105/125/250, NA=0.1). The length of the fiber was 50 m to minimize spatial and temporal intensity modulation caused by speckle formation at the distal fiber tip. This fiber was directly coupled to the slit-lamp fiber (Zeiss, Ø158 μ m, NA 0.1).
- 2) A 20 W (multiline) cw argon laser (Spectra Physics, 2030-15s) was used at the wavelength of 514 nm. The argon beam was modulated externally with an acousto-optical modulator. It was possible to produce any pulse duration, from 50 nanoseconds up to continuous irradiation; however, the peak power is limited to 7 W because of the laser's limitations. Any repetition rate up to several kilohertz could be used and any number of pulses could be delivered. The modulated beam was focused into the slit-lamp fiber of 2 m length (Ø158 μ m, NA 0.1). For the argon laser experiments, irradiation was performed using a 5 μ s and 200 ms pulse duration. The 5 μ s pulse energy was variable up to 12 μ J.

Speckle formation was measured at the fiber tip

with both laser systems. It is known that the transmission of laser light through multimode fibers causes interference to produce an intensity modulation called "speckles." This effect is based on the optical path length difference of the various fiber modes. If the path-length difference is smaller than the coherence length of the laser, speckles are produced by interference. The speckles can be characterized by their contrast K and the speckle factor F :

$$(1) \quad K = \frac{H_{\max} - H_{\min}}{H_{\max} + H_{\min}} \quad K = \text{contrast at fiber tip}$$

$$(2) \quad F = \frac{H_{\max}}{H_{\text{mean}}} \quad F = \text{speckle factor}$$

$H_{\max}$ = maximal radiant exposure

$H_{\min}$ = minimal radiant exposure

H_{mean} = mean radiant exposure

To measure these modulations, the fiber tip was imaged onto a CCD camera and the profile was recorded and saved with a laser beam analyzer (Spiricon, Inc.). The maximal, minimal and mean radiant exposures were calculated with the Spiricon-LBA-300PC software (Spiricon, Inc.). From each laser setting used in this study, 25 speckle measurements were performed and their means were calculated using the following equation:

$$(3) \quad ED_{50} = F * \frac{\text{pulse - energy}}{\text{spotsize}}$$

Because of using a contact lens, the speckle formation should be ideally imaged one to one to the retina. Thus, measured variations in the speckle formation at the fiber end must also be effective at the retina.

Settings

The laser beam was delivered to a clinically used ophthalmic laser slit lamp (30 SL/L, Zeiss, Oberkochen, Germany). The laser beam (tophat profile) was focused onto the retinal surface of the macular area in the rabbit eye using a contact lens (Goldmann three mirror contact lens) leading to uniform irradiance. Calculations showed that the use of a

planoconcave contact lens in cycloplegic emmetropic rabbit eyes translates the laser spot at the fiber tip of 158 μm into a retinal spot size of 102 μm .¹³ A green helium-neon laser beam (543 nm) was used as the aiming laser.

Animals

Five Chinchilla-breed rabbits were used for the experiments. Rabbits were chosen because the density and location of light-absorbing pigments in the fundus are rather uniform and similar to that of the human eye.⁸ The animals were anesthetized with ketamine hydrochloride (35 mg/kg of body weight) and xylazine hydrochloride (5 mg/kg of body weight). The animals were placed into a special holder system that allowed movement in all directions. A planoconcave contact lens was placed onto the mydriatic eye using methylcellulose as a contact gel. The lens was locked at the animal holder to prevent unfavorable movements.

The treatment of experimental animals in this study was in compliance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Laser Treatment and Documentation

Visible suprathreshold marker lesions were used in all eyes to enable orientation to place the particulate nonvisible selective laser spots. Therefore, 6 to 8 lesions were placed into the macular area of about 3 x 3 mm. These lesions were graphically documented. The laser power of the marker lesions was chosen to achieve conventional visible lesions. The treatment lesions were placed between these marker lesions and again documented graphically and in a table. We placed about 30 to 70 lesions into 1 eye. The first spots with higher energy led to visible lesions; step-by-step decrease of the energy led to ophthalmoscopically invisible laser lesions. Two hours after application of the laser lesions, fundus photographs were taken with a fundus camera (Carl Zeiss, Oberkochen, Germany). Afterwards, standard fluorescein angiography with injection of 10% fluorescein sodium into the ear vein was performed. If the RPE is damaged, the tight junctions of the RPE barrier will break up and fluorescein from angiography can pool from the choriocapillaris into the subretinal space. Thus, fluorescein angiography was used to detect a break of the RPE barrier and also define an exact endpoint. Two endpoints for RPE-damage were chosen for evaluation: the fluorescein angiographic and ophthalmoscopic visibility of the

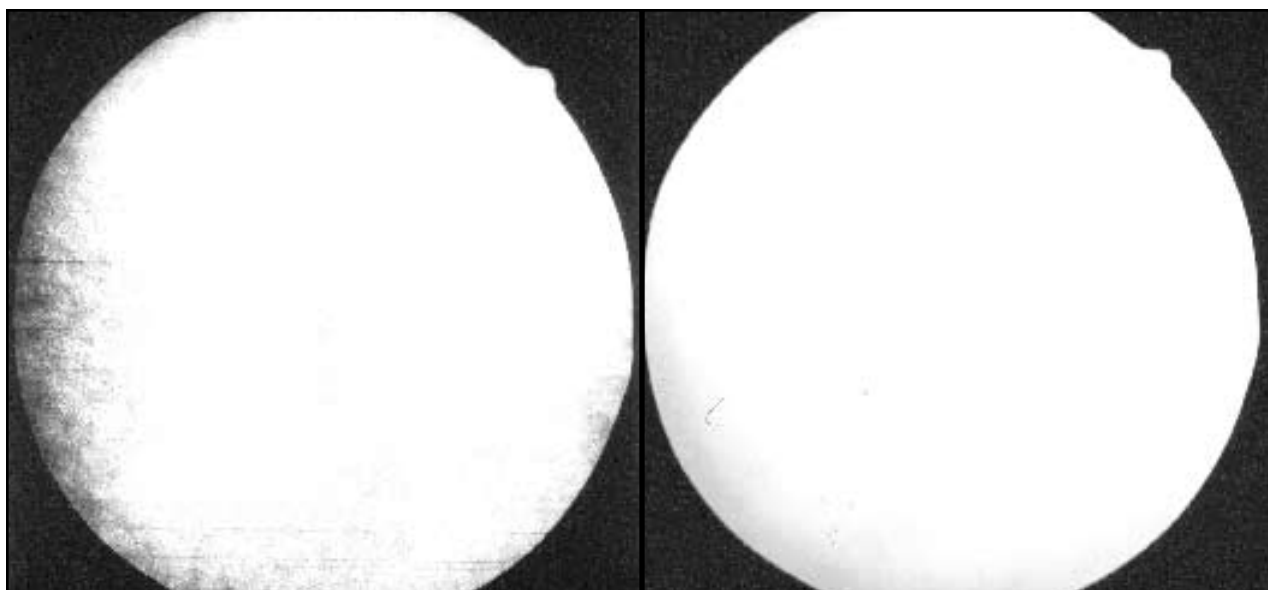


Figure 1. (Left) Angiogram of the fundus obtained 2 hours after exposure following irradiation with repetitive 1.7 μs and 200 ns laser pulses (100 pulses, 500 Hz) and decreasing pulse energy. For orientation, several large suprathreshold marker lesions were made. **(Right)** Corresponding fundus photograph obtained 2 hours after irradiation. Just suprathreshold pulsed lesions are visible ophthalmoscopically; none of the others were visible during and immediately after treatment. Two hours after treatment, the previously invisible laser spots became slightly visible because of retinal edema in the irradiated area.

lesion. For the ophthalmoscopic threshold, first, the visibility was noted, independent of white or greyish appearance. A typical irradiation pattern is shown in **Figure 1**.

For evaluation of the laser lesions, the threshold of fluorescein angiographic visibility and ophthalmic visibility was plotted versus the pulse energy for various numbers of pulses. From the plot, the mean threshold radiant exposures for the different pulse durations of the Nd:YLF and the argon laser system could be determined. The angiographic or the ophthalmoscopic threshold energy (ED_{50}) was defined as the necessary pulse energy to achieve fluorescein angiographically visible or ophthalmoscopically visible damage with a 50% probability. For calculations and presentations of the ED_{50} the y-axis was plotted as an inverse normal distribution function (probability plot). This leads to a logarithmically normal distribution resulting in a line $y = a \cdot x$.^{14,15} Thresholds were then calculated by linear fits (**Figures 2-6**). Calculations and graphic presentations were performed using the Origin 6.0 software (Microcal Software, Inc. Northhampton, MA 01060, USA).

Laser experiments were performed in 9 eyes from 5 animals. The ophthalmoscopic and angiographic thresholds were determined for each laser setting including Nd:YLF 5 μs , 1.7 μs , and 200 ns (100 pulses,

500 Hz) and Argon 5 μs (100 pulses, 500 Hz), as well as 200 ms quasi cw-irradiation (200 ms).

RESULTS

Nd:YLF 5 μs . Laser treatment was performed in 3 eyes with a total of 89 exposure sites. Large, long-lasting bubbles were noticed at energy levels higher than 60 mJ. The mean ophthalmoscopical ED_{50} threshold was 25.4 μJ (95% confidence interval: 23.8 μJ and

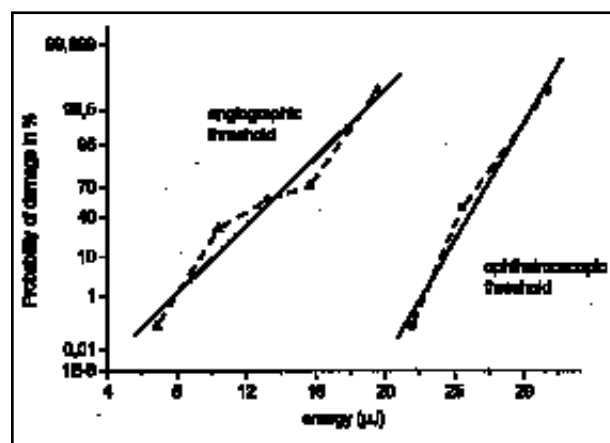


Figure 2. Graph of ophthalmoscopic and angiographic thresholds for Nd:YLF 5 μs (527 nm, 102 μm , 100 pulses, 500 Hz) and the linear fit. $\text{ED}_{50,\text{ophth}}$ was 25.4 μJ , $\text{ED}_{50,\text{ang}}$ was 13.4 μJ .

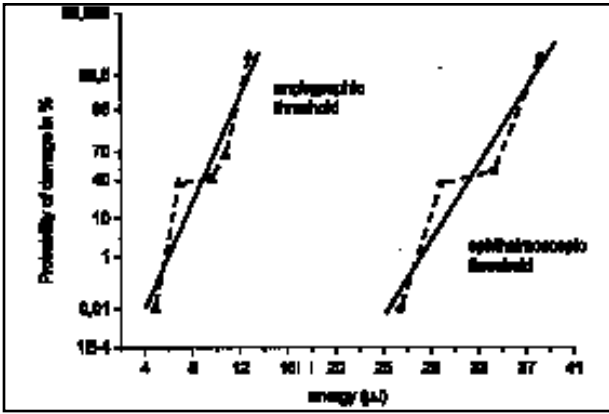


Figure 3. Graph of ophthalmoscopic and angiographic thresholds for Nd:YLF 1.7 μ s (527 nm, 102 μ m, 100 pulses, 500 Hz) and the linear fit. $ED_{50,oph}$ was 32 μ J and $ED_{50,ang}$ was 9.2 μ J.

28.2 μ J), the angiographic ED_{50} threshold was 13.4 μ J (95% confidence interval: 9.2 μ J and 16.8 μ J) (Figure 2).

Nd:YLF 1.7 μ s. Laser treatment was performed in 5 eyes with a total of 137 exposures. Bubble formation could be noticed at energy levels higher than 50 μ J. The mean ophthalmoscopic threshold was 32 μ J (95% confidence interval: 29.3 μ J and 35.5 μ J). The angiographic threshold was 9.2 μ J (95% confidence interval: 6.8 μ J and 11.4 μ J) (Figure 3).

Nd:YLF 200 ns. Laser treatment was performed in 4 eyes with a total of 87 exposures. Bubble formation could be noticed at energy levels higher than 38 μ J. The mean ophthalmoscopic threshold was 30 μ J (95% confidence interval: 25.4 μ J and 35.7 μ J). The angiographic threshold was 6.7 μ J, (95% confidence interval: 5.9 μ J and 7.6 μ J) (Figure 4).

Argon 5 μ s. Laser treatment was performed in 3 eyes with a total of 39 exposures. The maximal achieved energy with this laser system was 12 μ J, which was not high enough to produce visible laser spots and therefore no ophthalmoscopic threshold could be determined. The mean angiographic threshold was 5.5 μ J, (95% confidence interval: 4.2 μ J and 8.2 μ J) (Figure 5).

Argon 200 ms. Laser treatment was performed in 2 eyes with a total of 27 exposures. The mean ophthalmoscopic threshold was 20.4 mW and the angiographic threshold was 19.2 mW (Figure 6). No bubble formation was noticed.

Speckle Measurements. For the Nd:YLF system with the long slit-lamp fiber, the speckle factor $F_{Nd:YLF}$ was $F=1.225$ (no speckle: $F=1$) and the contrast $K_{Nd:YLF}$ was $K=0.668$ (no speckle: $K=0$). For the

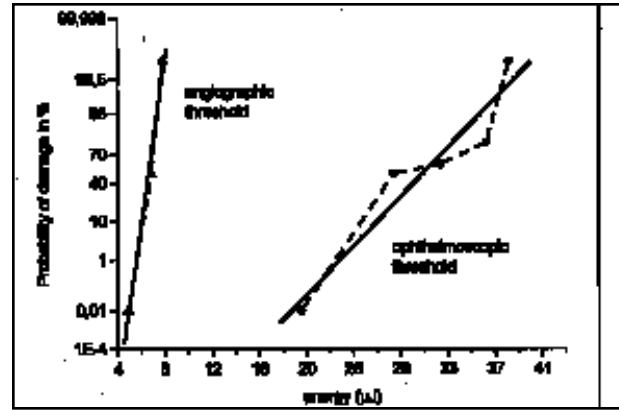


Figure 4. Graph of ophthalmoscopic and angiographic thresholds for Nd:YLF 200 ns (527 nm, 102 μ m, 100 pulses, 500 Hz) and the linear fit. $ED_{50,oph}$ was 30 μ J and $ED_{50,ang}$ was 6.7 μ J.

Argon system, F_{AR+} was $F=3.180$ and K_{AR+} was $K=0.860$. The speckle formation achieved with the Nd:YLF system is regular and homogenous, while the argon laser beam profile shows multiple high peaks suggesting a higher maximum radiant exposure. A typical speckle formation of both systems is displayed in Figure 7.

DISCUSSION

Presently the Nd:YLF is used selectively to treat the RPE for various macular diseases in a clinical pilot study (to date about 100 patients) with a train of repetitive 1.7 μ s pulses.¹⁰ The aim of this study was to investigate the ophthalmoscopic and angiographic thresholds of different repetitive pulsed laser settings as a function of the pulse duration, from significantly longer (5 μ s) to much shorter (200 ns) pulse durations. Additionally, speckle measurements were carried out to determine the influence of the spatial intensity modulation on the thresholds.

Origin of RPE Cell Damage by μ s Laser Pulse Duration

The selective RPE damage was primary postulated as a thermal effect caused by repetitively high temperatures generated by the train of single pulses applied to the retina.⁹ According to the model of Arrhenius, the rate of thermal damage to tissue increases strongly with temperature.¹⁶ The mechanism of the selective RPE destruction, therefore, was thought to be thermal necrosis of the cell. However, recent investigations showed that, most likely, a thermomechanical damage of the cells has to be taken into

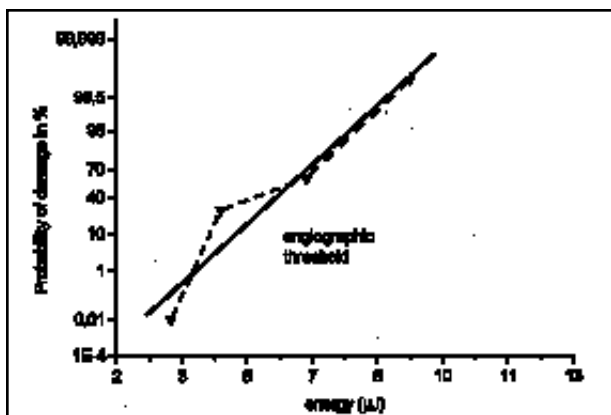


Figure 5. Graph of angiographic thresholds for Argon 5 μ s (514 nm, 102 μ m, 100 pulses, 500 Hz) and the linear fit. $ED_{50,opht}$ was above $>12 \mu$ J (no threshold because of less available laser energy) and $ED_{50,ang}$ was 5.5 μ J.

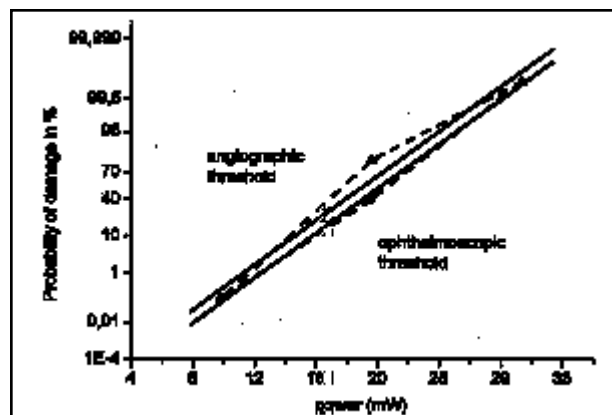
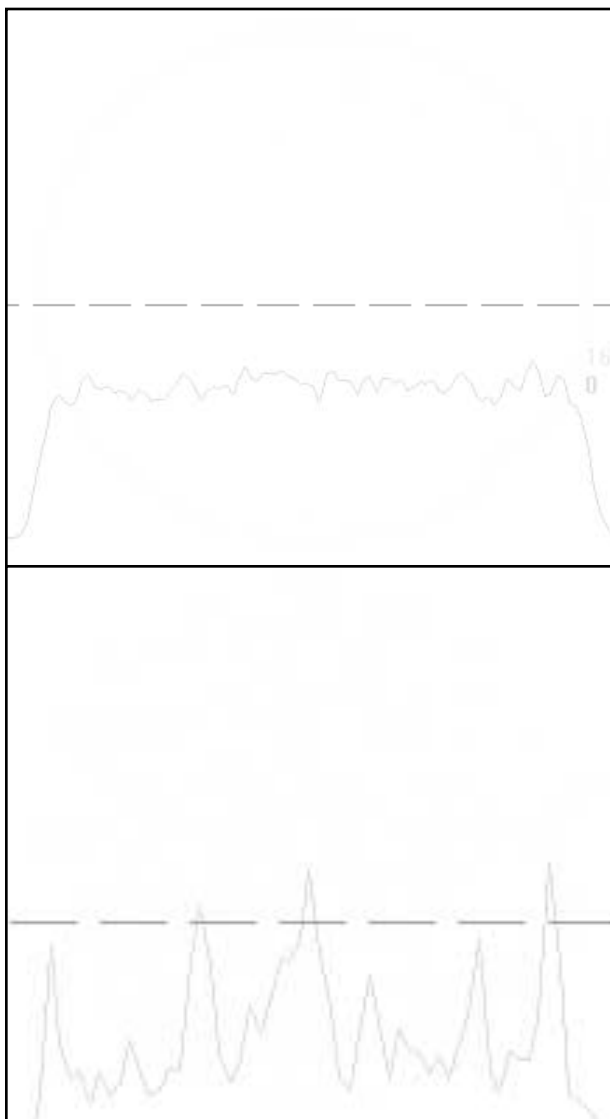


Figure 6. Graph of ophthalmoscopic and angiographic thresholds for Argon cw 200 ms (514 nm, 102 μ m) and the linear fit. $ED_{50,opht}$ was 20.4 mW and $ED_{50,ang}$ was 19.2 mW.



account.¹⁷ Thus microbubble formation around melanosomes in suspension with pulse durations of μ s were demonstrated. Brinkmann et al argued that cell death after applying a train of μ s pulses originated from microbubble formation instead of other mechanisms.¹⁷

Speckle Factor

As displayed in **Figure 7**, the speckle factors of the laser systems used were rather different. For the argon laser, it was nearly 3x higher (3.180) than for the Nd:YLF laser (1.225). This means a higher irregularity of the laser beam profile with significantly higher maximum radiant exposures in several areas with the argon laser compared to the top hat of the Nd:YLF laser. Thus, the several high peaks of the argon beam profile lead to lower thresholds than the regular beam profile of the Nd:YLF. Therefore, the maximum radiant exposure (H_{max}), explained by the speckle factor, has to be considered rather than the mean radiant exposure (H_{mean}) to determine the ED_{50} threshold radiant exposure.

In our study, the angiographic threshold of irradiation with the argon 5 μ s was 5.5 μ J, interestingly only half of the Nd:YLF 5 μ s (13.4 μ J). Corrected by the speckle factor, our data revealed that the maximum

Figure 7. (A) Speckle formation of the laser beams after Nd:YLF and the argon laser. **(B)** There are regular speckle formations for the Nd:YLF laser and multiple high peaks in the irregular speckle formation from the argon beam. This irregular speckle formation means higher maximum radiant exposure and therefore, an incorrect lower mean threshold.

Table. Summary of the Speckle Factor Considered to be the Ophthalmoscopic and Angiographic Thresholds for all Laser Settings Used in This Study

Lasers	Σ Eyes	Σ Spots	ED _{50,ophth}	ED _{50,ang}	Factor (No. 95% CI)	Factor (With 95% CI)
Nd:YLF 200 ns	4	87	438 mJ/cm ²	99 mJ/cm ²	~4.5x	~3.4x
Nd:YLF 1.7 μ s	5	137	472 mJ/cm ²	143 mJ/cm ²	~3.3x	~2.6x
Nd:YLF 5 μ s	3	89	362 mJ/cm ²	189 mJ/cm ²	~1.9x	~1.5x
Argon 5 μ s	3	39	>374 mJ/cm ²	170 mJ/cm ²	> ~2.2x	> ~1.5x
Argon cw	2	27	205 W/cm ²	165 W/cm ²	~1.2x	~1.2x

Note: Also displayed are the number of irradiated eyes, the number of laser spots applied, and the gap between both thresholds determined as the factor above angiographic threshold without and with consideration of the 95% confidence interval.

radiant exposure for the argon laser are $H_{\max, Ar+} = 170$ mJ/cm² and for the Nd:YLF laser $I_{\max, YLF} = 189$ mJ/cm², which is nearly the same. The different wavelength of both lasers also has to be considered, but it is relatively small and should not strongly influence the difference of both thresholds.

Roider et al performed rabbit trials using the argon laser (514 nm) with 5 μ s and the Nd:YAG laser (532 nm) with 200 ns (each 500 Hz, 100 pulses) and found mean angiographic thresholds of 19 mJ/cm² (5 μ s) and 26 mJ/cm² (200 ns) respectively.^{7,18} Notably, these thresholds were lower by a factor of 3 compared with our findings. Measurements of the speckle formation, as described above, could explain these results with the highly irregular speckle formation of the argon and Nd:YAG beam at the fiber tip. The maximal radiant exposure of these thresholds were comparable to our results. Corrected threshold results ($H_{\max}$) of the speckle factor for all the parameters used in this study are summarized in the **Table**. These values are used for further discussion.

Threshold Results

In our experiments, the angiographic ED₅₀ thresholds decreased with shorter pulse durations from 189 mJ/cm² (5 μ s) to 143 mJ/cm² (1.7 μ s) and 97 mJ/cm² (200 ns). A comparable decay was also found for RPE damage in vitro using a vitality stain to prove cell damage.¹⁷ Assuming a thermomechanical damage as described above, this result is expected and can be explained with heat contribution from melanosomes towards the periphery that increases towards longer pulse durations. Calculations showed that vaporization could be initiated when the surface temperature of melanosomes reaches 140°C.¹⁷ Therefore, if the same

energy is applied in a shorter time, higher temperatures are reached at the melanosomes. Thus, to achieve a certain temperature at the melanosomes to start vaporization, a lower radiant exposure is needed for shorter pulse durations.

Comparison of our threshold radiant exposure data with other works are difficult since they irradiate the eye with collimated beams, and the refractive power of the eye focuses the laser beam onto the retina.^{19,20} Only some articles provide information about the spot diameter and therefore radiant exposure can be calculated. In these cases, pulse durations were only 4 ns (wavelength: 532 nm), ophthalmoscopic thresholds of 707 mJ/cm² (spotsize: 30 μ m) in rabbits, and 1910 mJ/cm² (spotsize: 10 μ m) in monkeys were achieved.^{21,22} It is not clear why the thresholds were higher when lower thresholds would be expected with shorter pulses. This could be explained if a larger spot size than expected and a gaussian instead of a tophat beam profile were used. Additionally, no contact lens was used and a certain amount of energy delivered to the cornea is lost because of reflexion.

The described correlation between angiographic thresholds and pulse durations could not be found for ophthalmoscopic thresholds. These results ranged from 478 mJ/cm² (1.7 μ s) to 362 mJ/cm² (5 μ s) showing no significant correlation with the pulse duration, which could be caused by a greater inaccuracy in determining the whitening of the retina. It is also known that during the first hour after irradiation, presumably after seconds, a biological enhancement of the laser effect caused by occurrence of an intra- or intercellular edema appears.²³ This might lead to difficulties judging an ophthalmoscopically visible laser lesion, especially for threshold lesions applied with small energies.

Using argon irradiation with 200 ms the ophthalmoscopic threshold was 205 W/cm² and the angiographic threshold was 165 W/cm² suggesting that near the angiographic threshold even without retinal whitening a selective effect on the RPE could be performed. Roeder et al also found different ophthalmoscopic and angiographic thresholds that differed by a factor of 2 for long pulse irradiation with a 514 nm argon laser (50 ms, 100 ms, 500 ms, and 1 sec).⁷ However, histological findings revealed destruction of the choroid and the photoreceptors.⁷ In our study, a factor of 1.2 was obtained, too small to achieve selective treatment.

Therapeutic Window

For a new approach of a selective RPE laser treatment, it is important to have an adequate gap between the ophthalmoscopic and the angiographic threshold to prevent unintentional photoreceptor cell damage. Because of the intra- and the interindividual pigmentation that varies by a factor of 2 in humans,⁸ a factor of at least 2 should be ensured to treat patients with this new technique. Damaging Bruch's membrane by short pulses must also be avoided because it is known that new vessel formation can be promoted.²⁴⁻²⁶

Irradiation above the ophthalmoscopic threshold produces a visible laser lesion meaning denaturation of retinal tissue and laser scotoma. Irradiation lower than the angiographic threshold produces no immediate angiographic visible damage to the tissue. However, some destruction of the tissue might appear. Because angiography is the most sensitive tool to proof proper irradiation by leakage, treatment should be performed with energies above the angiographic threshold, but, as pointed out, beneath the ophthalmoscopic threshold. This should lead to a selective defect in the RPE, but does not lead to thermal damage to the neurosensory retina as determined by ophthalmoscopy.

It is possible with a single laser burn from a cw-laser to cause a relatively selective RPE damage,²⁷ but a slight increase of the energy leads to severe damage due to thermal heat conduction and following denaturation of the photoreceptors.^{6,28} In comparison, there is a fundamental difference using short-pulsed repetitive μ s-expositions. Even pulse energies exceeding a factor 2 above the angiographic threshold do not lead to damages of the photoreceptors in rabbits as shown by Roeder et al using trains of 5 μ s pulses (argon 514 nm, 500 Hz, 1-500 pulses).⁹

In our experiment, the use of Nd:YLF 5 μ s pulses shows the smallest window between angiographic and

ophthalmoscopic ED₅₀-thresholds with a factor of about 2x above ED_{50,ang}. **If the 95% confidence interval of the factor, calculated from the 95% value of the angiographic threshold, the 5% value of the ophthalmoscopic threshold is considered a therapeutic window with a factor of about only 1.5 results (Figure 2, Table).** Roeder et al could not determine a window when irradiating with argon 5 μ s pulses because of a lack of available power.⁷ However, the window had at least a factor of 2.

The Nd:YLF 1.7 μ s that is already used for the treatment of patients has a larger window with a factor of 2.6 times over ED_{50,ang} (95% confidence interval considered) (Figure 3, Table). Therefore, the Nd:YLF 1.7 μ s seems to be an appropriate treatment device for clinical trials. Also the Nd:YLF 200 ns pulses achieved a factor of 3.4x over ED_{50,ang} (95% confidence interval considered) (Figure 4, Table). No ruptures or hemorrhages occurred during treatment in between the "therapeutic window." However, histological evaluations have to prove whether no extensive mechanical rupture, because of strong vaporization, leads to a photoreceptor damage. Such damage might not necessarily be ophthalmoscopically visible. If no such effect is found up to the ophthalmoscopic threshold, the Nd:YLF 200 ns could also be an appropriate laser setting for the selective RPE treatment.

CONCLUSION

For threshold experiments it is important to measure the speckle factors since the maximum radiant exposure in the beam profile, instead of the mean radiant exposure, is responsible for the damage.

In vivo laser irradiation in rabbits revealed decreasing angiographic thresholds with decreasing pulse durations from 5 μ s to 200 ns for the Nd:YLF system. Thresholds for the 200 ns pulse durations and those of the 1.7 μ s pulse durations that are currently used in clinical trials were nearly the same.

Ophthalmoscopically, nonvisible laser lesions in our experiments underline the suggestion of the mechanical rupture rather than a thermal damage. Side effects of such a short pulsed irradiation could be hemorrhages and ruptures of the retinal tissue, but this was not noticed during our experiments. This suggests that selective treatment down to 200 ns may be safe.

However, histological evaluation has to prove whether 200 ns pulse durations are also appropriate for selective RPE treatment.

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