

## Intraocular light intensity and spectral analysis using 308-nm excimer lasers via quartz fiber

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### Abstract

**Background:** Different wavelengths of excimer lasers have been used successfully for ophthalmic surgery in clinical practice. While the 193-nm wavelength of ArF lasers have been applied for photorefractive keratectomy, 308-nm XeCl lasers have been tested as an alternative surgical technique in the treatment of glaucoma. The laser ablation of tissue caused extremely thin, smoothly limited zones of necrosis, and minimal irritation of the adjacent tissue. Cytotoxic and mutagenic tissue effects induced by ultraviolet excimer laser radiation are caused by photochemical reactions with the irradiated cellular molecules, particularly DNA, and are responsible for the risk of cataractogenesis and retinal toxicity following the intraocular use of 308-nm radiation.

**Material and methods:** To assess the risk of the clinical use of ultraviolet radiation, the authors analyzed the intraocular intensity of scattered light and spectral distribution of secondary radiation (e.g., fluorescence) of the 308-nm ultraviolet radiation applied by a quartz fiber (320  $\mu\text{m}$ ). The 308-nm radiation with an intensity of 4 mJ at the distal quartz fiber end was applied into the anterior chamber of enucleated porcine eyes. The fluorescence was collected by a detector fiber at various positions within the eye. Optical spectra were obtained by a polychromator and CCD array of a triggered optical multichannel analyzer (OMA).

**Results:** Fluences of as low as less than 0.1  $\mu\text{J}/\text{cm}^2$  were measured at the retina, due to high UV absorption of the lens. Lens damage is unlikely when quartz fibers with high numerical apertures are used. Laser-induced autofluorescence was detected at 330-360 nm, 380 nm and 430-440 nm.

**Conclusion:** Proper handling of high-aperture fibers will help prevent radiation damage to the lens or retina caused by excimer laser radiation.

### Introduction

In clinical practice, different wavelengths of excimer lasers have been used successfully in ophthalmic surgery. While the 193-nm wavelength of ArF lasers has been applied for photorefractive keratectomy, 308-nm XeCl lasers have been tested as an alternative surgical procedure for fistulating effects in the treatment of glaucoma. Production of a scleral canal of about 300  $\mu\text{m}$  in diameter permitted aqueous humor to flow from the

anterior chamber into the subconjunctival space, hence immediately decreasing intraocular pressure.

March *et al.*<sup>1-3</sup> performed so-called sclerostomy using an *ab interno* procedure with a pulsed Nd:YAG laser. The laser radiation was directed to the anterior meshwork by means of a gonio contact prism. In similar experiments with Nd:YAG lasers<sup>4</sup>, argon-ion lasers<sup>5</sup>, pulsed dye lasers<sup>6</sup>, and excimer lasers<sup>7</sup>, radiation was applied to the anterior chamber angle, using a light fiber. In these

cases it was necessary to make a prior opening at the limbus on the opposite side and to insert the fiber through the entire anterior chamber. Some authors stained the adjacent sclera to improve energy absorption<sup>8</sup>.

In contrast to the *ab interno* procedure, a quartz fiber, mechanically protected by a drain tube, was placed at the limbus by a transscleral opening of the anterior chamber. Due to the specific absorption characteristics of corneoscleral tissue, the ablative effects of the 308-nm radiation caused thin, smoothly limited borders of necrosis and minimal irritation of the adjacent tissue. At that time, the first clinical investigations showed a strong delay in the scarring tendency of the fistula<sup>9</sup>. However, 308-nm radiation of intraocular structures may cause cataractogenesis and retinotoxicity<sup>10</sup>, based on photochemical DNA changes.

It was our intention in this study to estimate the photostress of intraocularly applied 308-nm radiation in clinical use. To do this, we determined the absorption and fluorescence characteristics of isolated intraocular porcine eye tissue, and analyzed the radiation intensity and spectral characteristics in different eye compartments.

## Material and methods

The experiments were carried out on freshly enucleated porcine eyes or, in some cases, on isolated tissues.

The transmission characteristics of isolated globe tissues, placed in quartz cuvettes, were analyzed by a commercially available absorption spectrometer equipped with an Ulbricht sphere (UVICON, Fa. Kontron). Measurement of the spectral characteristics of the fluorescence was carried out with the SMF 25 spectrometer (Fa. Kontron).

Whole eyes were used for analysis of the optical distribution of laser radiation under clinical conditions. The 308-nm radiation of the XeCl laser was applied transcorneally in the direction of the optical axis with a 320- $\mu\text{m}$  quartz-quartz fiber (NA: 0.2). The detector fiber (core diameter: 600  $\mu\text{m}$ , NA: 0.2) was positioned under visual control in different eye compartments.

For the measurements of intraocular intensity, the distal fiber end was coupled to a photodiode.

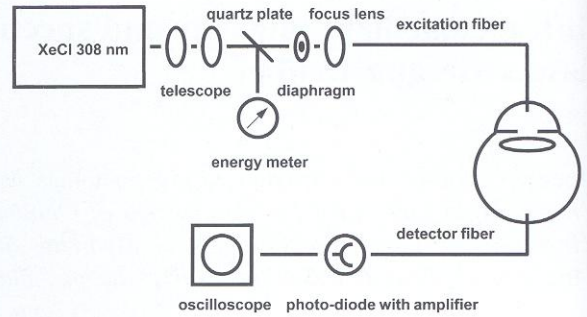


Fig. 1. Set up for fiberoptic measurement of intraocular distribution of radiation intensities.

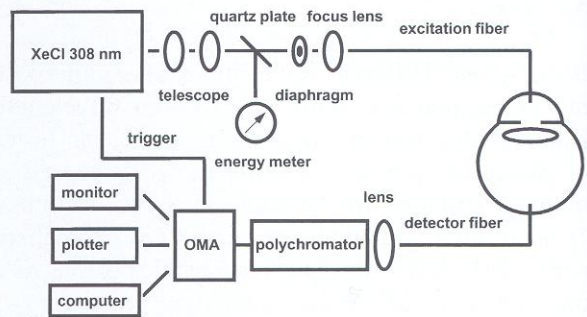


Fig. 2. Set up for fiberoptic measurement of intraocular spectral distribution of the primary and secondary radiation.

Amplified voltage signals were recorded by a storage oscilloscope (Fig. 1). To calibrate the diode, the 308-nm beam of known pulse energy was completely coupled into the detector fiber.

Autofluorescence and transmission spectra were obtained using a polychromator and the CCD array of a triggered optical multichannel analyzer (OMA) (Fig. 2). Each measurement lasted 20 msec. A black body radiator served as a relative spectral calibration device.

## Results

### *Transmission and fluorescence of isolated tissue probes*

Transmission measurements of isolated tissues from the cornea, aqueous humor, lens and vitreous body in the UV and visible spectral region, revealed high absorption at wavelengths of less than 300 nm (Fig. 3). Radiation of between 330 and 800 nm showed an extinction of less than 0.3

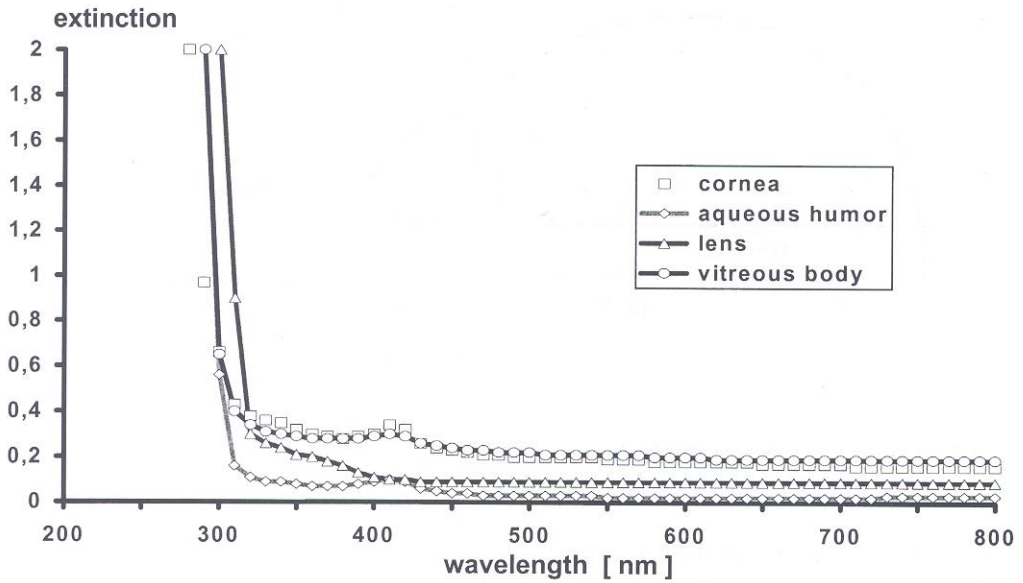


Fig. 3. Transmission spectrum of isolated eye tissue (measurement with UVICON absorption spectrometer).

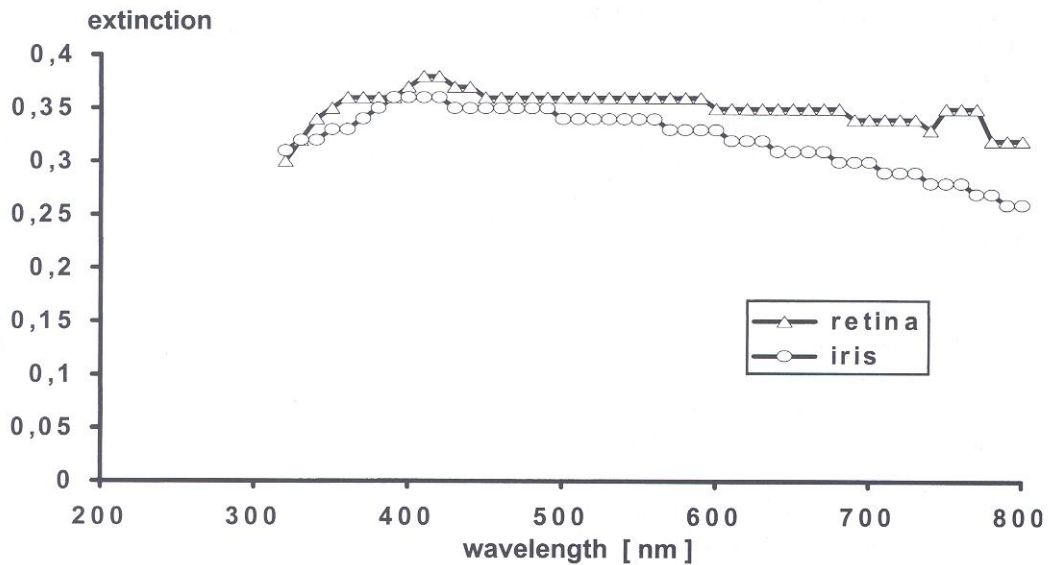


Fig. 4. Transmission spectrum of isolated eye tissue (measurement with Ulbricht sphere).

in the case of 1-mm thick tissue. Generally, transmission increased at longer wavelengths; only the cornea, aqueous humor and vitreous body showed a 410-nm absorption peak.

The absorption spectrum of the iris and retina exhibited unstructured spectral characteristics (Fig. 4).

The fluorescence spectra of the 308-nm excited tissue probes revealed relatively unstructured

characteristics in all the tissues. The aqueous humor, lens and vitreous body showed fluorescence in the blue spectral range below 450 nm. There was no visible fluorescence in the cornea, iris or retina.

A UV fluorescence maximum was found at 340 nm for the cornea, lens, vitreous body and aqueous humor; at 390 nm for the vitreous body; and at 360 nm for the retina.

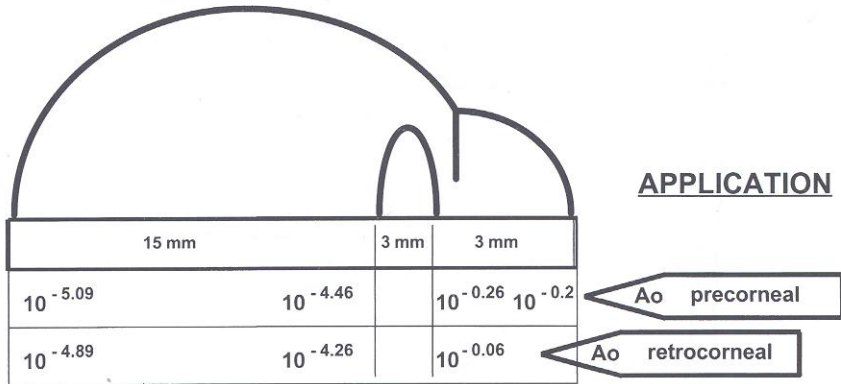


Fig. 5. Intraocular distribution of radiation intensities following transmission within different compartments of the globe.

*Estimation of the relative photon flux to retina and lens during ab externo sclerostomy*

The photon flux A depends on extinction E and thickness of the intraocular tissue layers. With the measured data

- E (cornea)  $\approx 0.2$  (D = 0.5 mm)
- E (aqueous humor)  $\approx 0.06$  (D = 3 mm)
- E (lens)  $\approx 4.2$  (D = 3 mm)
- E (vitreous body)  $\approx 0.63$  (D = 15 mm)

the photon flux at the retina and at the lens front can be estimated as

$$A \text{ (retina)} \approx A_o * 10^{-E} = A_o * 10^{-4.89} \approx A_o * 10^{-5} \tag{1}$$

$$A \text{ (lens)} \approx A_o * 10^{-0.06} \sim A_o * 0.87 \tag{2}$$

E: extinction;  $A_o$ : incident photon flux

In the case of precorneally applied radiation,  $A_o$  is slightly reduced (5-7%) due to Fresnel reflection.

It should be noted that, in *ab externo* sclerostomy, the cornea cannot act as an efficient UV barrier because of the retrocorneal release of radiation via the fiber. Therefore, the lens is highly exposed to UV.

The estimated intraocular decrease of the photon flux is shown in Figure 5. According to Equation (1), a total retinal radiation exposure energy of about 40 nJ may be calculated in the case of an applied pulse energy of 4 mJ at the fiber end.

*Distribution of intensity of the 308-nm radiation within the eye*

The radiation intensity in different compartments

of the eye was measured by fiberoptical detectors. The intensity depended on the application direction of the excitation fiber. A two- to three-fold increase in preretinal radiation intensity was measured when the excitation fiber was pushed horizontally from the angle to the center of the anterior chamber (Fig. 6A, sites a,b).

In the retrolental vitreous body, the radiation intensity decreased strongly according to the distance to the anterior chamber. The preretinal radiation intensities were less than 1/10 of the retrolentally measured intensities. Even with the excitation fiber in a prelental position and a direction along the optical axis, the fluence on the retina was less than  $0.10 \pm 0.03 \mu\text{J}/\text{cm}^2$  (detected energy divided by fiber surface) (Fig. 6B, sites a-c).

*Spectral distribution of the secondary radiation within the eye*

The measured fluence of  $0.1 \mu\text{J}/\text{cm}^2$  means the integral radiation exposure (*i.e.*, both UV- and laser-induced fluorescence were recorded). The appearance of autofluorescence in the blue spectrum was visually observed intraoperatively.

The excitation fiber was positioned retrocorneally along the optical axis, whereas the detection fiber was held at various distances from the excitation fiber (Fig. 6B). A preretinal autofluorescence was found in the vitreous body at 340 nm and 380-400 nm, with a relatively high intensity at 340 nm (Fig. 7A). With the detector fiber positioned in the lens tissue, the expected primary 308-nm radiation appeared in addition to the

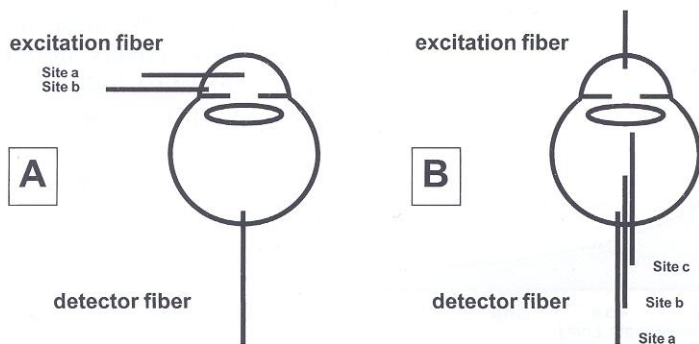


Fig. 6A and B. Measurement of intensities to different fiber sites.

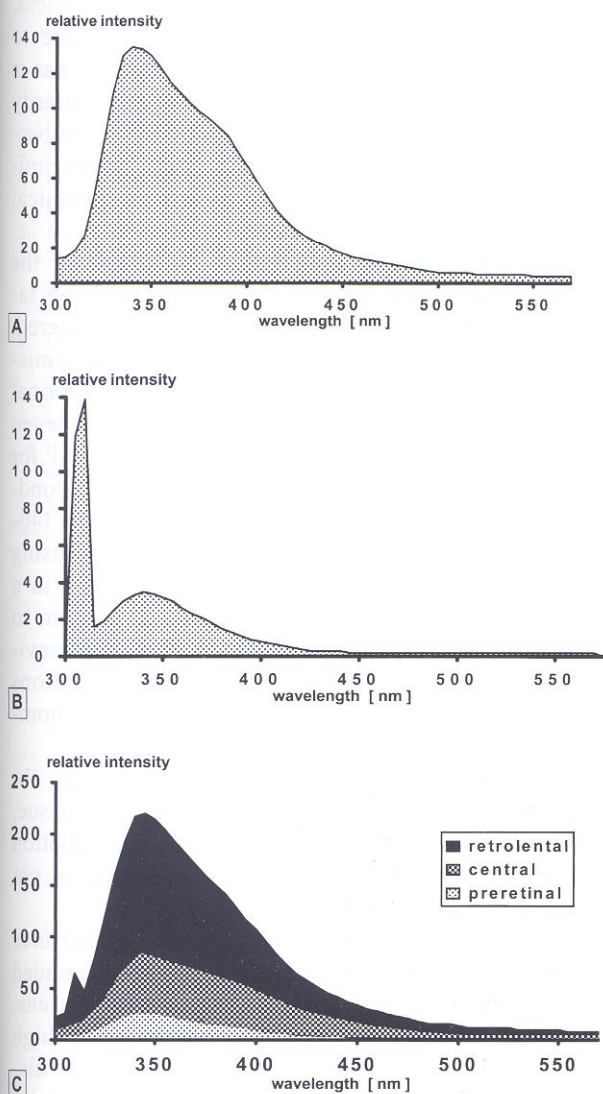


Fig. 7A, B and C. Spectral distribution in intraocular tissue (fiberoptic measurement). A. preretinal; B. lens; C. vitreous body.

autofluorescence (Fig. 7B). Only low, 308-nm intensities were found in the vitreous body (Fig. 7C).

With the same experimental set up, both excitation and detection fibers were positioned along the optical axis within the same tissue probe. The fluorescence spectra of the corneal consisted of a broad band with a maximum at 360 nm in addition to the 308-nm radiation (Fig. 8A). Lens autofluorescence was noted in peaks at 360 nm and 380 nm (Fig. 8B).

In the vitreous body and aqueous humor, the transmitted photon flux consisted of photons, primarily of the excitation radiation (Fig. 8C).

### Discussion

The 308-nm radiation of excimer lasers was used in conjunction with quartz fibers as an alternative microsurgical technique for glaucoma. The specific ablative tissue effect of the 308-nm radiation caused thin layers of necrosis and minimal irritation of the adjacent tissue. In addition, the postoperative inflammatory reaction was reduced by perforating the conjunctival tissue at a distance of 8-10 mm from the corneal limbus. In *ab externo* procedures, the limbus is opened only once, in contrast to *ab interno* approaches which require an additional opening<sup>5,7</sup>. Damage to the cornea, iris and lens might be avoided by pushing the light fiber into the anterior chamber. Using an energy emission of 4 mJ at the fiber end and a repetition rate of 80 Hz, paracentesis of the anterior chamber was performed within a few seconds. General and peribulbar anesthesia, with their well-known risks, were avoided, allowing

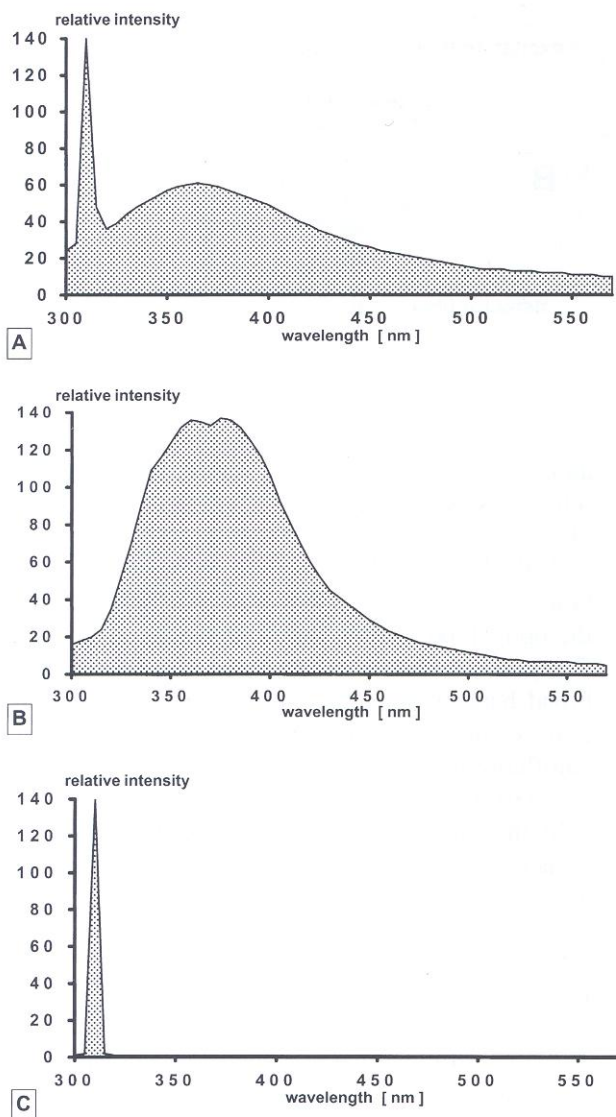


Fig. 8A, B and C. Spectral distribution of isolated tissue (fiber-optic measurement). A. cornea; B. lens; C. aqueous humor, vitreous body.

treatment to be performed on an out-patient basis. At that time, clinical investigations showed satisfactory postoperative results, even after a year<sup>9</sup>.

The disadvantage of using the excimer laser is the well-known risk to intraocular structures due to the mutagenic and carcinogenic effects of 308-nm radiation. A point of criticism is the negative influence on the metabolism of the corneal endothelium layer, lental and retinal tissues<sup>10,11</sup>. At 305 nm, the damage threshold for cataract induction was found to be 300-600 mJ/cm<sup>2</sup>.<sup>11</sup> It has been reported that fluences of 1.8 J/cm<sup>2</sup> damage the

retina when 308-nm radiation is used<sup>12</sup>. The photobiological effects induced by UV absorption have a negative influence on cell and tissue metabolism. The major chromophore for most UV-induced rechanges in cells and tissue is nuclear DNA, with an absorption maximum of about 190 nm<sup>13,14</sup>. In comparison, 308-nm radiation is assumed to be at least 1000 times less cytotoxic and mutagenic than, for example, 254-nm radiation<sup>15</sup>. But, in addition, 308-nm radiation induced sister chromatid exchanges about 125 times more efficiently than 254-nm radiation<sup>16</sup>. Excimer laser radiation at 308 nm is also known to be mutagenic or even lethal in *Escherichia coli*<sup>17</sup>. Nevertheless, it is still doubtful whether a single exposure to UV radiation during a medical procedure has a carcinogenic effect<sup>15</sup>. Damage is caused primarily by direct absorption of radiation or by the subsequent release of photoproducts such as pyrimidine-dimers<sup>18</sup>, both of which induce chemical changes in DNA.

The intraocular distribution of fluence, the wavelength of the applied radiation, and the laser-induced fluorescence, must be considered. The use of 308-nm radiation with an energy emission of 4 mJ at the fiber end will lead to retinal fluences of about 0.1  $\mu\text{J}/\text{cm}^2$ . This value is seven orders of magnitude less than the threshold for retinal damage<sup>12</sup>. Also, the formation of secondary radiation (fluorescence), ranging from 340-380 nm, does not seem to be in danger of causing retinal damage. Isolation of tissues resulted in a 20-nm shift of the fluorescence maximum to higher wavelengths. Changes in the microenvironment and modified physiological conditions could be a possible explanation for the phenomenon.

Transmission measurements showed high absorption of 308-nm radiation in the lens tissue. However, a substantial reduction of radiation over the distance of the aqueous humor is not expected.

The use of fibers with high numerical apertures and therefore higher divergence is recommended to minimize the intensity of radiation to the lens. In the case of contact procedures, the high fluence for ablation is required only at a low distance; at higher distances, UV radiation is intraocularly radiated with a wide angle of divergence. According to our experience, the long distance between the lens and the fiber end should

be controlled intraoperatively.

Proper handling of a high aperture fiber excludes radiation damage to the lens or the retina caused by excimer laser radiation. In the meantime, to avoid further UV radiation risks, fiber application and laser systems in the infrared range (e.g., the Er:YAG laser) have been tested.

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