
Introduction: Brief Review

Reflection, absorption, scattering, and fluorescence in living tissues and blood can be effectively controlled by various methods.^{1–197} Staining (sensitization) of biological materials is used extensively to study mechanisms of interaction between their constituent components and light, and also for diagnostic purposes and selective photodestruction of individual components of living tissues. This approach underlies the diagnosis and photodynamic therapy (PDT) of malignant neoplasm, UV-A photochemotherapy of psoriasis and other proliferative disorders, angiography in ophthalmology, and many other applications in medicine.

In the visible and NIR spectrums, tissues and bioliquids are low absorbing, but highly scattering media.⁷ Scattering defines spectral and angular characteristics of light interacting with living objects, as well as its penetration depth; thus, optical properties of tissues and blood may be effectively controlled by changes of scattering properties. The living tissue allows one to control its optical (scattering) properties using various physical and chemical actions such as compression, stretching, dehydration, coagulation, UV irradiation, exposure to low temperature, and impregnation by chemical solutions, gels, and oils. All these phenomena can be understood if we consider tissue as a scattering medium that shows all optical effects that are characteristic to turbid physical systems. It is well known that the turbidity of a dispersive physical system can be effectively controlled by providing matching of refractive indices of the scatterers and the ground material. This is a so-called optical immersion technique. Another possibility for controlling the optical properties of a disperse system is to change its packing parameter and/or scattered sizing.^{29,37,46}

Control of optical properties tissues *in vivo* is very important for many medical applications. A number of laser surgery, therapy, and diagnostic technologies include tissue compression and stretching used for better transportation of the laser beam to underlying layers of tissue. The human eye compression technique allows one to perform transscleral laser coagulation of the ciliary body and retina/choroid.^{35,41,57} The possibility of selective translucence of the upper tissue layers should be very useful for developing of the eye globe imaging techniques and for detecting local inhomogeneities hidden by a highly scattering medium in functional tomography. Results on the control of human sclera optical properties by tissue impregnation with osmotically active chemicals such as Trazograph (x-ray contrast), glucose, and polyethylene glycol (PEG), as well as hypaque-60 (x-ray contrast), have been reported.^{6,11,21,24,31,32,36,38,82,84–89,162,163,174}

In general, the scattering coefficient μ_s and scattering anisotropy factor g of a tissue is dependent on the refractive index mismatch between cellular tissue com-

ponents: cell membrane, cytoplasm, cell nucleus, cell organelles, melanin granules, and the extracellular fluid. For fibrous (connective) tissue (eye scleral stroma, corneal stroma, skin dermis, cerebral membrane, muscle, vessel wall noncellular matrix, female breast fibrous component, cartilage, tendon, etc.), index mismatch of the interstitial medium and long strands of scleroprotein (collagen-, elastin-, or reticulin-forming fibers) is important. The refractive index matching is manifested in the reduction of the scattering coefficient ($\mu_s \rightarrow 0$) and increase of single scattering directness ($g \rightarrow 1$). For skin dermis and eye sclera μ_s , reduction can be very high.^{32,36,45,90,198} For hematous tissue such as the liver, its impregnation by solutes with different osmolarity also leads to refractive index matching and reduction of the scattering coefficient, but the effect is not so pronounced as for skin and sclera, due to cells changing size as a result of osmotic stress.^{79,80}

It is possible to achieve a marked impairment of scattering by means of the intratissue administration of appropriate chemical agents. Conspicuous experimental optical clearing in human and animal sclera; human, animal, and artificial skin; human gastrointestinal tissues; and human and animal cartilage and tendon in the visible and NIR wavelength ranges induced by administration of x-ray contrast agents (Verografin, Trazograph, and Hypaque-60), glucose, propylene glycol, polypropylene glycol-based polymers (PPG), polyethylene glycol (PEG), PEG-based polymers, glycerol, and other solutions as has been described in Refs. 6–8, 11, 21, 24, 31–40, 45, 52–54, 82–98, 101, 102, 104, 105, 107–110, 116, 117, 127, 138, 139, 141–144, 161–175, 179–183, 186–189, 192, 196, and 197.

Coordination between refractive indices in multicomponent transparent tissues showing polarization anisotropy (e.g., cornea) leads to its decrease.^{5,10} In contrast, for a highly scattering tissue with a hidden linear birefringence or optical activity, its impregnation by immersion agents may significantly improve the detection ability of polarization anisotropy due to reduction of the background scattering.^{8,33,34,174,175,192,197}

Concentration-dependent variations in scattering and transmission profiles in α -crystalline suspensions isolated from calf lenses are believed to be related to osmotic phenomenon.⁷⁴ Osmotic and diffusive processes that occur in tissues treated with Verografin, Trazograph, glucose, glycerol, and other solutions are also important.³² Osmotic phenomena appear to be involved when optical properties of biological materials (cells and tissues) are modulated by sugar, alcohol, and electrolyte solutions. This may interfere with the evaluation of hemoglobin saturation with oxygen or identification of such absorbers as cytochrome oxidase in tissues by optical methods.^{79,80}

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. 7, 8, 19, 45, 54, 83, 85–92, 94–96, 101, 102, 104, 105, 108, 110, 118, 142, 167, 168, 172, 179, 180, 186–189, 192, 193, 196, and 197. The control of skin optical properties was related to the immersion of refractive indices of scatterers

(keratinocytes components in epidermis and collagen, and elastin fibers in dermis) and ground matter, and/or reversible collagen dissociation.¹⁰¹ In addition, some of the observed effects appear to have been due to the introduction of additional scatterers or absorbers into the tissue or, conversely, to their washing-out.

A marked clearing effect through the hamster⁴⁵ and the human^{86,90,92,102,105,142} skin, the human and rabbit sclera,^{21,84} and rabbit dura matter¹⁴³ was occurred for an *in vivo* tissue within a few minutes of topical application (eye, dura matter, skin) or intratissue injection (skin) of glycerol, glucose, propylene glycol, Trazograph, and PEG and PPG polymers.

Albumin, a useful protein for index matching in phase contrast microscopy experiments,^{65,75–77} can be used as the immersion medium for tissue study and imaging.^{20,28} Proteins smaller than albumin may offer a potential alternative because of relatively high scattering of albumin. Sometimes medical diagnosis or contrasting of a lesion image can be provided by the enhancement of a tissue's scattering properties by applying, for instance, acetic acid, which has been successfully used as a contrast agent in optical diagnostics of cervical tissue.^{20,28,128–133} It has been suggested that the aceto-whitening effect seen in cervical tissue is due to coagulation of nuclear proteins. Therefore, an acetic acid probe may also prove extremely significant in quantitative optical diagnosis of precancerous conditions because of its ability to selectively enhance nuclear scatter.^{20,28}

Evidently, the loss of water by tissue seriously influences its optical properties. One of the major reasons for tissue dehydration *in vivo* is the action of endogenous or exogenous osmotic liquids. In *in vitro* conditions, spontaneous water evaporation from tissue, tissue sample heating at a noncoagulating temperature, or its freezing in a refrigerator push tissue to loose water. Typically in the visible and NIR spectrums, far from water absorption bands, the absorption coefficient increases by a few dozen percent, and the scattering coefficient by a few percent due to closer packing of tissue components caused by its shrinkage. However, the overall optical transmittance of a tissue sample increases due to the decrease of its thickness at dehydration.^{43,44} Specifically, in the vicinity of the strong water absorption bands, the tissue absorption coefficient decreases due to less concentration of water in spite of higher density of tissue at its dehydration.

It is possible to significantly increase transmission through a soft tissue by its squeezing (compressing) or stretching it.⁷³ The optical clarity of living tissue is due to its optical homogeneity, which is achieved through the removal of blood and interstitial liquid (water) from the compressed site. This results in a higher refractive index of the ground matter, whose value becomes close to that of scatterers (cell membrane, muscle, or collagen fibers). Closer packing of tissue components at compression makes the tissue a redundant more organized system—what may give less scattering due to cooperative (interference) effects.^{37,46} Indeed, the absence of blood in the compressed area also contributes to altered tissue absorption and refraction properties. Certain mechanisms underlying the effects of optical clearing and changing of light reflection by tissues at compression and stretching were proposed in Refs. 21, 22, 35, 37, 46, 57, 145, and 198.

Long-pulsed laser heating induces reversible and irreversible changes in the optical properties of tissue.^{43,44,127} In general, the total transmittance decreases and the diffuse reflectance increases, showing nonlinear behavior during pulsed laser heating. Many types of tissues slowly coagulated (from 10 min to 2 hrs) in a hot water or saline bath (70–85°C) exhibit an increase of their scattering and absorption coefficients.

UV irradiation causes erythema (skin reddening), stimulates melanin synthesis, and can induce edema and tissue proliferation if the radiation dose is sufficiently large.^{18,56,121,122} All these photobiological effects may be responsible for variations in the optical properties of skin, and need to be taken into consideration when prescribing phototherapy. Also, UV treatment is known to cause color development in the human lens.

Natural physiological changes in cells and tissues are also responsible for their altered optical properties, which may be detectable and, thus, used as a measure of these changes. For example, measurements of the scattering coefficient allow one to monitor glucose^{52–54,153,154,156–159} or edema¹⁵² in the human body, as well as blood parameters.^{16,17} Many papers report optical characteristics of blood as functions of hemoglobin saturation with oxygen. The alterations of the optical properties of blood caused by changes of the hematocrit value, temperature, and flow parameters can be found in Refs. 7, 16, and 17.

As a particle system, whole blood shows pronounced clearing effects that may be accompanied by induced or spontaneous aggregation and disaggregation processes, as well as RBC swelling or shrinkage at application of biocompatible clearing agents with certain osmotic properties.^{8,47,64,111–115,142,160,177,178,184,185,192,197}

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Tissue and Blood Optical Immersion by Exogenous Chemical Agents

1.1 Tissue Structure and Scattering Properties

Soft tissue is composed of closely packed groups of cells entrapped in a network of fibers through which interstitial liquid percolates. At a microscopic scale, the tissue components have no pronounced boundaries, thus tissue can be considered as a continuous structure with spatial variations in the refractive index. To model such a complicated structure as a collection of particles, it is necessary to resort to a statistical approach.¹⁹⁹ The tissue components that contribute most to the local refractive index variations are the connective tissue fibers (either collagen or elastin forming, or reticulin forming) that form part of the noncellular tissue matrix around and among cells, and cell membrane; cytoplasmic organelles (mitochondria, lysosomes, and peroxisomes); cell nuclei; and melanin granules.^{28,78–80,199–204} Figure 1 shows a hypothetical index profile formed by measuring the refractive index along a line in an arbitrary direction through a volume of tissue and corresponding to the statistical mean index profile. The widths of the peaks in the actual index profile are proportional to the diameters of the elements, and their heights depend on the refractive index of each element relative to that of its surroundings. This is the origin of the tissue-discrete particle model. In accordance with this model, the index variations may be represented by a statistically equivalent volume of discrete particles having the same index, but different sizes.

The refractive indices of tissue structure elements, such as the fibrils, the interstitial medium, nuclei, cytoplasm, organelles, and the tissue itself can be derived using the law of Gladstone and Dale, which states that the resulting value represents an average of the refractive indices of the components related to their volume fractions:²⁹

$$\bar{n} = \sum_{i=1}^N n_i f_i, \quad \sum_i f_i = 1, \quad (1)$$

where n_i and f_i are the refractive index and volume fraction of the individual components, respectively, and N is the number of components.

The statistical mean index profile in Fig. 1 illustrates the nature of the approximation implied by this model. According to Eqs. (1), the average background index is defined as the weighted average of the refractive indices of the cytoplasm and

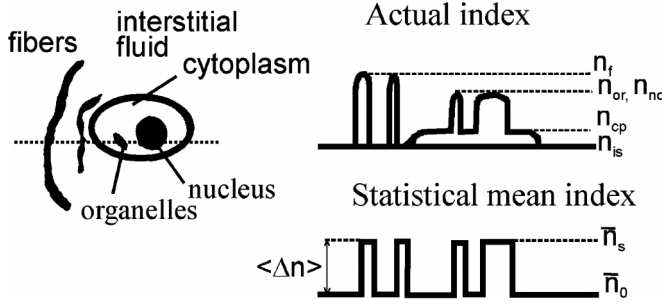


Figure 1 Spatial variations of the refractive index of a soft tissue. A hypothetical index profile through several tissue components is shown, along with the profile through a statistically equivalent volume of homogeneous particles. The indices of refraction labeling of the profile are defined in the text.¹⁹⁹

the interstitial fluid, n_{cp} and n_{is} , as

$$\bar{n}_0 = f_{cp}n_{cp} + (1 - f_{cp})n_{is}, \quad (2)$$

where f_{cp} is the volume fraction of the fluid in the tissue contained inside the cells. Since approximately 60% of the total fluid in soft tissue is contained in the intracellular compartment, in accordance with Refs. 20, 21, 27, 28, 199, 205–210, $n_{cp} = 1.37$ and $n_{is} = 1.35$, it follows that $\bar{n}_0 = 1.36$.

The refractive index of a particle can be defined as the sum of the background index and the mean index variation,

$$\bar{n}_s = \bar{n}_0 + \langle \Delta n \rangle, \quad (3)$$

which can be approximated by another volume-weight average,

$$\langle \Delta n \rangle = f_f(n_f - n_{is}) + f_{nc}(n_{nc} - n_{cp}) + f_{or}(n_{or} - n_{cp}). \quad (4)$$

Here, subscripts f , is , nc , cp , and or refer to the fibers, interstitial fluid, nuclei, cytoplasm, and organelles, which are identified as the major contributors to the index variations. The terms in parentheses in this expression are the differences between the refractive indices of the three types of tissue components and their respective backgrounds. The multiplying factors are the volume fractions of the elements in the solid portion of the tissue. The refractive index of the connective-tissue fibers is about 1.47, which corresponds to the approximately 55% hydration of collagen, its main component.³² The nucleus and the cytoplasmic organelles in mammalian cells that contain similar concentrations of proteins and nucleic acids, such as the mitochondria and the ribosomes, have refractive indices that fall within a relatively narrow range (1.38–1.41).^{20,28} The measured index for the nuclei is $n_{nc} = 1.39$.^{209,210} Accounting for this and supposing that $n_{or} = n_{nc} = 1.39$, the mean index variation can be expressed in terms of the fibrous-tissue fraction f_f

only:

$$\langle \Delta n \rangle = f_f(n_f - n_{is}) + (1 - f_f)(n_{nc} - n_{cp}). \quad (5)$$

Collagen and elastin fibers comprise approximately 70% of the fat-free dry weight of the dermis, 45% of the heart, and 2–3% of the nonmuscular internal organs.¹⁹⁹ Therefore, depending on the tissue type, f_f may be as small as 0.02 or as large as 0.7. For $n_f - n_{is} = 1.47 - 1.35 = 0.12$ and $n_{nc} - n_{cp} = n_{or} - n_{cp} = 1.39 - 1.36 = 0.03$, the mean index variations that correspond to these two extremes are $\langle \Delta n \rangle = 0.03\text{--}0.09$.

For example, the nucleus and cell membrane of fibroblasts have an index of refraction of 1.48, the cytoplasm has an index of 1.38, and the averaged index of a cell is 1.42.²¹¹ The collagenous fibrils of cornea and sclera have an index of refraction of 1.47, and the refraction index of the ground matter is 1.35.²⁰⁶ The relative index of human lymphocytes in respect to plasma varies from $1.01 < m < 1.08$.²¹² Additional information on the refractive indices of biological cells and tissues may be found in Refs. 1, 7, 29, 213, and 214.

The matter surrounding the scatterers (intercellular liquid and cytoplasm), the so-called ground substance, is composed mainly of water with salts and organic components dissolved in it. The ground matter index is usually taken as $n_0 = 1.35\text{--}1.37$. The scattering particles themselves (organelles, protein fibrils, membranes, protein globules) exhibit a higher density of proteins and lipids in comparison with the ground substance and, thus, a greater index of refraction, $n_s = 1.39\text{--}1.47$. This implies that the simplest way to model tissue is to consider the binary fluctuations in the index of refraction of the various tissue structures.

The refractive index variation in tissues, quantified by the ratio $m \equiv n_s/n_0$, determines the light scattering efficiency. For example, in a simple monodisperse tissue model, such as dielectric spheres of equal diameter $2a$, the reduced scattering coefficient is²¹⁵

$$\mu'_s \equiv \mu_s(1 - g) = 3.28\pi a^2 \rho_s \left(\frac{2\pi a}{\lambda} \right)^{0.37} (m - 1)^{2.09}, \quad (6)$$

where $\mu_s = \sigma_{sca}\rho_s$ is the scattering coefficient, σ_{sca} is the scattering cross section, ρ_s is the volume density of the spheres, g is the scattering anisotropy factor, and λ is the light wavelength in the scattering medium. This equation is valid for noninteracting Mie scatterers, $g > 0.9$, $5 < 2\pi a/\lambda < 50$, $1 < m < 1.1$.

For example, epithelial nuclei can be considered as spheroidal Mie scatterers with refractive index n_{nc} , which is higher than that of the surrounding cytoplasm n_{cp} . Normal nuclei have a characteristic diameter $d = 4\text{--}7\mu\text{m}$. In contrast, dysplastic nuclei can be as large as $20\mu\text{m}$, occupying almost the entire cell volume. In the visible range, where the wavelength $\lambda_0 \ll d$, the Van de Hulst approximation can be used to describe the elastic scattering cross section of the nuclei:^{216,217}

$$\sigma_{sca}(\lambda, d) = \frac{1}{2}\pi d^2 \left[1 - \frac{2 \sin \delta}{\delta} + \left(\frac{2 \sin \delta}{\delta} \right)^2 \right], \quad (7)$$

with $\delta = 2\pi d(n_{nc} - n_{cp})/\lambda_0$; λ_0 is the wavelength of the light in vacuum. This expression reveals a component of the scattering cross section, which varies periodically with inverse wavelength. This, in turn, gives rise to a periodic component in the tissue optical reflectance. Since the frequency of this variation (in inverse wavelength space) is proportional to particle size, the nuclear size distribution can be obtained from the Fourier transform of the periodic component.

Absorption for most tissues in the visible region is insignificant, except for the absorption bands of blood hemoglobin and some other chromophores.^{7,19,218} The absorption bands of protein molecules are mainly in the near-UV region. Absorption in the IR region is essentially defined by water contained in tissues.

The $n_s/n_0 \equiv m$ ratio determines the scattering coefficient. For example, in a simple monodisperse model of scattering dielectric spheres (Mie theory), the reduced scattering coefficient μ'_s is defined by Eq. (6), where $\mu'_s \sim (m - 1)^2$. It follows from Eq. (6) that only a 5% increase in the refractive index of the ground matter ($n_0 = 1.35 \rightarrow 1.42$), when that of the scattering centers is $n_s = 1.47$, will cause a sevenfold decrease of μ'_s . In the limit of equal refractive indices for nonabsorbing particles and background material, $m = 1$ and $\mu'_s \rightarrow 0$. In a living tissue, the relative refractive index is a function of the tissue's physiological or pathological state. Depending on the specificity of the tissue-state refractive index of the scatterers and/or the background change (increase or decrease); therefore, light scattering may correspondingly increase or decrease.

Light scattering and absorption of particles that compose tissue or blood can be calculated by Mie theory.^{219–221} The relevant parameters are the size (radius a) of the particles, their complex refractive index

$$n_s(\lambda_0) = n'_s(\lambda_0) + i n''_s(\lambda_0), \quad (8)$$

the complex refractive index of the dielectric host (ground material in tissues or plasma in blood) n_0 , and the relative refractive index of the scatterers and the ground materials, $m = n'_s/n_0$. The imaginary part of the complex refractive indices is responsible for light losses due to absorption. Mie theory yields the absorption and scattering efficiencies and the phase function from which the absorption and scattering coefficients, $\mu_s = \rho\sigma_{sca}$ and $\mu_a = \rho\sigma_{abs}$, and the scattering anisotropy g are calculated; ρ is the scatterers' (particles') density. The corresponding scattering and absorption cross sections σ_{sca} and σ_{abs} , scattering phase function $p(\theta)$, and g factor are described by²¹⁹

$$\sigma_{sca} = \frac{2\pi}{k^2} \sum_{n=1}^{\infty} (2n+1) (|a_n|^2 + |b_n|^2), \quad (9)$$

$$\sigma_{abs} = \frac{2\pi}{k^2} \sum_{n=1}^{\infty} (2n+1) [\text{Re}(a_n + b_n) - (|a_n|^2 + |b_n|^2)], \quad (10)$$

$$p(\theta) = \frac{1}{k^2 r^2} (|S_1|^2 + |S_2|^2), \quad (11)$$

$$g = \frac{4\pi}{k^2 \sigma_{sca}} \left[\sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} \operatorname{Re}(a_n b_n^*) + \sum_{n=1}^{\infty} \frac{n(n+2)}{n+1} \operatorname{Re}(a_n a_{n+1}^* + b_n b_{n+1}^*) \right], \quad (12)$$

where $k = 2\pi/\lambda$ is the wave number; a_n and b_n are the Mie coefficients, which are functions of the relative complex refractive index of the particles, $m = n_s/n_0$, and size parameter, $2\pi a n_0/\lambda_0$; θ is the scattering angle; r is the distance from the scatterer to the detector; S_1 and S_2 are the elements of the amplitude scattering matrix and the asterisk indicates that the complex conjugate is to be taken. The coefficients a_n and b_n are

$$\begin{cases} a_n = \frac{S'_n(y)S_n(x) - mS_n(y)S'_n(x)}{S'_n(y)\zeta_n(x) - mS_n(y)\zeta'_n(x)}, \\ b_n = \frac{mS'_n(y)S_n(x) - S_n(y)S'_n(x)}{mS'_n(y)\zeta_n(x) - S_n(y)\zeta'_n(x)}, \end{cases} \quad (13)$$

where

$$\begin{aligned} x &= \frac{2\pi a n_0}{\lambda_0}, \\ y &= \frac{2\pi a n_s}{\lambda_0}, \\ m &= \frac{n_s}{n_0}, \end{aligned} \quad (14)$$

where ζ_n and S_n can be written in terms of Bessel functions:

$$\begin{cases} S_n(z) = \left(\frac{\pi z}{2}\right)^{0.5} J_{n+0.5}(z), \\ \zeta_n(z) = S_n(z) + iC_n(z), \\ C_n(z) = -\left(\frac{\pi z}{2}\right)^{0.5} N_{n+0.5}(z), \end{cases} \quad (15)$$

where $J_{n+0.5}(z)$ is the Bessel function of the first kind and $N_{n+0.5}(z)$ is the Bessel function of the second kind. The derivatives of S_n and C_n can be obtained through

$$\begin{cases} S'_n(z) = \left(\frac{\pi}{8z}\right)^{0.5} J_{n+0.5}(z) + \left(\frac{\pi z}{2}\right)^{0.5} J'_{n+0.5}(z), \\ C'_n(z) = -\left(\frac{\pi}{8z}\right)^{0.5} N_{n+0.5}(z) - \left(\frac{\pi z}{2}\right)^{0.5} N'_{n+0.5}(z). \end{cases} \quad (16)$$