19th Congress of the International Commission for Optics

ICO XIX

Optics for the Quality of Life

25-30 August 2002
Florence, Italy

Technical Digest

Anna Consortini and **Giancarlo C. Righini**
Editors

Part 2 of Two Parts

Sponsored by
CNR – Consiglio Nazionale delle Ricerche
INOA - Istituto Nazionale di Ottica Applicata
SPIE - The International Society for Optical Engineering
Università degli Studi di Firenze

Firenze, 2002
SPIE Volume 4829

Reflectance, transmittance, and polarization of light interacting with immersed tissue: *in vitro* and *in vivo* study

(Poster)

Nina A. Lakodina, Alexey N. Bashkatov, Elina A. Genina, Georgy V. Simonenko, Valery V. Tuchin
Saratov State University, Russia

SUMMARY

Control of tissue optical properties is important for development of optical tomography, photodynamic therapy and selective photodamage of tissue components. As a scattering medium, tissue shows optical effects that are characteristic for turbid optical systems. Administration of the appropriate chemical agents, like solutions of *glucose, glycerol, propylene glycol, etc.* can effectively change scattering properties of a living tissue.[1]

Control of skin optical properties was related to the immersion of refractive indices of scatterers and ground matter. Skin optical properties in general are defined by dermis because of relatively big thickness of dermis (95% of the human skin). The dermis consists mainly of network of collagen fibers, elastic fibers, and an interstitial substance consisting of proteoglycans, salts, and water. Refractive indices of skin components differ from that of interstitial material. The variation in refractive index causes light scattering that decreases light penetration into a tissue.

Osmotic active agents (*glucose, dimethyl sulfoxide, trasograph, verografin, glycerol, propilene glycol,* etc.) have refractive indices higher than interstitial matter and can be used to reduce the scattering in tissues. Tissue – osmotic liquid interaction accompanies by a change of tissue thickness and sizes and packing density of scatterers, but the refractive index matching effect prevails over other processes.

Experimental results on *in vivo* immersion clearing dynamics of the skin under action of osmotic active agent are presented. The significant decrease of the human skin reflectance *in vivo* under action of *glucose* solution is demonstrated. Analysis of the results has shown that hypodermic injection is the most effective technique of the acting agent administration. The measurements of the reflectance spectra were performed using commercial available spectrometer LESA-6. *In vivo* reflectance measurements were performed using the fiber optical probe with system of optical fibers, which can be presented as the system of two fibers (radiation source and detector) with separation of r_{sd} = 0.28 mm. The reflectance spectra of the samples were measured against $BaSO_4$ plate as a reference. Recording of reflectance spectra of the human skin was provided by placing the fiber optical probe on the surface of the skin or by distant (1mm) apart probing of the skin site. The measurements were performed every 60 sec for 140 min. The volunteer's skin was used for *in vivo* experiments. Hypodermic injection of water, saline, and 20-40%-*glucose* solutions by volume 0.1 ml was made in the area of forearm. Measurements of the reflectance spectra of the human skin were done in the wavelength range 400 – 1000 nm. Dynamics of reflectance spectra and time-dependent reflectance at different wavelengths was studied. The abrupt decrease and followed gradual increase of the reflectance in the first twenty minutes at 40%-*glucose* solution injection were seen. The *glucose*-injected region became more transparent. The area around the injection site that was unaffected by the *glucose* solution remained turbid. Reflectance of skin decreases in about 4 folds in a hour after agent injection and then increases gradually, that shown beginning of *glucose* going out from the observed area and reduction of initial value of skin reflectance. On the base of the experiments it can be conclude that partial matching of refractive indices of both collagen fibers of dermis (n=1.46) and interstitial liquid (n=1.36) under action of 40%-*glucose* solution (n=1.396) makes the main contribution to tissue clearing in the first phase (in the first hour after injection). Obtained results have allowed estimating of diffusion coefficient of 40%-*glucose* solution in skin as $D_g = (2.56 \pm 0.13) \cdot 10^{-6}\ cm^2/\text{sec}$.

The imaging contrast of hemoglobin bands at skin clearing was calculated from reflectance spectra $(R_{max} - R_{min})/(R_{max} + R_{min})$, where R_{max}, R_{min} are maximal and minimal values of skin reflectance, respectively. It was found that contrast of hemoglobin bands in the immersed skin increased significantly during the first hour after injection.

The study of optical clearing dynamics of various connective tissues was carried out by means of polarization microscopy. Rate difference of optical clearing of such types of tissues as cartilage, meniscus, nasal septum tissues, sclera and tunica testis was found. The areas of unidirectional orientation of collagen fibers in cartilage and tunica testis were determined.

The study of structural and functional peculiarities of bradytrophic tissues are of great importance for prognosis of atherosclerosis formation.[2] Eye sclera and cornea, tendon and cartilage, dura mater, and other tissues with slow metabolism belong to the bradytrophic tissues. The nutrition of such tissues is the result of the diffusion of extravasal liquid. Such tissues are mostly composed from orientated or quisi-orientated collagen fibers, therefore, have to show polarization properties. Nevertheless, in the normal state collageneous tissues showing rather strong scattering preventing precise estimation of their polarization characteristics and causing light beams attenuation and distortion. These phenomena lead to serious limitations in optical diagnostics of a braditrophic tissue state and in application of laser therapeutic or surgery techniques in ophthalmology, otolaryngology and orthopedics.

The early studies of polarization properties of sclera, cornea, cartilage, bloodless muscular tissue at optical clearing showed that optical properties of these tissue look like optical properties of uni-axial or bi-axial crystals.[2] In this paper the study of optical clearing dynamics of various connective tissues was carried out by means of polarization microscopy. Difference in the rate of optical clearing of such tissues as cartilage, meniscus, nasal septum tissues, sclera and tunica testis was found. The areas of unidirectional orientation of collagen fibers in cartilage and tunica testis were determined.

To clarify optically a tissue sample via reduction of its scattering coefficient we have used *60%-trazograph* solution (X-ray contrasting drug for intravenous applications). The samples were of 1 cm x 1 cm in size, their thickness was 0.5 –0.7 mm. The temperature during all studies was kept at about 22°C. *Trazograph* solution was warmed up to 36 – 40°C and with the help of pipette was dripped on the surface of a tissue sample then it diffused into the sample. For characterization of tissue clearing the following value was used:

$$C(t) = T(t)/T_{max},$$

where $T(t)$ is the current sample brightness at transillumination, T_{max} is the maximal sample brightness. The mean brightness of a sample was evaluated from the image obtained by the digital camera with usage of the program MS Photo Editor. The program allows for evaluation of mean brightness of the image as a whole or its parts by a quantity of pixels having a given intensity level. To increase the contrast of the clearing curves the linear polarizer and analyzer in parallel orientation were used. For the quantitative description of the clarifying agent diffusion into a tissue the rate of contrast change in time $V(t)$ was used

$$V(t) = d[C(t)]/dt,$$

$$V(t) = A + B \times exp(-G \times t),$$

A, B and G are determined by a tissue structure. These parameters characterize an agent diffusion process and its saturation. In Table quantities of those parameters and initial contrast changing rate V(0) are presented.

Table.
Parameters characterizing rate of clearing for various tissue samples

Tissue	A	B	G	V(0)
Sclera	0.70	2.59	6.4	3.28
Testis	0.19	3.49	6.6	3.68
Cartilage	-0.32	3.29	6.1	2.97
Nose partition	-0.57	7.08	10.6	6.51
Meniscus	0.00	9.83	17.2	9.82

Authors acknowledge financial support of grants 00-15-96667 RBRF and REC-006 CRDF.

1. V.V. Tuchin, *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis*, SPIE Press, **TT38**, Bellingham, USA, 2000, 352 p.
2. G.V. Simonenko, T.P. Denisova, N.A. Lakodina, and V.V. Tuchin, "Measurement of an optical anisotropy of *Proc SPIE* **3915**, 152-157, 2000.