

# Noninvasive Dosimetry and Monitoring of TTT Using Spectral Imaging

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## ABSTRACT

Transpupillary thermo therapy (TTT) is a slow (60 seconds) photothermal treatment of the fundus with a near-infrared (780-810nm) laser irradiating a large spot (0.5- 1. mm) on the retina. Due to high variability in ocular tissue properties and the lack of immediately observable outcome of the therapy, a real-time dosimetry is highly desirable. We found that fundus spectroscopy and spectrally-resolved imaging allow for non-invasive real-time monitoring and dosimetry of TTT. A 795nm laser was applied in rabbit eyes for 60 seconds using a 0.86mm retinal spot diameter. The fundus was illuminated with a broadband polarized light, and its reflectance spectra were measured in parallel and cross-polarizations. The fundus was also imaged in selected spectral domains. At irradiances that do not create ophthalmoscopically visible lesions the fundus reflectance increases at the wavelengths corresponding to absorption of the oxygenated blood indicating the reduced concentration of blood in the choroid. Vasoconstrictive response of the choroidal and retinal vasculature during TTT was also directly observed using spectrally-resolved imaging. At irradiances that produce ophthalmoscopically visible lesions a rapid reduction of the fundus reflectance was observed within the first 5-10 seconds of the exposure even when the visible lesions developed only by the end of the 60 second exposure. No visible lesions were produced where the laser was terminated after detection of the reduced scattering but prior to appearance of the enhanced scattering.

Keywords: TTT, fundus reflectance, dosimetry, spectral imaging, vasoconstriction

## 1. INTRODUCTION

Transpupillary thermo therapy (TTT) is an optical treatment of the fundus aimed at thermal stimulation of cells associated with expression of heat shock proteins (HSP). Differences in optical transmission, light absorption and choroidal blood perfusion lead to strong variations of the fundus temperature. First clinical results (TTT4CNV study) showed that in selected cases TTT leads to significant vision improvement, however in most cases it seems to be ineffective. Strong variability of the retinal temperature and the lack of a real-time dosimetry is most probably the main cause of the poor clinical results. We have found that spectroscopy of the fundus reflectance and its spectrally-resolved imaging allows for non-invasive dosimetry and real-time monitoring of the ophthalmoscopically invisible effects in the tissue during TTT.

From in vitro[1, 2] and in vivo[3, 4] studies it is known that the margin between an effective expression of HSP and a cellular death is quite small. Typically the HSP expression starts roughly at 85 % of the temperature elevation which causes irreversible cellular damage [1, 2]. The high variability of retinal absorption, choroidal blood circulation and ocular light transmission from patient to patient and even within one eye makes it difficult to ensure a successful treatment with TTT. Strong differences in temperature result in large variability and poor predictability of the clinical outcomes of TTT. Several minimally-invasive techniques have been developed to measure the laser induced retinal temperature increase in order to provide an online dosimetry system for TTT.

Temperature induced vasodynamic reaction of choroidal blood vessels is a known, but poorly understood phenomenon. Earlier studies[5-7] indicate that choroidal vasodilatation can be induced as a response to intense light. This reaction can be found even in the unexposed fellow eye[5] however in that case it is restricted to the macular region[7]. Vasodilation increases heat removal from the optically heated retina, but plays a significant role only for the heating times longer than several seconds. A recent human study has shown a decrease in the choroidal blood flow and blood concentration upon heating of the eye, however the retinal blood flow increased at the same time[8]. Surprising is the fact that a choroidal vasodynamic reaction can be induced by the temperature elevation but not by hyperoxia, which induces vasoconstriction only in the retinal blood vessels[9]. Pericytes which are responsible for tone reaction in the retinal blood vessels are absent in the choroid.

Fundus reflectance is widely used in ophthalmology providing information about fundus pigments and cellular scattering. Besides the retinal light scattering and absorption, the retinal and corneal birefringence plays an important role in fundus reflectance if polarized light is used. Fundus reflectance at single wavelengths was also introduced to monitor the retinal photocoagulation [10-13]. In these studies the fundus reflectance increase during the laser pulse correlated well with the extent of the retinal burn seen ophthalmoscopically. No change of reflectance was found for the sub-threshold lesions, i.e. with the laser power below the visible retinal damage threshold.

In this study we use spectrally-resolved fundus reflection to evaluate the temperature induced tissue response during TTT *in vivo*.

## 2. MATERIAL AND METHODS

### 2.1. Setup

A simplified sketch of the experimental setup is shown in Figure 1. A diode laser operating at 790 nm wavelength (prototype, Lumenis Inc., Santa Clara, CA) was coupled to an ophthalmic slit lamp (Zeiss, SL10) using a laser adapter (LaserLink, Lumenis). The last mirror of the LaserLink was additionally coated with a thin layer of gold to increase the reflectivity in the IR spectral range. The laser was modified to allow turning off the aiming laser during the exposures, which would interfere with the reflection measurements. Distribution of the laser power in the focal spot (1.3mm in diameter in air) was measured with a CCD camera (Retiga 1300i, Q-Imaging) and had a nearly tophat profile. With a Mainster Standard contact lens on a rabbit eye the beam diameter on the retina was reduced to 0.86 mm. The Mainster Standard contact lens was chosen because it maintains polarization of light. Before each experiment the output laser power was calibrated using a laser power meter (LaserStar, Ophir Optronics Inc., Jerusalem, Israel). Laser pulses with 60 sec in duration and exposures of 1.7 to 17.0 W/cm<sup>2</sup> were applied to the retina. Ophthalmoscopically visible marker lesions were used to provide orientation for the non-visible exposures. About 12 to 18 lesions were placed in each eye. Immediately after the exposure an ophthalmologist evaluated visibility of the lesion.

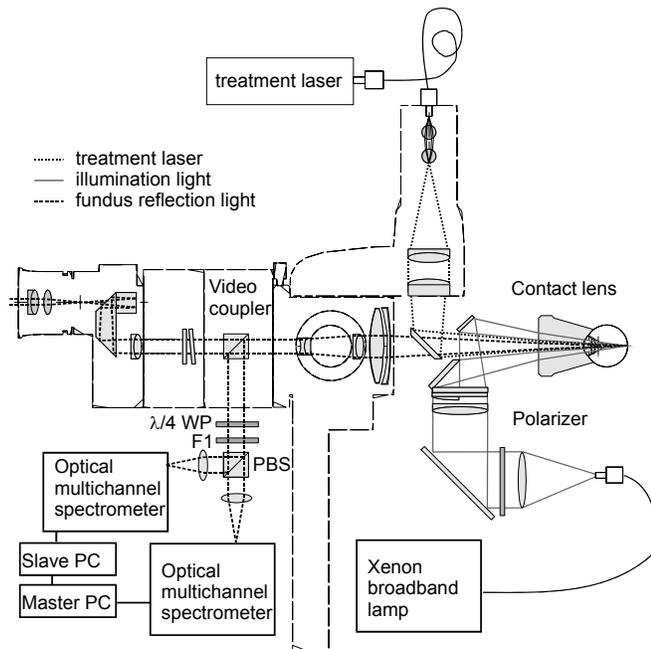


Figure 1: Experimental setup for spectroscopic fundus reflection measurements during TTT. (F1=short pass filter,  $\lambda/4$  WP= broadband quarter waveplate, PBS=polarized beam splitter)

For the fundus illumination the light from the fiber coupled xenon broadband lamp (Xe-lite, Ophthalmic Technologies Inc, Toronto, Canada) was polarized with a thin film polarizer and coupled to the slitlamp illumination path. The retinal illumination spot diameter was 1.5mm. The back-scattered light from the fundus was coupled into the spectrometer (USB2000, Ocean Optics Inc.) using the video port on the slit lamp. To suppress the laser light the scattered white light

was transmitted through a pass filter of 400nm-750nm. To compensate for the birefringence in the cornea and the retina the scattered light passed through an adjustable broadband quarter wave plate (Thorlabs). Then the beam was split by a polarizing beam splitter cube (Glan-Thompson, Newport) into parallel and crossed polarizations relative to the incident illumination polarization. The scattering spectra in both polarizations were analyzed using two fiber-based optical multichannel spectral analyzers (USB2000, Ocean Optics). Typically 20ms integration time was used in these spectrometers, with averaging over 15 consecutive spectra. To ensure simultaneous acquisition in both spectrometers the data was read out by two synchronized computers. The data acquisition and PC synchronization was controlled by LabView software (National Instruments). A barium sulfoxide scattering sample was used for spectral calibration of the setup.

For spectral fundus imaging the spectrometer for the cross-polarization was replaced with a cooled CCD camera (Retiga 1300i, reduced to 634\*512 pixels, 10 bit, Q-Imaging). An additional dielectric filter provided a spectral selection between 530 nm and 570 nm. The CCD was controlled and the images were acquired digitally using a PC, having an integration time of 500-1000 ms.

## 2.2. Animals

The treatment of experimental animals in this study was in compliance with the Association for Research in Vision and Ophthalmology on the Use of Animals in Research and approved by the Institutional Review Board of Stanford University. Rabbits from New Zealand Red breeds were used. The animals were anesthetized with the combination of ketamine hydrochloride (35mg/ml), xylazine hydrochloride(5mg/ml) and glycopyrrolate (0.1mg/ml) through intramuscular injection. The eye was isolated and a peribulbar string was placed around the eye bulb. The rabbits were positioned in front of the slit lamp, pupil was dilated and the laser contact lens was placed on the cornea using methylcellulose as a contact gel. To avoid the eye movement due to breathing the contact lens and the head of the animal were mechanically fixed during the experiment.

## 2.3. Image analysis

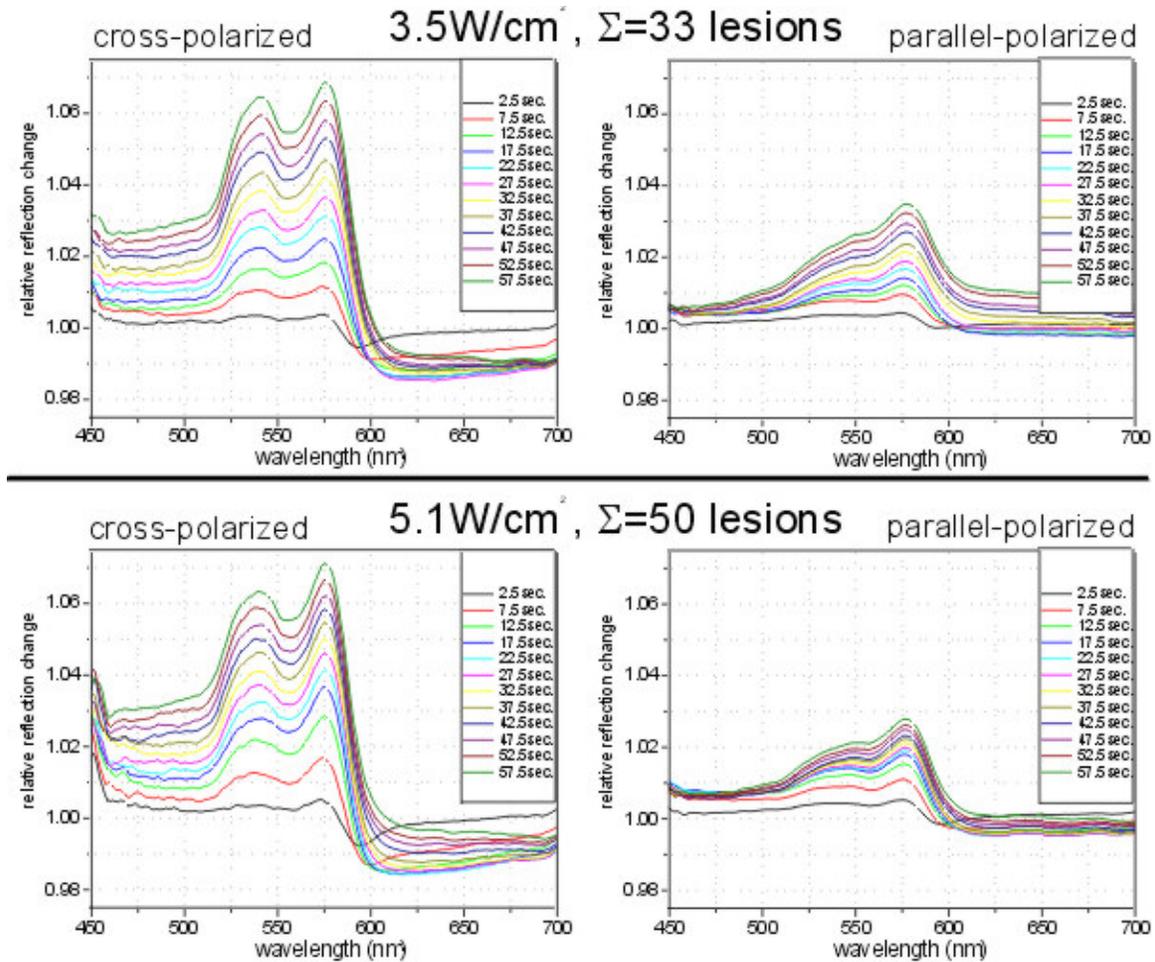
The image acquisition started 30 second prior to the laser exposure. The fundus image recorded prior to the laser exposure was used to normalize the following images. The change of the fundus reflectance intensity (pixel by pixel) was converted into the color coded intensity frames.

## 3. RESULTS AND DISCUSSION

### 3.1. Spectral fundus reflectance during TTT exposure

The spectra of the fundus reflectance have been measured for both polarizations during the TTT exposures. Figure 2 shows the averaged and normalized cross- and parallel-polarized fundus reflectance spectra at different power levels. The spectra were normalized on the fundus reflectance spectra acquired prior to the laser exposure. The right graph shows the cross-polarized, the left the parallel-polarized spectra. For each irradiance level the graph was averaged over the multiple measurements as indicated by the number of lesions on each plot. Every legend label indicates the corresponding time point of the laser exposure. All lesions were ophthalmoscopically invisible. The standard deviation for all spectra was about 15 %; the error bars are not shown in the graphs for better clarity of the plot.

Although all the lesions in Figure 2 were ophthalmoscopically invisible, significant changes in the fundus reflectance spectra could be detected. Two spectral peaks - at 540 nm and 575 nm - increased at all irradiance levels, with the 540nm peak being always lower than that at 575 nm. The response was more pronounced in the cross-polarization. At the first time point of 2.5 seconds there was always a dip at 590 nm that either disappeared or was obscured by other



**Figure 2.** Relative spectral changes during TTT exposure at different power levels. Each graph shows the averaged result of multiple measurements at a distinct power level. The right graph reflect the cross-polarized, the left the parallel-polarized spectra. The graph legend indicates the corresponding time point of the laser exposure. All lesions were ophthalmoscopically invisible. Error bars are not shown in the graphs to gain a better readability. The standard deviation for all spectra is about 15 %.

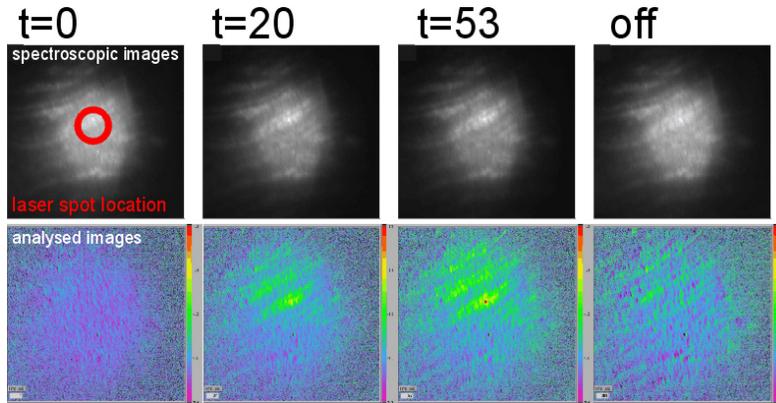
effects after about 10 seconds of exposure. Besides the peaks and dips there was a general increase in scattering in the range of 450 nm to 590 nm and decrease between 590 nm and 700 nm.

The two detected spectral peaks at 540 nm and 570 nm correspond well to the absorption peaks of oxy-hemoglobin. Since the rabbit retina is avascular the blood related spectral changes can only originate from the choroidal vasculature. This conclusion is further supported by the fact that the response is stronger in the cross-polarized spectrum, which is dominated by the multiple-scattered light originated at the deeper fundus layers. Thus increase of the back-reflection at the wavelengths corresponding to absorption in oxy-hemoglobin is due to decrease of oxy-hemoglobin concentration in the choroidal blood due to choroidal vasoconstriction. This interpretation is further supported by a recent human study that showed temperature induced choroidal vasoconstriction[8]. Although the spectral changes appeared in all exposures, the amplitude of these effects varied significantly in the range from 3 % up to 20 % from exposure to exposure. Such strong variability of the response and especially the lack of correlation with formation of the visible lesions made it impossible to use the vasodynamic retinal response for TTT dosimetry.

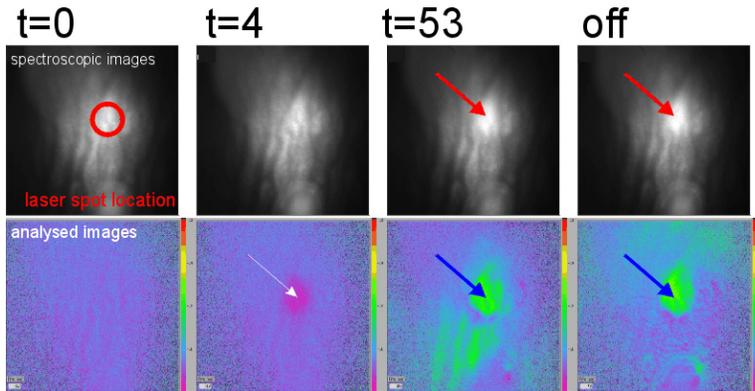
### 3.2. Monitoring of TTT by spectral fundus imaging

Figure 3 shows a typical example of the original spectrally-filtered images (in 8 bit grey scale) and the analyzed images (in a false color scale) at different time points for an ophthalmoscopically invisible lesion. The laser-induced

vasoconstriction-related changes are not clearly visible in the original spectroscopic images. The analyzed color-coded images, which reflect the relative intensity changes clearly indicate the increased signal (green and yellow color code) in between the big choroidal blood vessels. This indicates that the choroidal micro-vasculature constricts most.



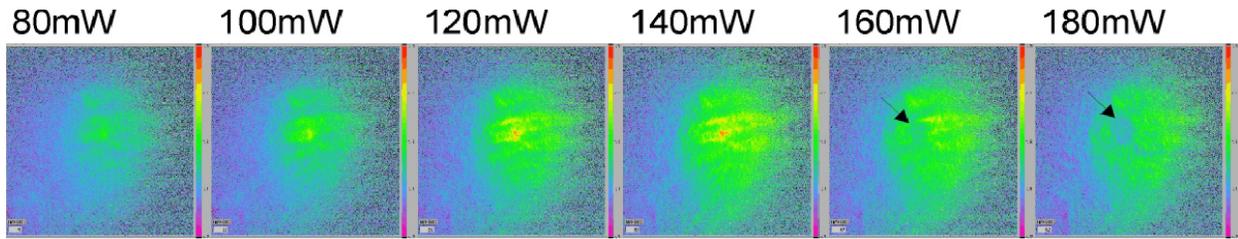
**Figure 3.** Original spectroscopic images (grey scale) and analyzed images (color-coded) at different time points for an ophthalmoscopically invisible lesion.



**Figure 4.** Original spectroscopic images (grey level) and the analyzed image data (color-coded) at different time points for an ophthalmoscopically minimally visible lesion.

In case of a minimally-visible lesion (Figure 4) the onset of thermal denaturation can be seen in the original spectroscopic images at the end of the laser pulse (red arrows), at  $t=53$  seconds. However, in the analyzed images a decrease of the scattering signal (white arrow) in the exposed area can be detected after only a few (4) seconds. At a later time point the choroidal vasodynamic reaction can be seen (as indicated by the increased color values) around the laser spot. The onset of denaturation (increased scattering) can also be seen in the analyzed images (blue arrows) at  $t=53$ seconds. When laser exposure was terminated after detection of the decreased scattering but prior to onset of increased scattering the visible burn did not develop. Thus reduction in scattering can serve as an early warning sign of the immanent tissue damage and can be used for a real-time TTT dosimetry. Since scattering is decreased in a round pattern which does not correlate with the structure of the vascular bed, the origin of this effect is probably not hemodynamic.

In a few spots the laser power was ramped up every 20 seconds from 80mW to 180mW with 10 seconds exposure time. The displayed analyzed images were taken at the different power levels direct at the end of the 10 second laser exposure. Starting at 160mW but clearly more pronounced at 180mW the analyzed images indicate a signal reduction in the exposed area (black arrows pointing at blue round spots) on top of the hemodynamic increase of the tissue scattering (large green areas). This lesion was ophthalmoscopic invisible after the exposure as the laser was turned off after the appearance of the reduced light scattering in the exposed spot.



**Figure 5.** Analyzed images each at the end of the 10 second exposure for different laser power settings. Note, the color change in the exposed area (black arrows) starting at 160mW which becomes more pronounced at 180mW.

#### 4. CONCLUSION

This study demonstrates that fundus reflectance spectroscopy allows for monitoring the choroidal vasoconstriction by detecting the increased reflectance at oxy-haemoglobin absorption peaks. However, this vasodynamic response is highly variable and not suitable for dosimetry. Spectral imaging allows for visualizing the vasodynamic response during the laser exposure. Decreased scattering can serve as an early warning of tissue damage and can be used for a real-time TTT dosimetry. The origin of the rapid decrease in the fundus scattering remains unclear and requires further investigation.

#### ACKNOWLEDGEMENT

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