Ultraviolet analysis of donated corneas: a portable prototype

APPLIED OPTICS, v.49, n.26, p.4890-4897, 2010
http://producao.usp.br/handle/BDPI/14662

Downloaded from: Biblioteca Digital da Produção Intelectual - BDPI, Universidade de São Paulo
Ultraviolet analysis of donated corneas: a portable prototype

Victor A. C. Lincoln,¹ Liliane Ventura,¹,* and Sidney J. Faria e Sousa²

¹Department of Electrical Engineering, EESC University of São Paulo, São Carlos, São Paulo, Brazil
²Department of Ophthalmology, Otorhinolaringology, Head and Neck, FMRP University of São Paulo, Ribeirão Preto, São Paulo, Brazil
*Corresponding author: lilianeventura@usp.br

Received 4 February 2010; revised 14 July 2010; accepted 4 August 2010; posted 4 August 2010 (Doc. ID 123799); published 3 September 2010

As technology improves human vision, some procedures currently performed may be causing a decrease of the natural UV protection of the cornea. A portable dual beam system prototype was assembled for physicians for clinical studies of these effects on the corneas endowing two types of 300–400 nm evaluations: 1, regularly donated corneas and 2, simulating refractive keratectomy by corneal lamellae removal. The system performs 500 measurements/s, providing \( \frac{1}{C_6} \times 0.25\% \) precision for the transmittance. The measurements performed on the prototype are 95% in agreement with Cary 17 and HR4000CG-UV-NIR Ocean Optics spectrophotometers. Preliminary studies on cadaveric corneas demonstrate that, as the stromal layer is reduced (~150 \( \mu \)m depth), there is significant loss—an average of 7.1%—of the cornea’s natural UV protection. The prototype is being tested in an eye bank for routine evaluation of donor corneas. © 2010 Optical Society of America

OCIS codes: 170.0170, 330.5380, 300.6540.

1. Background

Ultraviolet radiation may cause several damages to the human eye. However, the cornea has natural UV-A (320–400 nm) and UV-B (290–320 nm) protection to avoid further exposure of the inner optical components of the eye.

The corneal tissue is transparent and avascular, which permits incident light to transit to the more posterior ocular structures. As the cornea is continuously exposed to a wide spectrum of light, including the ultraviolet (UV) range, which is an environmental stress factor that generates free radicals and reactive oxygen species harmful to most cells and tissues [1], it is susceptible to damage from reactive oxygen species, because the cornea absorbs most of the UV entering the eye.

Although the cornea and aqueous humor of the human eye absorb significant amounts of UV-A and UV-B radiation; nearly 50% of radiation at 320 nm is transmitted through to the lens [2].

Transmittance decreases substantially below 320 nm, so that less than 1% is transmitted below 290 to 300 nm. Lembares et al. [3] showed that, although transmittance decreases for wavelengths below 300 nm, absorption in the band between 200 and 300 nm is weak. High corneal absorption occurs only for wavelengths below 220 nm. However, laboratory experiments on animals indicate that the shorter wavelength UV-B (i.e., below 290 nm) is perhaps 250 times more effective than the longer wavelength UV-B (i.e., 320 nm) in inducing cataract, which is an opacity in the normally transparent lens of the eye that produces an impairment of vision [4].

Recent studies show that exposure of the cornea to UV irradiation induces pathological changes in its structure [5]. The UV rays may cause corneal swelling, lens opacity (cataract), and damage to the retina. The UV exposures of the cornea to high levels or low levels of irradiation frequently cause irreversible damage, resulting in keratopathy affecting the anterior
part of the epithelium and the stroma. It has become evident in recent years that removal of the epithelial layer of the cornea makes the internal structures of the eye more susceptible to damage induced by UV irradiation [5]. This is particularly important, because the photorefractive keratectomy (PRK) has recently become a common ophthalmic procedure.

PRK is the application of ultraviolet high-energy photons (193 nm) generated by an argon fluoride excimer laser to the anterior corneal stroma to change its curvature and, therefore, correct a refractive error (myopia, hyperopia, and astigmatism, overall) [6]. The physical process of remodeling the cornea by PRK is called photoablative. In these types of surgery, tissue removal is performed for vision correction.

According to the Munnerlyn formula [7], 14 μm of stromal tissue is removed for each dioptr of vision to be corrected. Therefore, evaluating the UV-A and UV-B corneal natural protection, after tissue removal, is essential.

Furthermore, personnel in the eye banks are concerned with evaluating the cornea regarding its natural UV protection, since this kind of evaluation is not routinely performed. As PRK has currently become popular, patients who have undergone this procedure are not reported to the eye banks by the donor family. It is usually difficult (in some cases, it is impossible) to identify the corneas that have been submitted to PRK procedure. These corneas are presumably thinner (thickness measurements are not performed in the donor cornea, either) and there is an assumption that the natural UV absorption of the tissue might be altered.

Aiming to perform the evaluation of the UV-A and UV-B transmittance as corneal thickness decreases, reproducing the consequences of tissue removal from the PRK surgery, we have developed a portable UV-A/UV-B measuring prototype for evaluating donated corneas, as cornea lamellae are removed by a microkeratometer.

2. Methodology

Ethical Committee Statement: All the experiments performed in this work for the human corneas have been submitted to Conselho Nacional de Ética em Pesquisa—The National Council for Ethics in Research (CONEP) and has been approved under registration number HCRP 6788/2009 by the Ethical Committee at Hospital das Clinicas de Ribeirão Preto. The study was conducted in accordance with the provisions of the Declaration of Helsinki for experimentation involving human tissue.

The most proficient method for evaluating the mean transmittance of the corneal tissue is spectroscopy. However, in order to provide a portable device to be used in the eye bank, which supplies an instant transmittance of the corneal tissue, in a very intuitive way of performing the measurement, we have projected a dual beam device with special features to hand out the required needs of this kind of experiment.

A. Hardware Setup

The dual beam setup improves the control of fluctuations in the system, avoiding undesired errors in the measurements, as learned from a previous single beam system developed from some of the authors of this work [8,9].

Prior to developing the prototype, we evaluated 15 corneas in order to establish the working range for this study. The corneas elected for this experiment were usually rejected by positive serology, such as HIV and hepatitis, but had their optical properties intact. The protocol consisted of removing the cornea from a donor eye and then preserving it in Optisol. Its optical transmittance was evaluated by a USB Ocean Optics spectrophotometer, calibrated with a holmium oxide solution (Ho3O4) in the UV range, using a UV source in the range of 180–410 nm. Peaks at 333.8, 360.8, 385.8, and 418.5 nm were used for calibration (0.2 nm resolution).

Subsequently, the transmittance of the tissue was measured at the spectrophotometer for each of the following steps: 1, removal of the epithelium layer; 2, removal of the endothelium layer; and 3, removal of lamella of 150 μm using a surgical microkeratometer (measurements were performed after the removal of the lamella, simulating the photoablative). We observed that no alterations were detected in the 180–300 nm range. However, alterations in the range of 300–400 nm required attention. Therefore, the working range for the prototype was from 300 to 400 nm.

The optical system consists of a UV source (OSRAM 4 W fluorescent lamp, 300–450 nm emitting range), which permits the transit of the radiation toward two identical GaP sensors (EPD 365—spectral sensitivity at the 245–400 nm range; active area of 1.2 mm² and output current of the order of μA). The emitting spectrum of the UV source and the sensitivity spectrum of the UV sensor are presented in Figs. 1(a) and 1(b). The UV source is limited by a central aperture providing the same amount of radiation to be delivered to both sensors.

The radiant power on the cornea surface is 0.100 mw/cm², which is adequate for this kind of measurement, causing no damage to the endothelial cells. Wollensak et al. stated that [10] endothelial cells are not damaged in corneas with thickness greater than 400 μm, treated with surface epithelial UV-A irradiance less than 2.62 mW/cm² (4.7 J/cm²). Figure 2 illustrates the schematics of the setup: “(1)” is designated to be the reference sensor, and “(2)” is the effective measuring sensor.

The conducted experiment consists of positioning the cornea in front of the measuring sensor. The reference sensor is free of any obstruction. The UV source irradiates the two optical paths, as presented in the diagram of Fig. 2. The portion of the beam that passes throughout the cornea and reaches the photodiode provides the tissue transmittance.
The sensors measure simultaneously the delivered UV radiation, and their signals are divided to provide the total amount of light transmitted by the cornea, presenting the transmittance as percentage.

The analog electronics are composed by the amplifier stages of the type TEE transimpedance. The referred stages perform the conversion of the current generated by the photodiodes into voltage with tunable gain that will later be analyzed by the analog-to-digital (A/D) converter.

The digital part of the system consists of a microchip—PIC18F4550—and of a digital potentiometer of 100 kΩ. The PIC microcontroller that executes the A/D conversion performs all the mathematical data and manages every USB communication of the embedded system with a notebook.

The A/D conversion is executed by the 10 bit integrated converter and 5 V reference, supplying approximately 5 mV/bit of resolution in the measurements, which correspond to 0.1% of resolution for the present scale.

The data acquisition is performed 500 times/s, resulting in a simple arithmetical average \( \bar{y} \) as in Eq. (1):

\[ \bar{y} = \frac{\sum_{i=1}^{500} A_i}{500}. \]

\( A_i \) is the nominative value of measurement sensor (2) and \( R_i \) is the nominative value of reference sensor (1). Figure 3(a) presents a picture of the system itself, Fig. 3(b) presents the holder for the corneas, and Fig. 3(c) presents the LCD display.

As previously mentioned, the system was built so that both sensors (reference sensor and cornea measuring sensor) are delivered by the same amount of radiation, at the same angle, and from the same spot area, simultaneously, in order to avoid fluctuations from the signal of the grid and from natural heating of the lamp. However, the lamp requires 10 s to stabilize, which allows the system to work properly.

It was also mandatory that the whole area of the sensors should be covered by UV radiation. So as to avoid the focusing of the UV rays by the cornea, which has a high refractive power (order of 44.76 D; radius of curvature of the order of 7.259 mm front and 5.585 mm back [11]), the UV source and the UV sensors were closely positioned to the corneas. Furthermore, the cornea holder is half of a stainless

---

**Fig. 1.** (a) OSRAM UV lamp emission spectra and (b) spectral responsivity of the EPD 365 sensor.

**Fig. 2.** Schematics of the setup.

**Fig. 3.** (Color online) (a) Picture of the system itself, (b) detail of the cornea being positioned for tests, (c) LCD display.
steel sphere, with a radius of curvature of 7.895 mm and a 3 mm central hole, to allow the evaluation of the central portion of the cornea. Thus, the system works as if the cornea had parallel faces at these distances and area limitation.

1. **Calibration of the System**

The system requires a fine adjustment for the transimpedance amplifier gain in order to achieve the correct reference calibration of 100%. The fine adjustment is done by the digital potentiometer, which is constantly adjusted by the microcontroller to specific commands, increasing or decreasing its internal resistance in accordance with the required gain.

The C language programmed embedded software of the microcontroller establishes the USB connection and allows the control of the hardware using a notebook. The transmittance is presented on the notebook’s screen. The software saves all the information and extracts to “txt” file, as an organized table, allowing further analysis of the data. Additionally, the transmittance is also presented at the LCD display.

### B. Manipulating the Cornea and Tissue Preparing

Human corneal rims \( n = 16 \) were obtained from cadaveric eyes within up to 24 h after preservation. The eyeballs were transported and stored in a humid chamber at 10 °C, and then the corneas were removed.

The corneas used in this research were carefully selected. They were corneal tissues rejected by the eye banks due to their pathological positive results such as HIV and hepatitis, but with required optical conditions, such as good transparency evaluated on slit lamp microscopy, adequate endothelial cells counting (over 2000 cels/mm²), undamaged epithelium, and no scars. The time of death was also a factor to be considered.

As the cornea was elected to be tested in our prototype, it was removed from the eyeball and preserved in Optisol solution, the endothelium cells were recounted, and, finally, the cornea was ready to be tested. The cornea was washed out with saline solution in order to avoid residual Optisol solution (which absorbs UV radiation). It was tested in the
prototype and then immediately evaluated on the Ocean Optics HR4000CG-UV-NIR spectrophotometer. The light source was the same for both systems—prototype and spectrophotometer.

The protocol used for testing the UV protection of the cornea as a simulation of the photorefractive keratectomy tissue removal was:

2. Removal of the epithelial layers—the epithelium was carefully scraped off with a surgical blade.
3. Removal of the endothelial layer.
4. Finally, the remainder of the cornea was sectioned. Lamella of 150 μm thickness was removed with a microkeratometer (MASIK).

Transmittance measurements of the remaining corneal tissue were performed on the prototype.

Forty control corneas have been analyzed in both systems. The Ocean Optics HR4000CG-UV-NIR spectrophotometer was used due to its portability and straightforward measurements with the UV transmission optical fiber cable.

3. Results

Several acrylic samples have been tested and then compared to spectroscopic UV transmittance measurements performed in a Cary 17 Olis upgraded spectrophotometer to ensure the precision of the developed prototype. The average transmittance obtained for the samples at the spectrophotometer were moreover compared to the transmittance obtained on the prototype, providing consistent results. These measurements were performed in these types of samples to ensure that the elapsed time between tests over the two systems would not interfere with their optical properties, such as, for the human cornea, drying out with time.

Figure 4 presents one of the standard samples being measured in our system. Figure 5 presents the correlation between our system and the Cary spectrophotometer. The scatterplotting for the eight tested samples was obtained for the measurements.
ments performed on the prototype and at the spectrophotometer. Figure 6 presents the scatterplot for the acrylic samples with different thicknesses.

Also, difference plotting (Fig. 7) was obtained for the acrylic samples. In this plotting, the bias (visual difference between both systems) and the inaccuracy (plotting dispersion with reference to the average value) are shown. The agreement for the prototype measuring acrylic samples is in the 95% limits. As for the 40 control corneas, Table 1 presents the UV transmittance of the 16 human corneas as some layers from the cornea are removed. We compared these transmittance measurements in both systems, immediately after preservation, with no tissue removal, and obtained their correlation factor, as presented in Fig. 8.

Figure 9 shows the correlation diagram between the prototype and the Ocean Optics HR4000CG-UV-NIR spectrophotometer for the corneas submitted to steps 1-4 (layers and tissue removal) of the protocol previously described. The scatterplots for the cornea—intact and with tissue removal—are presented in Figs. 10 and 11. The difference plots are presented in Figs. 12 and 13. They show that both systems are 95% in agreement for corneal tissue UV transmittance measurements as well as they are for the acrylic samples. Although both systems are 95% in agreement, there is a wider range of limit between the prototype and the spectrophotometer as tissue is removed, as can be noticed in Figs. 12 and 13. That is due to the fact that tissue deteriorates as it becomes thinner. The histogram of the differences corroborates that the distribution of the measurements in the 95% limit of agreement between the two systems—the prototype and the spectrophotometer (Figs. 14 and 15)—is adequate.

4. Discussion

Preliminary results in 16 corneas have shown that the natural UV protection of the corneas drastically decreases as the lamellae are being removed, as observed in Table 1. The 95% agreement between both
systems indicates the reliability of the prototype if the histogram distribution is observed.

Regarding the wider range of limit (0.3–3.9) on the chart from Fig. 13 compared to the range in Fig. 12 (0.60–2.15), it can be attributed to the degradation of the tissue. As the lamellae are removed, the tissue becomes thinner and difficult to work with, regarding its centralization in the system and trying to avoid crimps. Nevertheless, it was possible to build a reliable system for the eye bank testing the corneas, as well as providing means for the clinicians to carry out experiments on the natural UV protection of the donated corneas. The prototype is a straightforward system for the clinician, with a learning curve of a few minutes; therefore, significant data must be obtained in the next months.

The accuracy of the system is 0.25%, which is in agreement with the accuracy required for these types of measurements, which is 0.5% [2]. The preliminary data show that, as the lamellae are removed, the natural protection of the corneas decreases, as well.

It is evident in Table 1 that each corneal layer influences some quantity of UV absorbance. Our results demonstrate the modest influence of the endothelium layer—an average of 2.1%, which is a considerable decrease as the epithelium (~10 μm of the corneal tissue) is removed (an average of 7%). However, this layer is able to restore itself in a few days, since this layer has a high density of DNA cells, and the strong, most important decrease in the UV absorbance of the cornea as the stroma is removed (~150 μm depth)—an average of 7.1%.

It should be considered that these are still preliminary results and conclusive statements for these clinical trials are still premature. Substantial clinical data should be available in the next months.

### 5. Conclusion

The system has presented a good performance for a portable prototype and straightforward use for the clinicians and personnel from the eye bank. The 0.25% instability error in transmittance is adequate in providing reliable results. The timing for warming up the system is also very satisfactory for the eye bank (10 s), and the parts that are in contact with the corneas are all able to be removed and sterilized.

As preliminary results from this work, the 16 tested human corneas demonstrate that, as the stromal layer is reduced, there is significant loss of the natural UV protection of the cornea. A larger sample of corneas is undergoing tests, and statistics will be available shortly in order to conclude about the degradation on the cornea’s natural UV protection as photoablation is performed and to establish the correspondence of loss of natural UV protection as a function of reduction of the stroma. Therefore, we will be able to understand the influence on the cornea’s natural protection relative to the small amount of tissue removed in the PRK procedure and its relevance.

Because many cornea donors may have an abnormal thinning of the cornea, either by an inherited disease such as keratoconus or an acquired condition by means of a photorefractive keratectomy, and this fact is frequently missed by the current eye bank examination of the donor tissue, this prototype could be used as an eye bank screener for corneal ectasies.

Tests will be done in the following months using this prototype as part of the eye bank protocol to follow up the outcome that the loss of natural UV protection might bring for the recipient patient. As previously mentioned, since currently, many donors have been submitted to photorefractive keratectomy

---

Table 1. UV Transmittances of Human Corneas for Steps 1-4 of the Protocol

<table>
<thead>
<tr>
<th>Cornea</th>
<th>Endothelium Cells Counting (cells/mm²)</th>
<th>Thickness (μm)</th>
<th>In Vitro Corneal Tissue Epithelium Removed</th>
<th>Endothelium Removed</th>
<th>Lamella Removed (150 μm ± 15 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epithelium Removed</td>
<td>Endothelium Removed</td>
<td>Lamella Removed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68.9</td>
<td>69.7</td>
<td>71.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61.2</td>
<td>66.7</td>
<td>69.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.1</td>
<td>67.3</td>
<td>69.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60.9</td>
<td>70.0</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.9</td>
<td>71.7</td>
<td>73.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60.3</td>
<td>73.4</td>
<td>75.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51.7</td>
<td>63.9</td>
<td>65.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.8</td>
<td>60.3</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63.1</td>
<td>71.7</td>
<td>74.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.2</td>
<td>66.2</td>
<td>69.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63.4</td>
<td>69.2</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>64.2</td>
<td>71.1</td>
<td>73.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.5</td>
<td>69.8</td>
<td>71.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58.0</td>
<td>62.1</td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>67.9</td>
<td>72.1</td>
<td>74.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61.4</td>
<td>71.3</td>
<td>73.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>61.5</td>
<td>68.5</td>
<td>70.7</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>55.9</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Min</td>
<td></td>
<td></td>
<td>4.5</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Max</td>
<td></td>
<td></td>
<td>68.9</td>
<td>73.4</td>
<td>75.5</td>
</tr>
</tbody>
</table>

---

4896  APPLIED OPTICS / Vol. 49, No. 26 / 10 September 2010
and this procedure has never been mentioned by the donor family to the eye bank, depending on further consequences (earlier cataract, etc.), this might become an additional regular procedure for corneal evaluation in the eye banks.

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo—Foundation for Research Support of the State of São Paulo (FAPESP)—for the financial support for this research; to Lino Misoguti from the Institute of Physics in São Carlos, University of São Carlos (USP), who kindly loaned the spectrophotometers; and to the eye bank of the Hospital das Clínicas de Ribeirão Preto for performing all the cornea management and dissection.

References


