# Color Plate Section

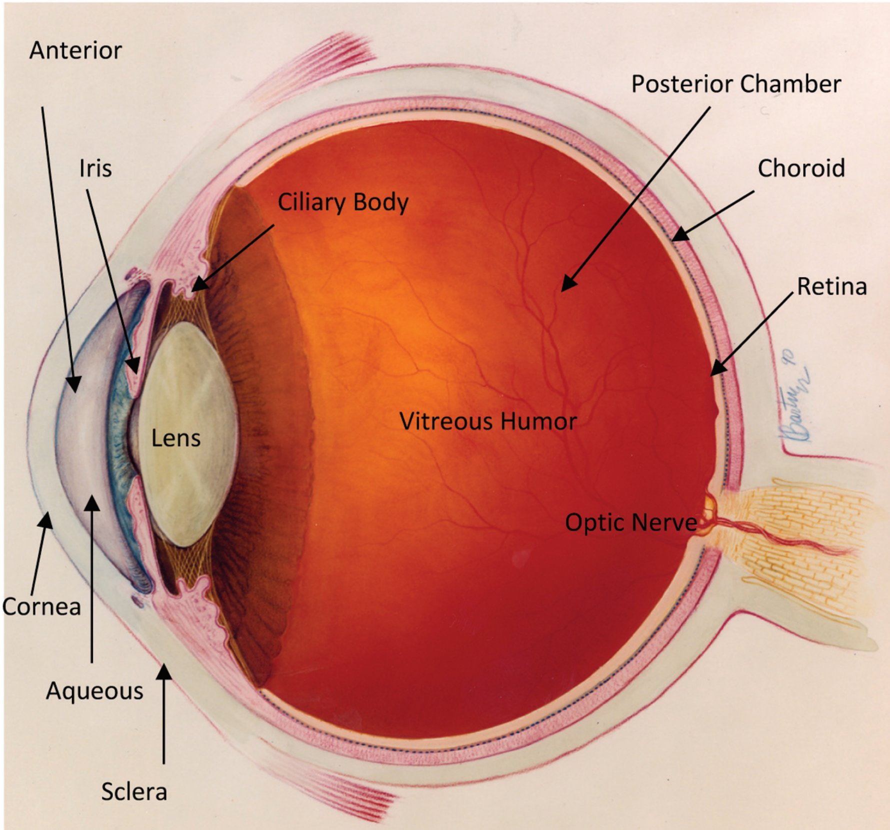



**Figure 3.2** Diagram of the human eye showing the locations of cornea, lens, sclera, and other eye components (see Ref. 776).

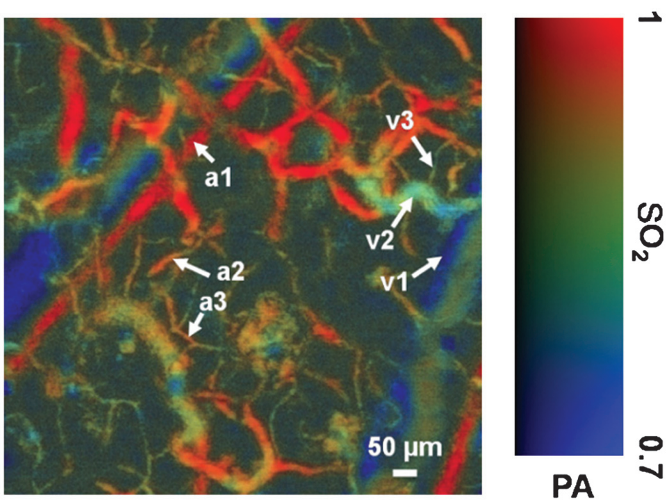



**Figure 4.9** Pseudo-color $sO_2$ mapping of a microvascular network in a nude mouse ear acquired *in vivo* by OR-PAM (see Fig. 4.8); a1–a3: arterioles of different diameters; v1–v3: venules of different diameters. PA: photoacoustic signal amplitude (see Ref. 897).

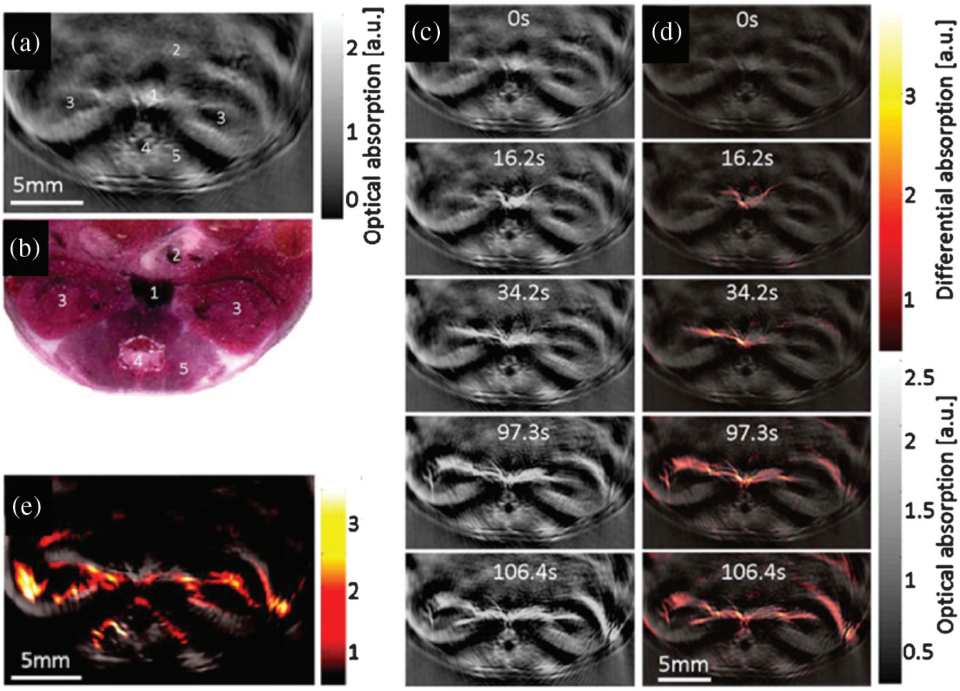
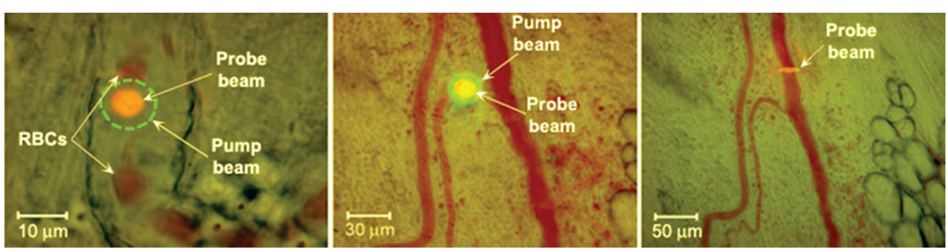



**Figure 4.10** ICG entering the kidneys of a mouse. OA slice image through the kidneys (a): (1) vena cava, (2) portal vein, (3) kidneys, (4) spine. Corresponding cryosection photograph showing the same structures (b). Selected images from single laser pulses at 800 nm during ICG injection showing increased contrast from the agent (c). Overlay of differential contrast highlighting the ICG enhancement (d). Overlay showing the distribution of ICG in the same animal by spectral unmixing after imaging at multiple wavelengths (750, 770, 790, 810, 830, 850, 870, 890, and 910 nm) (e) (see Refs. 924 and 955).




(a) (b) (c)

**Figure 4.18** Typical positions of probe (red) and pump (green) laser beams during PT imaging: circular beams in a blood capillary of rat mesentery (cell velocity, 0.5–2 mm/s; magnification,100×) (a); overlapping pump and probe pulses in an artery of rat mesentery (cell velocity, 2–5 mm/s; magnification, 10×) (b); a linear (or ellipsoidal) beam geometry in a blood vessel of rat mesentery (magnification, 10×) (c) (see Refs. 880 and 969).

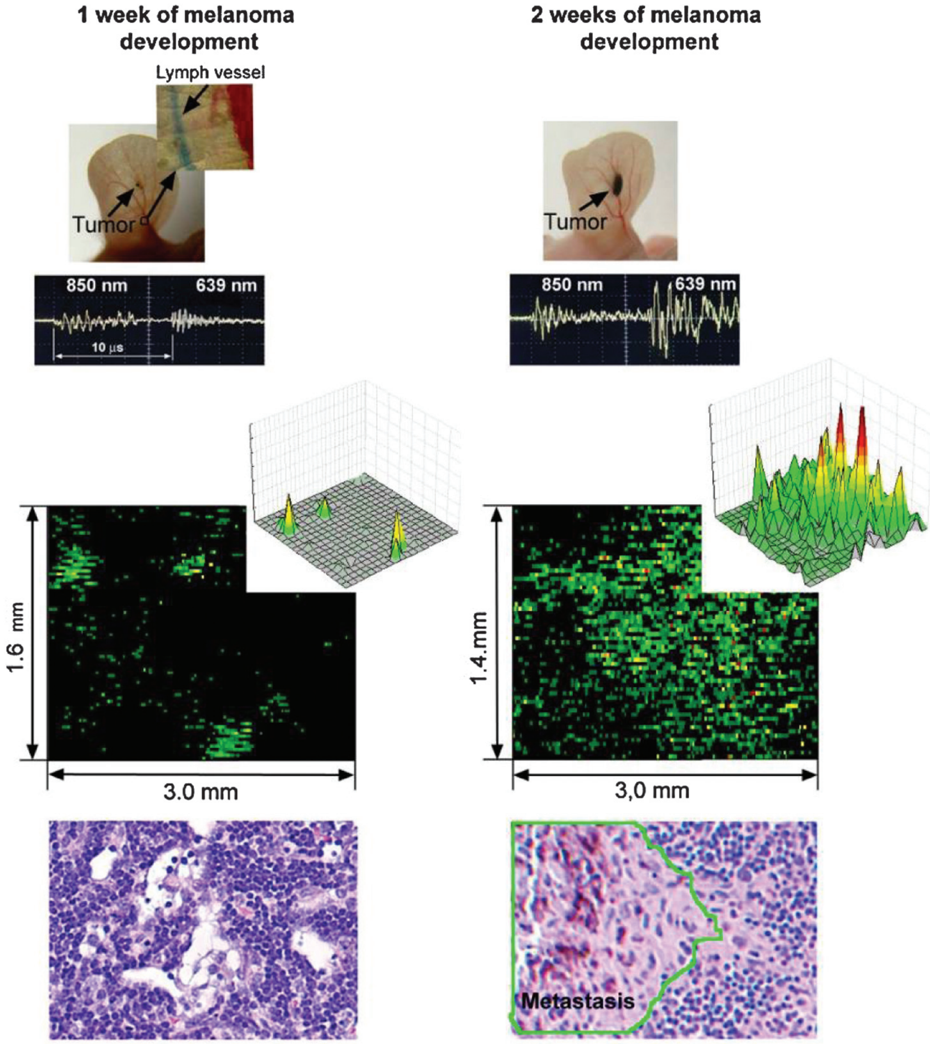



**Figure 4.33** PA detection and counting of melanoma metastasis during tumor development. Top row: photo of tumor with visualization of the lymph vessel (using Evans Blue dye) collecting lymph from primary tumor area (top, callout) and *in vivo* two-wavelength PA detection (bottom oscillogram) of melanoma metastasis with tumor progression in the SLN at first (left) and second (right) week. Middle row: *ex vivo* PA mapping of the SLN with melanoma metastasis at single cell level at 1 (left) and 2 (right) week(s) of primary tumor development. The data are presented as 2D high-resolution (bottom) and 3D low-resolution (top, callout) simulation. Each single spot on bottom is associated with single metastatic cells shown on the right panel (top, left callout). Red pseudo-color peaks indicate the photoacoustic signals with maximum amplitudes (bottom row). Histological images of the investigated SLNs demonstrating no histological changes at week 1 (left) and the detectable metastases, contoured by a green line, at week 2 after tumor inoculation (right) (see Ref. 1033).

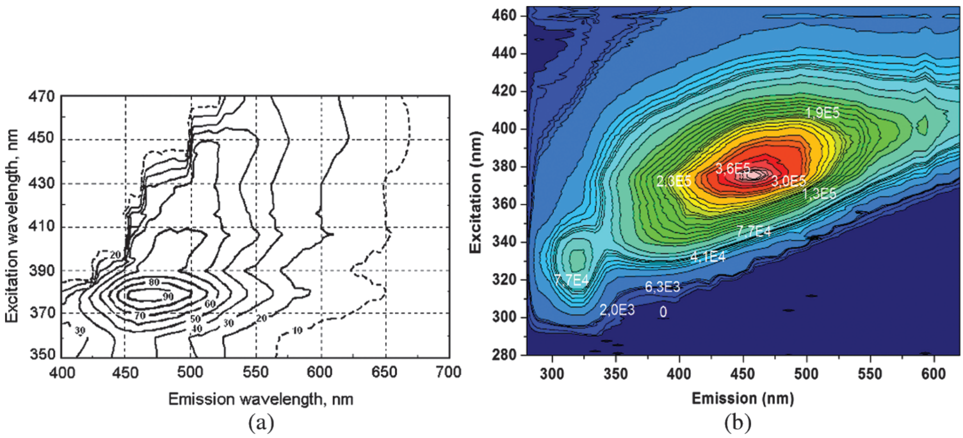



**Figure 5.5** Excitation-emission maps of the *in vivo* skin AF emission: see text and Ref. 1067 (a); Normal skin phototype II. Measurements were carried out using FluoroLog 3 with fiberoptic adaptor - F-3000 (HORIBA Jobin Yvon S.A., France), supported by the NSF-Bulgaria under grant #DMU-03-46/2011, courtesy of Dr. Ekaterina Borisova, Institute of Electronics, Bulgarian Academy of Sciences (b).

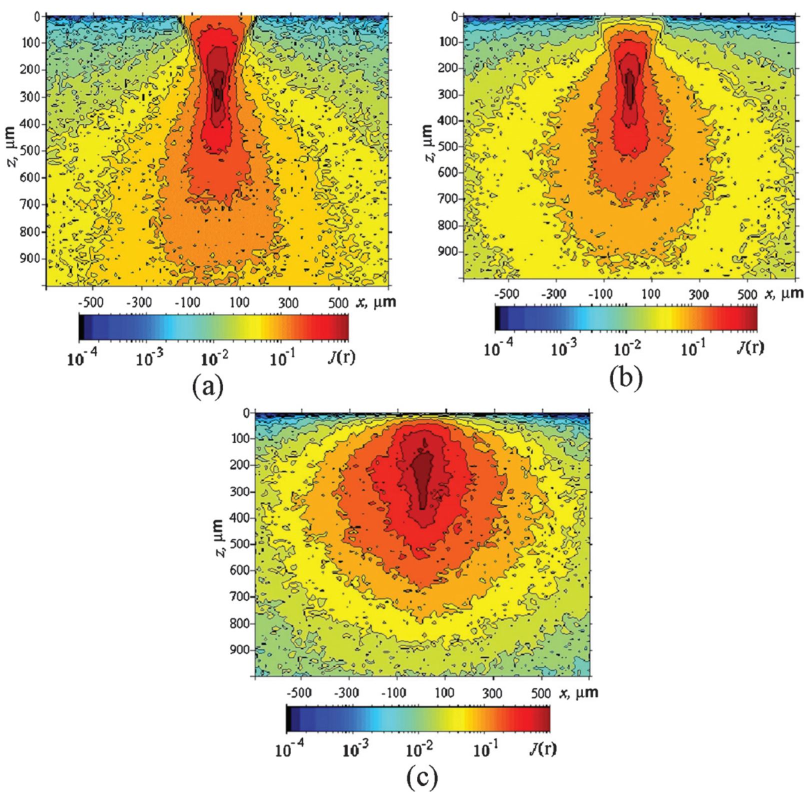
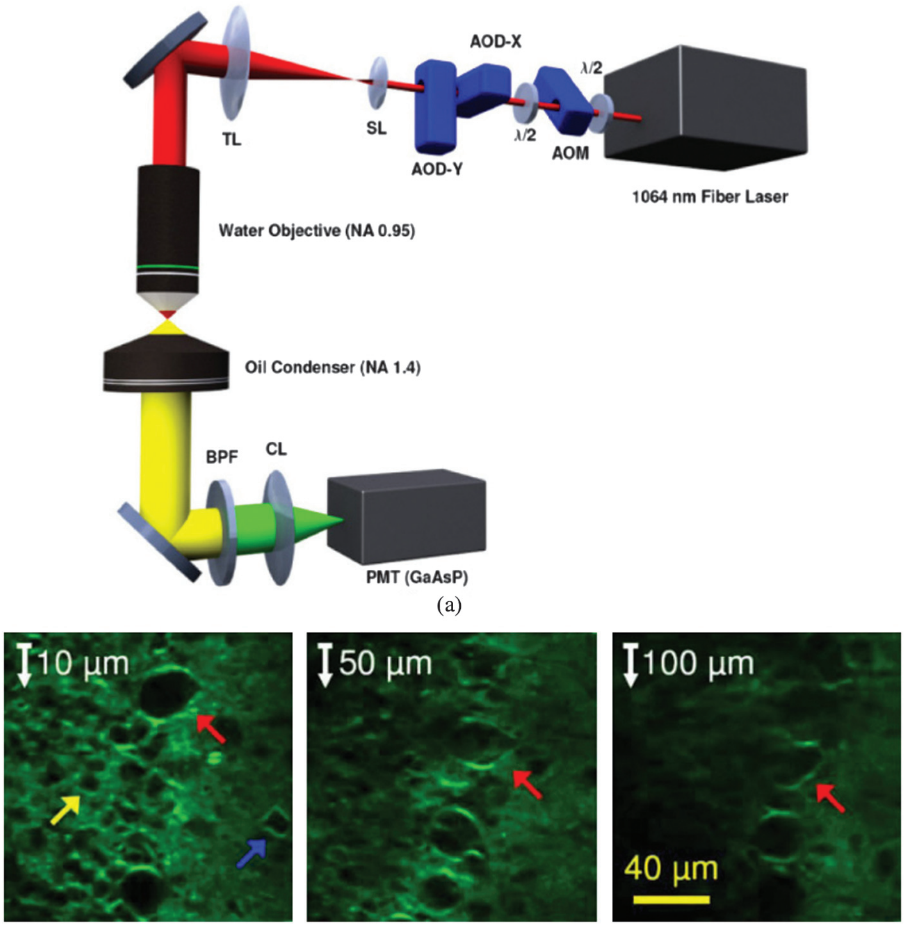



**Figure 8.20** Spatial distribution, $J(r)$, of the probability density of the effective photon optical paths calculated for homogeneous ($n_1 = 1.4$), multiply [$\mu_s = 10$ (a), 26.6 (b), and 100 $mm^{-1}$ (c)], and anisotropically ($g = 0.9$) scattering, and weakly absorbing ($\mu_a = 0.01$ $mm^{-1}$) media upon probing by reflection confocal microscopy in the geometry presented in Fig. 8.19 and $z_f = 300$ μm (see Ref. 1521).




(a)

(b)

**Figure 8.32** RA-SHG microscope (see Ref. 1591). RA-SHG microscope arrangement: a fiber laser ($\lambda = 1064$ nm) provided the excitation light, which comprised 200 fs width pulses at 80-MHz repetition rate. The laser beam was adjusted for optimal linear polarization via a half-wave ($\lambda/2$) plate. Beam passes were made through 45-deg AOM for angular spreading precompensation. A second half-wave ($\lambda/2$) plate was placed after the AOM to optimize the diffraction efficiencies of the two orthogonally mounted AODs: AOD-*x* and AOD-*y*. A scanning lens (SL) and a microscope tube lens (TL) expanded the beam before it was focused onto the specimen by the objective lens. The SHG signal was collected by an oil immersion condenser, band-pass filtered (BFP) and focalized by a collection lens (CL) into a GaAsP PMT (photomultiplier) (a). SHG image of rat cerebellar slice at three different depths of 10, 50, and 100 μm. Examples of SHG signals from a Purkinje cell (red arrow), granule cell (yellow arrow) and interneuron (blue arrow). The images were acquired with the same laser power across all three depths (b).

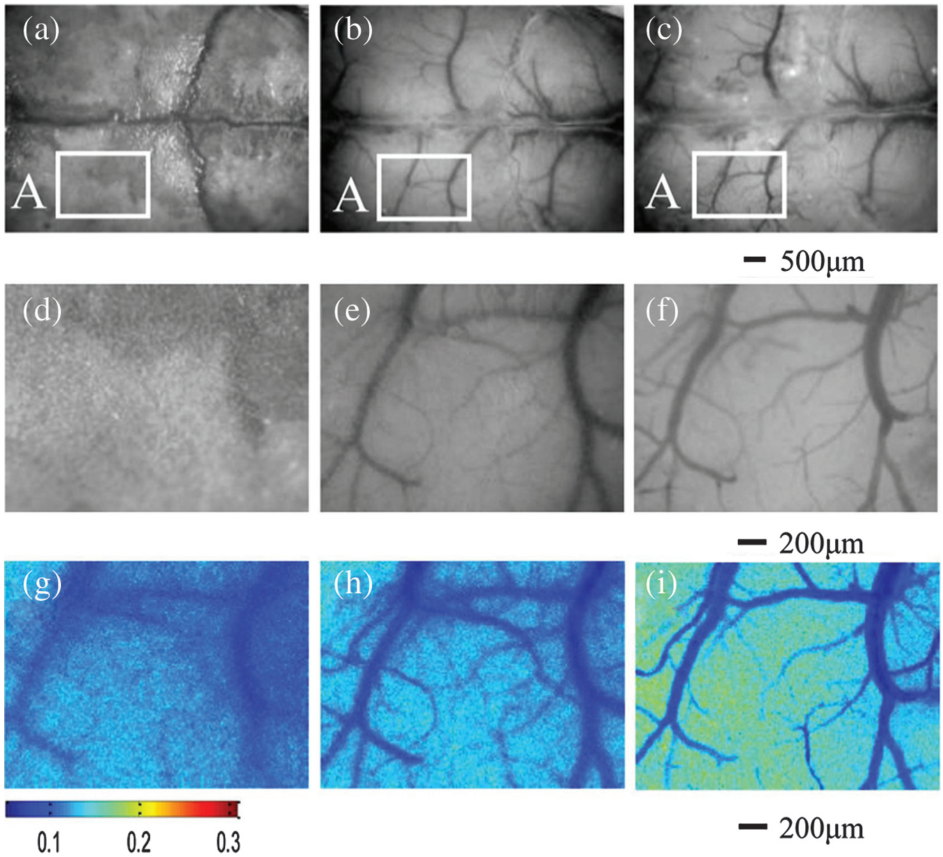
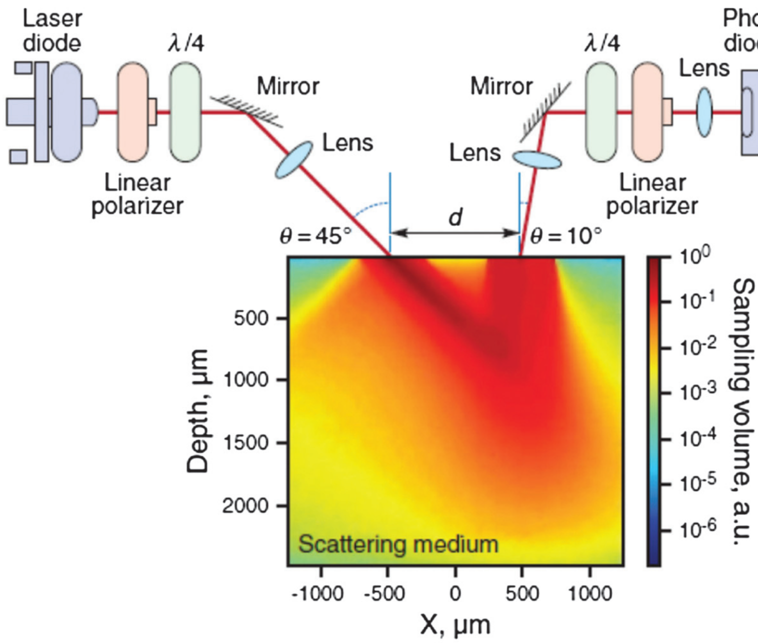
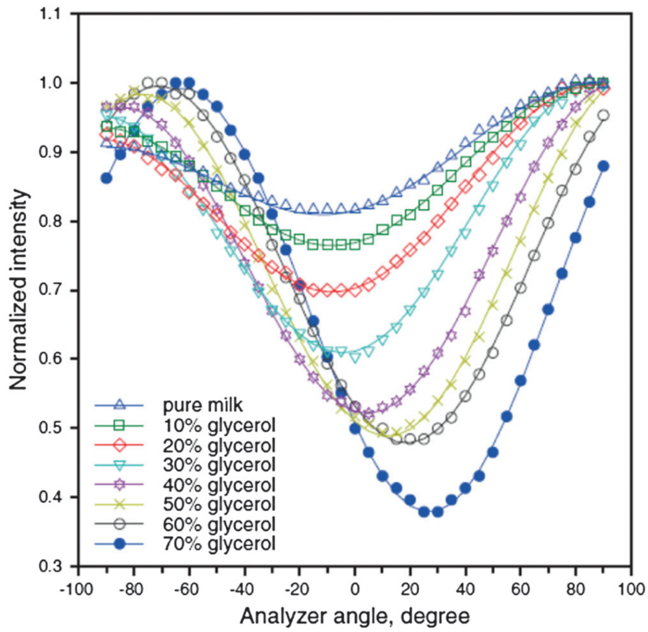



**Figure 9.50** White-light images of intact mouse skull (a), transparent skull after SOCS treatment for 25 min (b), and with removed rectangle area A (c). Corresponding magnified white images (d–f) and speckle contrast images and (g–i) are within rectangle area A shown in (a–c) (see Ref. 1784).




(a)




(b)

**Figure 9.57** Polarization-sensitive measurements at optical clearing (see Ref. 738): schematics of the experimental setup (a): the circularly polarized light is produced by a laser diode (Thorlabs, Inc., 635 nm), using linear polarizer and quarter wavelength plate (λ/4), and is focused onto the sample surface; backscattered optical radiation is collected at distance *d* away from the area of incidence and then passed through a quarter wavelength plate (λ/4) and linear polarizer (analyzer); normalized intensity of light backscattered from milk and detected using a quarter wavelength plate and rotating analyzer on the way to detector (b); each curve shows results for samples diluted by given amounts of water solution of glycerol: 10%, 20%, 30%, 40%, 50%, 60%, and 70%.

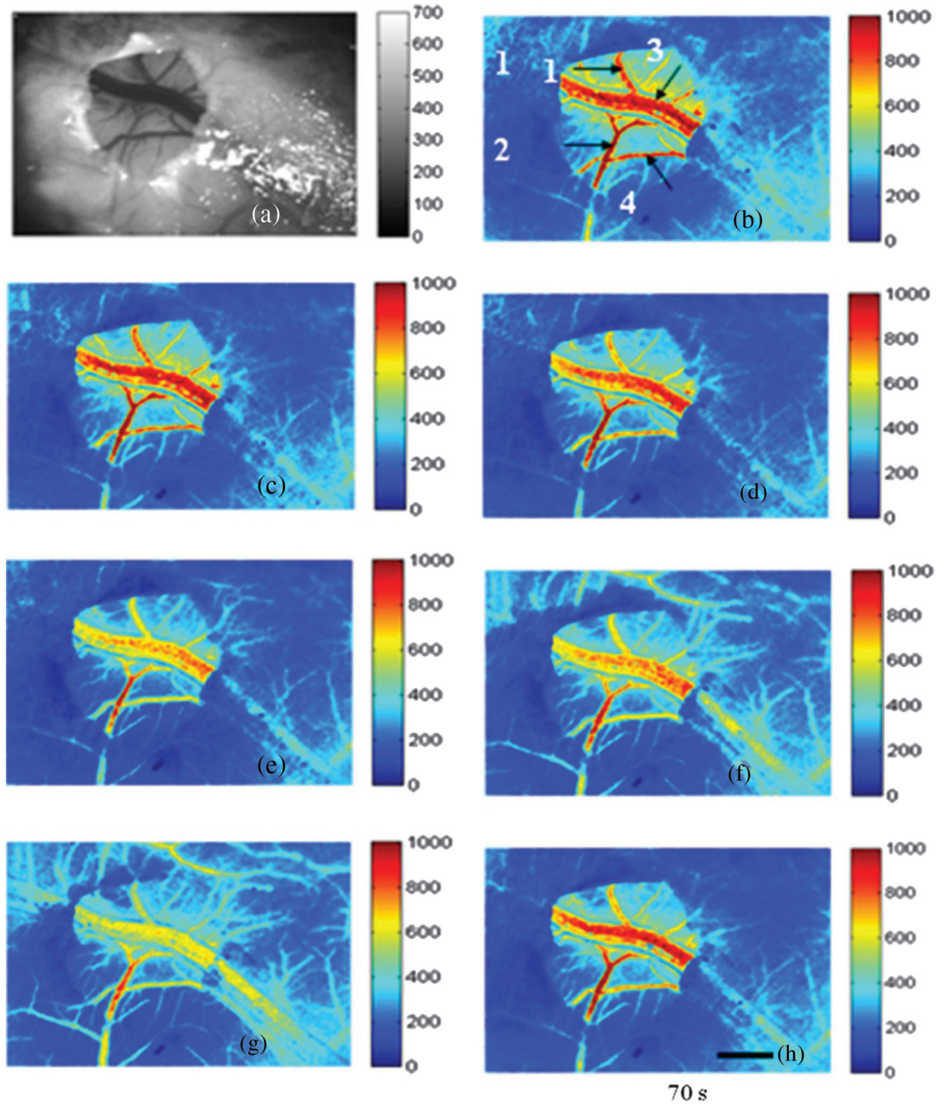



**Figure 9.65** Blood-flow images following the epidurally applied glycerol around the exposed area of *in vivo dura mater*. White-light image of the area of interest (a). Blood flow maps expressed as measured velocity, which is proportional to the blood flow velocity, during treatment with glycerol and represented by images at the time points shown in Fig. 9.66 (b)–(h): imaged blood flow before the application of glycerol (control), four vessels are indicated (b); 10 s application of glycerol, no obvious change in blood flow was observed (c); 20 s application of glycerol, blood flow began to decrease (d); 30 s application of glycerol, blood vessels underneath *dura mater* began to be clear (e); 40 s application of glycerol, blood flow decreased and the transparency of surrounding *dura mater* increased (f); 50 s application of glycerol, more blood vessels could be seen through *dura mater* and the blood flow decreased significantly (g); and 70 s application of glycerol, blood flow increased and *dura mater* became turbid again (h). Bar = 1 mm (see Ref. 1439).

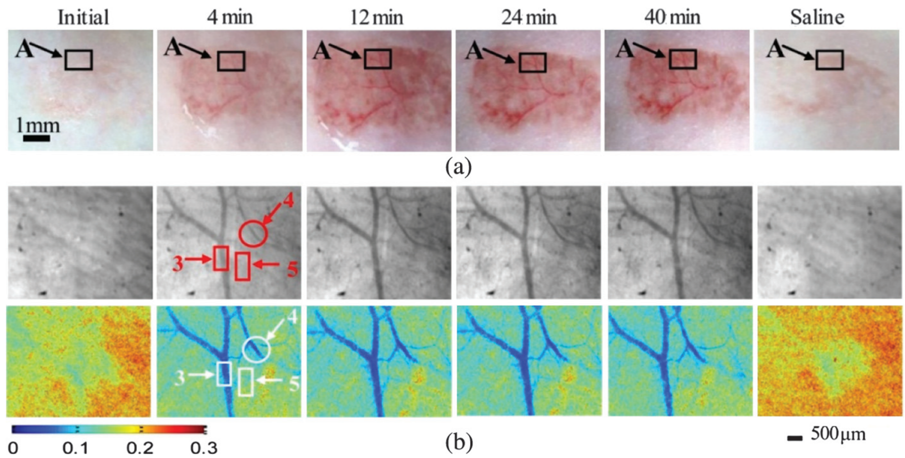



**Figure 9.68** Blood vessel visibility and dermal blood flow imaging through rat skin under topical treatment by a mixture of PEG-400 and thiazone: images of skin before and after treatment with the mixture (a); white-light (top row) and speckle temporal contrast images (bottom row) of rectangle area A in (a) after treatment (b) (see Ref. 1781).

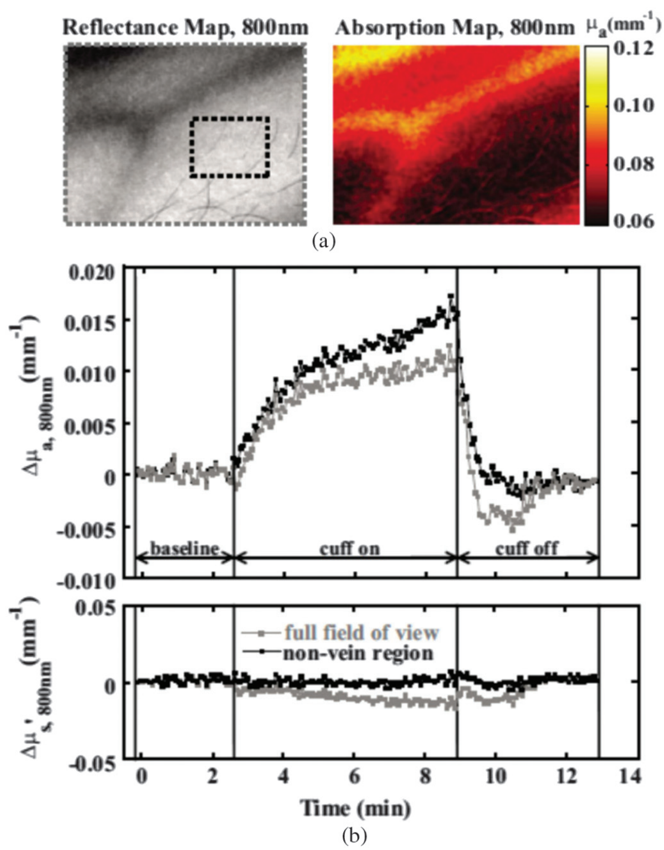



**Figure 11.20** SMI technique applied to venous occlusion data collection for the human volar forearm at 800 nm: diffuse reflectance map (left) and optical absorption coefficient map (right) measured at the baseline (a), dotted lines in the reflectance map indicate the regions of interest for time-course analysis; region-wise average changes in optical absorption (top) and reduced scattering (bottom) coefficients are shown for the whole image field (gray lines) and a region absent of any obvious large vessels (black lines) (b). Large increase in absorption observed in the microvascular region may be explained by the fact that the microvasculature is more susceptible to pooling, while the larger vessels are less reactive (see Ref. 479).

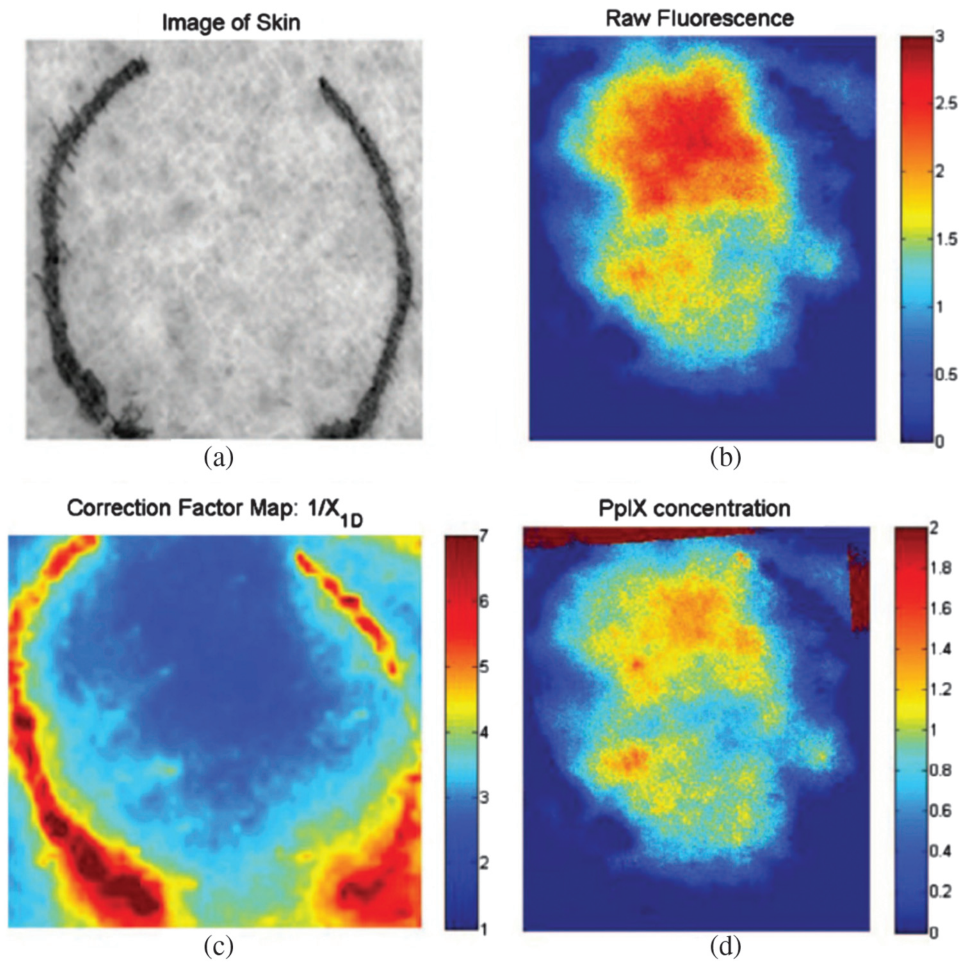



**Figure 11.22** Application of SMI technique for *in vivo* imaging of spatial distribution of ALA-induced PpIX in human volar forearm skin: image of skin tissue prior to topical application of ALA (a); raw fluorescence image (b); 7 h after application of ALA (scale bar: arbitrary units); correction map ($1/X_{1D}$) based on measured optical properties (c); and corrected fluorescence image (scale bar units: μg/ml) (d) (see Ref. 493).

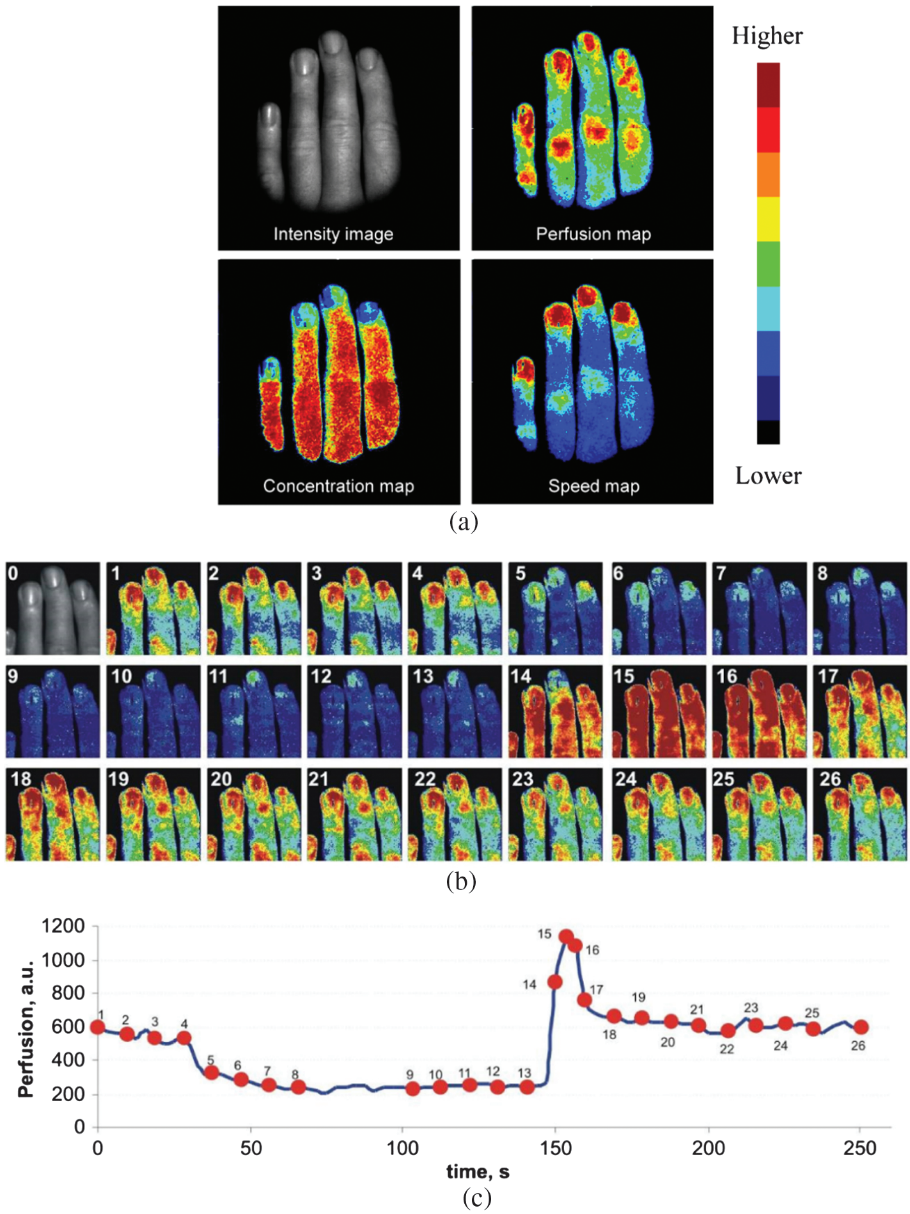



**Figure 13.15** Flow-related maps obtained with the CMOS integrated imager for finger skin (ROI = 512 × 512 pixels, imaging area is 11 × 11 $cm^2$) (a): image of the object (intensity image); perfusion map (lower is 200 a.u. and higher is 700 a.u.); blood concentration map (lower is 140 a.u. and higher is 310 a.u.); flow speed map (lower is 400 a.u. and higher is 1500 a.u.); capture time of each image is 5 s total. Sequence of images (b) and corresponding graph (c) of the averaged blood perfusion measured in real time at occlusion (ROI = 256 × 256 pixels; 5.5 × 5.5 $cm^2$); 0: normal finger image; 1–4: before occlusion; 5–13: during occlusion; 14–17: removal of occlusion, visible postocclusive hyperemia; 18–26: restoration of perfusion baseline; capture time of each image is 1.2 s. Increase of blood flow parameters displayed color from blue to red (see Ref. 1978).

# Conclusion

This monograph only addresses certain aspects of light-tissue interactions, primarily focusing on noncoherent and coherent light scattering by random and quasi-ordered structures as tissue models. The methods and results presented allow us to make certain conclusions and predictions concerning the direction of further investigations in the field of tissue optics and the development of optical diagnostic medical systems. Recently published monographs, book chapters, special issues of topical journals, review papers, and plenary talks also give prospects for future development of tissue optics and its biomedical applications.[130,136,156,2197–2267]

Results of numerous studies on light scattering emphasize the necessity of an in-depth evaluation of the optical properties of tissues with different structural organizations. At present, light propagation in tissues is fairly well described in quantitative terms, which provides a sound basis for the implementation of different diagnostic, therapeutic, and surgical modalities. At the same time, the estimation of optimal irradiation doses or the choice of correct diagnostic clues sometimes presents great difficulty because of the lack of reliable data on the optical parameters of tissues. Therefore, novel theoretical approaches and analyses of experimental data on the optical properties of tissues and blood are desirable.[2211–2214] In particular, on the basis of tissue optical properties reported in the literature, the wavelength-dependent behavior of scattering and absorption coefficients has been summarized.[2212] Equations have been derived for generating the optical properties of a generic tissue with variable amounts of absorbing chromophores (blood, water, melanin, fat, and yellow pigments) and a variable balance between small-scale and large-scale scatterers in the ultrastructures of cells and tissues. The use of a generic tissue can adequately mimic any real tissue, and has the advantage of generating smoothly predictable spectra for absorption and scattering. These generic equations allow one to calculate the expected optical properties versus wavelengths of tissues with varying chromophore content and ultrastructural character.

Traditional spectrophotometry, angular scattering, and polarization measurements are useful to characterize tissues, but need to be improved on the basis of more sophisticated tissue models. Such models must take into consideration the spatial distribution of scatterers and absorbers, their polydispersity and optical activity, along with the birefringence properties of the materials of which scatterers and base matter consist. At present, researchers are greatly interested in solving these problems.[2211–2213,2215–2223] *In vivo* quantitative multispectral and polarization imaging of tissue abnormalities is a new trend in biophotonics.[2215–2221] Tissue models represented as mixtures of spherical particles and long cylinders that account for the direction of alignment of cylinders, orientation angle distribution around the direction of alignment, particle number density, refractive index, birefringence, absorption properties, and for ambient media surrounding the scatterers, are under investigation for the quantification of tissue polarization properties.[2219–2223]

It is also necessary to further develop methods for the solution of inverse scattering problems with due regard for the real geometry of the tissue shape and the configuration of light beam and detecting arrangements. These solutions might be valid for the arbitrary values of scattering and absorption coefficients as well as anisotropy scattering factors. The inverse MC method is a useful instrument, and robust and accelerated (GPU-based) new technologies and internet-based approaches for the particular needs of biomedical optics and biophotonics are rapidly developing and attracting more users.[2224–2226] However, for many practical medical diagnostics and dosimetry tasks, the required fast computations should be based on approximate solutions of the radiative transfer equation.[2222]

Extensive studies are underway to better understand the role of temporal and spatial (structured illumination) photon-density waves and their use in phase modulation methods to obtain the optical characteristics of tissues and tissue imaging.[2227,2228] They are expected to generate novel algorithms for the reconstruction of 3D tissue images and practical applications of diffuse optical tomography in spatial and temporal frequency domains. Time-resolved fluorescence polarization spectroscopy of tissue-labeling dyes can be also used to enhance the image contrast.[2229]

The polarization properties of tissues to which this monograph is largely devoted are of primary importance for physiological polarization optics, and for early diagnosis of skin and epithelial cancer and other pathologies.[2218–2221] The scattering matrix technique has long been used in optics and is currently applied by many authors to investigate the properties of tissues and cell suspensions. The intensity matrix (Mueller matrix) is normally employed for this purpose, but the use of two-frequency lasers (e.g., a Zeeman laser) or quadruple-channel OCT allows for amplitude matrix elements to be measured, thus offering the possibility to simplify the inverse problem solution for many biological structures. Tracking the Stokes vector of the detected light on the Poincaré sphere is also applicable for characterization of tissue scattering anisotropy upon probing by elliptically polarized light, which is prospective for the differentiation of cancerous and noncancerous tissue.[2221]

The designing of laser photoacoustic (optoacoustic), acousto-optic, and photothermal (optothermal) imaging systems can be considered as a very prospective direction in biomedical optics, which allows one to provide impressive contrast and resolution in the imaging of both deep and superficial small tumors and cancer cells in lymph nodes and blood vessels.[955,2230–2233] It seems that these techniques should be optimal for the diagnosis of tissues at the middle depths (3 to 10 mm), where time-resolved diffusion, OCT, and multiphoton methods are not effective. The present photoacoustic computed tomography is a molecular-level noninvasive functional imaging modality offering deep penetration with optical contrast and ultrasonic resolution of 1 cm penetration depth or more, up to 7 cm.[2231] This opens up applications in whole body imaging, brain function, oxygen saturation, label-free cell analysis, and noninvasive cancer biopsies, including evaluating sentinel lymph nodes for breast cancer staging.

For many clinical applications, selective labeling of pathologically modified tissue regions is an urgent problem; thus, the pathways by which optical imaging agents are synthesized, qualified, and validated for preclinical testing, and ultimately translated for "first-in-humans" studies by using investigational optical imaging devices are under intensive discussion.[2234]

Optical speckle techniques, particularly the methods based on partially developed speckles emerging from the diffraction of focused laser beams, offer much promise for investigation into the structure of tissues and the analysis of tissue vibrations, mechanical properties, and motility of blood and lymphatic cells. The development of these techniques requires detailed research into the optics of speckles, speckle statistics, and the interference of laser beams in dense scattering media.[1455,2178,2235] Recording of speckles with digital cameras has advanced and facilitated measurements in laser speckle photography, speckle pattern interferometry, digital holographic interferometry, shearography, and tissue elastography.[1556,2145,2235] Real-time capability with extensive information capacity will push speckle techniques to become automated and reliable tools for measurements and inspection in tissues, with detection of deformations in a wide range from 10 nm to 100 μm.

Methods based on the dynamic scattering of light are useful for the analysis of both weakly scattering and dense biological media.[2197,2236,2237] Many of these methods have already found their specific areas of biomedical application. Considerable progress can be achieved with the use of the methods of diffusion wave spectroscopy or diffusion wave imaging, also termed speckle contrast optical tomography (SCOT), which allows one to probe heterogeneities in the dynamics of blood flow in deep tissues.[2237]

Coherent optical methods are promising tools presenting a novel multifunctional optical imaging platform for the development of new high-resolution and high-performance tomographic devices, allowing for the imaging of subcellular structural and functional states of a tissue.[136,156] Currently, investigation of superficial tissue layers with the use of OCT can provide important results and offers much promise for medical applications, especially for ophthalmology; early cancer detection of the skin and cervix; blood microcirculation analysis; and endoscopic/laparoscopic/needle-based studies of blood vessel wall, mucous of internal organs, breast, muscle, lung, and many other tissues and organs. The authors of Ref. 2238 conducted a systematic search of the electronic databases PubMed and Embase on OCT in the diagnostic process of (pre)malignant epithelial lesions and found that OCT can detect skin cancers, oral, laryngeal, esophageal cancer, and genital and bladder cancer. OCT technologies have perspectives in the quantification of tissue optical properties[2185] and quantitative parametric imaging;[2179–2183] tissue elastography;[2142–2145,2159,2160] imaging of breast cancer, dystrophic skeletal muscle, tendon, connective tissues, and air-filled lungs with OCT needle probes;[1412,2077,2081,2173,2174] monitoring the early development of mammalian embryos and their individual organs directly within the uterus;[2084,2085] label-free OCTOMAG for *in vivo* study of microcirculation and the vasculature of the retina, skin, and mucous;[2088,2089,2239] quantitative monitoring of microbubbles in blood;[2087] 3D visualization of coronary microstructures;[2103–2107] OCT-based tractography of myofibers;[2099,2136,2137,2166,2167] study of molecular/nanoparticle diffusion in healthy and pathological tissues;[1766,1768–1776,2086,2091–2097] and study of electrokinetic response of a soft tissue.[2122,2123,2187]

The well-known advantages of CM are very useful for the investigation of tissues and cells. Many coherent techniques and devices of medical diagnostics successfully employ the confocal principle of optical sectioning of an object under study.[2198] One of the important features of the optical imaging techniques is its feasibility to be integrated with other optical and nonoptical diagnostic and therapeutic methods to obtain enhanced diagnostic and therapeutic (theranostic) abilities. A number of dual OCT/CM systems are already available.[1564] For example, the combination of dual *en face* OCT/CM with fluorescence imaging creates a universal instrument in microscopy and ophthalmology;[1564] this OCT/fluorescence system is used for optical slicing and biochemical probing[2240] and combined OCT/fluorescence laminar optical tomography (FLOT) for enhanced multimodal tomography.[2241] Dual OCT/MPT for 3D multimodal *in vivo* imaging;[2184] OCT/PA[1045] and OCT/PT[2186] systems for nanoparticle imaging; adaptive optics-assisted OCT systems;[1564,2188,2189] dual OCT systems for imaging in ENT;[2169] and OCT complementary to standard laparoscopy[2172] are examples showing the major trends in the development of biomedical optical imaging.

Fourier phase microscopy with spatially coherent white light (wFPM) offers high spatial phase sensitivity due to the low temporal coherence and high temporal phase stability due to used common path geometry.[2242] This is a prospective tool of dynamic phase measurement in tissues and cells with enhanced contrast.

Multiphoton and SHG microscopies provide additional new facilities in studying tissues and cells separately and in combination with other imaging modalities.[2200,2218,2243–2246] Nonlinear optical techniques have been applied for skin and neural-tissue imaging, and the first generation of *in vivo* imaging apparatus has been developed and applied to animals and humans. The ability to see the morphology of the brain will lead to new understanding and new treatments.[2245]

The IOC technique allows one to effectively control the optical properties of tissues and blood. Such control leads to an essential reduction of scattering and improvement of scattering anisotropy factor; therefore, it causes much higher transmittance (optical clearing) and the appearance of a large amount of least-scattered (snake) and ballistic photons, allowing for the successful application of coherent-domain and polarization imaging techniques.[1615,2246–2259] It has great potential for noninvasive medical diagnostics using CM, OCT, OCM, TPEF, SHG, phase-resolved, and speckle techniques, due to the rather thin tissue layers usually examined by these methods, which allows for fast impregnation of a target tissue during topical application of an immersion agent.

It has been demonstrated that probing depth and image contrast of many optical imaging methods can be significantly improved, and that even a whole-organ or animal body image can be provided by using designed comprehensive optical clearing protocols and corresponding optical imaging facilities.[1615,2246,2248,2255–2259] Despite these achievements, which are extremely valuable in biology and especially in neural science, such clearing protocols are not suitable for *in vivo* tissue examination. For *in vivo* applications, the IOC method must provide a rapid treatment process (seconds and minutes, sometimes 1–2 hours), sufficient transparency, and safety for animals and humans, which makes it more difficult. Innovative optical clearing methods for *in vivo* use show a great potential for enhancing the contrast and resolution of different optical modalities[1615] including laser speckle contrast imaging (LSCI) for blood flow monitoring.[2247] It is very important that IOC can even be helpful to improve absorption-based technologies, such as PA microscopy[2249,2250] and flow cytometry.[2251] Future work should focus on developing highly effective and safe IOC methods and extending their *in vivo* applications; in particular, creating switchable transparent windows in skin, cranium, and internal organs that will permit the monitoring of tissue morphology, blood, and lymph flows with high resolution and contrast.

Because of the high sensitivity of IOC kinetics to the condition of tissue under investigation, such as inflammation, malignancy, atherosclerosis, glycation, and other pathological alterations, study of optical clearing kinetics, i.e., rate of OCA diffusion (permeation), will provide an additional marker of pathology detection and demarcation.[1769,1770,1772–1774]

IOC technology is prospective for application in a very wide spectral range from UV to terahertz. In UV, visible, and NIR ranges scattering properties can mostly be improved (reduced), and for IR and terahertz ranges, strong absorption properties can be reduced due to the dehydration abilities of the primary OCAs.[2260]

For *in vivo* applications, toxicity concerns are of great importance; thus, more studies of OCA impacts on tissue components (temporal dehydration, rehydration, and interactions of proteins and lipids with OCAs) are expected in the near future. At present, there are only a few studies that have directly investigated the problem, but they are encouraging the end users.[1741,2254] Molecular-sensitive spectroscopies, such as nonlinear TPEF and SHG, Raman, and CARS, are suitable tools for such investigations.

Additionally, optical elastography is well suited to another optical clearing technology, which is based on soft tissue compression or stretching. Understanding of this statement may stimulate further development of both tissue-mechanics-based technologies within a single platform.

Optical adaptive systems, particularly based on the optical phase conjugation (OPC) method, have enabled many optical applications, such as eye aberration correction and image transmission through optical microscopes.[2261–2267] This is also one way to induce less tissue scattering with less image blurring, i.e., optical clearing. Critically, this technique is free from any action on tissue itself (chemical agents or compression), therefore it is potentially a safer technology. However, the technique is comprehensive and costly, and will need further development.

In particular, implementation of digital optical phase conjugation (DOPC) has opened the possibility for the use of OPC for deep-tissue optical focusing owing to the ability to provide greater-than-unity OPC reflectivity (the power ratio of the phase conjugated beam and input beam to the OPC system) and to accommodate additional wavefront manipulations.[2264] The requirements for precise alignment (pixel-to-pixel matching) of the wavefront sensor and the SLM can be realized by autoalignment of a DOPC system that allows an axial displacement of up to $\sim$1.5 cm to be compensated.[2266] Harmonic holography combines the capability of holographic OPC with the SHG contrast-forming mechanism, which provides a unique opportunity for imaging through a turbid medium owing to efficient suppression of the overwhelming light scattering and the ability of holographic wavefront reconstruction.[2267]

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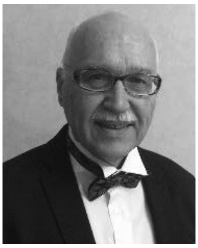

**Valery V. Tuchin** was born February 4, 1944. He received his degrees—M.S. in Radiophysics and Electronics (1966), Candidate of Sciences in Optics (Ph.D., 1973), and Doctor of Science in Quantum Radiophysics (1982)—from Saratov State University, Saratov, Russia. Prof. Tuchin holds the Optics and Biophotonics Chair and is a director of the Research-Educational Institute of Optics and Biophotonics at Saratov State University. He also heads the Laboratory of Laser Diagnostics of the Technical and Living Systems of Precision Mechanics and Control Institute, Russian Academy of Sciences and the Multidisciplinary Laboratory on Biophotonics of Tomsk State University. He was Dean of the Faculty of Physics at Saratov State University from 1982 to 1989.

Prof. Tuchin is a member of the Russian Academy of Natural Sciences and of the International Informatization Academy, as well as a member of SPIE, OSA, and IEEE. He was awarded the Honored Science Worker of the Russian Federation (1999), SPIE Fellow (2004), SPIE Educator Award (2007), and Chime Bell Prize of Hubei Province, China (2014). He was Vice-President of the Russian Photobiology Society (2005–2011). He is a FiDiPro Professor of the University of Oulu (Finland) (2011–2014), Guest Professor of HUST (Wuhan) and Tianjin Universities of China, and Adjunct Professor of the University of Limerick (Ireland) and National University of Ireland (Galway).

His research interests include biophotonics, tissue optics and laser medicine, tissue optical clearing, physics of optical and laser measurements, biosensing, photonic crystal fibers, nanobiophotonics, and theranostics. He has authored more than 50 patents and 400 peer-reviewed papers and books, including Tissue Optics: *Light Scattering Methods and Instruments for Medical Diagnosis* (SPIE Press, 2000), *Handbook of Optical Biomedical Diagnostics* (SPIE Press, 2002), translated to Russian (Fizmatlit, Moscow, 2007, two volumes); *Optical Clearing of Tissues and Blood* (SPIE Press, 2005); *Optical Polarization in Biomedical Applications* (L. Wang and D. Zimnyakov, co-authors, Springer, 2006); *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnostics*, 2nd ed. (SPIE Press, 2007); *Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues* (CRC Press, Taylor & Francis Group, 2009); *Lasers and Fiber Optics in Biomedical Science*, 2nd ed. (Fizmatlit, Moscow, 2010); *Handbook of Photonics for Biomedical Science* (CRC Press, Taylor & Francis Group, 2010); *Advanced Optical Flow Cytometry: Methods and Disease Diagnoses* (Wiley-VCH Verlag GmbH & Co. KGaA, 2011); *Handbook of Biophotonics* (3 vols., Wiley-VCH Verlag GmbH & Co. KGaA, J. Popp, A. Chiou, and S. H. Heinemann, co-editors, 2011–2012, three volumes); *Dictionary of Biomedical Optics and Biophotonics* (SPIE Press, 2012); *Coherent-Domain Optical Methods: Biomedical Diagnostics, Environmental Monitoring and Material Science*, 2nd ed. (Springer-Verlag, 2013, two volumes); *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis*, 2nd ed., translation to Russian (Fizmatlit, Moscow, 2013); and *Advanced Biophotonics: Tissue Optical Sectioning* (R. K. Wang, co-editor, CRC Press, Taylor & Francis Group, 2013).

Prof. Tuchin is also editor-in-chief of the *J. of Biomedical Photonics & Engineering*, associate editor-in-chief of the *J. of Innovative Optical Health Sciences* (JIOHS), vice editor-in-chief of the *News of Saratov University. Ser. Physics*; and associated editor of Frontiers of Optoelectronics. He is also a member of the editorial and international advisory boards of numerous biomedical journals.

He is founder, chair, or co-chair of the following international conferences: Saratov Fall Meeting (since 1996), Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine, SPIE, Photonics West, USA (co-chairs: J. G. Fujimoto and J. A. Izatt) (since 1997), Dynamics and Fluctuations in Biomedical Photonics, SPIE, Photonics West, USA (co-chairs: D. D. Duncan, K. V. Larin, M. J. Leahy, and R. K. Wang) (since 2005), Photonics and Imaging in Biology and Medicine (PIBM), China (co-chairs: Q. Luo and L. V. Wang) (since 2001), and Biophotonics: Photonic Solutions for Better Health Care, SPIE Photonics Europe (co-chairs: J. Popp, W. Drexler, D. L. Matthews, and F. Pavone) (since 2008).

Prof. Tuchin currently teaches courses on tissue optics, introduction to biophotonics, laser and fiber optics for biomedicine, optical measurements in biomedicine, optical cytometry, and microspectral analysis for biomedicine for undergraduate and postgraduate students. Since 1992, he has been an instructor of SPIE, OSA, and IEEE short courses on biomedical optics for an international audience of engineers, students, and medical doctors. Some of his Ph.D. graduates now hold prestigious chairs and departments at high-ranking U.S. and European universities and companies.