Handbook Of photomedicine

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Light–Tissue Interactions

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“ādityasya namaskaraṁ ye kurvanti dinedine ।
janmāṁ tarasahse śudrīdhryam nopajāyate ॥
akālamṛtyuharaṁ sarvavyādhivinasānam ।
sūryapādakāṁ tirtham jaṭharedhārayāmyaham ॥”

—Rigveda

(“Those who pay obeisance to Sun every day do not face poverty of health in life, early death or suffer from diseases. One should drink the water kept before the Sun.”)

In this chapter, we provide an overview of the fundamentals of the interaction of light with tissue and how this interaction is utilized in photomedicine.

3.1 Introduction

The interaction of light with biological matter plays an important role in our life; photosynthesis and vision are good examples. Therefore, studies on the use of this interaction to address an issue of utmost importance to mankind, the quality of its health care, have always been an important scientific pursuit. Use of sunlight for therapy has been explored since time immemorial by the ancient civilizations. For example, in India, the therapeutic potential of sunlight was well appreciated, and, for good health, exposure to light from the rising sun and consumption of water kept in sunlight were recommended. Indian medical literature dating back to 1400 B.C. documents the combined use of Psoralea corylifolia L. and sunlight for the treatment of non-pigmented skin lesions (vitiligo) (Pathak and Fitzpatrick 1992). In his book A History of Medicine: Medieval Medicine, Plinio Pioreschi (2003) notes the medieval practices of the use of red light for treatment of smallpox. A resurgence of the use of light in therapy happened in the second half of the 19th century when the role of sunlight for the treatment of rickets, tuberculosis, etc. was explored and the ability of the invisible ultraviolet (UV) radiation to kill microorganisms was scientifically established (McDonagh 2001). Perhaps the biggest thrust toward phototherapy was a result of the efforts of Niels Finsen, a Danish physician, who carried out several interesting experiments on the therapeutic effects of light and established the role of UV light in curing skin tuberculosis (lupus vulgaris) (Bie 1899) for which he was awarded the Nobel Prize in physiology or medicine in 1903. The use of light for the treatment of tuberculosis remained popular until 1946 when a more effective treatment and cure became possible with the development of the antibiotic streptomycin. Another important discovery made in the early 20th century was by Oscar Rabb, a student working in the laboratory of von Tappeiner in Munich, who found that a low concentration of acridine, which had no effect in the dark, led to rapid killing of the protozoan paramecium on illumination (Moan and Peng 2003). This discovery of photodynamic action also spurred a great deal of interest in investigating the effect of light on living systems and has led to the development of photodynamic therapy (PDT). Presently, PDT is an accepted modality for the treatment of several forms of cancer and some other diseases (Moan and Peng 2003). Other notable uses of light in medicine developed in the 20th century include treatment of neonatal jaundice using UV light (Cremer, Perryman, and Richards 1958), use of the UV-A spectrum of light to suppress the immune system and reduce inflammatory responses in psoriasis (Parrish 1977), and treatment of seasonal affective disorders (Lam et al. 2006).

The use of light in therapy received a major boost with the invention of lasers in 1960. Laser applications in medicine exploit one or more of the several rather remarkable properties of lasers, viz., high directionality, monochromaticity, brightness, the
ability to generate short-duration pulses, etc. Because of its high
directionality, a laser beam can be focused to very small spot
sizes, a few tenths of a micrometer for visible lasers. This micro-
irradiation capability of lasers, in conjunction with the control
of the laser pulse duration, energy, and intensity, helps the sur-
geon to elicit the desired response in the tissue, making possible
an ultraprecise surgery. Further, the laser light can be efficiently
coupled to thin optical fibers and thus guided endoscopically to
internal organs for therapeutic applications without any major
incision, considerably reducing the patient trauma and hospital-
ization time. The use of lasers in surgery that started in 1961
(within a year of its invention) most often exploits the heating of
the target tissue. It is pertinent to note that although tissue heat-
ing can be achieved by several other means, none can provide the
selectivity made possible by the exquisite control on laser
parameters. By use of laser pulses of duration shorter than the
thermal relaxation time of the target tissue, heat can be confined
within the target tissue so that it can be vaporized or coagulated
without significant thermal effect on surrounding tissue. Laser’s
monochromaticity provides further control for selective pro-
cessing of a constituent of a multicomponent tissue. For exam-
ple, before lasers, no satisfactory treatment existed for port-wine
stains (purple birthmark), a cutaneous vascular disorder. These
are now effectively managed by the use of pulsed lasers with a
wavelength tuned to the hemoglobin absorption peaks to selec-
tively destroy the vasculature without affecting the overlying or
nearby structures. More recently, there has also been interest in
the use of lasers as well as noncoherent light for promoting the
healing of wounds and for providing relief in pains of different
etiology, neuralgia, arthritis, etc. (Fulop et al. 2010; Woodruff
et al. 2004), presumably exploiting the photodynamic effect in
endogenous photosensitizers present in the tissue.

Apart from therapy, the observant ancient civilizations had
also used visual inspection of the patient through the light scat-
tered from the patient’s body as a tool to diagnose diseases. The
invention of the microscope in the 17th century helped evolve histology, and in the early 19th century, light penetration in tis-
ue was utilized for diagnosis of hydrocephalus [an increase in the
volume of cerebrospinal fluid (CSF) in the head] in children and
intraventricular hemorrhage (Gibson and Dehghani 2009). Other
important breakthroughs made in this period include the develop-
ment of endoscopes, which allowed noninvasive examination of the internals of a hollow organ or body cavity and the invention of the ophthalmoscope by Hermann von
Helmholtz (Keeler 2002), which made possible in vivo investiga-
tions of retinal disorders. During the early 20th century, the
use of transillumination for imaging the human breast was also
initiated (Cutler 1931). As a result of the significant difference in
the transmission characteristics of the constituents of the breast
tissue (fat being highly translucent; fibrous tissue less translu-
cent; solid epithelial masses, fibro-epithelial masses, and epithel-
ial debris being moderately opaque; and blood being intensely
opaque), the transillumination of the breast was found to be a
valuable aid in the interpretation of pathological conditions in the
mammary gland.

Since the early 20th century, with the advent of quantum
theory, the use of optical spectroscopic methods for disease
diagnosis also started getting attention. Differences in the light
absorption properties of oxyhemoglobin and deoxyhemoglobin
were used by several groups (Millikan 1942) for monitoring the
oxygen content of arterial blood. These measurements led to
the development of the earlobe-based oxymeter and formed the
basis for the present-day pulse oxymeter. Jobis (1977) demon-
strated the use of near-infrared (NIR) spectroscopy for monitor-
ing of myocardial oxygen saturation in the animal model as well
as in the neonatal head. Over the last few decades, there has been
considerable growth in the use of different optical spectroscopic
techniques, such as fluorescence, Raman, and nonlinear spec-
 troscopy, to probe the biochemical composition or the morphol-
ogy of the tissue (Tuchin 2011). Presently, the advances in optics
and instrumentation and the large image processing capability of computers are making possible a much more comprehensive
use of the information content of the light coming from the tis-
ue for quantitative; sensitive; and higher-resolution, noninva-
sive diagnosis.

In this chapter, we first provide a brief overview of light propa-
gation in tissue and then discuss the use of light for biomedical
imaging, diagnosis, and therapeutic applications.

3.2 Light Propagation in Tissue

When light falls on tissue, a part of it is reflected, transmitted, or
scattered from the tissue. Some of the incident light may also be
absorbed by the tissue constituents. The energy absorbed can be
reemitted as fluorescence or dissipated as heat in the tissue. The
light emerging from the tissue (as a result of reflection, transmis-
sion, scattering, or reemission) depends on its optical properties
and therefore can be used for tissue diagnostics.

The therapeutic effects arise as a result of the energy absorbed
in the tissue. The major contributors for absorption in the UV
spectral range are DNA and proteins. In the visible and NIR
wavelength range, the absorption in tissue is dominated by
hemoglobin and melanin. Although cytochromes, the termina-
lar member of the respiratory chain, have a very large molar
extinction coefficient (higher than that for oxyhemoglobin and
dehemoglobin in the NIR region), these do not have signifi-
cant influence on tissue absorption characteristics because their
relative abundance is very small. Absorption by water, the main
constituent of all tissues, becomes significant beyond ~1000 nm
and becomes dominant for wavelengths higher than ~2000 nm.
It should be emphasized that for a multicomponent tissue, the
absorption at a given wavelength is a weighted average of the
absorption by its constituents at the wavelength.

For wavelengths greater than ~650 nm and smaller than
2000 nm, the tissue absorption is weak, so the light can pene-
trate more deeply. For biomedical imaging and diagnostic appli-
cations, it is desirable to have minimal absorption in the tissue
for two reasons: First, it would allow probing larger depths of
the tissue, and second, deposition of energy in the tissue may
result in irreversible changes. Therefore, generally, light in the
so-called diagnostic window (700 to, say, 1500 nm) in which tissue absorption is minimal is used for biomedical imaging and diagnosis. It should also be noted here that for some diagnostic techniques, which utilize fluorescence of native tissue or a suitable biomarker for diagnosis, use of light that is absorbed by the fluorescing component(s) will be required.

Attenuation of light propagating in a nonscattering medium is completely described by Beer–Lambert’s law \( I = I_0 \exp (-\mu_a z) \), where \( \mu_a \) is the absorption coefficient. Because scattering removes photons from the direction of propagation, it also leads to attenuation of the light. One may think that replacing \( \mu_a \) with \( \mu_a + \mu_s \), where \( \mu_s \) is the scattering coefficient, in Beer–Lambert’s law can describe the variation of irradiance of the collimated light with depth. However, it should be noted that while photons may get scattered out of the beam path, multiple scattering events might also bring photons back into the beam path. These photons, although not part of the collimated beam, also add to the irradiance at a given point along the direction of propagation of the beam limiting the validity of Beer–Lambert’s law. The degree of attenuation arising as a result of the scattering depends on the angular distribution of the scattered photons, which, in turn, has a strong dependence on the size of the scatterer. For scatterers with size \( \ll \) wavelength, the phase of the electromagnetic field across the scatterer can be treated as constant. Therefore, the light scattered by all the induced dipoles in the scatterer adds up in phase, resulting in dipole-like scattering. Here, the angular distribution of the scattered light, often referred to as the “phase function,” shows no dependence on the angle of scattering in the plane transverse to the electric field of the incident light, but in the plane containing the electric field, it shows a cosine square intensity pattern with minima along the dipole axis resulting from the transverse nature of the electromagnetic wave (Figure 3.1a). Rayleigh was the first to show that for such small scatterers, the scattered intensity is inversely proportional to the fourth power of the wavelength and that this is responsible for the blue color of the sky (Bohren and Huffman 1983). For larger-sized scatterers (\( > \) wavelength), light scattered by all the induced dipoles in the scatterer do not add up in phase except only in the forward direction, making the angular distribution of the scattered light peak in the forward direction (Figure 3.1b and c). An exact mathematical description of scattering from spherical particles of size \( > \) wavelength was provided by Ludwig Valentine Lorenz and Gustav Mie (Bohren and Huffman 1983). This is, therefore, referred to as Lorenz–Mie scattering or often just Mie scattering. In the Mie regime, the wavelength dependence of scattering coefficients for different tissue is given as \( \lambda^{-4} \), where \( k \) typically varies from 1 to 2 (Tuchin 2007).

The first moment of the phase function is the average cosine of the scattering angle, denoted by \( g \). It is also referred to as the anisotropy parameter. The value of \( g \) ranges from –1 to +1, where \( g = 0 \) corresponds to isotropic scattering (Rayleigh scattering), \( g = +1 \) corresponds to ideal forward scattering, and \( g = -1 \) corresponds to ideal backward scattering. A photon acquires random direction after approximately \( 1/(1-g) \) scattering events, which is only 5 for \( g = 0.8 \). Typical values of \( g \) for biological tissues vary from 0.7 to 0.99. Another parameter frequently used to describe the scattering properties of the tissue is \( \mu'_s(=\mu_s(1-g)) \).

FIGURE 3.1 (a) Angular distribution of scattering from a dipole in the plane perpendicular to the incident electric field (dotted line) and in the plane containing the electric field (black line). (b) Angular distribution of scattering for a Mie scatterer (diameter = 2 \( \mu m \), r.i. = 1.59 wavelength 632 nm) and (c) the same in log scale to highlight the oscillation in angular scattering distribution.
This is referred to as the reduced scattering coefficient. It defines the path length over which the incident light loses its directional information; that is, the angular distribution of the scattered light becomes isotropic.

The dependence of the angular distribution of the scattered light on the size and shape of the scatterer is widely utilized in flow cytometers for cell identification and characterization. The forward scattering is correlated with the total volume of the cell while the side scattering is related to the inner complexity of the cell, such as granularities in cytoplasm, shape of nucleus, and membrane roughness. In addition to elastically scattered light, a very small fraction of the incident light can also be scattered inelastically by processes such as Raman scattering, which involves energy transfer to or from internal excitations of the medium. This inelastically scattered light is a very sensitive probe for the biochemical composition or morphology of the tissue and is therefore being utilized for biomedical diagnosis.

A rigorous, electromagnetic theory-based approach for analyzing light propagation in tissue will need to identify and incorporate the spatial/temporal distribution and the size distribution of tissue structures and their absorption and scattering properties. Quite evidently, this is not straightforward. Therefore, heuristic approaches with different levels of approximations have been developed to model light transport in tissues (Ishimaru 1978). The radiative transfer theory has been the most successful in modeling light transport in tissues. In this approach, originally developed to explain light propagation in the stellar atmosphere, light propagation in tissue is described by the transport of energy by the motion of photons through a medium containing discrete scattering and absorption centers. Because there are no exact general solutions to the transport equations in a tissue, for practical applications, it is convenient to work with some approximate solutions. Further, for tissues, the tenuous scattering approximation (volume density of scattering particles < \(10^{-3}\)) rarely applies. Tissues are therefore usually modeled either as intermediate scattering media or dense media (volume density > \(10^{-2}\)). The intermediate scattering case is the most difficult to handle rigorously. Several approaches, such as the Kubelka–Munk model, have been developed (Kubelka 1948); however, the range of validity of these models is not well established, and the formulation cannot be generalized to most cases of practical interest.

For weakly absorbing, dense media, in which scattering predominates (\(\mu_s' > \mu_s\)), the equation of radiation transfer reduces to a simpler photon diffusion equation (Ishimaru 1978), which, for an isotropic point source placed on an optically dense slab far away from the boundary, leads to the following expression for the diffuse flux density \(\phi_d(z)\):

\[
\phi_d(z) = k \phi_o \exp(-\mu_{\text{eff}} z) \tag{3.1}
\]

where \(\phi_o(0) = k \phi_o, \phi_o\) being the incident fluence, and \(\mu_{\text{eff}}\) is \((3(\mu_s + \mu_s' + \mu_t)^{1/2})\). The factor \(k\) accounts for the enhancement of fluence just below the boundary arising as a result of back scattering. It follows from Equation 3.1 that the penetration depth, the depth at which the fluence reduces to \(1/e\) of the incident fluence, is \([1 + \ln(k)]\mu_{\text{eff}}^{-1}\). For typical tissue parameters, the depth of penetration can be 1.5 to 3 times \((1/\mu_{\text{eff}})\). The typical values for the absorption coefficient, reduced scattering coefficient, anisotropy parameter, and effective attenuation coefficient for some human tissues at different wavelengths are listed in Table 3.1.

The diffusion approximation provides a fair representation for light transport in soft tissue in the visible and NIR spectral region. It is, however, only valid away from the source and boundaries. The other widely used method for modeling light transport in tissues is Monte Carlo simulation (Wang, Jacques, and Zheng 1995). In this statistical approach to radiative transfer, the multiple scattering trajectories of individual photons are determined using random numbers to predict the probability of each microscopic event. The superposition of many photon paths approaches the actual photon distribution in time and space. Although Monte Carlo simulation may require lengthy computation time, it can be performed for any experimental geometry and is considered the gold standard of tissue optics calculations. For a tissue with optical transport parameters \(\mu'_s = 2 \text{ mm}^{-1}, \mu_s = 0.05 \text{ mm}^{-1}\), and \(g = 0.9\) (typical of tissue in the visible wavelength range), the calculated depth distribution of fluence using one-dimensional diffusion approximation and exact Monte Carlo simulation is shown in Figure 3.2.

One can see that away from the boundary, the predictions from diffusion theory are in good agreement with the Monte Carlo simulations. One should also note that, contrary to normal expectations, the fluence just below the boundary (\(z = 0\)) is significantly larger than the incident fluence. Scattering also leads to another interesting consequence. For finite beams, for a given value of irradiance, the depth of penetration increases with an increase in the size of the area of illumination (Star 1997). Figure 3.3 shows the variation in penetration depth with increasing size of beam diameter for fixed-incident fluence. These results, which may appear counterintuitive, further emphasize the need to have a clear understanding of light propagation in tissue because, for most practical applications, the spatial distribution of light in tissue is expected to play an important role.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>(\lambda) (nm)</th>
<th>(\mu_s) (mm(^{-1}))</th>
<th>(\mu'_s) (mm(^{-1}))</th>
<th>(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (normal)</td>
<td>530</td>
<td>0.11</td>
<td>1.85</td>
<td>-0.88</td>
</tr>
<tr>
<td>Breast (malignant)</td>
<td>530</td>
<td>0.21</td>
<td>2.87</td>
<td>-0.96</td>
</tr>
<tr>
<td>Lung</td>
<td>635</td>
<td>0.81</td>
<td>8.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Liver</td>
<td>635</td>
<td>0.23</td>
<td>10.0</td>
<td>0.68</td>
</tr>
<tr>
<td>Myocardium</td>
<td>1064</td>
<td>0.14</td>
<td>1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Skin (dermis)</td>
<td>630</td>
<td>0.27</td>
<td>3.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Skin (epidermis)</td>
<td>630</td>
<td>3.5</td>
<td>8.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

3.3 Measurements of Tissue Optical Parameters

In order to be able to model light transport in tissue, reasonable estimates for tissue optical parameters are required. Multiple scattering in tissue scrambles the information content and makes direct measurement of these parameters difficult. An obvious approach to eliminate multiple scattering effects is to use a tissue sample with thickness $d \ll 1/\mu_a$. Using such thin tissue sections, the refractive index of tissue is usually determined by applying the traditional refractive index measurement techniques, such as white light interferometry or prismatic dispersion. More recently, optical coherence tomography (OCT) (Ding et al. 2006), digital holography (Bhaduri et al. 2012), and light interference microscopy (Wang et al. 2011) have been used to determine the refractive index of tissue. These studies suggest that refractive index distribution of tissue can provide valuable diagnostic information and may serve as a basis for label-free histopathology (Wang et al. 2011). Measurements at the cellular and subcellular level are usually carried out using light-scattering and phase microscopy (Beuthany et al. 1996). Although the refractive index of tissue constituents varies over a range of 1.3 to 1.6 (Table 3.2), the average refractive index of tissue is close to that of water because water is the major constituent of tissue (typically ~75% by weight).

For a tissue sample with thickness $d \ll 1/\mu_a$, the absorption coefficient can be determined by using an integrating sphere to measure all the photons transmitted or scattered by the sample, so that the loss is only a result of the absorption. Having determined $\mu_a$, $\mu_s$, can be determined from a measurement of the collimated fraction of transmitted light as this will provide an estimate for the sum total of both the absorption and scattering coefficients. This approach, however, has a fundamental limitation. Because the typical value of $\mu_s$ for soft tissues is in the range of 100–1000 cm$^{-1}$, the sample thickness must be less than approximately 10 μm. The methods used for the preparation of these thin sections may alter the tissue optical properties. Further, the signals are weak. For example, for soft tissues in the visible wavelength range, $\mu_a \sim \mu_s/100$. Therefore, the loss of photons as a result of absorption in sample thickness $d \ll 1/\mu_s$ is very small. The weak signal may easily be swamped by several artifacts, such as unavoidable fluctuations in incident light flux, nonuniformity of response of the integrating spheres, etc. Although methods have been developed to minimize these effects, this approach may not provide very accurate measurements. Estimation of the phase function and the anisotropy parameter requires measurement of angular distribution using a goniometer. A reasonable estimate of the anisotropy parameter of tissue can be obtained by fitting the experimentally measured scattering phase function to the Henyey-Greenstein phase function (Ghosh et al. 2001).

A more established method, which can also be used for in situ determination of tissue optical parameters, involves either spatial or temporal distribution of the diffuse radiance as both of these are strongly influenced by $\mu_a$ and $\mu_s'$. The lower the value of $\mu_s'$, the larger the spatial extent over which photons will be distributed, and consequently, the steady-state relative radiance curve $R(r)$ will be less steep. Similarly, with increasing absorption, the radiance at larger distances will be affected more than at shorter distances, leading to a steeper $R(r)$ curve with lower intensity. Similar considerations apply to temporal broadening of an incident light pulse. The slope of the tail of the local time-dependent reflectance curve gives $\mu_a$ directly (Patterson, Chance, and Wilson 1989). However, for finite geometries, the slope also depends on the tissue geometry and source–detector separation. The time-resolved measurements can be done in both time and frequency domain. The


Table 3.2: Refractive Index of Some of the Important Tissue Constituents

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tissue Constituents</th>
<th>Size (µm)(^a)</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nucleus (Choi et al. 2007)</td>
<td>3–12</td>
<td>~1.39, Nucleoli ~1.39, Nucleoplasm ~1.35</td>
</tr>
<tr>
<td>2.</td>
<td>Mitochondria (Wax and Backman 2010)</td>
<td>0.5–1</td>
<td>~1.4</td>
</tr>
<tr>
<td>3.</td>
<td>Lysosomes (Wax and Backman 2010)</td>
<td>0.25–0.8</td>
<td>~1.6</td>
</tr>
<tr>
<td>4.</td>
<td>Cell membrane (Wax and Backman 2010)</td>
<td>~1</td>
<td>~1.46</td>
</tr>
<tr>
<td>5.</td>
<td>Cytoplasm (Wax and Backman 2010)</td>
<td>~1</td>
<td>~1.37</td>
</tr>
<tr>
<td>6.</td>
<td>Collagen fiber (Choi et al. 2007; Leonard and Meek 1997)</td>
<td>0.5–3</td>
<td>1.32 to 1.45 (along axis) 1.40 to 1.61 (radial)</td>
</tr>
<tr>
<td>7.</td>
<td>Red blood cells (Tuchin 2007)</td>
<td>7.1–9.2</td>
<td>1.39–1.41</td>
</tr>
</tbody>
</table>

\(^a\) Largest dimension.

time domain measurements have the potential to yield \(\mu_s\) and \(\mu'_s\) directly. Frequency domain measurements by themselves and in combination with steady-state measurements have also been explored for determination of \(\mu_s\) and \(\mu'_s\) (Gurfinkel, Pan, and Sevick-Muraca 2004).

**In vivo** measurements of tissue parameters using the steady-state spatial radiance profile have received more attention. The parameters \(\mu_s\) and \(\mu'_s\) can be estimated by iterative fitting of the data either with an analytical solution of the diffusion theory when applicable or by Monte Carlo simulations when necessary. It has also been shown that the slope of the \(\ln R(r)\) versus \(r\) curve is strongly correlated to the reduced transport coefficient \(\mu'_s = \mu_s + \mu'_a\) for smaller values of \(r\) and to the effective attenuation coefficient \(\mu_a\) for larger values of \(r\). Although the technique has been successfully used for **in vivo** measurements, its sensitivity to local variations in tissue optical parameters can affect the accuracy of the estimates. Indeed, a large variation was observed in the estimated values of \(\mu_a\) and \(\mu'_a\) for the human esophagus from **in vivo** measurements (Bays et al. 1996). While this may represent real variance in tissue optical properties, measurement errors, in particular, those induced by tissue inhomogeneities, are also expected to contribute.

### 3.4 Use of Light–Tissue Interaction for Biomedical Imaging and Diagnosis

The motivation for the use of light for biomedical imaging and diagnosis arises because optical techniques not only can image tissue with a resolution down to a few micrometers but also can provide valuable biochemical and morphological information on the tissue. It is pertinent to note here that only for thin tissue sections can transmitted light be used for imaging or diagnosis. For **in situ** imaging or diagnosis, the light scattered or reemitted in the backward direction (with respect to the direction of the illuminating beam) is utilized.

The use of light for biomedical imaging has to contend with the problem that, in contrast to x-ray photons, visible light photons undergo multiple scattering in the tissue leading to a blurring of the image. This can be best seen if one shines a torch at one’s palm. One can see a pinkish glow but not the outline of the bones in the path of the beam. The pinkish glow arises because the red component of the light is least attenuated and is therefore dominant in the light that emerges from the palm. The bones are not visible because of the multiple scattering of light in the tissue.

For optical imaging of objects embedded in a turbid medium, basically two schemes have been used. One scheme is to filter out the multiply scattered light, and the other, referred to as the inverse approach, is to use the multiply scattered light emerging from various positions around the object for imaging. For filtering out the multiply scattered light, one may exploit the depolarization or the loss of coherence of the scattered light or the fact that the scattered light emerges from the tissue in all directions and also takes a longer time to emerge as compared to the unscattered (ballistic) or predominantly forward scattered (snake-like) components. The latter essentially travel in a forward direction and so arrive earlier. Coherence gating filters out the ballistic photons having the highest image information and hence can provide images with the best resolution (down to a few micrometers, limited by the coherence length of the source). However, the number of ballistic photons decreases exponentially on propagation through a turbid medium and will be of the order of \(e^{-100}\) of the incident photons on propagation through 1-cm-thick tissue with a scattering coefficient of \(~100\ \text{cm}^{-1}\). Therefore, coherence gating can only be used for imaging the full depth of transparent objects (such as ocular structure) or a few millimeter’s depth of turbid tissue. OCT, the approach that exploits coherence gating for optical imaging, has emerged as a rapid, noncontact, noninvasive, high-resolution imaging technique and is finding clinical applications in ophthalmology, dermatology, etc. (Zysk et al. 2007). The contrast in OCT images can be further augmented by incorporating polarization sensitive detection, which would provide information about the birefringent properties of the tissue. This helps monitor changes in the morphology of the birefringent constituents (collagen, tendon, etc.) of the tissue and thus can be used for noninvasive monitoring of the healing of wounds (Sahu et al. 2010). Similarly, OCT can be extended to incorporate other functional imaging parameters, such as absorption or flow velocities in the spectral and Doppler OCT, respectively (Bouma and Tearney 2002).
A larger depth of imaging, but with a poorer resolution, is possible by the use of polarization gating or time gating because in these approaches both ballistic and forward scattered components are utilized for imaging. The combined magnitude of these on propagation through 1-cm-thick tissue is proportional to \( \exp(-\mu_s) \). This, for an anisotropy parameter of 0.9 and \( \mu_s \) of 100 cm\(^{-1}\), implies that even after a depth of 1 cm, the signal strength is reasonable: \( \sim e^{-10} \) as compared to \( e^{-100} \) for the ballistic component. It may be noted that the size distribution of the scatterer also affects the temporal profile of the transmitted laser pulse and thus the contrast in time-gated optical imaging (Rao et al. 2005). Further, with the use of nonlinear optical time-gating techniques, such as stimulated Raman scattering, the gated light can be amplified to generate reasonable signal levels even after propagating through a few centimeters thick tissue. For polarization-gated imaging, the rate of depolarization and hence the depth of imaging as well as the contrast depends both on the size distribution of the scatterer and the incident polarization state (Ghosh, Patel, and Gupta 2003).

While the polarization-gated optical imaging normally utilizes the polarized fraction of the light, the depolarized fraction has also been effectively utilized for the imaging of tissue vasculature. If the tissue is illuminated with a linearly polarized light and the backscattered light is detected in a cross-polarization channel, not only is the specular reflection from samples surface eliminated but also the depolarized light coming from deeper layers effectively back illuminates the tissue for the imaging of microvasculature (Grönert et al. 1999). Such orthogonal polarization spectral (OPS) imaging has been used for visualization and quantitative imaging of microcirculation in a number of diagnostic applications (Cerný, Turek, and Parizkova 2007).

Imaging through still larger depths as required for imaging of the human brain or female breast necessitates the use of diffuse photons with a comcomitant decrease in the spatial resolution (at best, a few millimeters). In a typical diffuse optical tomography (DOT) system, data are acquired by placing the source and detectors at various locations around the object. Both time-domain and frequency-domain measurements are used for DOT. In the time-domain approach, the temporal profile of the detected signal following illumination by ultrafast pulse is used to reconstruct the three-dimensional map of the optical parameter distribution, whereas in the frequency-domain systems, demodulation and changes in phase of the intensity-modulated light are used for estimation of the tissue parameter distribution. Although frequency-domain DOT setups have a limitation in that measurements are made at only few discrete frequencies, it is still more widely used being less expensive and portable compared to time-domain setups (Gibson and Dehghani 2009). DOT provides useful information about the blood dynamics, blood volume, blood oxygen saturation, and water and lipid content of the tissues because the absorption spectra of major tissue chromophores, such as oxyhemoglobin and deoxyhemoglobin, cytochrome, and water, differ significantly in the NIR region.

While for imaging one exploits the intensity, coherence, or polarization of the scattered light, other parameters of the scattered light, such as its angular distribution and the spectral content, also contain significant diagnostic information. As noted earlier, the angular distribution of the scattered light can provide information about the size of the scatterers. Further, measurements on the polarized component of back-scattered light can also be used to filter out the multiply scattered light from deeper layers of the tissue and thus look at the light scattered from the superficial tissue layer. This approach has been used to extract the size distribution and the density of the nuclei in the superficial epithelial cell layer, which can be used to monitor neoplastic changes in biological tissues (Gurjar et al. 2001). Changes in the polarization parameters of the tissue arising as a result of its birefringent (collagen, tendon, etc.) and chiral (glucose) constituents can be separated from the change in polarization arising as a result of scattering (Manhas et al. 2006) and exploited for diagnostics. Indeed, significant differences were observed in the polarization parameters (retardance, diattenuation, and depolarization) of the normal and malignant sites of oral and breast tissue, and these were shown to correlate well with the changes expected in the structure of the collagen present in these tissues (Manhas et al. 2009). Similarly, there is interest in the estimation of the concentration of chiral molecules, such as glucose in tissue, by measuring the rotation of the plane of polarization of the back-scattered light (Manhas et al. 2006). The differences in the structural and functional properties of normal and malignant tissue lead to differences in their wavelength-dependent absorption and scattering characteristics (Ghosh et al. 2001), which can also be used for diagnostics by itself or as a result of the effect these changes have on the elastically and inelastically scattered light.

The inelastically scattered light is much weaker compared to the elastically scattered light and therefore requires use of high brightness sources, for example, lasers and appropriate light delivery and collection systems, for its use for practical applications. Both fluorescence and Raman spectroscopic (Vo-Dinh 2003; Tuchin 2011) approaches are being actively pursued for their diagnostic potential. The fluorescence of native tissue originates from a number of endogenous fluorophores that are present in tissue. The prominent fluorophores include aromatic amino acids, such as tryptophan; structural proteins, such as collagen and elastin; coenzymes, such as NADH and flavins; and the porphyrins. Their excitation maxima typically lie in the range 280–500 nm, whereas their emission maxima lie in the range 300–700 nm. The observed fluorescence emission of native tissue is essentially a convolution of the emission spectra of the endogenous fluorophores of tissue and therefore strongly depends on the wavelength of the light of excitation. Only those endogenous fluorophores are excited and emit fluorescence whose absorption bands have an overlap with the wavelength of the excitation light. Because the excitation light and the emitted fluorescence have to propagate through the turbid tissue, the measured tissue fluorescence is also influenced by the absorption and scattering at both the excitation and the emission wavelengths. This makes it difficult to extract, from the measured fluorescence, the intrinsic fluorescence of the tissue,
which may have valuable biochemical information about the tissue. Changes in the absorption and scattering characteristics of tissue can also lead to subtle changes in its fluorescence characteristics. For example, it has been shown that malignant breast tissue sites have a larger scattering coefficient as compared to normal. This has an interesting consequence. Whereas for thin tissue sections (thickness < optical transport length), the depolarization of fluorescence was observed to be smaller in malignant tissues compared to normal (as a result of the changes in the biochemical environment of the fluorophores), the reverse was observed for a thicker tissue section because there scattering is the major cause of depolarization (Mohanthy et al. 2001).

As a consequence, the fluorescence from the superficial layer of tissue is the least depolarized, and that originating from deeper layers becomes increasingly more depolarized. Therefore, measurements on the polarized component of fluorescence could be used for depth-resolved measurements on fluorescence (Ghosh et al. 2005).

While, because of significantly larger strength, the fluorescence-based technique is presently better developed for clinical applications, the utilization of Raman spectroscopy for diagnostic applications is also receiving a lot of attention. Because of its molecular specificity, the Raman technique makes obtaining specific biochemical information about the tissue so much easier. For further details on the use of optical spectroscopy for biomedical diagnosis, the reader is referred to Vo-Dinh (2003) and Tuchin (2011).

### 3.5 Surgical and Therapeutic Applications

Surgical and therapeutic applications depend on absorption of light. The absorbed laser energy can broadly lead to three effects (Gupta, Ghosh, and Patel 2007). The most common effect is a rise in tissue temperature (the photothermal effect). At high intensities associated with lasers operating in short-pulse durations (nanosecond, picosecond), absorption of laser radiation may lead to the generation of pressure waves or shockwaves (photomechanical effects). Short-wavelength lasers can cause electronic excitation of chromophores in the tissue and thus initiate a photochemical reaction (photochemical effect). The relative role played by the three depends primarily on the laser wavelength, irradiance, and pulse duration.

#### 3.5.1 Photothermal Effects

Most of the surgical applications of light exploit a photothermal effect, that is, a rise in tissue temperature following absorption of light. The biological effect depends on the level of rise in tissue temperature, which is determined by two factors: the tissue volume in which a given energy is deposited and the time in which the energy is deposited vis-à-vis the thermal relaxation time (the inverse of which determines the rate of flow of heat from heated tissue to the surrounding cold tissue). A small rise in temperature ($5\text{°C}$–$10\text{°C}$) can influence the activity of enzymes and lead to changes in blood flow and vessel permeability. Tissues heated to a temperature of $45\text{°C}$–$80\text{°C}$ may get denatured as a result of breakage of van der Wall bonds, which stabilize the conformation of proteins and other macromolecules. Thermal denaturation is exploited for therapy in several ways. For example, hemostasis occurs because of increased blood viscosity caused by denaturation of plasma proteins, hemoglobin, and perivascular tissue. When the temperature exceeds $100\text{°C}$, boiling of water in the tissue takes place. Because of the large latent heat of water, the main constituent of tissue, energy added to tissue at $100\text{°C}$ first results in generation of steam without further increase in temperature. A volume expansion by ~1670-fold occurs when water is vaporized isobarically. When this large and rapid expansion occurs within tissue, physical separation or “cutting” occurs. Tissue surrounding the region being vaporized will also be heated, resulting in coagulation of the tissue at the wound edges and thus preventing blood loss.

If the rate of deposition of energy is faster than that required for the boiling of water, the tissue is superheated and can be thermally ablated. Thermal ablation or explosive boiling is similar to what happens when cold water is sprinkled on a very hot iron. In contrast to the vaporization in which the tissue temperature is ~$100\text{°C}$, for ablation, the tissue temperature is much higher ($500\text{°C}$ or more), and the kinetics involved are considerably faster. In ablation, practically all the energy deposited in the tissue is converted into the kinetic energy of the ablation products with the result of minimal thermal damage to the adjoining tissues.

It is pertinent to emphasize that by appropriate choice of laser wavelength, it is possible to selectively deposit energy in a target site if it has greater absorption than the surrounding tissue. Further, by use of laser pulses of duration shorter than the thermal relaxation time, heat can be confined within the target tissue so that it can be vaporized without significant effect on surrounding tissue. Such selective photothermolysis has been exploited for several therapeutic applications, such as laser treatment of port-wine stains. Another approach that is receiving attention for controlled localized heating involves the use of near infrared light tuned to surface plasmon resonance of metallic nanoparticles. Such heating of metallic nanoparticles that have been selectively deposited in target cells can be used for applications such as hyperthermia for cancer treatment.

#### 3.5.2 Photomechanical Effects

Photomechanical effects are usually important only at high intensities typical of short-duration ($10^{-9}$–$10^{-12}$ s) laser pulses. The localized absorption of intense laser radiation can lead to very large temperature gradients, resulting in enormous pressure waves and localized photomechanical disruption. Such disruption is useful, for example, in the laser removal of tattoo marks. Tattoo ink has pigmented molecular particles too large for the body’s immune system to eliminate. Photodisruption of these into smaller particles enables the body’s lymphatic system to dispose them, resulting in removal of the tattoo mark.

At high intensities, the electric field strength of radiation is also very large (about $3 \times 10^7 \text{V/cm}$ at an intensity of $10^{12} \text{W/cm}^2$)
and can cause dielectric breakdown in the tissue. The resulting plasma absorbs energy and expands, creating shockwaves, which can shear off the tissue. These plasma-mediated shockwaves are used for breaking stones in the kidney or urethra (lithotripsy) and in posterior capsulotomy for the removal of an opacified posterior capsule of the eye lens.

### 3.5.3 Photochemical Effects

For laser irradiation at power levels in which there is no significant rise in temperature of the tissue, the photothermal and photomechanical effects are not possible. In such a situation, only photochemical effects can take place provided the energy of the laser photon is adequate to cause electronic excitation of biomolecules, which can be either endogenous or externally injected. The photoexcitation of molecules and the resulting biochemical reactions can lead to either bioactivation, exploited in various phototherapies (Karu 2010), or generation of some free radicals or toxins, which are harmful for the host tissue. The latter process is used for PDT of cancer (Agostinis et al. 2011). Shorter wavelength radiation of sufficient energy can also break molecular bonds and impart kinetic energy to the fragments by which they get ejected from the tissue. This photochemical removal (photoablation) can occur only from the area of tissue exposed to the light and will have no effect on the surrounding unexposed tissue. In photoablation, there is no rise in the tissue temperature, and the tissue removal can be achieved in an extremely precise manner because of the small penetration depth of light at these short wavelengths. It is for this reason that UV excimer lasers are being widely used for reprofiling the cornea to correct vision disorders. Though conventional light sources are also useful for phototherapy, a better control of light characteristics can often make phototherapy more convenient with the use of laser. PDT, because of its high selectivity, has received a lot of attention for the treatment of cancer. Its use for inactivation of microbes, referred to as antimicrobial PDT (APDT), is also attracting attention. The advantage of PDT over conventional antimicrobials is that the treatment is restricted to the light-irradiated regions of the drug-treated area and therefore reduces the risk of adverse systemic effects. Also, reactive oxygen species generated in the photochemical reactions cause nonspecific damage to cellular components, and therefore, it is highly unlikely for bacteria to develop resistance (Maisch 2007).

### 3.6 Concluding Remarks

Light has been used in photomedicine since time immemorial. This field has seen remarkable growth in the past few decades fueled by the invention of lasers that provide a light source with much better control on its parameters and other technological developments, such as sensitive detection systems, the large information-processing capability of present-day computers, etc. Optical techniques are contributing significantly toward the realization of noninvasive, near real-time biomedical imaging and diagnosis, and also therapeutic modalities providing higher selectivity than conventional modalities. Effective utilization of these techniques and further improvement in their performance requires a good understanding of light propagation in tissue. In spite of the complexities of the problem, significant advancements have been made. It is expected that continued interest in photomedicine will provide further impetus to this field and should lead to more innovative methods for furthering photomedicine.

### References


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