Conclusion

This book shows that optical immersion technology allows one to effectively control the optical properties of tissues and blood. Such control leads to the essential reduction of scattering and therefore causes much higher transmittance (optical clearing) and the appearance of a large amount of least-scattered (snake) and ballistic photons, allowing for successful application of coherent-domain and polarization imaging techniques. The dynamics of tissue optical clearing, defined by the dynamics of refractive index matching, is characterized by a time response of about 5–30 min, which in its turn depends on the diffusivity of the immersion agent in a tissue layer, water diffusion rate, and tissue layer thickness. The tissue and cell swelling or shrinkage may play an important role in the tissue clearing process at application of hyperosmotic agents.

In vivo reflectance spectrophotometry and frequency-domain measurements for immersed tissues show that the refractive index matching technique provided by the appropriate chemical agent can be successfully used in tissue spectroscopy and imaging when radical reduction of scattering properties is needed. Hyperdermal injection of glucose causes the essential clearing of human skin. For such tissues as sclera or cornea, some drops of glucose is sufficient to make up very high and rather prolonged tissue clearing. In *in vivo* experiments, the impregnation of a tissue by an agent is more effective than in *in vitro* studies due to the higher diffusivity of an agent at physiological temperature and by involvement of blood and lymph microvessels into the process of agent distribution. Optical clearing may play a significant role in the designing of optical technologies for imaging of small living animals in studies of gene expression.

Dynamic optical characteristics can be used for the determination of the diffusion coefficient and concentration of endogenous (metabolic) and exogenous (chemical agent) fluids in human sclera, skin, and other tissues.

The immersion technique has great potential in noninvasive medical diagnostics using OCT due to the rather small thickness of tissue layers usually examined by OCT, which allows for fast impregnation of a target tissue during a topical application of an immersion liquid. It has been demonstrated that the body's interior tissues such as the blood vessel wall, esophagus, stomach, cervix, and colon can usually be imaged at a depth of about 1–2 mm. For more effective diagnosis using OCT, a higher penetration depth can be provided by applying immersion substances.

The method of clearing tissue is convenient, inexpensive, and simple for diagnostic purposes; in particular, it can be applied for *in vivo* monitoring of microcirculation. It may be useful for the study of structure and function of blood microvessels—diameters of arterioles and venules, capillary density, bifurcation angles, etc.

Optical clearing might be a fruitful technique for various methods of tissue spectroscopy, microscopy, and imaging (Raman, fluorescence, confocal, laser scanning, near-field, multiphoton, SHG, etc.), where scattering is a serious limitation.

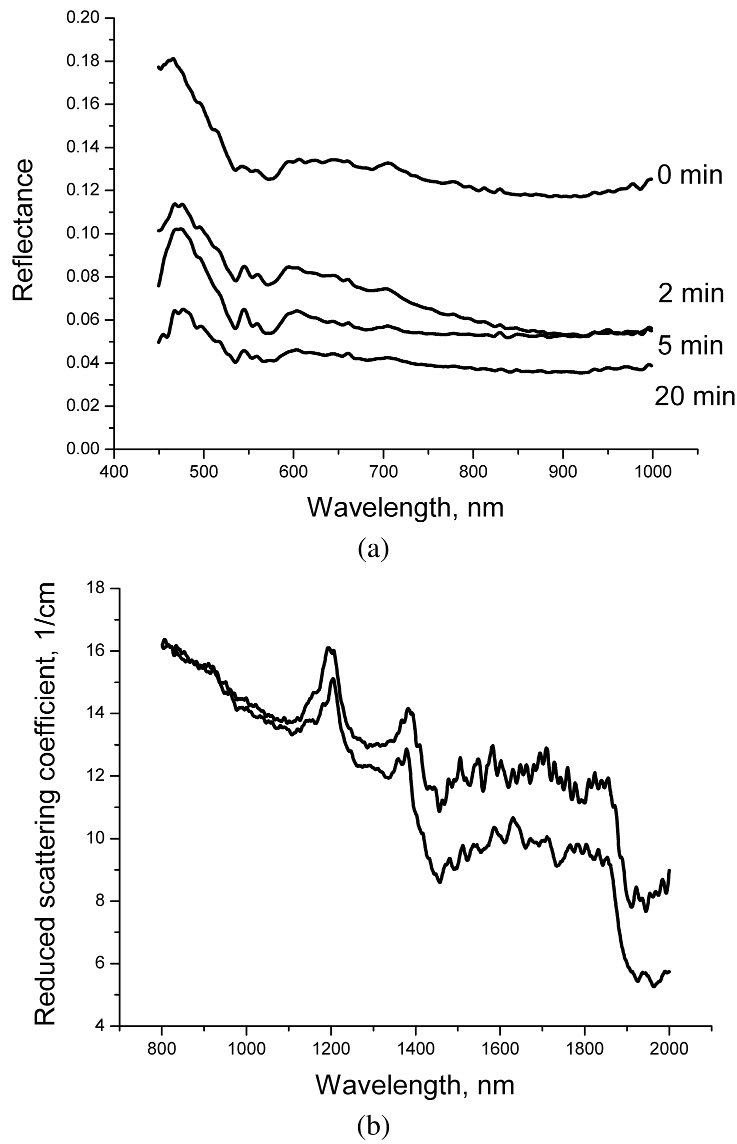

Figure 99 Optical clearing of the cranial bone samples.[181] (a) The reflectance spectra of porcine temple bone measured concurrently with administration of propylene glycol at different time intervals (a fiber-optical spectrometer with a source-detector separation of about 200 μm). (b) The human bone samples: the reduced scattering coefficient wavelength dependence before (upper) and after 1 hr administration of the glycerol (lower); integrating sphere spectrometer, inverse adding-doubling method.

Encouraging results were recently received in the enhancement of a single-photon fluorescent signal, as well as in improvement of image depth and contrast of two-photon fluorescence and SHG imaging at tissue immersion. It is important to note that the reduction of light scattering may help in the differentiation of various fluorophores in the depth of a tissue—for instance, of skin.

The concept of index matching improving the optical penetration depth of whole blood has been proved experimentally in *in vitro* studies. It should be accounted for that blood optical clearing is defined not only by the refractive index matching effect, but also by changes of red blood cells' size and their aggregation ability when chemicals are added.

Immersion optical clearing technology is applicable not only to soft tissues and blood, but also to hard tissues. At present, tendon (see Figs. 49 and 50),[33,34,101] cranial bones (see Fig. 99),[181] and tooth[182] have been tested. As it follows from Fig. 99(a), bone optical reflectance is very sensitive to impregnation of the superficial bone layers by clearing agents. This is a viable method for the study of bone demineralization (bone porosity). Figure 99(b) also demonstrates a high sensitivity of the scattering coefficient (reduction) to an immersion agent action, especially for the longer wavelengths, where water dispersion is essential.

Many of the tested agents and methods of their delivery have some advantages and disadvantages. The main disadvantages are the osmotic stress, which occurs at high concentration of osmotically active agent applied, and low permeability of tissue cell structures for the clearing agents. Therefore, the finding of new agents and delivery techniques is important. For example, it was recently proved that the mixture of prepolymers PPG/PEG has excellent clearing properties; the diffusivity in human skin is on the order of 0.2 mm/hr and it has no detectable influence on the tissue blood vascular system.[102,274,325] A few methods of enhancing skin permeability based on limited thermal damage in the SC was also recently proposed.[196,347,349–352]

Tissue optical clearing technology using an appropriate immersion agent can be applied for the temporal reduction of scattering needed for providing a precise laser photodisruption of the underlying tissue in ophthalmology[36] or skin tattoo removal.[374,375,389] Evidently, reversible tissue optical clearing technology has valuable features to be applied not only to tissue spectroscopy and diagnostics, but also to a variety of laser and photothermal therapies and surgeries.

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Valery V. Tuchin was born February 4, 1944. He received his degrees in radiophysics and electronics (1966), Candidate of Sciences in Optics (Ph.D., 1973), and Doctor of Science in Quantum Radiophysics (1982) from Saratov State University, Saratov, Russia. He is a Professor and holds the optics and biomedical physics chair, and he is a director of the research-educational Institute of Optics and Biophotonics at Saratov State University. Prof. Tuchin also heads the Laboratory of Laser Diagnostics of Technical and Living Systems at the Insitute of Precision Mechanics and Control, Russian Academy of Sciences. He was dean of the Faculty of Physics of Saratov University from 1982 to 1989.

His research interests include biomedical optics and laser medicine, nonlinear dynamics of laser and biophysical systems, and physics of optical and laser measurements. He has authored more than 250 peer-reviewed papers and books, including *Tissue Optics. Light Scattering Methods and Instrumentation for Medical Diagnosis* (SPIE Tutorial Texts in Optical Engineering, Vol. TT38, 2000), *Handbook of Optical Biomedical Diagnostics* (SPIE Press, Vol. PM107, 2002), *Coherent-Domain Optical Methods for Biomedical Diagnostics, Environmental and Material Science* (Kluwer Academic Publishers, Boston, USA, Vol. 1 & 2, 2004), and *Optical Polarization in Biomedical Applications*, Springer-Verlag, 2005 (Lihong Wang and Dmitry Zimnyakov—co-authors). He is a holder of more than 20 patents.

Prof. Tuchin currently teaches courses on optics, tissue optics, laser and fiber optics in biomedicine, laser dynamics, biophysics and medical physics for undergraduate and postgraduate students. Since 1992, Prof. Tuchin has been the instructor of SPIE and OSA short courses on biomedical optics for an international audience of engineers, students, and medical doctors; and he is an editorial board member of *J. of Biomedical Optics* (SPIE), *Lasers in the Life Sciences*, and *J. of X-Ray Science and Technology,* and the Russian journals *Izvestiya VUZ, Applied Nonlinear Dynamics* and *Laser Medicine*. He is a member of the Russian Academy of Natural Sciences and the International Academy of Information, a member of the board of SPIE/RUS and a fellow of SPIE (since 2004). He has been awarded the title and scholarship "Soros Professor" (1997–1999), and the Russian Federation Scholarship for the outstanding scientists (1994–2003); and Honored Science Worker of the Russian Federation (since 2000).