Terahertz Scanning for Evaluation of Corneal and Scleral Hydration

DOI: 10.17691/stm2018.10.4.17
Received February 2, 2018

E.N. Iomdina, DSc, Professor, Chief Researcher[1];
S.V. Seliverstov, PhD, Senior Lecturer[2];
A.A. Sianosyan, PhD Student[1];
K.O. Teplyakova, PhD Student[2];
A.A. Rusova, Student[2];
G.N. Goltsman, DSc, Professor, Head of the Department of General and Experimental Physics[2]

[1]Moscow Helmholtz Research Institute of Eye Diseases, 14/19 Sadovaya-Chernogryazskaya St., Moscow, 105062, Russia;
[2]Moscow Pedagogical State University, 1/1 Malaya Pirogovskaya St., Moscow, 119991, Russia

The aim of the investigation was to study the prospects of using continuous THz scanning of the cornea and the sclera to determine water concentration in these tissues and on the basis of the obtained data to develop the experimental installation for monitoring corneal and scleral hydration degree.

Materials and Methods. To evaluate corneal and scleral transmittance and reflectance spectra in the THz range, the developed experimental installations were used to study 3 rabbit corneas and 3 scleras, 2 whole rabbit eyes, and 3 human scleras. Besides, two rabbit eyes were studied *in vivo* prior to keratorefractive surgery as well as 10 and 21 days following the surgery (LASIK).

Results. There have been created novel experimental installations enabling *in vitro* evaluation of frequency dependence of corneal and scleral transmittance coefficients and reflectance coefficients on water percentage in the THz range. Decrease in corneal water content by 1% was found to lead to reliably established decrease in the reflected signal by 13%. The reflectance spectrum of the whole rabbit eye was measured in the range of 0.13–0.32 THz. The study revealed the differences between the indices of rabbit cornea and sclera, as well as rabbit and human sclera.

There was developed a laboratory model of the installation for *in vivo* evaluation of corneal and scleral hydration using THz radiation.

Conclusion. The preliminary findings show that the proposed technique based on the use of continuous THz radiation can be employed to create a device for noninvasive control of corneal and scleral hydration.

Key words: cornea; sclera; THz radiation; corneal hydration; backward-wave oscillator; avalanche transit-time diode (IMPATT diode).

Introduction

Water content in biological tissues is crucial for the living organism as a whole and for normal functioning of its individual structures, including the visual organ. The fundamental role of water explains high sensitivity to even a slight water balance impairment that can lead to the development of pathological conditions. Most of the water in the human body is associated with connective tissues able to retain water due to the presence of glycosaminoglycans in the intercellular matrix [1, 2]. The corneoscleral shell of the eye composed of connective tissue normally contains a significant amount of water: normal cornea — about 78%, normal sclera — about 65% [3]. If excessive water is accumulated in the cornea (corneal edema), its refractive properties change, but what is especially important, the cornea loses its transparence, which significantly reduces visual acuity. On the other hand, dehydration of the cornea leads to changes in its shape and refractive ability, prolonged loss of water resulting in a dystrophic process causing irreversible impairment of visual functions [4].

Corneal water balance impairment can be caused by various eye diseases (corneal inflammation, injury, corneal ectasia, etc.), treatment procedures such as surgical interventions (keratorefractive surgery for myopia and other refractive errors, cross-linking for keratoconus), therapeutic procedures (long-term instillation of certain drugs, in particular, hypotensive medications) or optical techniques (contact lens vision correction) [5].

Sclera performs supporting function for the internal shells (the vascular membrane and the retina) and other eye structures, plays an important role in maintaining the eye shape, therefore, impairment of its biomechanical properties due to dystrophic process in the scleral tissue is the leading cause of myopia progression and development of its disabling complications [6–9].

Corresponding author: Elena N. Iomdina, e-mail: iomdina@mail.ru

Besides, according to present-day ideas, changes in the structure and properties of the ocular corneoscleral shell prove to be a significant factor in glaucoma development [10–13]. Water balance impairment is supposed to be one of the links in this pathological process.

Thus, adequate control of corneal and scleral hydration is very important for early diagnosing and monitoring the progression of various ophthalmologic pathologies (corneal dystrophy, keratoconus, progressive myopia, primary open-angle glaucoma), identifying indications and contraindications to keratorefractive surgery, selecting effective and safe tactics of local medicament treatment, including tear replacement and hypotensive therapy, and developing an algorithm for contact lens vision correction.

Currently, corneal edema or corneal dystrophy supposedly associated with reduced hydration can be established only indirectly by measuring corneal thickness (topographic pachymetry), determining its outer surface shape (computer-assisted video keratography and corneal mapping) or by measuring the biomechanical parameters of the cornea using the ORA analyzer (Ocular Response Analyzer; ORA Reichert, USA). However, changes in corneal thickness or shape can be associated not only with its impaired hydration but also with other factors, therefore, the existing indirect methods for evaluation of water content in the corneal tissue are not informative enough. Measuring scleral thickness with contact methods such as ultrasound biomicroscopy (in the anterior) or echodensitometry (at the equator and posterior pole of the eye) is also insufficiently accurate and rarely used in clinical practice as an indirect hydration indicator.

At present, there is no direct noncontact method for determining corneal and scleral hydration in the arsenal of a clinical ophthalmologist. The use of electromagnetic radiation of the terahertz (THz) range (0.3–3.0 THz) for this purpose seems to be very promising, since water is known to exhibit high absorption and dielectric permeability in this frequency range, which determines high reflectance coefficient. This provides the possibility of using THz scanning in the light reflected from biological material surface in order to detect the slightest changes in the concentration of water contained there.

Only a few research groups in the world are involved in developing the system for THz scanning of biological tissue hydration. Pulse THz sources are mainly used for this purpose. For example, the paper [14] presents a method for THz scanning of porcine corneal hydration. The authors use a traditional scheme for obtaining pulsed THz radiation consisting of a photoconductive antenna and pumping femtosecond laser. A detector based on Schottky barrier diode serves as a receiver. This can be replaced in the proposed scheme by more sensitive high-speed detector based on hot-electron bolometer [15]. Other authors employed a similar technique earlier. In particular, the paper [16] presents a THz imaging system that allows high-precision differentiation of tissue areas with different water content. Other studies have successfully demonstrated the possibility of THz imaging in diagnosis of cutaneous carcinoma and melanoma and analysis of skin burns based on water concentration measurement [17–20]. The paper [21] presents the results of measuring reflectance coefficient of porcine cornea depending on its hydration in the range of 0.2–1.0 THz. The authors have revealed approximate linear dependence of reflectance coefficient on water concentration in the tissue with monotonically decreasing slope coefficient at increasing radiation frequency.

The work of Taylor et al. [22] shows the results of intravital measuring the reflectance coefficient of rabbit cornea using pulse THz imaging system (0.47–0.58 THz) and a millimeter-wave reflectometer (100 Hz). Positive correlations between corneal thickness and reflectance in the millimeter wave range have been obtained.

However, the method used in the above works is still difficult to implement in practical devices due to the fact that the implementation of the proposed scheme requires the use of powerful, bulky and extremely expensive femtosecond laser. Besides, these works provide no data on the reflectance and transmittance spectra of tissues in the millimeter wave range (less than 0.3 THz).

A research group from Lomonosov Moscow State University is currently working in the same direction. [23]. These researchers use special material in their investigations: pressed Al_2O_3 plates as models of the cornea. This material is characterized by high moisture absorbing ability making it possible to control water content in the material affected by changes in ambient humidity. By varying moisture content in the sample, its transmittance and reflectance coefficients are investigated in the THz range using time resolution spectrometer. However, it is important to note that this approach is far from being applicable in real practice at this stage as the cornea model developed by the authors is very rough and created with no regard for the internal corneal structure, its use is actually reduced to the analysis of one integral parameter: the average water concentration throughout the sample.

Liu et al. [24] have studied transmittance and reflectance coefficients of the extracted cornea depending on water content. They show that complex dielectric permeability of the cornea monotonically decreases with a decrease in corneal water content. In their investigation, the authors use time-resolution THz spectrometer with optimized scanning speed. In another work, the same authors evaluate the dependence of corneal transmittance and reflectance coefficients on radiation frequency in the range of 0.1–1.5 THz using time resolution THz spectroscopy technique *in vitro* [25]. The results show that reflectance coefficient decreases with increasing radiation frequency, while absorption coefficient, on the contrary, increases almost linearly.

The results obtained so far [26] prove the feasibility and prospective value of carrying out research in this direction in order to create an informative method for evaluation of corneal and scleral hydration degree *in vivo*.

The aim of the investigation was to study transmittance and reflectance spectra of the cornea and the sclera in the THz frequency range, to evaluate the prospects of using continuous THz scanning for measuring water concentration in these tissues and on the basis of the obtained data to develop the experimental installation for monitoring corneal and scleral hydration degree.

Materials and Methods

To evaluate corneal and scleral transmittance and reflectance spectra in THz range, 3 rabbit corneas and 3 scleras, 2 whole rabbit eyes, and 3 human scleras were studied. Besides, two rabbit eyes were studied *in vivo* prior to keratorefractive surgery as well as 10 and 21 days following the surgery (LASIK).

The order No.199н "On the Approval of the Rules of Good Laboratory Practice" (Russia, 2016) and the International Guiding Principles for Biomedical Research Involving Animals (CIOMS and ICLAS, 2012) were strictly observed. The work was performed in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg, 2006). The permission from the Ethics Committee of Moscow Helmholtz Research Institute of Eye Diseases to conduct these specific experiments was obtained.

To study corneal and scleral transmittance coefficients in the THz frequency range, there was created an experimental installation with a programmable generator in the range of 129.2–145.5 GHz and the minimum frequency step of 10 MHz used as a radiation source. The signal was amplitude-modulated by electric square-wave signal from an external sound generator at the frequency of 10 Hz (Figure 1).

The effective diameter of the beam in the focus of the mirror system was less than 1 cm.

The passed signal was detected using the Golay cell, from which an electric signal was sent to a phase-sensitive detector. The reference signal was fed to the detector from synchronization output of the sound generator. Schematic structure of the experimental installation is shown in Figure 2. It is important to note that the radiation power of the employed generator depends significantly on frequency. For this reason, calibration of the radiation path was performed with a Petri dish, this calibration was taken into account later when transmittance spectra of the material were obtained.

To measure corneal and scleral reflectance coefficients in the THz range, depending on their water content, there was also created an experimental installation with an avalanche transit time diode (IMPATT diode) of about 95 GHz radiation frequency used as a radiation source. This radiation was directed with a special horn to the investigated sample placed in a Petri dish (Figure 3).

The generated radiation is amplitude-modulated by the power supply unit at the frequency of 16.6 kHz. The reflected signal was measured using a detector based on Schottky barrier diode from which the electrical signal is fed to the phase-sensitive detector. The reference signal was fed to the detector from synchronization output of the power supply unit of the IMPATT diode. The second horn was used to match the signal reflected

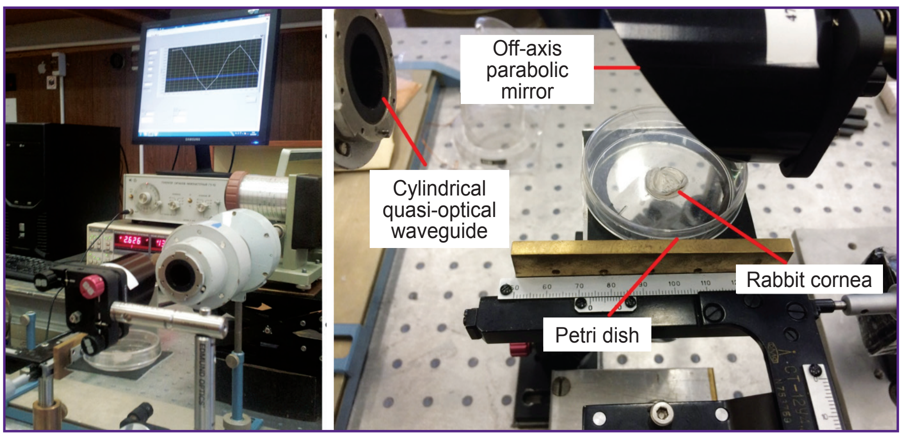

Figure 1. Evaluation of transmittance spectra of corneal samples in the THz range

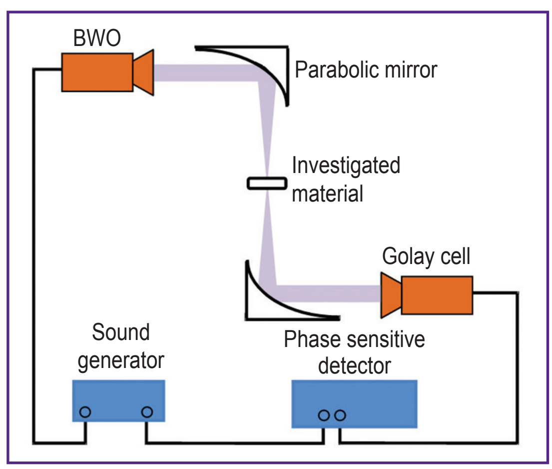

Figure 2. Schematic structure of the experimental installation for measuring corneal and scleral transmittance in THz frequency range

BWO — backward-wave oscillator as a source of radiation

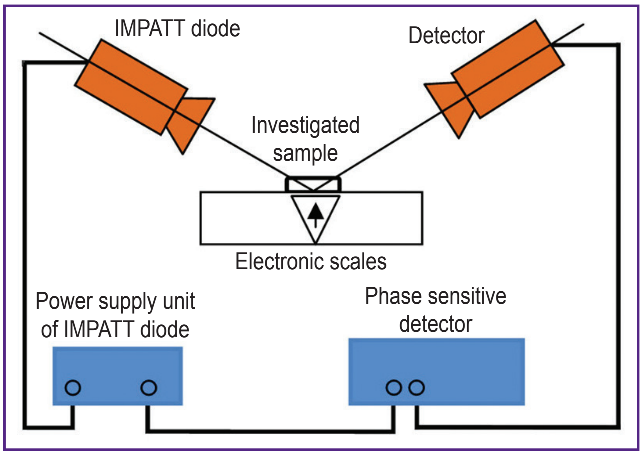

Figure 3. Schematic structure of the experimental installation for measuring corneal and scleral reflectance coefficients in the THz range, depending on their water content

IMPATT diode — avalanche transit-time diode as a source of radiation

from the tissue under study with the detector. The Petri dish containing the sample was mounted on scales with measurement accuracy of 10^{-4} g. This provided the possibility to measure the dependence of the reflected signal on water content in corneal or scleral sample in dehydration process.

Measurements were taken while the signal kept changing, the original shape of the cornea and the sclera being preserved. Absorbing material was glued to the opposite side of the Petri dish to reduce the parasitic radiation reflection from the metal surface of the scales used in the experiment. However, it appeared to be impossible to get rid of this parasitic reflected radiation completely. In the experiment, it accounted for about 30% of the total detected power. For this reason, the value of the received reflected signal was expressed in relative units.

Results and Discussion

The created experimental installations (see Figures 1–3) enabled us to evaluate frequency dependence of transmittance coefficients of rabbit and human cornea and sclera as well as the dependence of reflectance coefficients of these tissues on water percentage in the THz frequency range.

As we can see from the presented diagrams (Figure 4), transmittance coefficients of corneal and scleral samples expectedly increased while drying, since the content of unbound water being the main absorber of THz frequency range radiation decreased in the tissues under study.

However, alternating transmittance peaks spaced by about 1 GHz frequency were clearly visible in the corneal transmittance spectrum in the THz range, their origin has yet to be investigated in more detail in the future.

Similar measurements were performed for human sclera (Figure 5).

The study showed that water percentage decrease in rabbit cornea by 1% leads to reliably established decrease in the reflected signal by 13% (Figure 6), which is consistent with the results presented in paper [21].

Linear dependence of reflectance coefficient on water percentage in the corneal area with weak dehydration was also confirmed. Stronger dehydration led to changes in the curve shape, which was most likely due to corneal deformation caused by water loss.

The main disadvantage of the setup used to study reflectance coefficient was the fact that reflection from

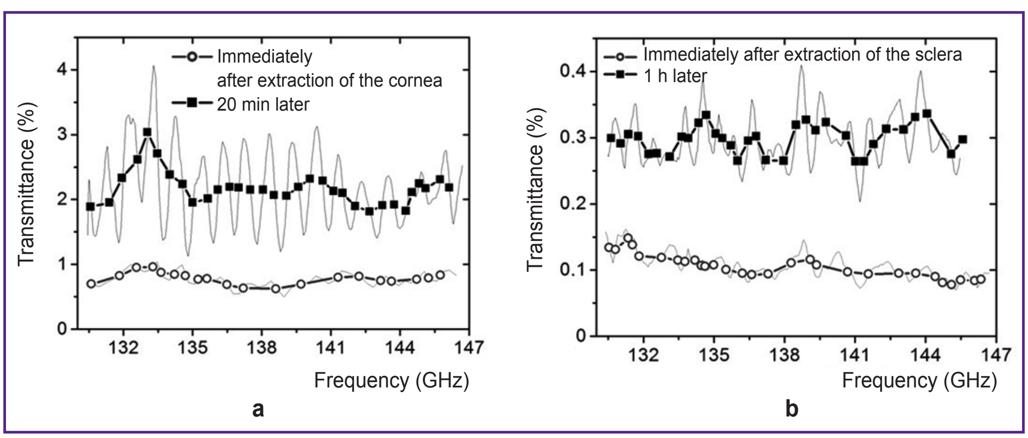

Figure 4. THz radiation transmittance spectra:

(a) of rabbit cornea; (b) of rabbit sclera. In both cases, the curve with empty round markers corresponds to the transmittance spectrum of the material immediately after its extraction from the rabbit eye, the curve with black square markers shows the findings obtained some time later (about 20 min for the cornea, about 1 h for the sclera) when certain amount of water has evaporated from the tissue

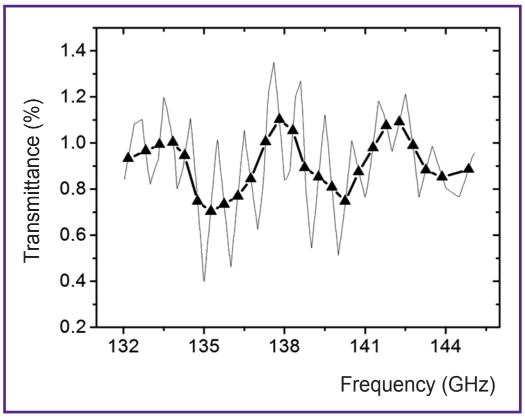

Figure 5. Transmittance spectrum of human sclera in the THz frequency range

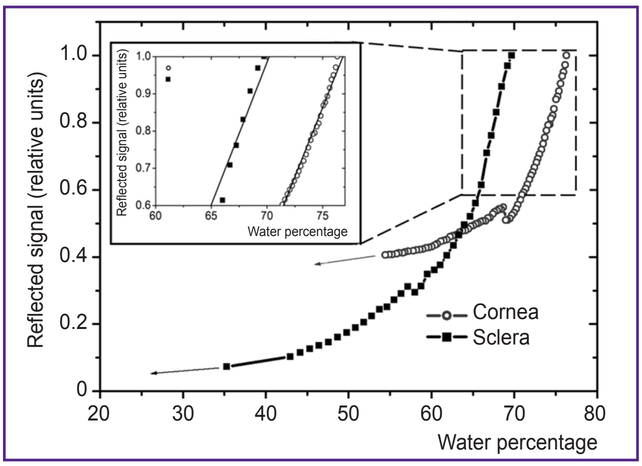

Figure 6. The diagram of reflected signal dependence on water percentage:
in rabbit cornea (a curve with empty round markers); in rabbit sclera (a curve with black square markers)

the tissue surface was measured integrally. Therefore, it was impossible to study reflection from the whole eye as most of incident radiation was scattered due to eye sphericity and the power reaching the detector was insufficient for reliable registration. For this reason, in the experiment on evaluation of corneal reflectance spectrum in 0.13–0.32 THz range with the whole eye, we directed radiation produced by a generator with a backward-wave oscillator to the eye from an open 2-mm waveguide placed in the immediate vicinity of the corneal surface and recorded scattered reflected radiation. Calibration was performed based on radiation reflected from the metal plate. The results of the experiment on evaluation of whole rabbit eye reflectance spectrum in 0.13–0.32 THz range are shown in Figure 7.

The results seem to be promising with regard to the possibility of developing a device for noninvasive control of corneal and scleral hydration degree in clinical practice.

So far, there has been developed an experimental installation (laboratory model) for *in vivo* evaluation of corneal reflectance coefficient in laboratory animals (rabbits) in the THz range (Figure 8). IMPATT diode with 95 GHz frequency is used in this installation as a source of THz radiation. Radiation from the source is directed to the rabbit cornea using a quasi-optical horn (Figure 9). The power of scattered radiation reflected from the eye and modulated with a mechanical obturator is measured with the Golay cell, from which an electric signal is fed to a lock-in amplifier.

It should be noted, living rabbits were used in our work in conditions close to real practice in contrast to the study [23] performed on a model of the cornea (plates of pressed material Al_2O_3).

This installation was employed to study temporal dynamics of reflectance coefficient of rabbit cornea in the THz range before and after (days 10 and 21) keratorefractive surgery (LASIK) was performed. The results showed that the coefficient values increased with each subsequent postoperative measurement for all examined rabbit eyes, which may be due to structural changes in the cornea caused by surgery as well as

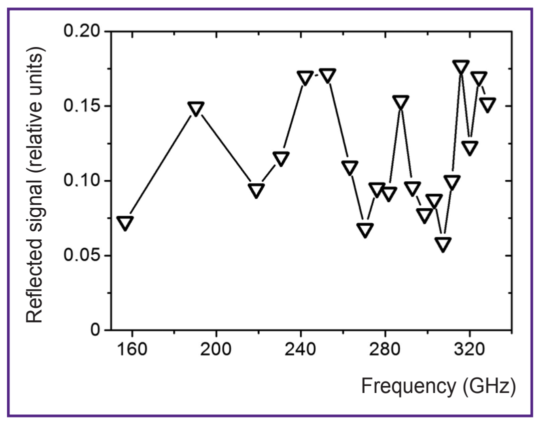

Figure 7. Reflectance spectrum of whole rabbit eye in the THz range

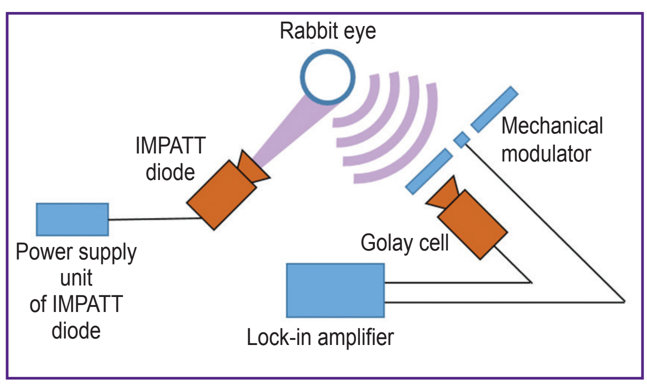

Figure 8. Principle diagram of the experimental installation for *in vivo* evaluation of rabbit corneal reflectance coefficient in the THz range with avalanche transit-time diode (IMPATT diode) as source of radiation

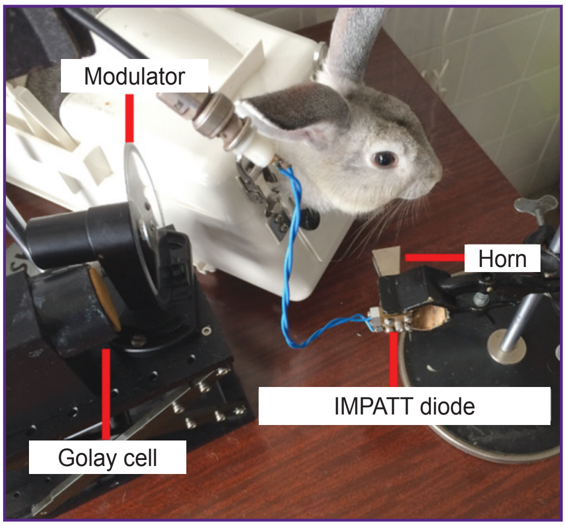

Figure 9. Part of the experimental installation for *in vivo* evaluation of rabbit corneal reflectance coefficient in the THz range

changes in corneal water balance. It is important to note, however, measurement of reflectance coefficient using the developed installation has a large error and requires further optimization of its operation in terms of matching incident radiation to the eye and the reflected radiation to the detector.

Conclusion

There have been created novel experimental installations for *in vitro* evaluation of frequency dependence of corneal and scleral transmittance coefficients and reflectance coefficients on water percentage in the THz range. The installations provide the possibility to measure reflectance spectrum of the whole rabbit eye in the range of 0.13–0.32 THz. Corneal and scleral transmittance spectra have been obtained and dependence of corneal and scleral reflectance coefficients on water percentage has been evaluated. The study revealed the differences between the indices of rabbit cornea and sclera as well as rabbit and human sclera. There has been developed a laboratory model of the installation for *in vivo* evaluation of corneal and scleral hydration using THz radiation. The preliminary findings show that the proposed technique based on the use of continuous THz radiation can be employed in prospect for noninvasive control of corneal and scleral hydration.

Study funding. The research has been carried out with the support of the Russian Science Foundation (project No.17-72-30036).

Conflict of interests. The authors declare that materials or methods used in the study do not involve conflicting interests.

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