In vivo and *in vitro* study of control of rat skin optical properties by acting of 40%-glucose solution

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ABSTRACT

We present experimental results on optical properties of the rat skin controlled by administration of osmotically active chemical, such as the *40%-glucose* solution. *In vivo* reflectance and *in vitro* transmittance spectra of the rat skin were measured. Result of the experimental study of influence of the *40%-glucose* solution on reflectance and transmittance spectra of the rat skin are presented. The significant increase of transmittance and decrease of reflectance of the rat skin under action of osmotical agent are demonstrated. The average value of *glucose* solution diffusion coefficient was estimated as $1.101 \cdot 10^{-6} \pm 0.153 \cdot 10^{-6}$ cm^2/sec.

Keywords: *in vivo* measurements, light scattering, skin, osmolytes, refractive index matching.

1. INTRODUCTION

Absorption and scattering properties of living tissues, particular in skin, can be effectively controlled by matching of the refractive indices of scattering centers and ground matter by means of intra-tissue administration of the appropriate chemical agents.[1-18] Experimental studies on optical clearing of normal and pathological skin and its components (epidermis and dermis) and the management of reflectance and transmittance spectra using *water*, *glycerol*, *glycerol-water* solutions, *glucose*, *sunscreen creams*, *cosmetic lotions, gels* and *pharmaceutical products* were carried out in Refs. 5, 7, 11-22. Controlling of skin optical properties was related to the immersion of refractive indices of scatterers (keratinocyte components in epidermis, collagen and elastin fibers in dermis) and ground matter. For some tissues as the secondary effect, drying of connective tissue fibers and swelling or shrinkage of cells should be taken into account.[10,14]

A marked clearing effect through the rat skin (reduced back reflectance) was occurred for an *in vivo* tissue within a few minutes of intra-dermal injection of *glycerol*.[5] *In vivo* topical impregnation of the human skin by *glycerol*, *glucose*, *trazograph* (x-ray contrasting substance), *cosmetic lotions* and *gels* also made skin more translucent in a few minutes.[7,11,12,14,16,22] The loss of water by the skin or its impregnation by water or moisturizing substances seriously influences skin optical properties.

In this paper, we have presented the results of experimental *in vivo* and *in vitro* study of the rat skin optical properties changes under action of *40%-glucose* solution.

2. MATERIALS AND METHODS

2.1 Experimental setups

The measurements of the light collimated 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 - 700 nm spectral range was used in these measurements.

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

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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. The measurements were performed every 60 sec for 400 min.

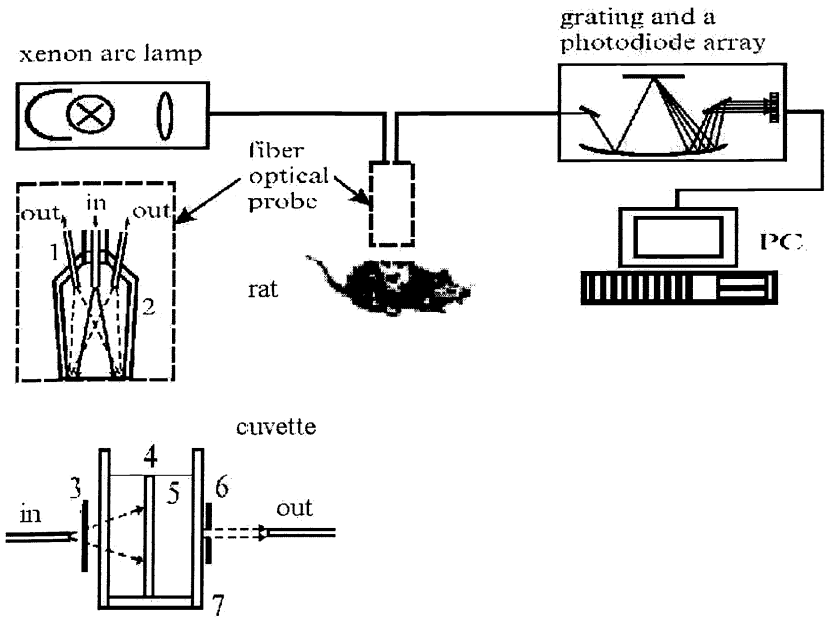

Figure 1. Experimental setup for measurements of the collimated transmittance and the reflectance spectra: 1 – optical fiber; 2 – aluminum jacket; 3 – neutral filters; 4 – rat skin sample; 5 – osmotically active solution (*40%-glucose* solution); 6 - the 0.5 mm – diaphragm; 7 – cuvette.

In vivo reflectance measurements were performed using the fiber optical probe with seven fibers. 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 (also 400 μm core diameter) collected radiation reflected from the illuminated surface. Collecting fibers are mounted at small angles regarding the central fiber, so each fiber collects emission from the surface area a little bit 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 tissue surface. The distal ends of six collecting fibers were arranged as a vertical structure and imaged at the entrance slit of multi-channel spectrometer. The reflectance spectra of the samples were measured against $BaSO_4$ plate as a reference. The measurements were performed every 60 sec for 105 min.

Electron microscopy was used to determine structure of the rat skin tissue. These measurements were performed for tissue samples obtained by autopsy within an hour *post mortem*. Samples of the rat skin were prepared using standard technology for electron microscopy of the tissues. Each sample of the rat skin was cut into pieces with the thickness 100 nm using commercially available microtome (LKB company), which then were negatively colored by a phosphotungstic acid. These experiments were performed using electron microscope JEM-7A at accelerated voltage 80 kV.

2.2 Tissue samples preparation

Table 1. Thickness of the rat skin tissue samples, averaged for ten measurements.

Sample	*Thickness, mm*	*Standard deviation*
Sample 1	0.73	0.04
Sample 2	0.57	0.02
Sample 3	0.7	0.03

In vitro experiments were performed with the three samples of the rat skin tissue extracted from various rat specimens. Tissue samples were obtained by autopsy within an hour *post mortem*. Before experiments hairs were removed using tweezers.

Samples thickness was measured just before experiments (see Table 1). For samples, 2 and 3 hypodermic fatty layer was removed. For *in vitro* measurements, the *40%-glucose* solution was used as a chemical agent for the skin optical clearing. Before experiments, the rat skin tissue was cut into pieces with the area about 1 cm^2. All experiments were performed at room temperature.

2.3 *In vivo* measurements

In vivo measurements were done for white rat (*rat wistar*) specimen. Rat age was about 9 months and weight was about 200 g. Rat was anaesthetized by an intraperitoneal injection of the *1%-natrium ethaminal* solution with doze 40 mg/kg of the animal weigh. Removing the hairs was done before experiments. Firstly, we measured the reflectance from the untreated rat skin. Then the injection of the *40%-glucose* solution was done in the same place. Injection of osmotically active agent was done into subdermis of the rat skin. The measurements of dynamics of the optical clearing for rat specimen were started after 60 sec after the injection. The *40%-glucose* solution was used as a chemical agent for the skin optical clearing. Refractive index of the *40%-glucose* solution was measurement by Abbe refractometer. It is 1.390 at wavelength 589 nm. Recording of the rat skin 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 rat skin.

2.4 Estimation of the glucose diffusion coefficient from *in vivo* measurements

As it was mentioned above administration of the osmotical active substance into tissue occurs decreasing of the tissue scattering coefficient because of matching of the refractive indices of the scatterers and surrounding medium. According to diffusion theory, the spatial dependence of the diffuse reflectance, $R(\rho)$, of continuous light remitted from a semi-infinite scattering medium at a separation of ρ from the source is[23-25]

$$R(\rho)=\frac{I_0}{4\pi\mu_t'}\left[\left(\mu_{eff}+\frac{1}{r_1}\right)\frac{exp\left(-\mu_{eff}\cdot r_1\right)}{r_1^2}+\left(\frac{4}{3}A+1\right)\left(\mu_{eff}+\frac{1}{r_2}\right)\frac{exp\left(-\mu_{eff}\cdot r_2\right)}{r_2^2}\right]$$

where $r_1=\sqrt{\left(1/\mu_t'\right)^2+\rho^2}$, $r_2=\sqrt{\left(\left(\frac{4}{3}A+1\right)/\mu_t'\right)^2+\rho^2}$, $\mu_t'=\mu_a+\mu_s'$, $\mu_{eff}=\sqrt{3\mu_a\left(\mu_a+\mu_s'\right)}$, μ_a is the absorption coefficient of the medium, μ_s' is the reduced scattering coefficient of the medium, I_0 is the initial light source intensity, and A is an internal specular reflection parameter, depending only on the relative refractive index of the tissue and surrounding medium. For matching of this formulae with geometry used in our experiment we have integrated the function $R(\rho)$ over all area from which reflected radiance was collected. Absorption coefficients of the skin were presented in Ref. 26. Then a nonlinear least-squares fit to this equation with the Levenberg-Marquardt method yielded the reduced scattering coefficient, μ_s' in dependence on wavelength and time of osmotical active substance influence.

Reduced scattering coefficient depends from the number density of scatterers, sizes of the scatterers, and values of the refractive indices of the scatterers and surrounding medium.[27] For native skin all these characteristics were obtained in Ref. 26. When the osmotical active liquid influences on the tissue change of the refractive index value of the medium surrounding of the scatterers of skin takes place. Using the method described in Ref. 26, we can calculate change of the value of the interstitial fluid refractive index in dependence on the time of action of *40%-glucose* solution on the skin.

As was presented in Refs. 1, 7, and 8 refractive index of the interstitial fluid is connected with concentration of osmotical active liquid by following relationship.

$$n = (1-C)n_0 + Cn_{gl}$$

$$C = C_0\left(1-\exp\left(-\frac{t}{\tau}\right)\right)$$

where n is the refractive index of the interstitial fluid of the skin; n_0 is initial value of the refractive index of the interstitial fluid of the skin (untreated skin); C is the concentration of osmotical active liquid (*40%-glucose* solution), volume fractions; n_{gl} is the refractive index *40%-glucose* solution; C_0 is the initial concentration of *40%-glucose* solution $(C_0 = 1)$; t is the time of influence of osmotical liquid on the tissue, and τ is the diffusion constant, $\tau = \frac{d^2}{D}$; d is the thickness of the skin, and D is the diffusion coefficient. Thus, if we know as the refractive index of the interstitial fluid changes under action of the osmotical liquid we can reconstruct the value of the diffusion coefficient of *40%-glucose* solution into the rat skin.

3. RESULTS AND DISCUSSION

Because of relatively big thickness of dermis (up to 1-2 mm for the human skin) and comparable scattering coefficients of epidermis and dermis the optical properties of the skin is defined by the optical properties of the dermis. Dermis is a typical fibrous tissue. Figure 2 presented the typical electron-microscope photograph of the dermal tissue. From comparison of this photograph with typical electron microscope photographs of the scleral tissue[28] we can see that both dermis and sclera have the similar fibrous structure.

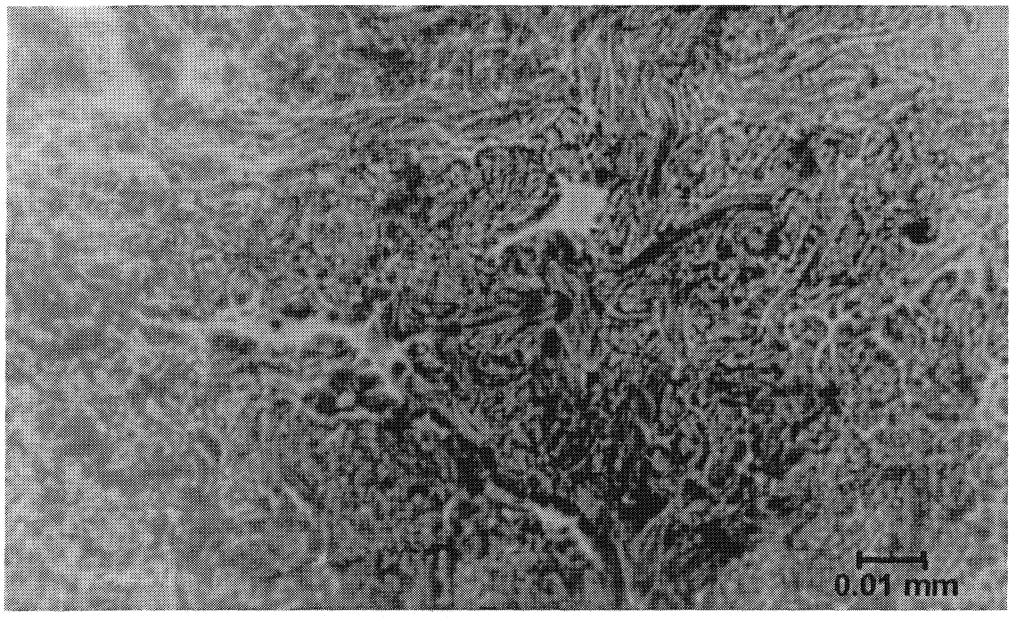

Figure 2. Electron microscope photograph of the dermal tissue (magnification x960)

3.1 *In vitro* investigation of the optical clearing of the rat skin

To understand the mechanisms of the rat skin tissue optical clearing we have investigated the collimated transmittance spectra concurrently with administration of *40%-glucose* solution. Figure 3 illustrates the typical collimated transmittance spectra (sample 1). It is well seen that the untreated rat skin is poorly transparent for the visible light. The *40%-glucose* solution administration makes this tissue highly transparent. For example, transmittance increases up to 15 times at 700 nm for the sample kept in the *40%-glucose* solution during in t = 60 min (Fig. 4). Figures 5 and 6 present the time-dependent collimated transmittance at different wavelengths for samples 2 and 3, respectively. They show the dynamics of tissue clearing for various skin samples.

Presented experimental results well show that the rat skin transmittance spectra can be substantially changed by administration of osmotically active chemical agent (*40%-glucose* solution). Analyzing the data presented in a Figs. 4-6 and comparing them with data obtained for clearing of another fibrous tissue, it can be concluded that permeability of the agent into the skin less than that into the sclera[8] or into the *dura mater*[9]. Representative clearing time for scleral tissue is about 8 min. Thus, we can connect the prolonged clearing effect up to 1-4 hours with protective properties of epidermis and fat layer, which prevent the penetration of osmotically active agent into dermis.

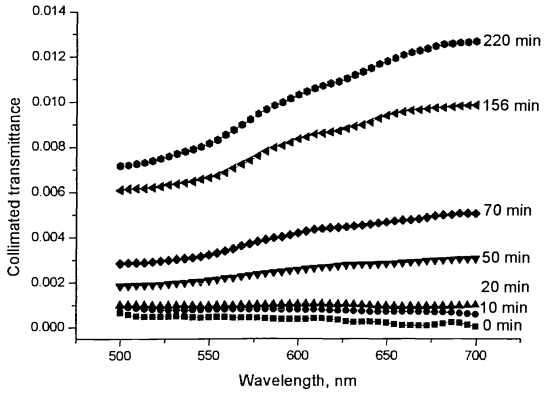

Figure 3. Typical collimated transmittance spectra of the rat skin sample (sample 1) measured concurrently with administration of the *40%-glucose* solution at different time intervals.

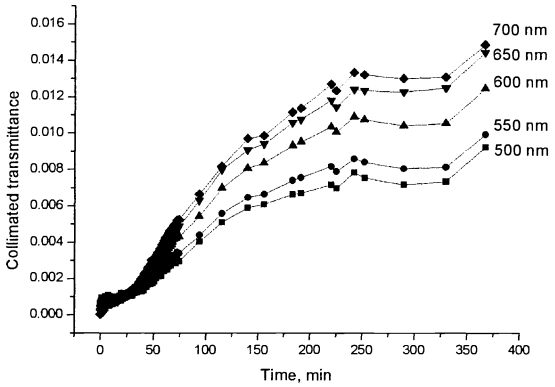

Figure 4. The time-dependent collimated transmittance of the rat skin sample (sample 1) measured at different wavelengths concurrently with administration of the *40%-glucose* solution.

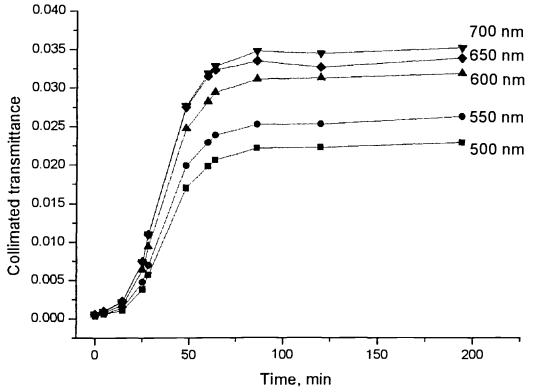

Figure 5. The time-dependent collimated transmittance of the rat skin sample (sample 2) measured at different wavelengths concurrently with administration of the *40%-glucose* solution.

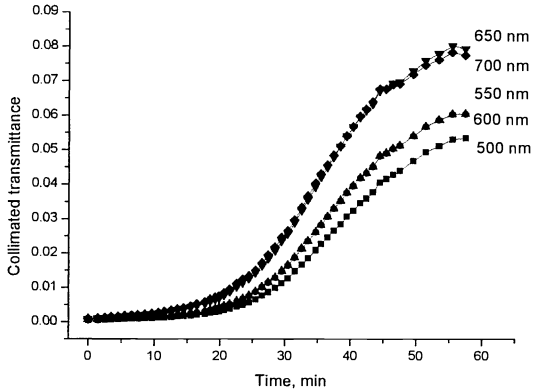

Figure 6. The time-dependent collimated transmittance of the rat skin sample (sample 3) measured at different wavelengths concurrently with administration of the *40%-glucose* solution.

To confirm this hypothesis we compared the graphs presented the dynamics of the rat skin samples clearing. Analyzing the clearing dynamics of the sample with unremoved hypodermic fatty layer (Fig. 4) it is seen that the clearing of tissue continuous after 400 min of agent action. The maximum value of collimated transmittance is only 0.015 at wavelength 700 nm. In contrast, for samples with removed hypodermic fatty layer the clearing process is finished after 60 min of *glucose* action (Figs. 5 and 6). In this case, the maximum values of the collimated transmittance are 0.035 and 0.075 for samples 2 and 3, respectively.

3.3 *In vivo* investigation of the optical clearing of the rat skin

Figure 7 shows the *in vivo* back reflectance spectra measured in the area of hypodermic injection of the *40%-glucose* solution. Figure 8 presents the dynamics of the change of reflectance at different wavelengths.

Analyzing the results of this experiment, it can be seen that the dynamics of the reflectance change has monotone character (Fig. 8). The decreasing of the reflectance at the beginning and then its increasing we connect with a slight change of experimental geometry, when fiber top contacted with skin surface. Because of the bubble appearance on the skin surface after hypodermic injection of the osmotical active liquid. It disappears during 5-10 minutes and then we see the skin clearing induced by matching of refractive indices of the skin scatterers (collagen and elastin fibers of dermis, cells of epidermis, fat cells, etc.) and interstitial fluid which surrounding them. It has be, noted that process is stabilized after 60-70 minutes after injection, when reflectance does not change. This time (60-70 min) is well matched with data obtained from *in vitro* experiments described above (see Figs. 5 and 6). From this, we can conclude that these data well describe the process of the living tissue clearing.

Using the method, which was described in Section 2.4 we have estimated the value of the *40%-glucose* solution diffusion coefficient into the rat skin. It is equal $1.101 \cdot 10^{-6} \pm 0.153 \cdot 10^{-6}\ cm^2/\text{sec}$. Using the data, which were presented in Ref. 26 we also have estimated the value of the diffusion coefficient of the *glycerol* into the rat skin. It is equal $1.157 \cdot 10^{-6} \pm 0.026 \cdot 10^{-6}\ cm^2/\text{sec}$.

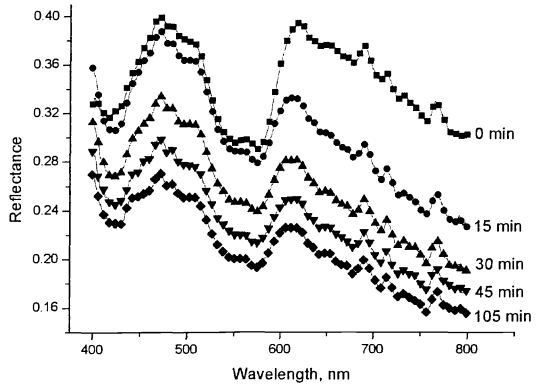

Figure 7. The *in vivo* time-dependent reflectance spectra of the rat skin measured in the area of hypodermic injection of the *40%-glucose* solution.

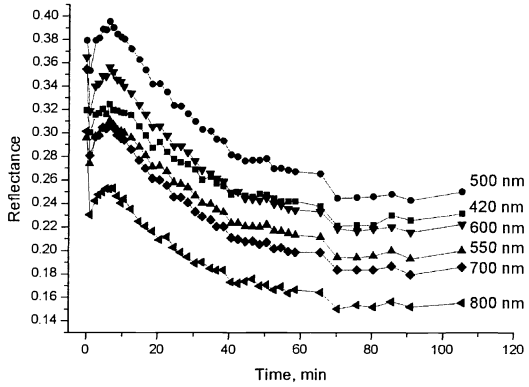

Figure 8. The *in vivo* time-dependent reflectance of the rat skin measured at different wavelengths in the area of hypodermic injection of the *40%-glucose* solution.

It should be also noted that 1) we have watched a white ring around the injection puncture of about 1 cm in radius, when *glucose* or after osmolyte (*glycerol*) injection. The origin of this ring is due to osmolyte and tissue water interaction, which diffuse from surrounding tissue occupies some area around osmolyte injection. Because of additional amount of water in this ring. The additional mismatch of refractive indices of the scatterers and interstitial fluid is occurring, that is why this ring looks, like white. 2) It was shown that in the case of *glycerol* use as an active agent the irritation of hypodermic tissues after 13.5 min takes place.[26] It is expressed in the rush of blood to the place of injection. 3) In the case of using of the *40%-glucose* solution, as a clearing agent the rush of blood is not observed, as is well seen in Fig. 8. Hence, we can conclude that the *40%-glucose* solution is more applicable than *glycerol* for *in vivo* optical clearing of the tissue in spite of the fact that *glycerol* has larger refractive index that the *40%-glucose* solution.

4. CONCLUSION

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

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REFERENCES

1. V.V. Tuchin, I.L. Maksimova, D.A. Zimnyakov, I.L. Kon, A.H. Mavlutov, A.A. Mishin, "Light propagation in tissues with controlled optical properties," *J. Biomed. Opt.* **2** (4), pp. 401-417, 1997.
2. E.K. Chan, B. Sorg, D. Protsenko, M. O'Neil, M. Motamedi, A.J. Welch, "Effects of compression on soft tissue optical properties," *IEEE Journal of selected topics in quantum electronics* **2** (4), pp. 943-950, 1996.
3. B. Chance, H. Liu, T. Kitai, Y. Zhang, "Effects of solutes on optical properties of biological materials: models, cells, and tissues," *Anal. Biochem.* **227**, pp. 351-362, 1995.
4. P.O. Rol, *Optics for transscleral laser applications*, Ph.D. Dissertation: Swiss Federal Institute of Technology, Zurich, Switzerland. ETH N. 9665, 1991.
5. G. Vargas, E.K. Chan, J.K. Barton, H.G. Rylander III, A.J. Welch, "Use of an agent to reduce scattering in skin," *Laser in Surgery and Medicine* 24, pp. 133-141, 1999.
6. A.N. Bashkatov, V.V. Tuchin, E.A. Genina, Yu.P. Sinichkin, N.A. Lakodina, V.I. Kochubey, "The human sclera dynamic spectra: in vitro and in vivo measurements," *Proc.* SPIE **3591**, pp. 311-319, 1999.
7. V.V. Tuchin, A.N. Bashkatov, E.A. Genina, V.I. Kochubey, N.A. Lakodina, G.V. Simonenko, Yu.P. Sinichkin, Yu.M. Proshina, N.A. Razumikhina, "Optics of living tissues with controlled scattering properties," *Proc.* SPIE **3863**, pp. 10-21, 1999.
8. A.N. Bashkatov, E.A. Genina, Yu.P. Sinichkin, N.A. Lakodina, V.I. Kochubey, V.V. Tuchin, "Estimation of glucose diffusion coefficient in scleral tissue," *Proc.* SPIE **4001**, pp. 345-355, 1999.
9. A.N. Bashkatov, E.A. Genina, V.I. Kochubey, Yu.P. Sinichkin, A.A. Korobov, N.A. Lakodina, V.V. Tuchin, "*In vitro* study of control of human *dura mater* optical properties by acting of osmotical liquids," *Proc.* SPIE **4162**, pp. 182-188, 2000.
10. H. Liu, B. Beauvoit, M. Kimura, B. Chance, "Dependence of tissue optical properties on solute-induced changes in refractive index and osmolarity," *J. Biomed. Opt.* **1** (2), pp. 200-211, 1996.
11. V.V. Tuchin, Yu.M. Proshina, N.A. Razumikhina et al., "Dosimetry of laser radiation for immersed skin," *Proc* SPIE **3601**, pp. 491-495, 1999.
12. V.V. Tuchin, "Controlling of tissue optical properties," *Proc* SPIE **4001**, pp. 30-53, 2000.
13. R.R. Anderson and J.A. Parrish, "Optical properties of human skin," *in The Science of Photomedicine*, Eds. J.D. Rogan and J.A. Parrish, Plenum Press, New York, pp. 147–194, 1982.
14. V.V. Tuchin, *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis*, SPIE Tutorial Texts in Optical Engineering, **TT38**, 2000.
15. V.V. Tuchin, "Light scattering study of tissues," *Physics-Uspekhi* **40**, pp. 495-515, 1997.
16. V.V. Tuchin, J. Culver, C. Cheng, S.A. Tatarkova, M. DellaVecchia, D.A. Zimnyakov, A. Chaussky, A.G. Yodh, and B. Chance, "Refractive index matching of tissue components as a new technology for correlation and diffusing-photon spectroscopy and imaging," *Proc.* SPIE **3598**, pp. 111-120, 1999.
17. V.V. Tuchin, "Coherent optical techniques for the analysis of tissue structure and dynamics," *J. Biomed. Opt.* **4**, pp. 106-124, 1999.
18. V.V. Bakutkin, I.L. Maksimova, P.I. Saprykin, V.V. Tuchin et al., "Light scattering by the human eye sclera," *J. Appl. Spectroscopy (USSR)* **46**(1), pp. 104-107, 1987.
19. V.V. Tuchin, S.R. Utz, and I.V. Yaroslavsky, "Tissue optics, light distribution, and spectroscopy," *Opt. Eng.* **33**, pp. 3178–3188, 1994.
20. F.K. Kolmel, B. Sennhenn, and K. Giese, "Investigation of skin by ultraviolet remittance spectroscopy," *British J. Dermatol.* **122**(2), pp. 209–216, 1990.
21. J. Lademann, H.-J. Weigmann, W. Sterry, V. Tuchin, D. Zimnyakov, G. Mueller, and H. Schaefer, "Analysis of the penetration process of drugs and cosmetic products into the skin by tape strippings in combination with spectroscopic measurements," *Proc* SPIE **3915**, pp. 194-201, 2000.
22. E. Lankenau, J. Welzel, R. Birngruber, and R. Engelhardt, "*In vivo* tissue measurements with optical low coherence tomography," *Proc.* SPIE **2981**, pp. 78-84, 1997.

23. H. Liu, D.A. Boas, Y. Zhang, A.G. Yodh, B. Chance, "Determination of optical properties and blood oxygenation in tissue using continuous NIR light," *Phys. Med. Biol.* **40**, pp. 1983-1993, 1995.
24. T.J. Farrell, M.S. Patterson, B.C. Wilson, "A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the noninvasive determination of tissue optical properties *in vivo*," *Med. Phys.* **19**, pp. 879-888, 1992.
25. A. Kienle, L. Lilge, M.S. Patterson, R. Hibst, R. Steiner, B.C. Wilson, "Spatially resolved absolute diffuse reflectance measurements for noninvasive determination of the optical scattering and absorption coefficients of biological tissue," *Appl. Opt.* **35**(13), pp. 2304-2314, 1996.
26. A.N. Bashkatov, E.A. Genina, I.V. Korovina, V.I. Kochubey, Yu.P. Sinichkin, V.V. Tuchin, "*In vivo* and *in vitro* study of control of rat skin optical properties by acting of osmotical liquid," *Proc.* SPIE **4224**, 2000.
27. C.F. Bohren and D.R. Huffman, *Absorption and Scattering of Light by Small Particles*, Willey, New York, 1983.
28. Y. Komai, T. Ushiki, "The three-dimensional organization of collagen fibrils in the human cornea and sclera," *Investigative Ophthalmology & Visual Science* **32** (8), pp. 2244-2258, 1991.