Damage mechanisms for near-infrared radiation induced cataract

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Abstract

Purpose: 1) To estimate the threshold dose and the time evolution for cataract induction by near infrared radiation (IRR) in seconds exposure time domain; 2) to determine the ocular temperature development during the threshold exposure; 3) to investigate if near IRR induces cumulative lens damage considering irradiance exposure time reciprocity; 4) to experimentally estimate the temperature in the lens indirectly from the measurement of temperature-induced light scattering increase.

Methods: Before exposure, 6-weeks-old albino rats were anesthetized and the pupils of both eyes were dilated. Then the animals were unilaterally exposed to 1090 nm IRR within the pupil area. Temperature was recorded with thermocouples placed in the selected positions of the eye. At the planned post-exposure time, the animal was sacrificed and the lenses were extracted for measurements of forward light scattering and macroscopic imaging (Paper I-III). In Paper IV, the lens was extracted from six-weeks-old albino Sprague-Dawley female rats and put into a temperature-controlled cuvette filled with balanced salt solution. Altogether, 80 lenses were equally divided on four temperature groups, 37, 40, 43 and 46 ºC. Each lens was exposed for 5 minutes to temperature depending on group belonging while the intensity of forward light scattering was recorded.

Results: The in vivo exposure to 197 W/cm² 1090 nm IRR required a minimum 8 s for cataract induction. There was approximately 16 h delay between exposure and light scattering development in the lens. The same radiant exposure was found to cause a temperature increase of 10 ºC at the limbus and 26 ºC close to the retina. The in vivo exposure to 96 W/cm² 1090 nm IRR with exposure time up to 1 h resulted in an average temperature elevation of 7 ºC at the limbus with the cornea humidified and no significant light scattering was induced one week after exposure. Arrhenius equation implies that the natural logarithm of the inclination coefficient for light scattering increase is linearly dependent on the inverse of the temperature. The proportionality constant and the intercept, estimated as CI(0.95)s, were 9.6±2.4 x10³ K and 22.8±7.7. Further, it implies that if averaging 20 measurements of inclination coefficients in a new experiment at constant heat load, the confidence limits for prediction of temperature correspond to ±1.9 ºC.

Conclusions: It is indicated that IRR at 1090 nm produces thermal but not cumulatively photochemical cataract, probably by indirect heat conduction from absorption in tissues surrounding the lens. Applying the Arrhenius equation the in vivo temperature in the lens can be determined retrospectively with sufficient resolution.

Keywords: infrared radiation, photochemical, thermal, forward light scattering, lens, cataract, temperature, Arrhenius equation, heat diffusion

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List of papers

This thesis is based on the following papers and manuscript, which are referred to in the text by their Roman numerals.


Papers not included in this thesis:


Yu Z, Steinvall O, S Sandberg, U Hörberg, R Persson, F Berglund, K Karsslsson, J Öhgren, Söderberg P, Green light exposure at 532 nm near the exposure limit during a human volunteer vehicle driving task does not alter structure or function in the visual system. Journal of Laser Applications, 2014 May; 26(2).


Contents

Introduction ........................................................................................................................................... 8
Transmission and absorption of IRR in the ocular media ............................................................... 8
Characteristics of photochemical and photothermal effects ......................................................... 8
Evidence of IRR induced photochemical damage in the lens ....................................................... 9
Current safety guideline for IRR exposure ..................................................................................... 10
Aims .................................................................................................................................................... 10
Materials and methods ................................................................................................................... 11
Animals ................................................................................................................................................ 11
Radiation source (Paper I-III) ......................................................................................................... 11
Temperature measurement (Paper II and III) .................................................................................. 12
Temperature-controlled cuvette ....................................................................................................... 12
Measurements of forward light scattering in the lens ..................................................................... 13
Macrophotography .......................................................................................................................... 14
Experimental procedure .................................................................................................................. 14
Paper I-III .......................................................................................................................................... 14
Paper IV ............................................................................................................................................ 14
Experimental Design ....................................................................................................................... 14
Paper I ................................................................................................................................................ 14
Paper II ............................................................................................................................................. 14
Paper III ........................................................................................................................................... 15
Paper IV ........................................................................................................................................... 15
Statistical parameters ....................................................................................................................... 15
Results and discussion ..................................................................................................................... 16
Paper I ................................................................................................................................................ 16
Paper II ............................................................................................................................................. 16
Paper III ........................................................................................................................................... 17
Paper IV ........................................................................................................................................... 18
Conclusion ......................................................................................................................................... 20
Further study ..................................................................................................................................... 20
Acknowledgements .......................................................................................................................... 21
References ......................................................................................................................................... 22
**Introduction**

Since the late 1800s, surveys of workers in the glass and steel industries have implied an association between infrared radiation (IRR) and cataract (Meyenhofer 1886; Wallace et al. 1971; Lydahl 1984). Vogt (Vogt 1932) proposed that IRR cataract results from direct absorption of IRR in the crystalline lens. Goldmann (Goldmann 1933) hypothesized that IRR cataract is due to temperature rise induced by IRR in the iris and heat transfer to the lens from the iris. This was supported by Verhoff and Bell (Verhoeff et al. 1915). Wolbarsht advocated that near infrared radiation cataract can be photochemically induced (Wolbarsht 1991).

**Transmission and absorption of IRR in the ocular media**

The sunlight incident on earth contains approximately 313 W/m² IRR (mainly from 780 to 3000 nm) (Mecherikunnel & Richmond 1980). IRR within the waveband 3000 to 10000 nm is absorbed by the atmosphere. The cornea receives IRR from sunlight with an irradiance of the order of 10 W/m² (Sliney & Freasier 1973). In general, the cornea absorbs most radiation with a wavelength above 1400nm. The crystalline lens absorbs some radiation between 700 nm and 1400 nm (near IRR) and the retina absorbs most of the remaining near IRR (Fig 1) (Boettner & Wolter 1962).

![Fig 1 Near IRR transmittance through the eye.](image)

**Characteristics of photochemical and photothermal effects**

For exposures to optical radiation with exposure times exceeding microseconds, photochemical damage and thermal damage are the
dominating damage mechanisms (ICNIRP et al. 2013). Photochemical damage is characterized by a delay between exposure and expression of biological damage. Further, the threshold for photochemical damage expresses reciprocity for irradiance and exposure time, the threshold radiant exposure thus being independent of exposure time. As a consequence, photochemical damage may be accumulated at low irradiance over very long exposure time. Moreover, a photochemical damage has a characteristic action spectrum. A high irradiance of near IRR on the cornea, causes thermal damage that is expressed immediately after the exposure (Pitts & Cullen 1981). A high irradiance on the lens, causes an immediate onset of cataract (Goldmann 1933). Although immediate onset of a detectable thermally induced effect holds for a high irradiance, there may be a delay between exposure and onset of damage at low irradiance. In 1960, Langley et al. observed appearance of anterior subcapsular dots at 24 hrs after exposure of the iris to 0.8 W/cm² for 30 s (27 J/cm²) to broad band IRR (Langley et al. 1960). In 1983, McCally observed corneal damage 48 hrs after exposure to 10.6 µm IRR derived from a CO₂ laser (McCally et al. 1983). In the retina, a time delay was observed between exposure to visible optical radiation close to the threshold and development of an observable thermal reaction in the tissue (Lund et al. 2007). Due to heat diffusion, the threshold radiant exposure increases with increasing exposure time and if the exposure time is long enough the threshold will never be attained. Thus, photothermal damage does not occur after accumulated exposures at low enough irradiance. Moreover, photothermal damage has no characteristic action spectrum.

Evidence of IRR induced photochemical damage in the lens

Findings of two previous studies implied reciprocity between irradiance and exposure duration, which is characteristic of a photochemical effect. Wolbarsht (Wolbarsht et al. 1977; Wolbarsht 1978; Wolbarsht 1991) stated cataract formation after in vivo exposures of rabbits to approximately 1.4 kJ/cm² within the dilated pupil with a CW Nd:YAG (1064 nm) laser using irradiances ranging between 1.4 and 28 W/cm². Pitts et al.(Pitts et al. 1980; Pitts & Cullen 1981) claimed a threshold dose for in vivo exposure to low irradiance IRR of 3.5 kJ/cm². This was based on in vivo exposure of rabbits to wide band IRR derived from a Xenon arc source, 715-1 400 nm (mainly below 1100 nm) using irradiances ranging between 2 and 4 W/cm². Furthermore, the epidemiological finding that steel and glass workers exposed to daily doses of 80-400 mW/cm² for 10-15 years
developed cataract also indicates a photochemical effect (Lydahl 1980).

**Current safety guideline for IRR exposure**

Presently, it is believed that IRR damage in the lens is wavelength independent (Sliney 1986). Based on Goldmanns (Goldmann 1930) and Wolbarshts (Wolbarsht 1980) findings and Scott's heat transport model (Scott 1988), Vos et al. (Vos & Van Norren 1994) calculated a threshold temperature rise of 5 °C in the lens and stated that an irradiance of 1 kW/m² would not increase the temperature of the anterior segment of the eye more than 5 °C. The current safety guideline for IRR exposure in the crystalline lens is based on thermal damage (ICNIRP et al. 2013; ICNIRP et al. 2013).

Probably, due to the lack of generally used low intensity near IRR sources, a potential photochemical effect of near IRR has not been further elucidated. However, the recently rapidly increasing use of near IRR emitting diodes in remote controls and remote sensing presents a potential for accumulation of high doses over long period of time and therefore has created a need for a more solid basis for safety estimation (Sliney 2006).

**Aims**

- **Paper I:** To determine the just above threshold exposure time for the near IRR exposures in the seconds time domain at a constant irradiance of 197 W/cm² within the pupil, and to establish the time evolution of lens damage after the estimated threshold exposure.

- **Paper II:** To determine the temperature time evolution in the eye during an 8 s *in vivo* exposure to 197 W/cm² at 1090 nm, and the heat diffusion associated with exposure.

- **Paper III:** To investigate if 1090 nm IRR induces cataract photochemically considering irradiance exposure time reciprocity.

- **Paper IV:** To experimentally estimate the temperature in the lens indirectly from the measurement of increase rate of temperature-induced light scattering, based on Arrhenius equation.
Materials and methods

Animals
The experimental animal used was 6-weeks-old albino Sprague-Dawley female rat. The animals were kept and treated according to the ARVO Statement for the Use of the Animals in Ophthalmic and Vision Research. Ethical permission was obtained by Uppsala Djurförsöksetiska Nämnd (C29/10, C380/12, and C29/16).

Radiation source (Paper I-III)
The infrared radiation was generated with a single mode CW fiber laser, emitting at 1090 nm (Model SP-10C-0011, SPI Lasers, UK) with a maximum output power of 10 W and a 5 mm exit diameter. Schematic diagram for exposure setup was shown in Fig. 2.

![Fig. 2 Schematic of optical configuration used for exposures](image)

The laser beam was first expanded with a negative lens, focal length 50 mm, projecting the beam on a second positive lens, focal length 50 mm (Fig 1). The distance between the lenses was approximately 340 mm. The second lens focused the beam in front of the eye aiming for a 2 mm spot size on the cornea with a vergence of -400 D (22 ° planar angle of divergence, focal point corneal plane distance = 2.5 mm). The position of the negative lens was adjusted to obtain the desired vergence and spot size on the cornea while measuring the three-dimensional distribution of the beam with a 0.3 mm pinhole in front of the detector (3A-SH, Ophir Optics, North Andover, Massachusetts, USA). Finally, the center of the corneal plane in space was indicated with the cross-over between two diode laser beams. The anterior surface of the cornea under exposure was centered on the cross-over between two diode laser (TIM-201-5D/650, New Taipei City, Taiwan) beams. The optical axis of the eye under exposure was adjusted to coincide with the beam optics. The beam propagation through the eye was calculated based on Hughes schematic rat eye model (Hughes 1979) and resulted in a close to collimated beam between lens and retina.
The power incident on the cornea was measured with a thermopile radiometer (L40(150)A-SH-V2, Ophir Optics, USA) calibrated by the manufacturer. The spatial irradiance profile of the beam incident on the cornea was measured by moving a pinhole (0.3 mm) through the beam. The spatial irradiance distribution on the cornea was found to be pseudo-flat top (Fig. 3).

Temperature measurement (Paper II-IV)

Temperature was measured with thermocouples (HYP0, OMEGA, USA) connected to an amplifier with an integrated analogue-digital converter (TC-08, OMEGA, USA). The thermocouples with an external diameter of 0.2 mm and a length of 25 mm, and LabVIEW (National Instruments, USA) were used for acquisition, processing and storage of measurement data.

Temperature-controlled cuvette (Paper IV)

The exposure setup consisted of a cuvette which has an inner water channel bypassing a central well (Fig. 4). A pump (MD-10, IWAKI CO., Japan) drives water from a temperature controlled water bath (VWB 12, VWR, Germany) in a closed loop through a heater, indirectly regulating the temperature in the lens cuvette filled with balanced salt solution (BSS).
Measurements of forward light scattering in the lens

The intensity of forward light scattering in the lens was measured on a dark field source (Fig. 5). Measurements were calibrated to a commercially available standard light scattering lipid emulsion of Diazepam (Stesolid Novum, Actavis AB, Sweden). In order to make measurements normal distributed, the concentrations of Diazepam are log transformed, as transformed Equivalent Diazepam Concentration (tEDC) (Söderberg et al. 1990).
**Macrophotography**

The morphology of the lens after IRR exposure was imaged by macrophotography using a dark-field illumination. A circular illumination source emitted the light at an angle from below the lens. Then, the lens image was recorded with a Nikon camera (Coolpix 4500, Nikon, Japan).

**Experimental procedure**

**Paper I-III**

The animals were anesthetized with ketamine 95 mg/kg plus xylazine 14 mg/kg intraperitoneally, ten minutes before exposure. The pupils of both eyes were dilated with tropicamide, 5 mg/ml. Five minutes after pupil dilation, the animals were unilaterally exposed to 1090 nm IRR within the pupil area. Temperature was recorded with thermocouples placed in the selected positions of the eye (Paper II and III). At the planned post-exposure time, the animal was sacrificed and the lenses were extracted for measurements of forward light scattering and macroscopic imaging.

**Paper IV**

The animal was sacrificed. Then one lens was extracted and put into the preheated BSS in the temperature-controlled cuvette and intensity of forward light scattering in the lens was measured for 5 minutes.

**Experimental Design**

**Paper I**

*Light scattering as a function of exposure time*

Altogether, 12 animals were equally divided into four exposure time groups, 5, 8, 13 and 20 s. The animals were unilaterally exposed to 197 W/cm$^2$ 1090 nm IRR. At 24 h after exposure, the light scattering was measured three times in both lenses of each animal and the lens was photographed.

*Light scattering evolution after in vivo exposure to near IRR*

The exposure time of 8 s was selected, based on the results of the first experiment. Then, altogether 16 animals were equally divided into four post exposure time groups. At 6, 18, 55 and 168 h after exposure to 1090 nm IRR of 197 W/cm$^2$, the intensity of light scattering was measured three times in both lenses of each animal and the lens was photographed.

**Paper II**

Altogether, 24 animals were equally divided into two groups. In one group, the temperature was measured by five thermocouples placed in the exposed eye; externally at the limbus, in the vitreous just behind
the lens and on the outer sclera next to the optic nerve, respectively and in the contralateral not exposed eye; at the limbus and on the outer sclera next to the optic nerve, respectively. In the other group, two thermocouples were placed externally at the limbus and on the sclera next to the optic nerve in both eyes. The animals were unilaterally exposed to 197 W/cm² 1090 nm IRR for 8 s, resulting in a radiant exposure of 1.6 kJ/cm². Immediately after the temperature descended back to the baseline (within 5 min after exposure), the intensity of forward light scattering in the lens was measured and the lens was photographed.

**Paper III**

Altogether, 80 animals were randomly divided into four radiant exposure groups of 10, 18, 33 and 60 minutes resulting in a total dose of 57, 103, 198 and 344 kJ/cm² respectively. All animals were unilaterally exposed to 96 W/cm² IRR at 1090 nm within the dilated pupil. During exposure, temperature was recorded at the limbus of the exposed eye and the cornea of the exposed eye was humidified. One week after exposure, light scattering in the lens was measured and the lens was photographed.

**Paper IV**

Altogether, 80 lenses from 80 animals were equally divided into four temperature groups, 37, 40, 43 and 46 ºC.

**Statistical parameters**

The significance limit and the confidence level were set to 0.05 and 0.95, respectively, considering the sample size.
Results and Discussion

Paper I

To study photochemical effect of near IRR, our strategy is to begin with near IRR induced thermal damage and to determine the threshold temperature for cataract induction. Then with control of temperature below threshold, we did the low irradiance long term exposure to further investigate cumulative photochemical effect.

Prior to elucidate the threshold temperature for cataract induction, it is necessary to firstly investigate the threshold dose at high irradiance short time exposure (Paper I). We found that the in vivo exposure to 197 W/cm\(^2\) 1090 nm IRR required a minimum 8 s for cataract induction (Paper I Figure 4) and there was approximately 16 h delay between exposure to 197 W/cm\(^2\) 1090 nm of 8 s and light scattering development in the lens (Paper I Figure 5, Table 2). Based on Hughes schematic rat eye model (Hughes 1979) and the attenuation coefficients of the human eye (Boettner & Wolter 1962), the maximum possible energy absorbed at exposure to 197 W/cm\(^2\) 1090 nm IRR for 8 s was calculated as 3.5 J in the lens. Considering a specific heat capacity of water of 4.18 J·g\(^{-1}\)·K\(^{-1}\), the estimated maximum temperature rise in the lens was around 25 °C. Besides the direct radiation absorption in the lens, the radiation absorbed in the retina might also cause a heat increase in the lens through heat diffusion. Thus, it is possible that the temperature increase in the lens due to the exposure to IRR led to denaturation of functional proteins and that it took some time for that damage to become biologically expressed.

Paper II

With the estimated threshold exposure (Paper I), Paper II aimed to determine the threshold temperature in the eye for cataract induction. The in vivo exposure to 197 W/cm\(^2\) 1090 nm IRR for 8 s was found to cause a temperature increase of 10 °C at the limbus and 26 °C close to the retina (Paper II Figure 2, Table 1). The temperature increase in the lens was probably higher than the 10 °C recorded at the limbus since there was a temperature increase on the outer sclera close to the optic nerve of approximately 26 °C. The rate constants estimated for the increase of temperature (Paper II Table 1) was of the order of 10 times higher than the rate constants estimated for decrease of temperature (Paper II Table 2). This asymmetry is seemingly explained by less heat conduction off the surface of the eye during temperature increase than heat conduction within the eye after the end of the exposure. The
finding that there was no increased light scattering in the lens soon after the exposure, implicates that the lens temperature rise due to the exposure does not cause immediate denaturation of lens proteins. The lack of increased light scattering shortly after the exposure is consistent with our previous finding that there is a 16 h latency before the onset of light scattering after in vivo exposure at the equivalent exposure conditions (Paper I).

**Paper III**

Since the threshold dose (Paper I) and the threshold temperature (Paper II) for 1090 nm IRR induced thermal lens damage were determined, Paper III aimed to further investigate if 1090 nm IRR induces cataract photochemically considering irradiance exposure time reciprocity.

The in vivo exposure to 96 W/cm$^2$ 1090 nm IRR was found to result in an averaged temperature elevation of 7 °C at the limbus of the exposed eye in all the radiant exposure groups (Paper III Figure 2, Table 1). With the currently used irradiances without the corneal cooling caused by the humidifying agent, the temperature elevation would probably have exceeded 7 °C. The finding that no significant light scattering (Paper III Figures 3 and 4) was induced one week after exposure illustrates that near IRR does not induce cataract provided that the temperature rise at the limbus is less than 8 °C.

The highest radiant exposure used in the current experiment when keeping the temperature rise at the limbus below 8 °C, was far higher than that claimed for photochemical cataract induction in rabbits by Wolbarsht et al (Wolbarsht et al. 1977; Wolbarsht 1978; Wolbarsht 1991) and that reported for photochemical cataract induction by Pitts et al (Pitts et al. 1980; Pitts & Cullen 1981). We reported a temperature rise below 8 °C, while no temperatures were given by Wolbarsht and Pitts. Our finding that despite using a considerably higher radiant exposure than Wolbarsht and Pitts, we did not observe any cataract development, suggests there was a temperature rise in the previous experiments (Wolbarsht et al. 1977; Wolbarsht 1978; Pitts et al. 1980; Pitts & Cullen 1981; Wolbarsht 1991). Thus, our observations strongly suggest that an exposure below 0.35 MJ/cm$^2$, 1090 nm IRR does not cause photochemical damage in the lens.

We previously showed that exposure on the cornea within the dilated pupil to 197 W/cm$^2$ of 1090 nm IRR requires at least 8 s to induce cataract and the 8 s exposure induced a temperature increase of 10 °C in the anterior segment (Paper I and II). The current findings that, 96
W/cm² 1090 nm IRR with 1 h exposure does not result in direct damage in the lens if the temperature rise is below 8 °C, then implies that IRR induced cataract is caused by indirect heat absorption in tissues surrounding the lens or alternatively possibly a local inflammatory reaction induced by temperature rise in tissues surrounding the lens, or both. Thus, our findings are consistent with the hypothesis that IRR causes cataract thermally (Verhoeff et al. 1915; Goldmann 1933) but inconsistent with photochemical effect suggested by Wolbasht (Wolbarsht 1991).

However, the possible reason for no photochemical effect of near IRR at 1090 nm is that 1090 nm is not the action spectrum for near IRR induced cataract. Therefore, other wavelengths within near IRR band need be investigated.

**Paper IV**

From our previous study (Paper I-III), it is known that a thermocouple probe damages the lens and decreases the heat load due to heat conduction in the probe. Fiber optic sensors are disturbed by near infrared waveband. Thus, it is important to develop a new method for temperature measurement without disturbance from temperature sensors (Paper IV). The Arrhenius equation models denaturation rate as a function of temperature. On the assumption that light scattering in the lens above threshold thermal exposure depends on denaturation of lens proteins, the Arrhenius equation can be applied to estimate the temperature exposure corresponding to a measured intensity of light scattering if the denaturation rate dependence on temperature is known (Paper IV Appendix 1).

The finding that the inclination coefficient for light scattering increase increased with temperature (Paper IV Figure 3, Table 1) agrees with the Arrhenius equation. With 20 additional measurements of inclination coefficients, the resolution is on the order of ±2 °C (Paper IV Figure 4). The confidence interval for the inclination coefficient as a function of temperature (Paper IV Table 1) and the individual estimates of the natural logarithm of the inclination coefficient plotted (Paper IV Figure 4) reflects a substantial variation of scattering-time response among lenses from different animals. Considering the Arrhenius equation, denaturation rate is directly dependent on temperature. Therefore, it is possible that a very small heat load over long time may accelerate cataract formation (Sliney 1986). The activation energy calculated for temperature-induced aggregation of protein in whole lens, 8.0±2.0 x101 kJ·mol⁻¹, based on the outcome
depicted in Figure 4 is in the range of what has previously been reported for temperature induced conformational change of \( \gamma \)-crystalline tryptophan (Borkman, Douhal & Yoshihara 1993), temperature-induced \( \alpha \)-crystalline aggregation (Maulucci et al. 2011), and chemically induced aggregation of \( \gamma \)F-crystallin (Das & Liang 1998).

In our current study the in vitro dependence of denaturation rate on temperature has been determined. Further, it is stimulating to determine the dependence of denaturation rate on heat load in vivo. Therefore, it would be necessary to measure denaturation rate as heat induced rate of back scattering. In vitro determined dependence of denaturation rate on temperature can be used to estimate the heat load induced lens temperature. Consequently, the relationship between temperature in the lens and in vivo heat load exposure of the eye can be determined. Then, the critical temperature in the lens can be estimated by in vivo exposures at incrementing heat load exposure of the eye with subsequent post-exposure measurements of permanent light scattering in the lens (Paper IV Figure 6). The experimentally determined critical temperature in lenses from warm-blooded animals like rats used in current experiment can be extrapolated to that in human lens.
Conclusion

- The threshold exposure time, for cataract induction after in vivo exposure to 197 W/cm\(^2\) IRR at 1090 nm within the pupil, is 8 s and the cataract is expressed with a time delay.

- In vivo exposure to 197 W/cm\(^2\) of 1090 nm for 8 s induces an exponentially declining temperature increase during exposure and an exponential decrease after exposure.

- IRR at 1090 nm causes cataract by a thermal mechanism, probably by indirect heat conduction from absorption in tissues surrounding the lens. There is no cataract development on the condition that the temperature increase at the limbus is below 8 °C.

- Applying the Arrhenius equation, the in vivo temperature in the lens can be determined retrospectively with sufficient resolution. The presented method for measurement of lens temperature during heat load allows temperature measurement without disturbance from the measurement sensor.

Further study

Establish if there is any empirical evidence for an action spectrum for near IRR cataract by repeating the same experiment as in Paper III with exposure to near IRR wavelengths other than 1090 nm.

Develop a device for in vivo measurement of back scattering in the lens (Fig 6).

![Fig 6 Schematic of device for in vivo measurement of back scattering in the lens.](image)
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References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)

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