Optics of living tissues with controlled scattering properties

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ABSTRACT

Results of in vitro and in vivo experimental studies of the optical properties of the rabbit eye, the human eye sclera, and the human skin controlled by administration of osmotically active chemicals, such as glucose, trazograph, and the 50% - Glycerol + DMSO solution are presented. Glucose and other solutions administration induces the diffusion of matter and as a result the equalization of the refractive indices of collagen fibrils and ground material, and corresponding changes of reflectance and transmittance spectra of living tissue. Transmittance and reflectance spectra measurements of tissues, as well as optical anisotropy determination using color measurements were provided. The diffusion coefficient for glucose within scleral tissue was estimated; the average value is $5.62 \times 10^{-5} \pm 1.64 \times 10^{-4}$ cm$^2$/sec. The results are general and can be used to describe many other fibrous tissues impregnated by osmotically active chemical agents.

Keywords: light scattering, in vitro and in vivo measurements, glucose, DMSO, sclera, skin, optical anisotropy

1. INTRODUCTION

Transscleral diagnostics, therapy and surgery are of the importance for laser ophthalmology. The solution of the problem is connected with the success in the development of robust techniques for the control of the optical properties of the human sclera. Such control means the change of the scattering or absorption properties of a tissue. In general, a number of laser surgery, therapy, and diagnostic technologies use the tissue compression and stretching for a better transport of the laser beam to underlying layers of tissue. Compression of the human eye sclera allows for performing of transscleral laser coagulation of the ciliary body and retina/choroid. Selective clearing of the upper tissue layers should be very useful for performing of transscleral laser coagulation of the ciliary body and retina/choroid, as well as for detecting of local inhomogeneities hidden by a highly scattering medium in the human eye tomography.

Recently a number of results on noninvasive in vivo monitoring of glucose concentration using near infrared (NIR) light scattering techniques in application to skin surface examination were reported. The main idea of such measurements is based on the dependence of tissue scattering properties on the refractive index mismatch between collagen fibers and the extracellular substance. In general, an increase in tissue glucose concentration reduces index mismatch and correspondingly decreases the scattering coefficient. Therefore, measurement of scattering coefficient allows for estimation of glucose concentration in the tissue. Osmotic effects play a key role in such measurements and can dramatically change tissue optical response on glucose concentration. The possibility of scleral reflectance measurements for in vivo monitoring of glucose concentration was discussed in our previous paper. It was shown that turbidity of sclera can be effectively controlled using above discussed immersion effect and that osmotic effects are very important.

In this paper, we present the results of in vitro and in vivo experimental study of scleral, and skin optical reflectance and transmittance controlled by administration of glucose, trazograph, glycerol, and DMSO. Glucose and other osmolytes having a higher refractive index than that of tissue ground (extracellular) substance diffuses into a tissue (water diffuses from a tissue to the surrounding solution) and affects corresponding equalizing of the refractive indices of scatterers (collagen fibers) and the ground substance. As a result the tissue optical clearing is observed. The model of chemical agent diffusion is suggested and values of an agent diffusion coefficient in the human scleral tissue are estimated. In vitro experiments were performed using samples of the human sclera obtained from autopsy human eyes post mortem. In vivo measurements were done with rabbit eye and the human skin.

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2. PHYSICAL PROPERTIES AND STRUCTURE OF FIBROUS TISSUE

A nontransparent fibrous tissue like eye sclera, skin dermis mainly consists of collagen fibers (fibrils), which can be packed in lamellar bundles that are immersed in an amorphous ground substance containing glycosaminoglycans, proteins, and protein-polysaccharide complexes. These fibrils are arranged in individual bundles in parallel fashion. Collagen bundles show a wide range of widths and thickness. They cross each other in all directions but remain parallel to the tissue surface. All these inhomogeneities give a high scattering of a tissue in normal state. For example, fibers in the scleral stroma have a diameter ranging from 30 to 300 nm. The thickness of the sclera in dependence on the age and region of the eye is in the range 0.3 - 1.8 mm. Hydration of the human sclera is 68%. About 75% of its dry weight are due to collagen, 10% - to other proteins, and 1% - to mucopolysaccharides.

The average value of the refractive index of a fibrous tissue
\[
n_s = n_{col} V_{col} + n_{gr} V_{gr},
\]
where \( n_{col}, n_{gr} \) and \( V_{col}, V_{gr} \) are the refractive indices and volume fractions of collagen and ground material, respectively; \( V_{col} + V_{gr} = 1 \).

Measurement of the average refractive index of the human sclera, \( n_s = 1.385 \pm 0.005 \) at \( \lambda = 589 \) nm, for the estimated volume fraction of hydrated collagen, \( V_{col} = 0.31 \), and refractive index of ground substance, \( n_{gr} = 1.345 \), allows for evaluation the refractive index of the scleral fibrils as \( n_{col} = 1.474 \).

3. OPTICAL AND MATTER DIFFUSION MODELS OF A FIBROUS TISSUE

Model of a fibrous tissue in a local region can be represented as a slab with a thickness \( d \) that is filled by thin and long dielectric cylinders (collagen fibers). For the human sclera the average diameter \(-100\) nm and refractive index \( n_{col} = 1.474 \). The cylinders are located in planes that are parallel to the slab surface, but within each plane their orientations are random. The interstitial space is filled by homogeneous ground substance with the refractive index \( n_{gr} = 1.345 \). This refractive index is a controlled parameter and can be changed in the range from 1.345 to 1.474. For \( n_{col} = n_{gr} = 1.474 \) the medium becomes totally homogeneous and optically transparent.

The transmission of collimated light by a tissue layer of thickness \( d \) is defined as
\[
T = I/I_0 = \exp(-\mu_d d),
\]
where \( I_0 \) and \( I \) are the intensities of the incident and detected light, respectively; \( \mu_s \) is the extinction coefficient, where \( \mu_a \) and \( \mu_s \) are the absorption and scattering coefficients, respectively. For the human sclera at the wavelength \( \lambda = 650 \) nm the absorption coefficient \( \mu_a \approx 0.08 \) \( \text{cm}^{-1} \) and reduced scattering coefficient \( \mu_s(1-g) \approx 25 \) cm\(^{-1} \), where \( g \) is the scattering anisotropy factor. For \( g = 0.9 \), \( \mu_s \approx 250 \) cm\(^{-1} \).

For a system of noninteracting thin cylinders with a number of fibrils per unit area \( \rho_s \), the scattering coefficient can be estimated as
\[
\mu_s = \rho_s \sigma_s \equiv \rho_s \left( \pi^2 a^4 k^3 / 8 \right) \left( m^2 - 1 \right)^2 \left( \frac{1}{2} + \frac{1}{2} \left( m^2 + 1 \right) \right) \]
where \( \sigma_s \) is the scattering cross section for unpolarized incident light; \( a \) is the cylinder’s radius; \( k \) is the wave number of light in the tissue; \( m = n_{col} / n_{gr} \) is the ratio of the refractive indices of the cylinders and ground materials; \( \Omega_s \) is the surface fraction of the cylinder’s faces. We assume that the radii and density of the fibrils are unchangeable and only refractive index of interstitial (ground) substance is controlled by the chemical agent administration.

To describe dynamics of the refractive index and corresponding alterations of the scattering coefficient when the chemical agent diffuses within the interstitial substance of the tissue we can consider it as a diffusion through a partially permeable
membrane. The model of diffusion can be represented as two spaces separated by a partially permeable membrane: internal volume is filled by interstitial substance of the tissue and external one is filled by the chemical agent solution. Flux of matter diffused through membranes is described by Fick’s first law, which limits the flux of matter to the gradient of its concentration.

Assuming that both water (interstitial space) and chemical agent have the same paths for diffusion, concentration \( c_1 \) of the chemical agent in the space which surrounds the tissue can be considered as a constant (enough large external volume), the time dependent concentration of the chemical agent \( c(t) \) within tissue can be expressed as:

\[
c(t) \equiv c_1 \{1-\exp(-t/\tau)\},
\]

where \( \tau = d^2/D \) – diffusion constant, \( D \) is the diffusion coefficient, \( d \) is the thickness of the tissue sample.

**4. MATERIALS AND METHODS**

4.1. Experimental Setup

The measurements of the light transmittance and the reflectance spectra were performed using OMA (spectrometer LESA-6med, BioSpec, Russia). The scheme of the experimental setup is shown in Fig. 1. A 250 W xenon arc lamp with filtering of the radiation in the 400 - 800 nm spectral range was used in these measurements.

![Fig. 1. Experimental setup for measurements of the collimated light transmittance and the reflectance spectra: 1 – optical fiber; 2 – aluminum jacket; 3 – neutral filters; 4 – scleral sample; 5 – osmotically active solution (glucose-40%); 6 - the 0.5 mm – diaphragm; 7 – cuvette.](image)

In the case of *in vitro* light transmission measurements the cuvette with the sample was placed between two optical fibers (400 µm core diameter). One fiber transmitted the excitation radiation to the sample, and another fiber collected the transmitted radiation. The 0.5 mm diaphragm placed 20 mm apart from the tip of the receiving fiber was used to provide collimated transmittance measurements. Neutral filters were used to attenuate the incident radiation.

*In vivo* reflectance measurements were performed using the fiber optical probe with seven fiber holders. The centrally placed fiber (400 µm core diameter) delivers incident light to the surface of the sample and used for its illumination, and the six surrounding fibers (400 µm core diameter) collected reflected from the illuminated surface radiation. Fibers for collection of reflected light are mounted at small angles regarding the central fiber, so each fiber collects emission from the surface area some larger than the excitation light spot. All fibers are enclosed in aluminum jacket (6 mm outer diameter) to provide a fixed distance between the fibers and the sample surface. The distal ends of six collecting fibers were arranged as a vertical structure and imaged at the entrance slit of multichannel spectrometer. The reflectance spectra of the samples were measured against BaSO₄ as a reference. The measurements were performed every 30 sec for 30 – 60 min.
4.2. Tissue samples preparation

The samples of the human sclera were extracted correspondingly from the eye of men *post mortem*. After enucleating, eye was inflated with saline. During *in vitro* measurements the conjunctiva and the ciliary body, as well as the retina with choroid was removed. Samples were cleaned and cut into pieces of about 10×10 mm². Samples were fixed on a plastic plate with a square aperture 5×5 mm² and placed in a 5-ml cuvette filled with the chemical agent.

4.3. *In Vivo* Measurements

*In vivo* measurements were done for rabbit. Its age was 5 month and weight was 1.5 kg. The surface temperature of the rabbit eyes was ~38°C. The rabbit was anaesthetized by an injection of 0.1% *Natrium Ethaminal* solution. The doze was about 4 ml. The measurements were started in 30 min after the injection. *Glucose-40%* was used as a chemical agent for the scleral optical clearing. About 0.3 ml of glucose was put into the eye by drops. Recording of the scleral tissue reflectance spectra in the spectral range from 400 to 800 nm was provided by placing the fiber optical probe on the surface of the eye sclera.

For the human skin back reflectance measurements were done at 830 nm using the fiber optical diode laser photometer.

5. EXPERIMENTAL RESULTS

5.1. *In Vivo* Measurements for the rabbit eye

One of the goals of this paper was *in vivo* studying the change of the optical reflectance spectra of scleral tissue under administration of the osmotically active solution (*glucose-40%*). The results of *in vivo* measurements for the rabbit eye are shown in the Fig. 2. As seen from this figure the time dependence of the reflectance spectra is nonlinear. The main feature of the change is a significant decrease of the reflectance during the first five minutes of glucose administration. Dips appeared in the spectra at 420, 530 and 570 nm are caused by the influence of blood perfusion. Received spectra for untreated sclera at the wavelengths far from blood absorbing bands are well match to data presented in Ref. 19 for *in vitro* spectral reflectance measurements of the rabbit eye scleral samples. Plots for the time-dependent reflectance at three wavelengths are presented in Fig. 3. The lower reflectance at 420 nm is caused by the strong absorption of blood. Evidently, more fast decay reflects the blood perfusion dynamics due to the eye conjunctiva and sclera inflammation caused by light irradiation and osmotic action of glucose. Because of less influence of blood at 630 and 700 nm measured dynamic responses can be used for estimation of diffusion coefficient for glucose in scleral tissue.

![Fig. 2](image)

**Fig. 2.** The *in vivo* time-dependent reflectance spectra of the rabbit eye sclera measured concurrently with administration of glucose-40% solution: 1 - 60 sec; 2 - 240 sec; 3 - 1260 sec; 4 - 1500 sec; and 5 - 1800 sec after drop of glucose-40% solution into the rabbit eye.
5.2. In vivo measurements for the human skin

For the in vivo measurements of the back reflectance of the human skin the source-detector separation of laser diode photometer was fixed at 0.28 cm. The measurements were carried out on the human forearm. We used solution of 50% Glycerol and DMSO as the source of the changes of optical properties of skin. This substance was applied to the skin before the measurements. The dynamics of reflectance was monitored for 10 minutes (see Figs. 4 and 5). We also studied diffuse reflectance of the skin after the removal of the top layer with scrub cream. Since the reflectance is lower in the case of solution applied we conclude that this solution causes immersion of the skin, i.e. the skin becomes more transparent for the light. The preliminary scrub cream processing makes skin less responding to the application of osmotically active substances. The possible explanation of this experimental fact is that scrub removes the top layer of the skin which easily absorbs the immersion liquid, while the underlying layer less permeable for it.

![Reflectance Dynamics](image)

**Fig. 3.** The in vivo time-dependent reflectance of the rabbit eye measured at 420 nm (solid line, down triangles), 630 nm (dash line, squares) and 700 nm (dot line, up triangles) concurrently with administration of glucose-40%.

**Fig. 4.** The reflectance dynamics of non-processed and scrub-processed skin in vivo.
5.3. *In Vitro* Measurements

To understand the mechanisms of the fibrous tissue optical clearing we have investigated the collimated transmittance spectra of the human scleral samples concurrently with administration of glucose solution. Figure 6 illustrates the corresponding transmittance spectra.

**Fig. 5.** The immersion by 50% Glycerol and DMSO solution of non-processed and scrubbed skin *in vivo*.

**Fig. 6.** The time-dependent collimated transmittance of the human sclera sample of thickness 0.5 mm impregnated by a glucose-40%: 1 - 10 sec; 2 – 60 sec; 3 – 120 sec; 4 – 180 sec; 5 – 240 sec; 6 – 300 sec; 7 – 390 sec; 8 – 510 sec after the scleral sample was immersed in a glucose-40%.
Fig. 7. The time-dependent collimated transmittance of the human scleral sample measured at 420 (1); 630 (2); and 700 nm (3) concurrently with administration of glucose-40%.

It is easily seen that the untreated sclera is poorly transparent for visible light. Glucose administration makes this tissue highly transparent, for example, up to 18% at 750 nm for the sample kept in solution for $t = 8.5$ min. These spectra are well match to spectra presented in Refs. 1 and 4, respectively, for untreated and treated samples. The corresponding plots for time-dependent collimated transmittance at 420, 630, and 700 nm are presented in Fig. 7. They show the dynamics of tissue clearing. The registration of the collimated transmittance can be used to estimate the diffusion coefficients of glucose. Figure 7 shows that characteristic time response of human optical clearing is about 5 minutes.

5.4. Estimation of diffusion coefficient

For estimation of diffusion coefficient we used experimental data for the collimated transmittance, which are presented in Fig. 7. The transport of glucose in tissue sample can be described in the framework of the matter diffusion model. We assume that the following approximations are valid for description of glucose transport:

a) The diffusion coefficient is constant over the entire scleral volume.

b) Volume fraction of glucose in interstitial fluid is defined as $C_{gl}(t) = \frac{V_{gl}(t)}{V_{gl}(t) + V_{if}(t)} = C_{gl}^0 \left( 1 - \exp \left( -\frac{t}{\tau_{gl}} \right) \right)$, where $V_{gl}(t)$ is the time-dependent volume of glucose in tissue interstitial space; $V_{if}(t)$ is the time-dependent volume of water and proteins constitute the interstitial matter; $C_{gl}^0$ is the concentration of glucose in the external volume (expressed in volume fractions); $\tau_{gl} = d^2/D_{gl}$ is the diffusion constant; $D_{gl}$ is the diffusion coefficient; $d$ is the thickness of the scleral sample.

c) The volume fraction of water and proteins: $C_{if}(t) = 1 - C_{gl}(t)$.

d) Volume of the interstitial matter is defined as $V_{i}(t) = S \cdot d - V_{c}$, where $S$ is the area of the scleral sample and the $V_{c}$ is the volume of the collagen fibrils, respectively. The volume of collagen fibrils $V_{c}$ is defined as 0.31 of scleral sample volume.$^4$

e) Volume of water and proteins of interstitial space: $V_{if}(t) = C_{if}(t) \cdot V_{i}(t)$.

f) The volume of glucose dissolved in interstitial fluid: $V_{gl}(t) = \frac{C_{gl}(t)}{1 - C_{gl}(t)} \cdot V_{if}(t)$.
g) The refractive index of the interstitial fluid: \( n_f(t) = \frac{V_g(t)}{V_f(t)} \cdot n_{base} + \frac{V_g(t)}{V_f(t)} \cdot n_{gl} \), where \( n_{base} = 1.345 \).

h) The scattering coefficient dependence on refraction index is defined by Eq. 3.

i) The refractive index of the scleral sample: 
\[
\left( n_{scl}(t) = \frac{V_c}{S \cdot d} \cdot n_c + \frac{V_g(t)}{S \cdot d} \cdot n_{base} + \frac{V_g(t)}{S \cdot d} \cdot n_{gl}, \right.
\]
where \( n_c \) is the refractive index of collagen \((n_c = 1.474)\), \( n_{gl} \) is the refractive index of glucose \((n_{gl} = 1.4854)\).

j) The specular reflectance can be estimated from the collimated transmittance of the scleral sample impregnated by a glucose solution: 
\[
T_s(t) = \left( 1 - R_s(t) \right)^2 \cdot \exp \left( - (\mu_a + \mu_s(t)) \cdot d(t) \right),
\]
where \( R_s(t) \) is the specular reflectance and \( \mu_a \) and \( \mu_s \) are the absorption and the scattering coefficients, respectively.

This set of relations describing the glucose concentration in dependence on time represents the direct problem. The reconstruction of the diffusion coefficient of the scleral sample were carried out on the basis of measured time evolution of the collimated transmittance. The solving of the inverse problem were done by minimization of the target function: 
\[
F(t) = (T_c(t) - T_c^*(t))^2,
\]
where \( T_c(t) \) and \( T_c^*(t) \) are respectively the theoretical and experimental values of the time-dependent collimated transmittance.

The Levenberg-Marquardt method described in Ref. 20 was used for the minimization of the target function. The Levenberg-Marquardt method is a quasi-Newton method (a variation of the gradient method). At each step, we estimate the first partial derivatives of the errors with respect to the variables to be solved to create a Jacobian matrix. Ordinarily, we can determine the next estimate to make by computing the Gauss-Newton step \( s \) for each variable. In matrix notation, we solve the matrix equation: 
\[
J \cdot s = -f(x)
\]
in this equation, \( J \) is the Jacobian matrix, \( s \) is the step to take, and \( x \) is the vector of current estimates for unknown variables. For the first step, \( x \) is the vector of guesses; at each subsequent step, the new \( x \) is the old \( x \) plus \( s \), the vector of steps. Notice that computing of this step involves inverting the Jacobian matrix \( J \).

Computing of this step is not always possible. It is fail when the Jacobian matrix cannot be inverted or when there are more constraints than variables to be solved. In these cases, we add the additional condition that the following quantity be reduced to a minimum: 
\[
\sum_j \left( D_j \right)^2 \left( s_j \right)^2.
\]
Here \( D \) is a vector of weight factors computed from the norms of the columns of the Jacobian matrix. In these cases, \( s \) is computed to satisfy this minimization criteria as well as solving the Newton equation with the Jacobian.

In results we have estimated the average value of the diffusion coefficient of glucose transport in the scleral sample as 
\[
5.62 \cdot 10^6 \pm 1.64 \cdot 10^6 \text{ cm}^2/\text{sec}.
\]

5.5. Measurement of the optical anisotropy of biotissues

Due to fibrous structure of many tissues, like sclera, skin dermis or breast, there is expected that such tissues have to show optical anisotropy of form (birefringence and dichroism) caused by light scattering by a system of quasi-regular distributed collagen fibrils. The transmission spectrum substantially depends on the orientation of polarization vector of the linear polarized light relative to the collagen fibrils; the light polarized along fibrils is scattered more effectively. In the Rayleigh limit \((\lambda >> 2a, a \text{ is the fibril radius})\), the form birefringence is defined as
\[
n_e - n_o = V_1 V_2 (n_1 - n_2)^2 / (V_1 n_1 + V_2 n_2),
\]
where \( V_1 \) is the fractional volume of the cylinders which are arranged in parallel to each other; \( V_2 \) is the ground substance volume; and \( n_1, n_2 \) are the respective indices of refraction. The birefringence can be high for a small diameter cylinders and goes to zero for a system consists of parallel cylinders with large diameters \((2a \geq \lambda)\).

Such anisotropy properties should be more pronounced for a translucent tissue. We have studied transmittance and optical anisotropy properties of the human sclera, skin (epidermis and dermis), for fresh samples of tissue. Measurements of the optical anisotropy were done using method of a comparison of color coordinates of a sample under study and reference (see Figs 8 and 9).
Fig. 8. Scheme of the experimental setup for *in vitro* measurements of tissue sample time-dependent translucence caused by its impregnation by osmotically active agents. Setup contains white lamp or laser, cuvette with a sample located between two polarizers, and a digital camera with a personal computer.

Fig. 9. Color triangles. The coordinates of color of the reference (the liquid crystal at various controlling voltages) are shown by a line of black squares. The color coordinate of translucent human sclera is shown by a circle.

For example, for the translucent human scleral sample by its impregnation by a highly concentrated *trazograph* solution (about 70%) the optical anisotropy, defined by Eq. (5), \( n_e - n_o = 0.001 \) (Fig. 10). The additional measurement of the collimated transmittance allowed for estimation of the refractive index of the ground substance of the translucent tissue, \( n_r = 1.39 \). Using a well known value of the refractive index of hydrated collagen, \( n_f = 1.474 \), the mean diameter and mean length of collagen fibrils were estimated, respectively, as 100 nm and 0.5 mm. These data well correlate with electronic microscopy study in.
Fig. 10. Dependence of the optical anisotropy (difference of index of refraction for two polarization states) of the human sclera on index of refraction of the ground matter.

6. DISCUSSION

Presented results well show that fibrous tissue transmission and reflection spectra can be substantially changed by administration of osmotically active chemical agent. From experimental data presented in Figs. 3 and 7 one can see that for \textit{in vivo} measurements reflectance decreases up to 1.44–2.09 folds that is considerably less than that for \textit{in vitro} measurements of collimated transmittance, increasing of transmittance up to 14.6–25.8 folds. From the optical point of view we have to compare the same quantities. Unfortunately, it is not easy to perform \textit{in vivo} measurements of collimated and especially total transmittance. The living tissue seems to be more effectively controlled by immersion phenomenon due to more strong influence of absorbing layers which reduce the fluence rate of the back scattered photons as the light penetrates more deeply inside tissue (due to reduction of scattering) where absorption is maximal. Another reason for more effective control is the blood perfusion and metabolic activity of leaving tissue which allows for more effective impregnation of tissue in spite of that the osmolyte effects only the exterior surface of the tissue.

Measurements of the time-dependent collimated transmittance allowed us, basing on presented tissue refractive index matching model, to estimate the diffusion coefficients of the chemical agent (glucose – 40\%). The mean value of the diffusion coefficient for glucose solution transport in the human scleral sample is equal to $5.62 \cdot 10^{-6} \pm 1.64 \cdot 10^{-6}$ cm$^2$/sec. This value is not far from values for small molecules (like sucrose, glucose, etc.) diffusion in water at zero concentrations $(3.6–5.2) \cdot 10^{-6}$ cm$^2$/sec at 12–15 °C. We believe that this experimental result confirms the correctness of the refractive index matching model.\footnote{4}

\textit{In vitro} experiments show the intensive optical clearing at first few minutes, then transmittance saturates and even goes down after approximately 8 min after a sample placed in solution (Fig. 7). Besides refractive index matching the intensive clearing at first minutes is determined by tissue shrinkage due to osmotic stress of glucose solution. In its turn, the saturation of refractive index matching with time leads to saturation of tissue transmittance, but for a long time of sample kept in solution its swelling causes growing of tissue thickness and corresponding reduction of transmittance.
CONCLUSION

The results of this paper show that administration of osmolytes to a fibrous tissue allows for effectively control of its optical characteristics. The scattering properties of the sclera are effectively reduced by the refractive indices matching of the collagen fibrils and interstitial substance.

The dynamics of tissue optical clearing by used chemical agents is defined by a characteristic time response of about 5–8 min. Besides the refractive indices matching effect the tissue shrinkage plays an important role in tissue translucence. For more prolonged administration of the osmolyte (from 8 to 30 min for 40%-glucose solution) tissue swelling saturates and even slightly reduces the tissue transparency.

Dynamic optical characteristics can be used for determination of diffusion coefficient of endogenous (metabolic) and exogenous (chemical agent) fluids in the human sclera and other tissues. Received values for diffusion coefficient of glucose in the human scleral tissue are well match to values of diffusion coefficient of small molecules diffusion in water.

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