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Letter

In vivo THz sensing of the cornea of the eye

Ilya Ozheredov[1,4], Mikhail Prokopchuk[1], Mikhail Mischenko[1], Tatiana Safonova[2], Petr Solyankin[1,3,4], Andrey Larichev[1], Andrey Angeluts[1], Alexei Balakin[1,3,4] and Alexander Shkurinov[1,3,4]

[1] Faculty of Physics and International Laser Center, Lomonosov Moscow State University, Moscow, Russia
[2] Research Institute of Eye Diseases, Moscow, Russia
[3] Institute on Laser and Information Technologies—Branch of the Federal Scientific Research Center Crystallography and Photonics of Russian Academy of Sciences, Shatura, Russia
[4] Terahertz Technology Innovation Research Institute, Shanghai Key Lab of Modern Optical System, Terahertz Spectrum and Imaging Technology Cooperative Innovation Center, University of Shanghai for Science and Technology, Shanghai, People's Republic of China

E-mail: ozheredov@physics.msu.ru

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Abstract

Measurement of the absolute value of the humidity of the cornea of the human eye and its dynamics is of paramount importance for the preservation of eyesight. In the present paper we have demonstrated that terahertz technologies can be practically applied for quantitative measurement of the physiological dynamics of tear film and sensing of corneal tissue hydration. We suggest uses of the equipment for application in clinics and a method for absolute calibration of the values for measurement. The proposed method is fundamentally different from existing and currently available methods of ophthalmological diagnosis. This suggests that the developed technique may have high diagnostic significance and can be used in the study and treatment of several diseases of the ocular surface.

Keywords: terahertz technology, eye, corneal tissue, tear film

(Some figures may appear in colour only in the online journal)

1. Introduction

The cornea is one of the most important structures of the human eye. The major functions of the cornea include maintaining a precise curved shape ensuring the refractive function of the eye's optical system and a smooth surface for transmitting over 90% of the incident light at visible wavelengths [1]. Corneal transparency is a substantial factor, and like the other biochemical and physical properties of the cornea this is mainly maintained by a proper water content in the corneal stroma (about 78% water). This depends on accurate functioning of the epithelial and endothelial pumps and the correct organization of collagen fibers in a matrix consisting of proteoglycans (with attached glycosaminoglycans), glycoproteins, inorganic salts and water. The proteoglycans play the most significant role because they constitute up to 10% of the dry weight of the cornea and promote regular spacing and packing of the collagen fibrils by binding extracellular water [2].

The structure from the outer toward to the inner layers the cornea consist of five stages: the epithelium, Bowman's membrane, the stroma, Descemet's membrane and the endothelium [3]. The major component the stroma, makes up 90% of the total thickness of the cornea. The typical water content in stroma is 75–80%, which exceeds the water content in any other type of connective tissue. This is determined by the presence of negative charges on the chains of glycosaminoglycans, which attract a positively charged dipole of the water molecule. Impaired hydration of glycosaminoglycan chains reduces their size, changes the distance between collagen fibers and thus breaks the well-ordered structure, leading to loss of corneal transparency. Metabolic processes in the cornea should maintain the transparency of the corneal tissue and prevent its deterioration [2].

The cornea is coated by a pre-corneal tear film—a three-layer film that transports metabolic products to and from the cornea, prevents the cornea from drying, lubricates the ocular surface and has antibacterial properties. This film, formed after the blink, has a thickness of 4–7 μm. The middle aqueous layer of the tear film is represented by water solution and makes up 90% of the tear film volume. Under normal conditions, the tear fluid of the ocular surface and in the aqueous humor of the anterior chamber is isotonic with the corneal stroma. However, the hygroscopicity of glycosaminoglycans regulates the flux of water from the tear film through the corneal epithelium (passive transport) into the corneal stroma. The metabolism of the cornea must ensure the removal of excess water that is provided by active transport of ions across the cell membranes of the endothelial cells. This active ionic pumping creates an osmotic gradient providing a flux of water from the corneal stroma into the anterior chamber, i.e. the water is removed due to the endothelial pump. Thus, the normal corneal functioning and its transparency are achieved as a dynamic equilibrium state between the hygroscopic attraction of water by glycosaminoglycans in the corneal stroma and the activity of the corneal endothelial ions and water pumping.

If the above-described mechanism of the metabolic transport is broken—in cases of oxygen deprivation or oxidative stress or under the influence of a wide range of substances that disrupt corneal homeostasis—the stroma hydration level changes. This leads to the disturbance of spatial collagen organization and, consequently, to a decrease of corneal transparency. The epithelial corneal layer allows passive transport of water. Proteoglycans of the corneal stroma matrix, and glycosaminoglycans in their composition, are responsible for the inflow of water from the tear film into the corneal tissue as well as for the correct arrangement of collagen fibers in the form of flattened lamellae (layers of collagen fibrils). Endothelial cells of the cornea are specialized in pumping water out of the corneal stroma into the anterior eye chamber (water outflow). Thereby both of these essential metabolic processes create an active flow of water from the front to the back of the cornea and ensure the homeostasis, fluid balance, normal hydration level and optical transparency of the cornea. In various diseases and pathological conditions, or as a consequence of surgical interventions and the use of medicines, changes in the epithelium, endothelium or stromal matrix of the cornea result in loss of the ability to regulate stromal hydration.

The basis of progression of dry eye syndrome is dysfunction of the pre-corneal tear film. Its assessment is a leading indicator in diagnosis of this disease. In clinical practice the most common method for determining tear film stability is the Norn test [4]. For this test a 0.1% solution of fluorescein sodium is typically used. The study is conducted using a slit lamp with a cobalt filter. The time of appearance of the gaps in the painted tears is recorded. Conjunctival disease which develops as a result of hypolacrimia usually manifests itself by tearing of the tear film in corneal epitheliopathy [5].

Tear meniscus evaluation can help estimate tear volume on the ocular surface [6]. The tear meniscus can be analyzed by several parameters such as its height (tear meniscus height (TMH)), radius, depth and cross-sectional area. However, it was shown that the conventional methods for evaluating TMH suffer from large variation and poor repeatability [7]. Novel techniques such as optical coherence tomography show better performance [8]. The stability of the tear film can be assessed by measuring the time elapsing between the execution of a normal blink and the break-up of the tear film; this is known as the tear break-up time (BUT). The commonly accepted explanation [9] of the process of tear break-up is as follows. The hydrophobic lipid migrates down to the mucous layer and interferes with the hydrophilicity of the epithelial surface. Tears depart from this region and a dry spot forms. Subsequent intermixing of lipid and mucus occurs at the receding edge and the field of hydrophobicity increases; this increases the dry area and the process continues.

Several methods are available for the evaluation of BUT, including non-invasive techniques based on interferometry [10], distortions of reflected grid patters [11] and real time aberrometry [12]. Such tests are considered to be rather useful [13].

Another diagnostic method is based on observation of the lipid layer which is produced by the oily secretion from the meibomian glands, located in the tarsal plates of the lids. This secretion prevents the evaporation of tears and enhances the stability of the tear film. Since the lipid layer is extremely thin, white light interference within the air–lipid and lipid–aqueous boundaries occurs, forming colored fringes. The thickness of the lipid layer can be found by acquisition of such a fringe pattern. Several instruments [14] can be used for this, including modern topographers with white Placido rings.

Despite the existence of numerous methods for studying dry eye symptoms, as well as their different aspects and manifestations, direct assessment of dryness on the ocular surface and undersurface layers may be extremely beneficial.

Confocal microscopy expands the possibilities of studying the anatomy of the cornea at the level of its microstructure [15]. The method allows real-time study in detail of each corneal layer and detection of changes in the cellular structure of the cornea. This is especially important in the subclinical course of dry eye syndrome, when traditional methods of research are poorly informative. It has been established that with dry eye syndrome the main changes affect the epithelial layer of the cornea in the form of edema and cell polymorphism. The drawbacks of this method are the physical contact required and the likelihood of negative effects of analgesic on the surface epithelium of the eye during the study.

Recent progress in the development of terahertz (THz) spectroscopy and imaging systems allows the use of THz diagnostic methods for the study of biological objects [16]. Due to the high sensitivity of THz techniques to water content in biological tissues, the non-invasive nature of the radiation used, the high homogeneity of water distribution in the corneal tissue of the eye and the low physiological variability of the cornea compared with other human tissues, the development of corneal hydration monitoring techniques using THz radiation has great potential.

In recent publications [17, 18] a THz imaging and spectrometry technique was used with *ex vivo* porcine corneas with different water concentrations by mass. Comparison of

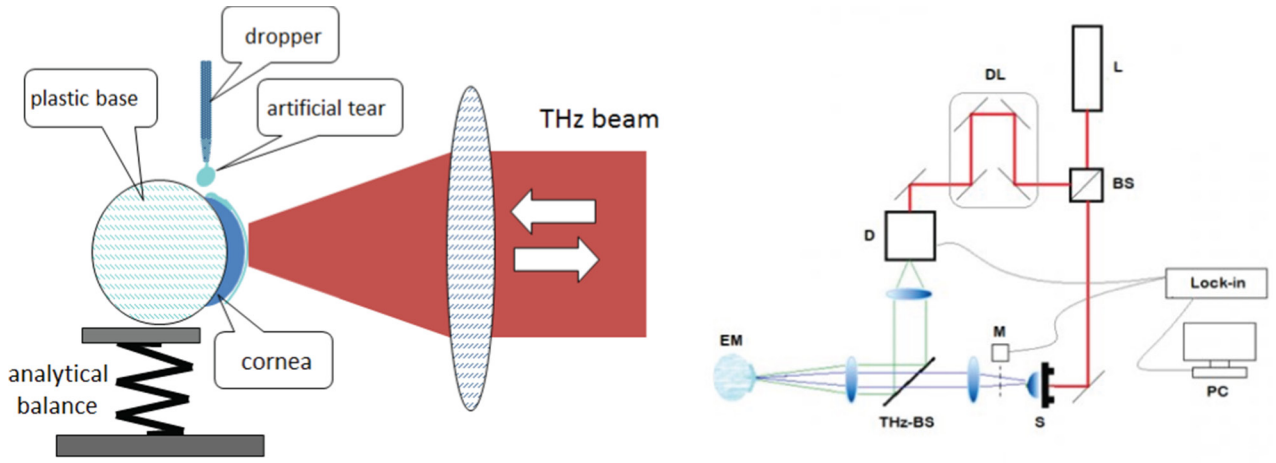

Figure 1. Eye phantom on the analytical balance (left) and block diagram of THz reflectometer used in the experiments (right): L, femtosecond laser; BS, optical beam-splitter; S, THz source; M, modulator; DL, delay line; THz-BS, beam-splitter for THz beam; D, THz detector; PC, personal computer; EM, eye phantom.

nine corneas with different hydration levels demonstrated an approximately linear relationship between THz reflectivity and water concentration, with a monotonically decreasing slope as frequency increases. The first *in vivo* application of the THz technique for sensing of corneal tissue hydration was reported in [19]. The authors used pulsed THz imaging and millimeter-wave reflectometry systems to acquire data on five rabbit corneas. The paper reported a good correlation between corneal thickness and reflectance coefficients in a millimeter-wave region. For slight dehydration of healthy cornea a gentle stream of air and a Mylar window was employed. However, the method used in the work described above is still far from being ready for application in practical devices.

A THz optical design proposed in [20] allows acquisition of THz reflectivity maps of *in vivo* cornea without any flattening window. The authors suggested a beam optics design for scanning of a curved eye corneal surface at normal incidence while keeping the source detector and target stationary. Such a THz system might be used for non-contact THz imaging of animal and human eyes.

In this paper we show the preliminary results of application of a THz reflectometry technique for monitoring the physiological dynamics of tear film and sensing corneal hydration. A developed phantom eye with implemented eyelid blinking function is used for testing the approach and monitoring the long-time dynamics of corneal tissue drying. In addition we pay special attention to scattering losses in the specular direction. Nanostructured aluminum oxyhydroxide pressed into the pellets emulating the human eye shape are suggested for the absolute calibration of the measured hydration values. The THz time-domain approach is not a good choice for real clinical applications because of its complexity and the need to use a delay-line. For such applications we propose a frequency-domain THz apparatus and give some examples of *in vivo* THz sensing of the human cornea. The data on the physiological dynamics of tear film open a path to the study of ophthalmological diseases and pathological conditions related to physiological disturbances of the eye and corneal tissue moisture content.

2. Phantom eye

A human eye phantom developed for experimental study was based on a human cornea sample. We used a cadaver corneal disc with no abnormalities. The cadaver eye was enucleated 12 h after death of the donor; trepanned corneal buttons were preserved in a special corneal storage solution (SEP EM Ltd., Russian Federation). These corneal samples were mounted on a hermetically sealed hollow plastic chamber with a spherical surface and aperture of diameter 8 mm. The holder design allows it to be filled with NaCl 0.9% solution to create pressure on the sample from the inside. Thus, the 'sample–holder' system means that the posterior endothelium of the corneal sample is wetted. Due to pressure created by the liquid in the holder, the sphericity of the corneal sample is maintained during the experiment. One of the important features of the developed eye phantom is an imitation of the blinking effect, moisturizing the anterior surface of the cornea. Figure 1 shows a schematic of the eye phantom installed on the analytical balance. The sensitivity of the balance was better than 0.1 mg, allowing for precise control of the weight of the eye phantom and to monitor of its change due to the evaporation of water from the cornea. Such a change in weight might be recalculated as the level of corneal dehydration.

For experimental study of the sensing of corneal tissue hydration we developed a pulsed THz reflection spectroscopy system. This was based on a THz time-domain spectrometer operated in reflection geometry, described in detail elsewhere [21]. The system allows one to measure the instantaneous electric field amplitude of a THz pulse of $\approx$1 ps duration. The shape of the temporal profile of the alternating electric field provided information about both the amplitude and the phase of the THz radiation in a wide spectral range (from tenths of THz to several THz). The average power level of the incident THz radiation did not exceed 100 nW, which is clearly below the damage value [22]. Figure 1 shows a block diagram of THz reflectometer used and indicates the major system components. The THz beam from the experimental setup was focused into the cornea under study by a wide-aperture

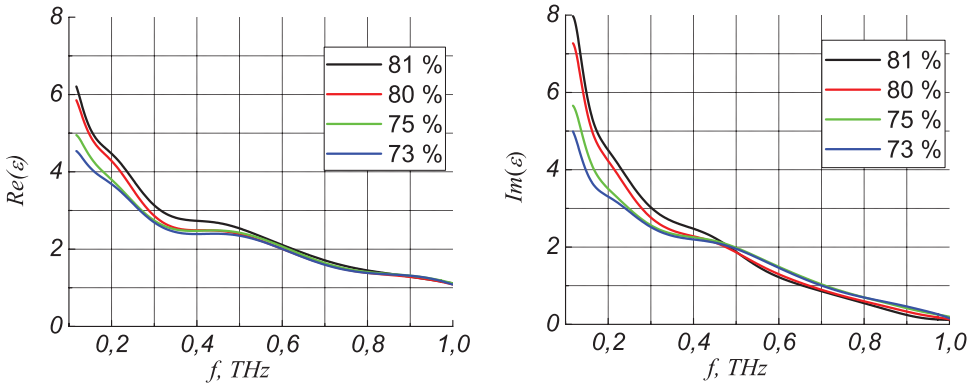

Figure 2. Real (left) and imaginary (right) part of the dielectric permittivity of the corneal sample for different hydration levels indicated in the graph.

lens with a focal distance of 50 mm. The diameter of the THz spot at the sample surface was about 4 mm. The reflected THz signal was collimated by the same lens and collected by a detector. Thus we monitored the changes in corneal weight and changes of the amplitude and phase of the THz reflected signal simultaneously.

By using proposed eye phantom in the sample position we obtained a series of THz reflection spectra from the cornea with simultaneous control of the sample weight. The falling sample weight due to the reduction in corneal tissue water content caused a reduction of THz-amplitude spectra. When discussing the cornea hydration–dehydration cycle one has to distinguish two types of water: unbound water and water coupled to the cells of the corneal tissue. In case of low levels of tissue dehydration the unbound water content decreases but coupled water remains unchanged [23]; i.e. the changes in the THz reflection coefficient due to corneal dehydration are primarily associated with a decrease in the unbound water content in the corneal matrix.

3. Reflected signal normalization procedure

The value of the dielectric constant of the corneal tissue is determined by three main components: the dielectric permittivity of collagen fibers, the permittivity of coupled water and the dielectric constant of free water in the corneal media. To evaluate the effective dielectric constant of hydrated corneal media the effective media approach, in which the effective dielectric constant of hydrated corneal media is related to dielectric constants of dry cornea and water, was suggested [24]. Such an approach based on the Bruggeman model [25] considers a medium composed of two equivalent components (uncoupled water and collagen fibers) with permittivities ϵ_1, ϵ_2 and fill factors f_1, f_2, respectively. The fill factors are taken to obey $f_1 + f_2 = 1$. The dielectric permittivity of the entire medium ϵ_{eff} obeys the relation

$$f_1 \frac{\epsilon_1 - \epsilon_{\text{eff}}}{\epsilon_1 + 2\epsilon_{\text{eff}}} + f_2 \frac{\epsilon_2 - \epsilon_{\text{eff}}}{\epsilon_2 + 2\epsilon_{\text{eff}}} = 0. \tag{1}$$

Since the determination of the dielectric constant of hydrated corneal tissue is based on determination of the values of both corneal collagen fibers and coupled water this complicates the procedure. To simplify the approach we propose the use of a highly porous transparent material—nanostructured aluminum oxyhydroxide (NAO). The dielectric properties of NAO were studied in [21], accounting for water chemically bonded and adsorbed on NAO fibrils. NAO pressed into pellets was used as a reference for calculation of the dielectric function of the hydrated corneal tissue under study. To minimize the influence of spatial dispersion on the results while pressing the pellets we used a specially shaped press emulating the shape of the human eye. Measured THz spectra for each hydration level were normalized on the spectra obtained for the NAO pellet. This process can be described by

$$A_{\text{w}} = \frac{A_{\text{c}}}{A_{\text{NOA}}}, \varphi_{\text{w}} = \varphi_{\text{c}} = \varphi_{\text{NOA}}, \tag{2}$$

where $A_{\text{w}}, A_{\text{c}}, A_{\text{NAO}}$ and $\varphi_{\text{w}}, \varphi_{\text{c}}, \varphi_{\text{NOA}}$ are spectral amplitudes and phases of water in corneal matrix, entire cornea and NAO media, respectively,

$$\epsilon_{\text{w}} = \left(\frac{1 + z_{\text{w}}}{1 - z_{\text{w}}}\right) \tag{3}$$

where

$$z_{\text{w}} = \frac{A_{\text{w}}}{\sqrt{1 + \tan^2 \varphi_{\text{w}}}} + \text{i}\frac{A_{\text{w}} + \tan \varphi_{\text{w}}}{\sqrt{1 + \tan^2 \varphi_{\text{w}}}}. \tag{4}$$

Such a normalization procedure allowed us to exclude the contribution of coupled water and the corneal collagen matrix from the dielectric permittivity of the corneal sample. The results for real and imaginary parts of the dielectric permittivity of the corneal sample for different hydration levels experimentally determined using the proposed normalization are shown in figure 2. The curve corresponding to the highest hydration level of 81% was measured immediately after taking the eye phantom out of the corneal storage solution. The next scan related to 80% was taken 12 min after the first one. The remaining two graphs corresponding to 75% and 73% corneal hydration were obtained at 82 and 100 min, respectively. A clearly detectable difference in the measured dependences shows the high sensitivity of the technique, especially in the low-frequency range.

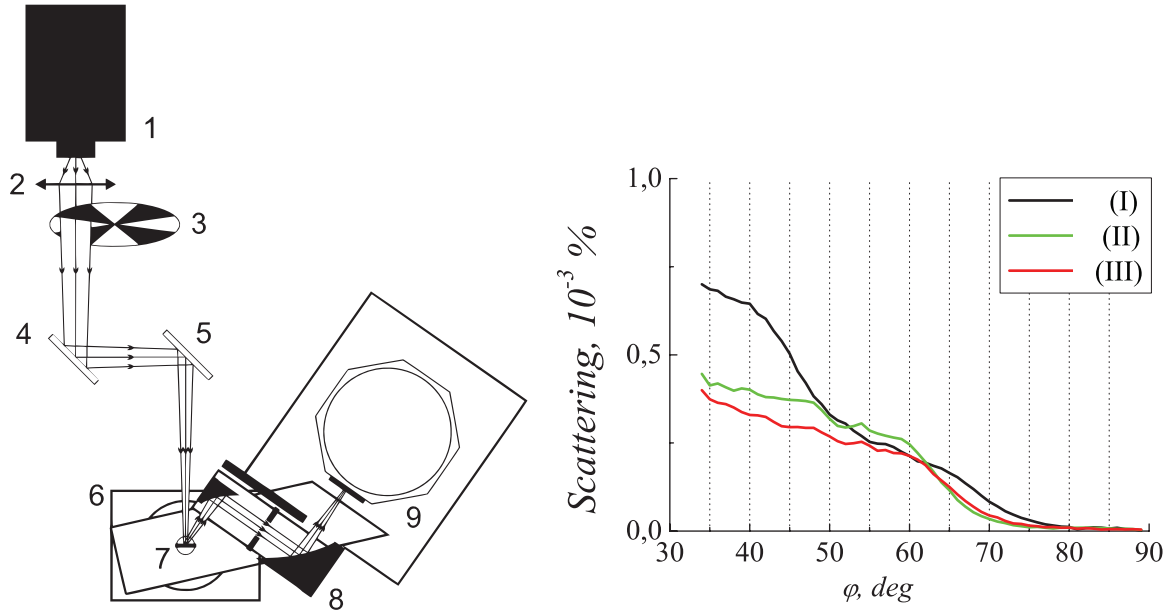

Figure 3. Left: setup of the scattering experiment: 1, quantum-cascade laser; 2, silicon lens; 3, modulator; 4 and 5, mirrors; 6, rotational arm; 7, phantom eye; 8, off-axis parabolic mirrors; 9, He-cooled bolometer. Right: angular distribution of back-scattered THz radiation on the corneal surface for different hydration levels: (I) immediately after eye blinking; (II) 10 min after blinking; (III) 15 min after blinking.

It should be noted that despite the fact that all the data were obtained for a sample whose hydration varied within the physiological norm [2], the change in phantom hydration level occurred over a long time (an hour or more). During this time, a change in the hydration of the corneal tissue will be associated with an additional change in the sample geometry. In order to correctly separate the mechanisms that lead to a change in the reflected signal, additional control measurements are necessary, for example to control the thickness of the cornea, as was suggested in [26].

4. The impact of the scattered component on the reflected THz signal

The distribution of light in the corneal tissue depends on the optical properties of its components. Healthy corneal tissue and other bio-tissue of the front of the human eye are transparent to visible light due to the well-ordered structure and lack of absorbing chromophores [27]. For visible light the scattering is of great importance because the size of the scattering components in corneal tissue and the distance between them are less than or of the order of magnitude of the wavelength of visible light. At the same time the refractive index is relatively small [28]. A typical eye tissue model is collagen fibers—long dielectric cylinders with a refractive index n_c regularly distributed in an isotropic substance with a refractive index $n_0 < n_c$. Due to the low scattering cross-section of corneal tissue the scattering process is well described by a single scattering model [29].

In order to account for scattering losses in the specular direction, the reflection coefficient, derived from Fresnel equations, can be multiplied by a Rayleigh roughness factor [30]:

$$\rho = \mathrm{e}^{-\left(\frac{2\pi\cdot\sigma\cdot\cos\Theta}{\lambda}\right)^2}. \tag{5}$$

Here Θ is the angle of incidence and reflection, σ is the standard deviation of surface roughness and λ is the free space wavelength of the incident wave. The modified reflection coefficient r' that models the reduction of the signal power in the specular direction is then $r' = \rho \cdot r$.

The stromal tissue consists of several hundred stacked lamellae, which differ in width (0.5–250 μm) and thickness (0.2–0.5 μm) [31]. Each lamella consists of a set of parallel collagen fibers. The length of the fibers in human stroma is almost 30 nm. They are distributed regularly with a period of about 55 nm. The intermolecular distance is 1.6 nm [32]. Thus, stromal tissue possesses three stages of structural ordering: a lamellar structure parallel to the surface of the cornea, a fiber structure inside each lamella and the intramolecular structure of the collagen fiber. The wavelength of the THz radiation is significantly higher than the typical size of corneal collagen fibers, but might be of order of magnitude of the lamella size. This could significantly reduce the signal power in the specular reflection r'. Therefore the THz scattering process has to be taken into account and the impact of the scattered component on the reflected THz signal has to be determined.

For experimental verification of the influence of THz radiation scattering from the cornea we measured the back-scattering diagram. The corneal sample was exposed to a beam of polarized monochromatic THz radiation from a quantum-cascade laser [33] at a frequency 3.06 THz under normal incidence (figure 3). Back-scattered THz radiation was collected by a highly sensitive detector system based on a helium-cooled bolometer installed on the rotational arm. This arm was rotated in the setup plane, allowing us to measure the angular distribution of the back-scattered THz light in the

range from 30° to 90° (where 0° corresponds to the angle of incidence). The results of observation of the back-scattered THz signal are shown in figure 3.

The angular distribution behavior changes when the cornea is drying. Under large angles (up to 40°) the amplitude of the scattered THz signal on corneas with different degrees of hydration doubles. Such a difference can be partly explained by the reduced level of hydration level and the change in the cornea surface profile during dehydration. At the same time the efficiency of THz scattering under large angles is low enough (less than 10^{-3}% of the incident THz radiation amplitude). The wavelength of the quantum-cascade laser used in the scattering experiments was 98 μm, which is shorter than that of other typical THz sources. So, the major change in the reflected amplitude caused by dehydration should be obtained from the reflection measurements.

5. *In vivo* measurements

The time-domain THz reflectometry technique described above was shown to be a good tool for monitoring the water content of corneal tissue. The proving experiments with an eye phantom using a pulsed THz system required a long measurement time. During this time, the thickness, composition and other parameters of the tear film were significantly altered. Moreover, a certain time after the start of eye phantom measurements, the tear film was thinned, ripped up and evaporated. This moves the eye phantom scenario very far from *in vivo* measurements of human corneal tissue.

The tear film is in fact an integral part of the cornea. Currently, pre-corneal tear film is considered as one of the corneal layers. The physiological connection between the cornea and the tear film is due to the peculiarities of the formation of the tear film itself, in which corneal epithelial cells play an active part. On the other hand, the formation of glycocalyx by microvilli of epithelial cells and transmembrane mucins allows a tear film to be maintained on the ocular surface and hinders the development of xerotic changes. Therefore, the problem of studying changes in corneal hydration is impossible without taking into account the state of the pre-corneal tear layer. The best solution to this is *in vivo* study.

For *in vivo* sensing of the tear layer in the human eye a frequency-domain reflectometry setup might be used. In such approach neither a delay line nor a complicated laser source are required. This might make the system potentially interesting for solving problems in clinical diagnostics. A proposed THz emitter/coherent receiver concept [34] allows a compact and low-cost cw THz reflectometer to be constructed. On the source side two fiber-based distributed feedback cw diode lasers with average power of 22 mW each are precisely temperature controlled for the generation of a narrow (10 kHz) line in the range 1530–1608 nm. Radiation was combined by an X-type fiber optical beam splitter with a splitting ratio of 50/50. The outputs of the splitter were connected to the THz emitter and receiver, respectively. We used an 'off-the-shelf' low-temperature InGaAs bow-tie photoconductive antenna (PCA). The pulsed DC bias voltage for the THz emitter (0/6 V, ~40 kHz) was provided by the function generator. The THz signal was guided through the polyethylene-lens (PE-lens) to the sample. The specularly reflected THz signal through a second PE-lens was guided to the receiver PCA module. The resulting photocurrent was measured by a digital lock-in amplifier SR 830 (Stanford Research Systems). The difference frequency range of distributed feedback lasers allowed tuning within a range of 50–300 GHz. For this range our PCA-based frequency-domain system delivered only sub-microwatt power, but that was enough for our study.

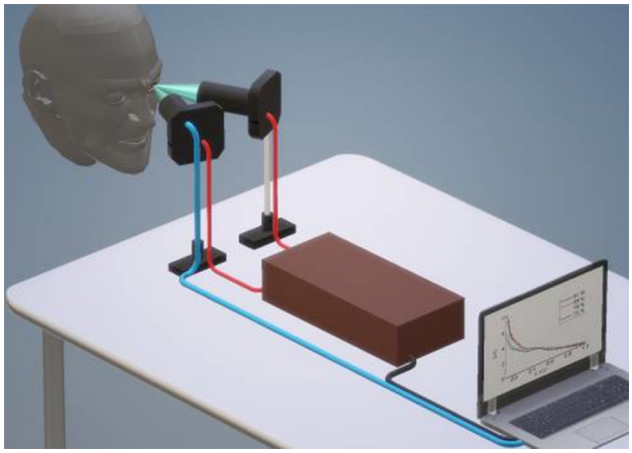

Figure 4. Reflectometry setup based on a frequency-domain THz system for *in vivo* measurements of human corneal hydration.

During *in vivo* experiments the head of a human subject was fixed by a special ophthalmological stage. The entire corneal surface was exposed to radiation at the frequency of 100 GHz focused by the PE-lens with a focal distance of 30 mm. The ophthalmological stage ensured matching of the examined cornea and the focal plane of the THz lens. The specularly reflected signal was collected by the lens with a similar focal distance and detected by the second PCA. A sketch of the experimental setup is shown in figure 4.

Test trials of the proposed method were performed on the eyes of several male humans aged 22 to 45 years, some of whom were wearing glasses. For each human eye we measured the reflected signal versus time. Each measurement started at the time of eye opening. During the measurement series subjects were requested to keep their eye open as long as possible. At the same time subjects were able to close their eye as soon as they needed to. Measurements continued until the eye closed.

The typical observed dynamic of eye reflection coefficient is shown in figure 5. There are two well-recognized areas corresponding to an opened and a closed eye. The THz reflectivity of an opened eye demonstrates the dehydration dynamics. Such a dependence may be fitted by a linear function and decreases with time. At a time of eye closure the reflectivity decreases significantly and remains constant while the eye is closed. Then after the eye is opened the reflectivity is restored to a value similar to that in the previous cycle. Thus, the pictures show the physiological thinning of the pre-corneal tear film between blinks, followed by restoration of the thickness of the tear film to the original values. Based on the results

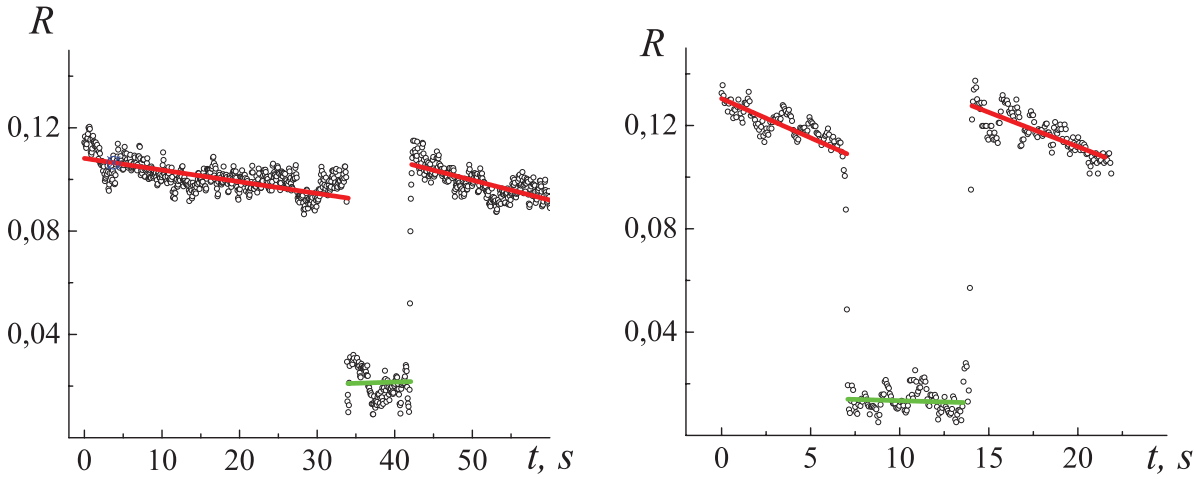

Figure 5. Results of the *in vivo* measurements of corneal THz reflectivity. Points, experimental data; lines, linear approximation. Left: 45 year-old man not wearing glasses. Right: 26 year-old man wearing glasses.

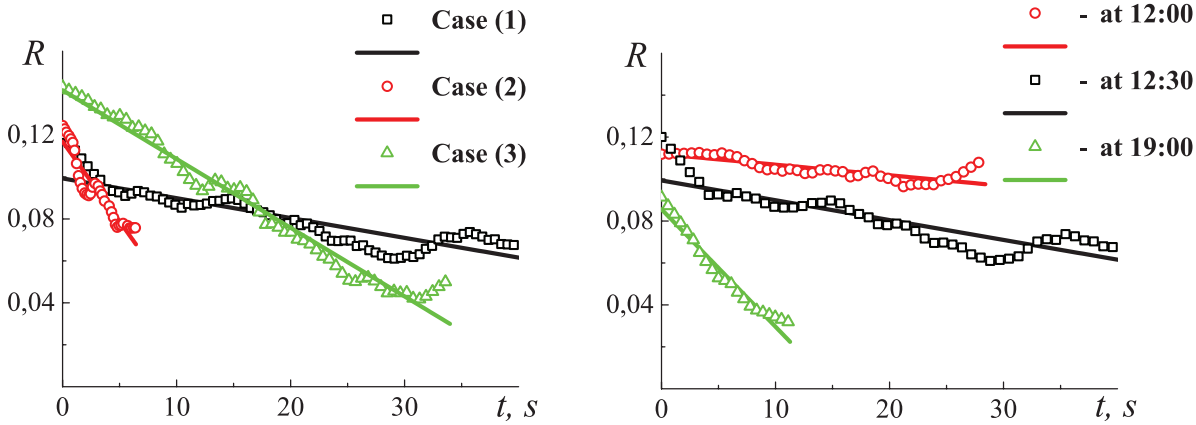

Figure 6. Results of *in vivo* measurements of corneal THz reflectivity. Points, experimental data; lines, linear approximation. Left: measurements for three different subjects taken at the same time during the day: case 1, 26 year-old man, not wearing glasses; case 2, 26 year-old man wearing glasses; case 3, 45 year-old man not wearing glasses. Right: measurements for a 26 year-old man taken at different times during the day.

of preliminary studies, it should be possible to measure pre-corneal tear film dynamics.

Figure 6 illustrates the results of *in vivo* test measurements of THz reflectivity dynamics for different human subjects and at different times during the day. The left graph of figure 6 demonstrates the sensitivity of the approach in the case of subjects of different ages with measurements taken at the same time of day. The right part of figure 6 shows the results for a 26 year-old man measured at different time periods during the day. The curves for 12:00 and 12:30 demonstrate similar behavior but the curve corresponding to 19:00 shows faster recession. Such behavior might be explained by deterioration of the physiological state of the eye associated with fatigue.

Our preliminary test measurements showed sensitivity to the various physiological dynamics of the tear film thinning. Dysfunction of the pre-corneal tear film is mainly related to the progression of dry eye syndrome. The results of *in vivo* measured change of THz reflectivity due to the variation of pre-corneal film thickness allow the control of such progression. The tear film is an integral part of the cornea and is considered as one of the corneal layers. Thus, the problem of studying changes in corneal tissue hydration is impossible to solve without taking into account the state of the pre-corneal tear layer.

For a real evaluation and estimation of the criteria for determination of dysfunction further tests need to be conducted accompanied by approved clinical measurements. That would be a topic for further studies.

6. Conclusion

The applicability of the THz time-domain reflectometry technique has been examined on an eye phantom with an implemented blinking function. The THz reflectometry approach allowed control of the water content in the corneal tissue of the human eye. For calibration measurements we propose to use nanostructured aluminum oxyhydroxide as a material with a stable level of hydration and well-known dielectric parameters. In this case, a sample of such material with a special shape was used as a reference object to mimic the shape of the human eye. Using a THz quantum-cascade laser we

experimentally demonstrated the negligible amount of backscattering and have shown the importance of the reflected signal. For real applications we proposed a diagnostic approach based on a frequency-domain THz apparatus. Some examples of *in vivo* human corneal THz reflectivity measurements demonstrated very good sensitivity and might be suggested as a diagnostic tool for further clinical tests. The developed method of evaluating the dynamics of tear film thinning allows information about the physiological state of the corneal tissue and human eye to be obtained. The proposed method is fundamentally different from existing and currently available methods of ophthalmological diagnosis. This suggests the prospects of high diagnostic significance of the developed technique and shows the research value of the study of ophthalmic diseases and pathological conditions characterized by a change in moisture content in the corneal tissues.

Acknowledgments

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