Chapter 11

Reflectance and transmittance spectroscopy

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Abstract

This chapter describes several approaches to the optical study of biological tissue using reflectance and transmittance spectroscopy. This topic has spurred significant research efforts as a result of the relevant physiological and metabolic information provided by the optical data, in conjunction with the safe, non-invasive, and cost-effective optical approach to the study of tissue. We give a brief historical introduction in Section 11.1, followed by a description of the optical absorption and scattering properties of tissue in Section 11.2. Section 11.3 is devoted to continuous-wave (CW) techniques, which can be applied to the study of relatively superficial tissue layers (as is the case for diffuse reflectance imaging and localized measurements using short separations between the illuminating and collecting optodes), as well as deep tissues on the basis of a modified Beer–Lambert law, transport theory, or diffusion theory. The latter model is commonly employed in time-domain and frequency-domain techniques, which are described in Sections 11.4 and 11.5, respectively. Three major application areas of near-infrared tissue studies, namely tissue oximetry, optical mammography, and functional imaging of the brain, are presented in Sections 11.6, 11.7, and 11.8, respectively. Finally, Section 11.9 discusses potential future directions for optical techniques in a number of clinical areas.

11.1 Introduction

The potential of reflectance and transmittance optical techniques for the study of biological tissue has long been recognized. For example, applications in medical diagnostics and monitoring of physiological parameters in vivo were developed as far back as 1929 for optical mammography [1] and 1942 for tissue oximetry [2]. In particular, in the early 1970s, the optical oximetry approach developed into pulse oximetry [3,4], which is routinely used today in hospitals and intensive care units to monitor the oxygen saturation of arterial blood. The demonstration of the applicability of reflectance spectrophotometry to the study of the brain [5] has opened up new opportunities to investigate the cortical architecture in animal models [6] and the brain functional activity in human subjects [7–10]. The introduction of a physical model, namely diffusion theory, to quantitatively describe light propagation in tissue [11], and the development of time-resolved techniques either in the time-domain [11,12] or in the frequency-domain [13–15] to the optical study of tissue have resulted in a further refinement of reflectance and transmittance spectroscopy and imaging of tissue.

The optical study of tissue in vivo is typically performed in reflectance, i.e. with the illumination and collection performed on the same side of the tissue. In many cases, this is dictated by the lack of adequate optical signal transmitted through thick tissues. However, body parts that are relatively thin (for instance, fingers) or present relatively low levels of optical attenuation (for instance, the female breast or the infant’s head) lend themselves to transmittance optical studies. In this
chapter, we will describe applications based on both reflectance and transmittance geometries, as well as continuous-wave (CW), time-domain (TD), and frequency-domain (FD) optical techniques.

11.2 Optical properties of tissue

The propagation of light in biological tissue can be described in terms of the flow of discrete particles called photons. According to this representation, a light source introduces a given number of photons per unit time at a given tissue location and these photons travel inside the tissue following individual paths. The collective propagation of photons along these paths is called photon migration. After a photon is introduced inside the tissue by the light source, it can interact with tissue through several mechanisms, including absorption, elastic scattering, and fluorescence. When a photon is absorbed it essentially disappears and transfers its energy to the absorbing center (chromophore). When a photon is elastically scattered by a scattering center in the tissue (for instance a cellular membrane, nucleus, or organelle), its direction of propagation changes while its energy (and wavelength) is essentially not affected. In a fluorescence process, a photon at wavelength $\lambda_x$ (excitation wavelength) is absorbed and a photon at a longer wavelength $\lambda_m$ (emission wavelength) is emitted. The fluorescence emission process is not immediate, but takes place with an average delay $\tau$ (fluorescence lifetime), typically on a time scale of nanoseconds, with respect to the time of absorption of the excitation photon. These three processes are schematically illustrated in Figure 1. In this chapter, we describe techniques of reflectance and transmittance spectroscopy for the optical study of tissue, which are based on absorption and elastic scattering processes. Fluorescence spectroscopy is described elsewhere in this book.

![Figure 1](image)

**Figure 1.** Schematic illustration of (a) absorption, (b) elastic scattering, and (c) fluorescence processes of interaction of photons with chromophores, scattering centers, and fluorophores, respectively, in tissue. In panel (b) $\lambda$ is the photon wavelength, which is largely unaffected by elastic scattering processes, and $\theta$ is the scattering angle. In panel (c), the wavelength of the emission photon $\lambda_m$ is longer than the wavelength of the incoming (or excitation) photon $\lambda_x$, and the emission photon is emitted with an average delay $\tau$ with respect to the absorption of the excitation photon.
11.2.1 Absorption

The main absorbers of visible/near-infrared light in bloodperfused tissues are oxy-hemoglobin, deoxy-hemoglobin, and water, with further absorption contributions, which sometimes can be of interest, from myoglobin, lipids, cytochrome oxidase, melanin, bilirubin, etc. The absorption spectra of oxy-hemoglobin (50 μM concentration), deoxy-hemoglobin (50 μM concentration), and water are shown in Figure 2, which is derived from published data of the absorption coefficients of water [16] and hemoglobin [17]. The so-called “medical spectral window” extends from approximately 700 to 900 nm, where the combined absorption of light from hemoglobin and water is minimal (Figure 2). As a result of the relatively smaller absorption, light in this spectral window penetrates deeper in tissues, making it possible to investigate deep tissue sites noninvasively.

The absorption properties of tissue are described by the absorption coefficient ($\mu_a$), which is defined as the inverse of the average photon path length before absorption. From this definition it follows that $1/\mu_a$ is the average distance traveled by a photon before being absorbed. In the near-infrared, typical values of $\mu_a$ in tissues range from 0.02 to 0.30 cm$^{-1}$, so that the mean photon path before absorption thus ranges between 3 and 50 cm.

11.2.2 Scattering

The scattering of photons is due to localized gradients in the refractive index caused by particles that act as scattering centers. The scattering properties are mainly

![Figure 2. Absorption spectra of three of the most important near-infrared chromophores in tissues, namely oxy-hemoglobin (HbO$_2$), deoxy-hemoglobin (Hb), and water (H$_2$O). The absorption coefficient is defined to base $e$. The concentrations of Hb and HbO$_2$ are both assumed to be 50 μM, a typical value for blood-perfused tissues. Spectra obtained from compiled absorption data for water [16] and hemoglobin [17].](image-url)
determined by the dimensions of the particles relative to the wavelength of light, and by the difference between the indices of refraction of the particles and the surrounding medium. In biological tissues, the scattering centers include cell nuclei, cell organelles, and cells themselves. In particular, cell organelles such as mitochondria have dimensions comparable to the wavelengths in the medical spectral window (700–900 nm), and their index of refraction is not dramatically different than that of the cytosol. Under these conditions, light scattering is mainly forward directed (i.e. the scattering angle $\theta$ shown in Figure 1 (b) is smaller than 90°), as is the case in most biological tissues.

The scattering properties of tissues are described by two parameters: (1) the scattering coefficient ($\mu_s$), defined as the inverse of the average photon path length between successive scattering events; (2) the average cosine of the scattering angle $\theta$, $g = \langle \cos \theta \rangle$. From the definition of $\mu_s$ it follows that $1/\mu_s$ is the average distance traveled by a photon between successive scattering events. Even though each scattering event is mainly forward directed, after a large number of collisions a photon loses memory of its original direction of propagation. Under these conditions, the scattering angle $\theta$ cannot be measured and we can describe photon scattering in terms of effectively isotropic scattering processes. In tissue spectroscopy, it is customary to define the reduced scattering coefficient ($\mu_s' = (1-g)\mu_s$) which represents the inverse of the average distance over which the direction of propagation of a photon is randomized. In other words, we can say that $1/\mu_s'$ is the average distance between effectively isotropic scattering events. Of course $\mu_s'$ coincides with $\mu_s$ in the case of isotropic scattering ($g = 0$). In the optical study of thick tissues, $\mu_s'$ is the only measurable scattering parameter. Typical values of $\mu_s'$ in biological tissues range from 1 to 50 cm$^{-1}$, while $g$ is typically 0.8–0.9 [18] (so that $\mu_s'$ is about one order of magnitude smaller than $\mu_s$). The average distance traveled by a photon in tissue between effectively isotropic scattering events is typically a few millimetres or less.

### 11.3 Continuous-wave techniques

Continuous-wave (CW) methods employ light sources (for example arc lamps, light emitting diodes, or laser diodes) with emission properties that are time-independent. While CW methods are typically unable to separate the absorption and scattering contributions to the optical absorbance measured in tissue, they present the advantage of being technically straightforward. For instance, charge coupled device (CCD) cameras can be readily used for reflectance imaging, while photo-multiplier tubes and avalanche photodiodes are typically employed for single-point detection.

#### 11.3.1 Diffuse reflectance imaging

Optical imaging of biological tissue with diffuse illumination and CCD camera detection is a powerful tool to investigate superficial tissue layers with high spatial resolution. A typical experimental setup is illustrated in Figure 3. The general idea
is to uniformly illuminate a broad area of the sample (or tissue) to be investigated, and to collect the diffusively reflected signal over a portion of the illuminated area. Linearly polarized light and a linear analyzer can be used to suppress the detection of specular reflections at the tissue surface. This approach has been used to obtain 50–100 \( \mu \text{m} \) resolution in vivo images of the cerebral cortex in animal models [6,19,20] and in humans [21]. The basic approach (see for example Ref. 22) consists of illuminating the cerebral cortex (after removal of the skull and dura) with light of proper wavelength. Spatial uniformity of the cortex illumination is achieved by using multiple light guides. Visible wavelengths (in the 500–600 nm range) are typically used to obtain a high sensitivity to blood vessels and to oxygenation changes, while longer wavelengths (>700 nm) are used to increase the optical penetration depth (in this configuration, the cortex can be investigated up to depths of about 1 mm) and the sensitivity to changes in light scattering. The light reflectance images are analyzed to obtain differential maps that represent the effect of specific cortical activity and provide information on the functional architecture of the cerebral cortex. For example, this method has been used to map the ocular dominance domains in the visual cortex [19,23], and the functional architecture of the somatosensory cortex [24] in animal models. Diffuse reflectance measurements on the skin of human subjects may allow for the discrimination of cutaneous melanoma from other pigmented lesions of human skin [25,26].

The theoretical description of the diffuse reflectance measured from a turbid medium (such as biological tissue) under uniform illumination conditions has been performed according to several different models. The objective of these models is to describe the relationship between the diffuse reflectance \( R \) and the optical properties of the turbid medium, namely the absorption coefficient \( (\mu_a) \), the
scattering coefficient \( (\mu_a) \), and the anisotropy scattering factor \( g \). We recall that \( \mu_a \) is defined as the inverse of the mean distance traveled by the photons in the medium before being absorbed, \( \mu_s \) is defined as the inverse of the photon mean free path between successive scattering events, and \( g \) is defined as the average of the cosine of the scattering angle for photons in the medium. The main theoretical models used to describe the diffuse reflectance are the following.

### 11.3.1.1 Kubelka–Munk theory

This model is based on a two-flux description of the light propagation in turbid media [27,28], which leads to the following expression for the diffuse reflectance under conditions of perfectly diffuse irradiation of the medium and isotropic light scattering \( (g = 0) \) [28,29]:

\[
R = 1 + \frac{K}{S} - \left( \frac{K^2}{S^2} + 2 \frac{K}{S} \right)^{\frac{1}{2}}.
\]

where \( K \) and \( S \) are the Kubelka–Munk absorption and scattering coefficients, respectively, which are related to \( \mu_a \) and \( \mu_s \) by the relationship \( K/S = 2.67 \, \mu_a/\mu_s \) [30].

### 11.3.1.2 Transport theory

Transport theory provides a general description of light propagation in absorbing and scattering media on the basis of the Boltzmann transport equation. This general approach does not provide an analytical expression for the diffuse reflectance. Tabulated values of the diffuse reflectance obtained by solving the Boltzmann transport equation for several values of the ratio \( \mu_a/\mu_s \) and for either isotropic scattering \( (g = 0) \) or particular cases of anisotropic scattering have been reported [31].

### 11.3.1.3 Diffusion theory

Even though, strictly speaking, the diffusion approximation to the transport equation is not applicable to the description of the diffuse reflectance obtained under uniform illumination conditions, it has nevertheless been used to obtain an analytical approximation for the diffuse reflectance [32,33]. For collimated, normal irradiation, and a 1.33 refractive index mismatch at the boundary between non-scattering and scattering media, the expression for \( R \) derived with diffusion theory is [33]:

\[
R = \frac{a'}{1 + 2k(1 - a') + (1 + 2k/3)[3(1 - a')]^{\frac{3}{2}}},
\]

where \( a' \) is the optical reduced albedo defined as \( \mu'_s/(\mu_a + \mu'_s) \), with \( \mu'_s = (1-g)\mu_s \) as the reduced scattering coefficient, and \( k = (1-r_d)/(1+r_d) \), with \( r_d \) as the internal reflection coefficient of the turbid medium.

### 11.3.1.4 Adding-doubling and Monte Carlo methods

Combining an adding-doubling solution to the transport equation [34] and Monte Carlo methods has resulted in an analytical expression for the diffuse reflectance.
from a turbid medium where the light scattering is described by the Henyey–Greenstein phase function [35]:

\[ R = \frac{1}{\alpha \nu_1 + \nu_2} \quad \text{(3)} \]

### 11.3.2 Spectral measurements insensitive to scattering changes

As mentioned above, CW methods are usually unable to perform measurements of both absorption and scattering properties of tissue. For instance, the CW reflectance expressions of Equations (1)–(3) all contain the ratio of absorption to scattering coefficients, which prevents the separations of the contributions from these two coefficients to the diffuse reflectance. One way to overcome this limitation of CW tissue spectroscopy is to develop measurement techniques that are relatively insensitive to the scattering properties of tissue. For example, when an illumination optical fiber (or optode) is used to deliver light at one specific location, and a collection optode is used to collect the optical signal at a distance \( r \) from the illumination point, there is an optimal optode separation, \( r \), for which the detected optical signal is not affected by changes in the scattering properties of the tissue [36]. The basic idea behind the existence of an optimal source–detector separation that minimizes the sensitivity to the scattering coefficient is that, in the limit of very small separations, the optical signal increases with the scattering coefficient, while the optical signal decreases in the diffusive limit of large separations. Therefore, it is reasonable to expect that, at some intermediate point, the optical signal does not change with the scattering coefficient. Such an optimal source–detector separation, for typical optical properties of biological tissue, was found to be 1.7 mm [36], a value that lends itself to being implemented in a clinical endoscope. The reason that the insensitivity of the optical signal to the scattering coefficient is relevant is the following. The detected optical intensity \( I \) in reflectance and transmittance spectroscopy can be described in terms of the Beer–Lambert law:

\[ I = I_0 e^{-\mu_a L} \quad \text{(4)} \]

where \( I_0 \) is the incident intensity and \( L \) is the photon pathlength. In the presence of scattering, \( L \) is not the same for all detected photons, so that \( L \) in Equation (4) should be replaced by an effective pathlength (which in general differs from the average photon pathlength). If \( L \), or the effective pathlength, is known from calibration measurements or look-up tables and is not affected by the scattering properties of tissues, then one can translate a spectral or temporal variation in \( I \) into a corresponding variation in \( \mu_a \):

\[ \mu_{a2} - \mu_{a1} = \ln \left( \frac{(I/I_0)_1}{(I/I_0)_2} \right) / L \quad \text{(5)} \]

where the subscripts 1 and 2 indicate measurements at different wavelengths or different times. Strictly speaking, the pathlength \( L \) also depends on the absorption
coefficient itself, but for relatively small values of $\mu_{a2} - \mu_{a1}$ such dependence may be neglected. Being able to apply Equation (5) regardless of the scattering properties of tissue is an important feature that allows, for example, the non-invasive quantitative measurement of drug concentration in tissue [37].

11.3.3 Modified Beer–Lambert law

Another common approach to measuring changes in the tissue absorption using CW methods is based on a generalization of Beer–Lambert law (Equation (4)) in conjunction with the assumption that the scattering properties of tissue do not vary with time. The modified Beer–Lambert equation is the following [12]:

$$ I = I_0 e^{-B \mu_a r - G} $$

(6)

where $B$ is a pathlength factor that depends on the optical properties of tissue, $r$ is the inter-optode distance (i.e. the geometrical separation between the points of illumination and light collection), and $G$ is an unknown geometry-dependent factor that also accounts for the effect of scattering. If the factor $G$ is constant, then a relatively small temporal variation in the absorption coefficient can be written as:

$$ \mu_a(t_2) - \mu_a(t_1) = \frac{1}{Br} \ln \left[ \frac{I(t_1)}{I(t_2)} \right] $$

(7)

The pathlength factor $B$ is usually estimated from literature values [38–40]. From diffusion theory (see Section 11.3.4), it can be shown that the pathlength factor $B$ is given by the following expressions in terms of the absorption and reduced scattering coefficients [41]:

$$ B_{\text{inf}} = \frac{\sqrt{3} \mu'_s}{2 \sqrt{\mu_a}} $$

(8)

$$ B_{\text{semi-inf}} = \frac{\sqrt{3} \mu'_s}{2 \sqrt{\mu_a}} \frac{r \sqrt{3 \mu_a \mu'_s}}{r \sqrt{3 \mu_a \mu'_s + 1}} $$

(9)

where the subscript “inf” refers to an infinite geometry, and the subscript “semi-inf” refers to the semi-infinite case where the optodes are located at the boundary of a scattering medium that extends indefinitely in one direction.

11.3.4 Diffusion theory

Diffusion theory describes the propagation of photons in optically turbid media. Mathematically, the diffusion equation is a limiting case of the general Boltzmann transport equation [42,43], which describes the propagation of photons in scattering and absorbing media. The key condition that leads to the diffusion approximation, which is often (but not always) fulfilled in the optical study of tissue, is that light
propagates in a strongly scattering regime, i.e. $\mu'_s \gg \mu_a$. This condition means that a photon, on the average, undergoes many effectively isotropic scattering events before being absorbed. Under this condition, the CW photon density in the tissue $[U_{CW}(r)]$ resulting from a point light source at $r = 0$ obeys the standard diffusion equation [44]:

$$-\nu D \nabla^2 U_{CW}(r) + \nu \mu_a U_{CW}(r) = P_0 \delta(r),$$

(10)

where $\nu$ is the speed of light in the tissue, $D$ is the diffusion coefficient defined as $1/[3(\mu_a+\mu'_s)]$, $P_0$ is the source power, and $\delta$ is the Dirac delta. The solution to Equation (10) for an infinite medium yields the CW photon density as a function of the distance $r$ from the illumination point [44]:

$$U_{CW}(r) = \frac{P_0}{4\pi \nu D} \frac{e^{-r\sqrt{3\mu_a\mu'_s}}}{r}$$

(11)

This expression is often used to guide the interpretation of optical data collected in tissue. Data collected in a reflection geometry is often modeled using a semi-infinite model [44], while data in transmission may be modeled using a slab geometry [11], leading to more accurate predictions of the optical signal with respect to the infinite-medium solution of Equation (11).

11.3.5 Determination of tissue optical properties using the spatial non-linearity of diffuse reflectance at short source–detector separations

The solution to the diffusion equation for an infinite medium, which is given by Equation (11), shows that the $\ln(r U_{CW})$ is a linear function of $r$. It can be shown that, for a semi-infinite geometry, a linear relationship holds between $\ln(r^2 U_{CW})$ and $r$, provided that $r \gg (3\mu_a\mu'_s)^{-1/2}$ [44–46]. The non-linearity of the $\ln(r^2 U_{CW})$ at relatively short source–detector separations ($<1 \text{ cm}$) can be used to measure both the absorption and the scattering properties of tissue from spatially-resolved CW data. By using this approach, Farrel et al. measured the absorption and the reduced scattering coefficients of skin in human subjects in vivo [44].

11.4 Time-domain techniques

Time-domain (TD) studies employ a pulsed light source that emits short light pulses (typically on the order of picoseconds) with a repetition rate of about 1 MHz [11,12]. Mode-locked solid state lasers, or fast laser diodes are used as the light sources. Photomultiplier tubes and microchannel plates in photon counting mode, or streak cameras, are usually employed as the detectors. In the time-domain, one directly measures the time-of-flight distribution of the collected photons, which travel along a set of trajectories that are collectively indicated as a light bundle (see Figure 4(a)).
Figure 4. (a) Photons that are incident at one point of the tissue (intensity $I_0$) and that are detected at another point, in transmission in the case illustrated in the figure, (intensity $I_t$) travel along a collection of trajectories within the tissue. (b) In the time-domain, $I_0$ is a short (ps) pulse, while $I_t$ is broadened and delayed on a time scale of nanoseconds as a result of propagation over a tissue thickness $L$ of the order of centimetres.

11.4.1 Diffusion theory in the time domain

In the diffusive regime, which is often established when light propagates over several millimeters or more into tissue, the distribution of photon times-of-flight is given by the solution to the diffusion equation for a point photon source which emits $N_0$ photons at time $t = 0$. The corresponding source term is written as $N_0 \delta(r) \delta(t)$, where $\delta$ is the Dirac delta, resulting in Equation (12) for the diffusion equation:

$$\frac{\partial U_{TD}(r,t)}{\partial t} - vD \nabla^2 U_{TD}(r,t) + \nu \mu_a U_{TD}(r,t) = N_0 \delta(r) \delta(t)$$  \hspace{1cm} (12)

where $U_{TD}(r,t)$ is the time-domain photon density at point $r$ and time $t$, and $D$ is the diffusion coefficient defined in Equation (10). The solution to Equation (12) for an infinite medium is Equation (13) [11]:

$$U_{TD}(r,t) = \frac{N_0}{(4\pi vDt)^{3/2}} e^{-\frac{v^2 r^2}{4Dt} - \mu_a t}$$  \hspace{1cm} (13)

The time dependence of $U_{TD}$, which represents the delay and broadening of the input pulse as it propagates into tissue, is qualitatively shown in Figure 4(b). From Equation (13) one can see that the behavior of $U_{TD}$ at short times is dominated by $\mu_a'$ (because of the inverse dependence on $t$ of the first term in the exponent, which contains $\mu_a'$), whereas the behavior at large times is dominated by $\mu_a$ (because of the direct dependence on $t$ of the second term in the exponent, which contains $\mu_a$). A fit of Equation (13), or its extension to appropriate geometries such as semi-infinite, slab, cylindrical, or spherical [47], to the experimental data enables one to independently recover $\mu_a$ and $\mu_a'$ of tissue [11].
11.4.2 Time gating

In spectroscopy applications, time-domain techniques can separately measure the absorption and reduced scattering coefficients of tissue. In imaging applications, where the goal is to measure the spatial distribution of tissue heterogeneities, time-domain methods offer the capability of time-gating to select photons that are associated with a time-of-flight (or, equivalently, with a pathlength) in tissue that is within a given range. Usually, the idea is to select the photons that have traveled the shortest paths, thus narrowing the light bundle illustrated in Figure 4(a) in an attempt to improve the spatial resolution [48]. However, the paucity of weakly scattered photons through tissues that are several centimetres thick poses serious limitations to the use of short time gates. As a result, the increase in spatial resolution afforded by time-gating with respect to CW data does not usually exceed a factor of 2 for a sample thickness of 4 cm and optical properties typical of tissue [49]. An alternative approach to using a fixed-interval time window is to select a variable-interval time window to detect a fixed percentage of the total transmitted photons [50]. Using this variable amplitude of the time window as an imaging parameter, Benaron and Stevenson obtained images of a rat that identified organs such as the heart, liver and spleen [50]. It has also been proposed to use as image parameters the photons collected during various consecutive time windows [51]. This has the advantage that different time windows are affected differently by boundary conditions and geometrical factors, so that one can identify the optimal time window according to the specific requirements of each application.

11.5 Frequency-domain techniques

Steady-state measurements are inadequate to determine the optical parameters of turbid media in the multiple scattering regime. Steady-state measurements only provide the amount of light that has been attenuated after emerging from the tissue. As seen in the previous sections, this amount of light depends on several processes, including absorption, scattering and the index of refraction of the medium. We need additional measurements to determine the optical parameters with measurement at a point. One possibility is to measure the time a photon employs to travel from the light source to the detector, as discussed in Section 11.4. When averaged on many photons, the time course can provide the necessary information to separate the contribution to the light transmitted arising from different processes in the medium. In the simple case of a homogeneous, uniform scattering and absorbing medium we need to measure only the average time it takes the photons to travel from the source to the detector. There are several techniques that have been employed to measure this average time. In general, they fall into two major categories: the time-domain techniques and the frequency-domain techniques.

In this section we discuss the frequency-domain methods [52,13–15]. The major difference between the frequency-domain and the time-domain methods is in the technique used to perform the actual measurements rather than in substantial physical differences. Both for the time-domain and the frequency-domain method
we need some sort of modulation of the source intensity, with harmonic content in the frequency-range that is affected by the light transport through the medium. In the frequency-domain method, the light is generally modulated sinusoidally in the frequency range between 50 MHz and 1–2 GHz. This range is necessary to produce appreciable changes in the characteristic modulation of the light as it travels throughout the medium. In the frequency-domain approach, the various harmonics of the light modulation are isolated electronically [53]. Each component is characterized by an amplitude and phase term referred to the amplitude and the phase of the source.

This richer information content with respect to steady state spectroscopy, where the intensity is the only measurable parameter, permits the determination of the optical parameters of the medium.

In principle it is possible to obtain information about the optical parameters of a multiple scattering medium without recurring to time-resolved techniques [44]. For example, the accurate measurements of how the light attenuates as a function of the distance from the light source at the surface of a tissue could be used to determine the optical parameters of the medium. In fact, the light distribution depends both on the scattering and absorption coefficient. Also the angular dependence of the light emitted by the surface depends on these parameters. However, the mathematical relationship of these parameters renders the separation of scattering and absorption problematic and a very high signal-to-noise ratio is needed to separate the contribution of scattering from absorption. For this reason, the most common approach to separately measure scattering and absorption coefficients is based on time-resolved methods.

From the theoretical point of view, there is no basic difference between the frequency-domain and the time-domain. A Fourier transformation will change the expression from one domain into the other. However, there are some important practical differences. In the time domain, the method of the correlated single photon counting is capable of tagging every photon detected with the time of arrival after the laser pulse. However, to process this signal, the detector must separately measure each photon. This is relatively simple to achieve at low photon fluxes but impossible at high fluxes. In a typical experiment, of the order of millions photons per second are collected by the detector fiber and even the faster detectors and counting electronics are unable to resolve them individually. Instead, the frequency-domain method is essentially an analog system. The photons detected are converted in the detector photocurrent. Only the average phase and amplitude modulation is measured. There is no need to separate the contribution of each photon. This technical difference provides much better linearity at high counting rate and much faster measurements for the frequency-domain method.

In the frequency domain technique the light source is modulated sinusoidally at a frequency \( f \) in the 50–2000 MHz range depending on the particular implementation [53]. There are two alternative methods for modulating the light intensity. For light sources such as light-emitting diodes (LEDs) or solid-state diode lasers, the current injected into the device is directly modulated [54,55]. Since the diode characteristics (light-emitted versus input current) is highly nonlinear, the light intensity contains the fundamental frequency of modulation and several harmonics.
This is not a problem since the detection system measures each harmonic separately. This is also true for the other type of light sources used in frequency-domain instrumentation, namely mode-locked lasers that produce a very narrow light pulse at high repetition rate. In this case, the source contains a wide range of harmonics that can be filtered and processed individually by the detection electronics [56]. Whatever is the source or method used to modulate the light, the key feature of frequency-domain technology is the modulation of the intensity of the light source. If the intensity is sinusoidally modulated at a frequency $f$, one can define three parameters: the average intensity ($I_{dc}$ or dc intensity), the amplitude of the intensity oscillations ($I_{ac}$ or ac amplitude), and the phase of the wave ($\phi$) with respect to some arbitrary phase, generally the phase of the electronic driving circuit. These parameters, which completely describe and characterize the sinusoidally modulated wave, are the quantities of interest in frequency-domain spectroscopy. On the basis of these definitions, the intensity modulated signal can be written as

$$I = I_{dc} + I_{ac}\cos(\Phi - \omega t)$$

(14)

where $\omega$ is the angular modulation frequency ($\omega = 2\pi f$ with $f$ modulation frequency). The ratio ($I_{ac}/I_{dc}$) is called modulation ($m$). In some applications, phase and modulation are the only parameters of interest.

In a typical experiment, the modulated light source is coupled to an optical fiber which is then put in contact with the tissue of interest. A second optical fiber collects the light at the tissue surface after it has traveled some distance and then is processed by the detection electronics. In most instruments, there are several light sources emitting at different wavelengths and several fibers to collect the light at different points on the tissue surface.

Optical detectors employed in frequency-domain spectroscopy include photomultiplier tubes (PMTs), avalanche photodiodes (APDs), and CCD cameras with image intensifiers. In all cases, the signal at a given frequency is not measured directly. Rather the detector gain is modulated at a frequency that is slightly different from the frequency of the light source. This process, called heterodyning, results in the creation of additional frequencies, in particular at the sum and difference between the frequency of the source and the frequency used to modulate the detector gain. Generally it is the difference frequency that is used. It is possible to show that this difference frequency contains the same phase and amplitude information as the original high frequency, but it appears in a frequency range that is very easy to measure, digitize and process with modern electronic components [56]. Figure 5 shows a typical frequency-domain instrument.

When working in the frequency-domain, it is useful to think of light propagating in a turbid medium in terms of photon density waves. These waves of light intensity modulated at high frequency travel at constant velocity in the medium. Photon density waves display some of the optical phenomena associated with waves such as reflection, refraction and diffraction. The velocity of the propagation of the photon density waves and their attenuation in the turbid medium depend on the medium optical parameters and on the light modulation frequency. In particular, the photon density $U_{FD}(t)$ of the wave at a detector point at the distance $r$ from
the source is given by Equation (15), which is valid in the diffusion regime in an infinite and homogeneous medium [57]:

$$U_{TD}(r, t) = \frac{S}{4\pi v D r} \left[ U_{ac} e^{-r \sqrt{\mu_a/D}} + U_{ac} e^{-kr} e^{i\phi_0} \right]$$

(15)

where $\omega$ is the angular modulation frequency, $r$ is the source–detector separation, $v$ is the speed of light in the medium, $D$ is the diffusion coefficient which is defined as $1/3(\mu_a + \mu'_s)$ with $\mu'_s$ as the reduced scattering coefficient, $\mu_a$ is the absorption coefficient, $k = [(\nu \mu_a - i\omega)/(\nu D)]^{-\frac{1}{2}}$, $\phi_0$ is the phase of the source and $S$ its strength. Equation (15) is the frequency-domain equivalent of Equation (13) in the time domain. From Equation (15), the phase and the amplitude of the wave can be calculated taking the argument (for the phase) and the magnitude (for the amplitude) of Equation (15). In particular, Equation (15) leads to the following expressions:

$$\Phi = r \sqrt{\frac{\mu_a}{2D}} \left( \sqrt{1 + x^2} - 1 \right)^{\frac{1}{2}}$$

(16)

$$\ln(r U_{DC}) = r \left( -\sqrt{\frac{\mu_a}{D}} \right) + \ln \left( \frac{S}{4\pi v D} \right)$$

(17)
\[
\ln(rU_{AC}) = r \left( -\frac{\mu_a}{\sqrt{2D}} \left( \sqrt{1 + x^2} + 1 \right)^\frac{1}{4} + \ln \left( \frac{SA}{4\pi vD} \right) \right)
\]

(18)

where

\[
x = \frac{\omega}{v\mu_a}
\]

(19)

\[
P = \frac{1}{3(\mu_a + \mu_s')}
\]

(20)

What is important is that from the phase \(\Phi\) and amplitude \(U_{AC}\) of the wave it is possible to extract the reduced scattering and absorption coefficients of the medium. In the next section we discuss how to measure accurately the value of the phase and attenuation of the photon density wave.

11.5.1 Spectroscopy: the multi-distance method

One of the major problems encountered in the practical application of the measurement of the absorption and scattering parameters of turbid media using the front of the photon density wave is that the so-called “source terms”, i.e., the phase and the amplitude of the source are not known exactly. In addition, even if they were known exactly, the description of the light transport in the multiple scattering medium is well described by the diffusion approximation only far from the source. We have shown that the extrapolation of the photon density wave phase measured far from the source back to the source can give values of the phase that are unphysical. This apparent contradiction is due to the approximation used to describe the propagation of the photon density wave. However, after traveling for a distance of about 7–8 mm from the source, the propagation of the photon density wave is well described by the diffusion approximation [58]. Since at those larger distances, the photon density wave travels at constant velocity and attenuates like an exponential divided by the distance, if we use the slope of the phase \((S_\Phi)\) as a function of distance and the slope of the logarithm of the amplitude (DC or AC) multiplied by the distance \((S_{dc} \text{ and } S_{ac}, \text{ respectively})\), we obtain three straight lines (see Equations (16)–(18)). From each one of a pair of slopes, we can extract the values of the absorption and scattering coefficients in the region in which the photon density wave is propagating according to the following expressions [59]:

using DC, Phase: \[
\mu_a = -\frac{\omega}{2v} \frac{S_{dc}}{S_\Phi} \left( \frac{S_{\Phi}^2}{S_{dc}^2} + 1 \right)^{-\frac{1}{2}}
\]

(21)

using Ac, Phase: \[
\mu_a = \frac{\omega}{2v} \left( \frac{S_\Phi}{S_{ac}} - \frac{S_{ac}}{S_\Phi} \right)
\]

(22)
using DC, AC: \( \mu_a = \frac{\omega S_{dc}}{2\nu S_{ac}} \left(\frac{S_{ac}^2}{S_{dc}^2} - 1\right)^{-\frac{1}{2}} \) \hspace{1cm} (23)

using DC, Phase or using DC, AC: \( \mu'_s = \frac{S_{dc}^2}{3\mu_a} - \mu_a \) \hspace{1cm} (24)

using AC, Phase: \( \mu'_s = \frac{S_{ac}^2 - S_{\Phi}^2}{3\mu_a} - \mu_a \) \hspace{1cm} (25)

There are additional practical advantages of the multi-distance method, one of the most important being the relatively independence of the slope measurement on the curvature of the surface. For practical purposes, we have implemented the measurement of the slope of the phase and amplitude by measuring the amplitude and the phase of the photon density wave at four selected distances from the source. A computer algorithm calculates the average scattering and absorption from those slopes. The recovery of \( \mu_a \) and \( \mu'_s \) is very accurate and given the nature of the measurement, i.e., measuring a slope rather than a value, are largely independent of the absorbance of the skin or other local factors [59–61]. Figure 6 shows a typical probe exploiting the multi-distance method. This specific probe was designed to be applied to the forehead. Although the equations we have reported are valid in the infinite medium, similar equations can be written for the semi-infinite medium, which better approximate actual measurement techniques in vivo [45].

The crucial point in the determination of the optical parameters of tissues using the multi-distance method is in how well the actual anatomy realizes the conditions of a semi-infinite medium. We have demonstrated that the effect of the skin absorption is negligible in the multi-distance method, but the effect of large underlying structures, such as bones or fat layers, may strongly modify the modalities of light propagation. We have performed measurements on layered

Figure 6. Light from 16 diode lasers at different wavelength are brought to the tissue surface at several distances from the two detector fibers. The detection algorithm automatically calculates the optical parameters using the multi-distance equations.
structures [62,63] to assess how much the multi-distance method is affected by the different layers. The results are quite complex and cannot be easily generalized, since they strongly depend on the optical properties and thickness of the different layers. However, for reasonable parameters of the intervening layers, the multi-distance method may provide one of the most robust approaches. As a general comment, only the full solution of the inverse problem will provide the correct medium parameters for a non-homogeneous medium. In the absence of a fast and reliable solution of the inverse problem, and for measurements of small variations of the optical parameters of one of the layers, the multi-distance method is one of the less affected by systematic errors due to layered structures. In measuring the optical parameters of muscle it appears that the assumptions of a relatively homogeneous mass are well satisfied.

11.5.2 Spectroscopy: multi-frequency

The equations describing the attenuation of photon density waves as they propagate in a multiple scattering medium show that the attenuation and the phase shift, for a given distance \( r \), depend also on the modulation frequency \( f \) (or, equivalently, the angular modulation frequency \( \omega \)). The measurements of phase shift and the modulation ratio as a function of the frequency provide another method to determine the optical parameters of the medium [64,65]. The signal-to-noise ratio analysis and a comparison between the multi-distance and multi-frequency method has been performed [66]. The conclusion is that the two methods are comparable provided that a relatively wide range of frequencies are measured. However, the electronics needed to implement the multi-frequency method is different. The multi-frequency method has the distinct advantage that all the measurements are performed at the same distance. Therefore, artifacts due to local skin heterogeneity are absent. However, the requirement of wide band detectors and electronics reduces the sensitivity of the measurement. In practice, the current implementation of the multi-frequency method utilizes avalanche photodiode detectors that limit the sensitivity [67]. Also the modulation electronics and the frequency analysis are done differently. As a result of the different electronic components, the current instruments have limited penetration. However, they have better capability to accurately recover the optical parameters of the medium.

11.5.3 Imaging: diffuse optical tomography

Once we realized that one can measure the optical properties of tissue, the next step is to build a map of the properties over a large region of the tissue. The basic idea is that the optical parameters are locally different and that each type of tissue has different optical properties. The major complication is that in regions in which there is a superposition of contributions from different tissues it is not possible, with a single frequency-domain measurement, to distinguish the different tissues. In this case, numerous measurements are performed and then an algorithm of inversion is used to estimate the original map of the optical parameters. As computational
methods have become faster and more precise, several labs have been working on this possibility. Again, the methods used are classified as CW methods, in which steady-state light is used and time-resolved methods, either in the frequency or time domain. The tomography issue will be further discussed in the section on optical mammography.

11.6 Near-infrared assessment of oxygenation and pharmacokinetics

One of the unique capabilities that the near-infrared technique provides is the possibility to continuously monitor physiological parameters such as hemoglobin saturation, total hemoglobin content, and blood flow and oxygen consumption. This is due to the non-invasive character of the determination and of the physical principle of the measurement that allows absolute measurements. In this section we discuss the measurements of physiological parameters, their accuracy and under which assumptions we expect that the values measured are accurate.

One of the most important applications of the near-IR technique is the determination of the concentration of the oxy- and deoxy-hemoglobin in tissue. When these two concentrations are known then other parameter such as tissue hemoglobin oxygen saturation and tissue total hemoglobin content can be determined. We have shown in the previous section that it is possible to determine the absolute absorption coefficient at the source wavelength in a uniform homogeneous medium. In tissue, the absorption value is determined by the sum of the contributions of all substances that absorb at each wavelength. To proceed with the measurement of concentrations, we need to account for the relative contributions of the major absorbors. In practice, only a few chromophores are concentrated enough or their extinction coefficient is large enough to substantially contribute to the absorption in the wavelength range from 650 to about 900 nm where most of the measurements are performed. In this region, the major contributions arise from hemoglobin, water and fat. Hemoglobin has two major forms, oxygenated and deoxygenated. There are also other chromophores that can potentially contribute to the absorption, myoglobin, melanin, cytochrome and other substances [68]. However, their contribution in a normal tissue is less than a few percent and we will not discuss their detection in this chapter. Figure 2 shows the spectrum of water, oxy-hemoglobin and deoxy-hemoglobin.

Since in most of the wavelength range considered we have three major contributions to the absorption, we need to determine the absorption coefficient in at least three different bands. However, the contribution of water is less than that of hemoglobin in the wavelength range 700 to 900 nm. In many instruments two wavelengths are used with the purpose to quantify only the hemoglobin and the contribution of water at these wavelengths is estimated and a correction is used. Of course, this method does not provide a measurement of the water content of tissue. A more accurate approach is to determine the absorption at many wavelengths. An interesting approach has been developed in Tromberg’s laboratory that uses a
combination of frequency-domain and cw technique to effectively expand the number of wavelengths [69]. In this ingenious approach a CW light source is used in addition to the frequency-modulated light source. The absolute absorption coefficient is determined using the frequency-modulated light and a continuous spectrum is measured as provided by the CW white light source. Then an algorithm is used to scale the CW spectrum to match the absolute absorption at these wavelengths that were measured by the frequency-domain method. This methodology allows also the determination of the scattering coefficient at many wavelengths and therefore to measure the wavelength dependence of the scattering coefficient.

The use of near-infrared spectrophotometry (NIRS) to measure hemodynamics and oxygenation in the brain [70,71], the skeletal muscle, muscle flaps [72,73] and individual organs [74] has increased. Measurements on the skeletal muscle have been performed in sports medicine [75], in patients with myopathies [76–78], heart failure [79–81] and peripheral vascular disease (PVD) [82–88]. NIRS has also been used during surgery [89] and in intensive care medicine [90,91]. NIRS is based on the principle of light attenuation and has the unique ability to non-invasively measure at a depth of several centimetres tissue oxygenation and hemodynamics at the level of arterioles, capillaries and venules [92]. Real time measurements (6 Hz), good spatial resolution (5 mm), bedside feasibility, high reproducibility and inexpensiveness could make this method a valuable tool in a clinical setting.

Most of the NIRS-instruments measure at one discrete location and the result is considered to be representative for the entire region of tissue under investigation. However, hemodynamics and oxygenation in the muscle shows a considerable spatial distribution at rest [93–95] and during exercise [96].

11.6.1 Absolute vs. relative measurements

One of the distinctive advantages of frequency-domain near-infrared methodology is the possibility to obtain absolute measurements of concentrations in highly scattering media. The principles of the method are presented in the previous sections. In a uniform and semi-infinite medium, the recovery of the optical parameters can be done using the diffusion approximation of the Boltzmann transport equation. Under this assumption, the method is accurate, in the sense that, with no other prior knowledge, we can obtain the absolute value of the absorption coefficient. If the extinction coefficient is known, we can calculate the concentration of the absorbing substance. To this level, the method is only based on physical principles and it does not require validation. Only when the basic assumption of uniformity and extension is not satisfied do we need to be concerned about the accuracy of the method. Of course, biological tissue is neither homogeneous nor extended. Therefore, in each case we need to re-evaluate our assumption to determine the limit of accuracy of the method. One important consideration is the length scale of the intrinsic heterogeneity of tissues. At the micron level, tissues are composed of cells and sub-cellular components. Those are the elements that give rise to the elementary process of absorption and emission. However, photons travel very fast and visit a relatively large volume of the tissue
(in the multiple scattering regime). Their absorption and scattering will depend on some sort of spatial averaging of the intrinsic microscopic heterogeneity. Depending on the local properties of the tissue it is estimated that averaging occurs at least over volumes of several millimetre. Using this type of reasoning we can model the tissue assigning average optical properties corresponding to relatively large structures. For example, when investigating muscles, we divide the tissue into two or more layers, corresponding to the skin, fat layer and muscle. Another important modeling consideration applies to the macro and microvasculature. The large blood vessels appear completely opaque and only the microvasculature contributes to the absorption. Therefore, the accuracy of the determination of the optical parameters depend on how accurate is the modeling rather than on the accuracy of the near-IR instrument per se.

A different kind of approximation is used when only changes in the optical parameters are measured. In this case, the accuracy in the baseline determination is less crucial and the changes in optical parameters can be determined more accurately than the absolute values.

For tissue measurements, the model used should be validated and the accuracy in the determination of the optical parameters should be assessed in every individual situation.

### 11.6.2 Arterial oxygenation

To separate the contributions of the different vascular compartments, we need some method to distinguish the arterial, venous and tissue blood contribution. The arterial compartment can be distinguished from its characteristic pulsatility. This principle has been largely exploited in the common pulse oxymeter. We describe here a similar approach based on absolute measurement of the oxy- and deoxy-hemoglobin. In fact, one of the possible artifacts of the pulse oxymeter is that it needs a calibration and also an estimate of the differential pathlength factor $B$ defined in Equation (6). Since these parameters can be directly measured by the frequency-domain method on a case by case basis, the measurement of the concentration of oxy- and deoxy-hemoglobin is absolute. However, the total absorption at any given wavelength is due to the contribution of all vascular compartments, not only the arterial blood. The basic idea is to measure only the changes of oxy- and deoxy-hemoglobin associated with the pulse. Since these changes are presumably due only to the addition of arterial blood, the corresponding saturation is related to the saturation of the arterial component. An instrument that implements this principle was constructed [97]. This particular instrument measures the arterial oxygenation in every tissue that has the pulse signal, including the brain. The value of the arterial saturation is compared with the saturation measured by a commercial pulse oxymeter applied to the finger of the same subject.

### 11.6.3 Venous oxygenation

To distinguish the venous compartment from the tissue and arterial compartment we perform a simple manipulation of the circulation. When a venous occlusion is
applied, for example to the calf of a subject, the muscle blood efflux is stopped. As a result there is an accumulation of blood in the muscle. The saturation of this blood is much lower than the arterial saturation and also lower than that of the tissue compartment. Typical accumulation of the blood in a muscle of a subject is shown in Figure 7. The accumulation proceeds through different steps, which also involve the plasticity of the blood vessels. However, when the occlusion is released, the blood rapidly leaves the muscle and the total hemoglobin returns to the baseline level.

We applied venous occlusion to provoke changes in hemodynamic and oxygenation parameters. The venous occlusion method, in comparison to ischemia tests, enables one to measure venous oxygen saturation (SvO₂), hemoglobin flow (HF) and oxygen consumption (VO₂) simultaneously. Moreover, it is less traumatizing to the tissue and can be easily repeated. The calculation of VO₂ by venous occlusion has been validated against an invasive technique [98] with both the venous and arterial occlusion methods providing similar results [99]. The measurements of SvO₂ [100] and BF [101] have been validated against other methods.

For the venous occlusion measurements, a particular distribution of sources and detectors was used to cover a relatively large area of the tissue under study. An area of 18.5 × 6 cm² (Figure 8) is covered by the sensor and permits for simultaneous

![Figure 7](image_url)

**Figure 7.** The green line shows the pressure used to producing the venous occlusion. As the occlusion is applied, blood accumulates until a maximum value is achieved. Upon release of the pressure, the oxy- and deoxy-hemoglobin concentration returns to the base value. Note the difference between the top panel (normal subject) and the bottom panel (subject with peripheral vascular disease).
measurements at 22 locations. The four detector fibers are equidistantly located along the centerline of the sensor. The source fibers are arranged at three different distances from the corresponding detector fibers. In the center of the sensor the source–detector distances are 2.4 and 3.5 cm. The inner group of detector and source fibers form three symmetrical parallelograms, which provide a multi-distance approach to determine absolute values for absorption and scattering from which the DPF can be calculated [102]. The distance between a detector and the other corresponding sources is 3.0 cm. Maps over the entire region covered by the sensor were generated at a sample rate of 6 Hz.

The sensor was placed and secured with an elastic band along the lateral side of the calf (lateral gastrocnemius muscle) and a pneumatic cuff was wrapped around the subject’s thigh. Venous occlusion was induced (within 2 s) by inflating the pneumatic cuff to a pressure of 60 mmHg. Venous occlusion was maintained for 60 s for the initial two, three or four venous occlusions and for 180 s long for the
Table 1. Mean values ± SD of the hemodynamic and oxygenation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Healthy subjects</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of legs</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>6.67 ± 3.36</td>
<td>6.85 ± 4.57</td>
<td>ns</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>4.04 ± 1.13</td>
<td>2.36 ± 1.79</td>
<td>0.0002</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>10.71 ± 3.99</td>
<td>9.21 ± 6.33</td>
<td>ns</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>8.70 ± 5.04</td>
<td>9.80 ± 4.55</td>
<td>ns</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>9.87 ± 2.82</td>
<td>6.38 ± 3.07</td>
<td>0.0003</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>18.57 ± 6.27</td>
<td>16.18 ± 7.32</td>
<td>ns</td>
</tr>
<tr>
<td>HF (( \mu \text{mol}(100 \text{ml})^{-1} )) min⁻¹</td>
<td>1.34 ± 0.97</td>
<td>1.44 ± 1.17</td>
<td>ns</td>
</tr>
<tr>
<td>BF (ml(100 g)⁻¹ mm⁻¹)</td>
<td>0.63 ± 0.29</td>
<td>0.62 ± 0.50</td>
<td>ns</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>0.033 ± 0.015</td>
<td>0.022 ± 0.020</td>
<td>0.003</td>
</tr>
<tr>
<td>SV ( \text{O}_2 ) (%)</td>
<td>65.2 ± 13.5</td>
<td>80.8 ± 4.51</td>
<td>0.000004</td>
</tr>
</tbody>
</table>

\(^a\text{ns} = \text{not significant}\)

Absolute values for absorption and scattering and the DPF were calculated for the three center regions according to the geometry of the sensor (Figure 8) and the frequency-domain equations [41,102]. By combining the attenuation changes with the DPF data [12] quantitative values for relative changes in \( \Delta \text{O}_2 \text{Hb} \) and \( \Delta \text{HHb} \) in all 22 locations were obtained. The global mean values for the oxygenation and hemodynamic parameters for both groups, the patients and the healthy subjects, are shown in Table 1. There were significant differences between patients and healthy subjects for \( \Delta \text{HHb} \), \( \text{VO}_2 \), and \( \text{SvO}_2 \), while HF and BF were nearly the same. In the patients we found a decrease in \( \Delta \text{O}_2 \text{Hb} \) during the later part of the 180 s venous occlusion in 44.7% of the measured locations, which is much greater than in healthy subjects (15.3%). Regional mean values for the hemodynamic and oxygenation parameters for the patients are given in Table 2. There were significant differences between the proximal region on the one hand and the two intermediate and distal regions on the other hand. However, the proximal–distal differences were much less pronounced in patients than in healthy subjects (Table 3).

Table 2. Regional mean values ± SD of the hemodynamic and oxygenation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proximal</th>
<th>Intermediate I</th>
<th>Intermediate II</th>
<th>Distal</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>8.40 ± 5.36</td>
<td>6.05 ± 3.74</td>
<td>5.98 ± 3.07</td>
<td>6.54 ± 3.67</td>
<td>ns</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>4.90 ± 1.81</td>
<td>3.74 ± 1.47</td>
<td>3.75 ± 1.23</td>
<td>3.87 ± 1.62</td>
<td>b</td>
</tr>
<tr>
<td>( \Delta[Hb] ) (( \mu \text{mol L}^{-1} ))</td>
<td>13.30 ± 6.41</td>
<td>9.80 ± 4.64</td>
<td>9.73 ± 3.77</td>
<td>10.41 ± 4.63</td>
<td>b</td>
</tr>
<tr>
<td>HF in [( \mu \text{mol} \text{(100 ml)}^{-1} \text{min}^{-1} )]</td>
<td>1.70 ± 1.18</td>
<td>1.21 ± 0.86</td>
<td>1.26 ± 0.65</td>
<td>1.26 ± 0.70</td>
<td>ns</td>
</tr>
<tr>
<td>BF in [ml(100 g)⁻¹ mm⁻¹]</td>
<td>0.78 ± 0.46</td>
<td>0.56 ± 0.37</td>
<td>0.60 ± 0.31</td>
<td>0.59 ± 0.31</td>
<td>ns</td>
</tr>
<tr>
<td>( \text{VO}_2 ) in [ml(100 g)⁻¹ mm⁻¹]</td>
<td>0.042 ± 0.028</td>
<td>0.030 ± 0.017</td>
<td>0.032 ± 0.014</td>
<td>0.029 ± 0.018</td>
<td>b</td>
</tr>
<tr>
<td>SV ( \text{O}_2 ) (%)</td>
<td>64.2 ± 14.8</td>
<td>66.2 ± 15.8</td>
<td>64.2 ± 17.2</td>
<td>66.0 ± 16.9</td>
<td>ns</td>
</tr>
</tbody>
</table>

\(^a\text{ns} = \text{not significant}; ^b P < 0.05\)

Final venous occlusion. At the end of each venous occlusion the pneumatic cuff pressure was quickly released. The pressure curve was recorded with a digital manometer. Consecutive venous occlusions were separated by 2 min rest periods.
Table 3. Proximal–distal mean differences for the parameters in patients and healthy subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔΔO₂Hb (µmol L⁻¹)</td>
<td>1.86 ± 4.18</td>
<td>2.92 ± 2.96ᵃ</td>
</tr>
<tr>
<td>ΔΔHHb (µmol L⁻¹)</td>
<td>1.03 ± 1.76ᵇ</td>
<td>1.21 ± 0.65ᵃ</td>
</tr>
<tr>
<td>ΔΔtHb (µmol L⁻¹)</td>
<td>2.89 ± 5.20ᵇ</td>
<td>4.13 ± 3.18ᵃ</td>
</tr>
<tr>
<td>ΔΔtHb (µmol L⁻¹)</td>
<td>1.06 ± 6.26ᵇ</td>
<td>3.76 ± 4.54ᵃ</td>
</tr>
<tr>
<td>ΔΔO₂Hb (µmol L⁻¹)</td>
<td>1.36 ± 6.02ᵇ</td>
<td>2.30 ± 2.05ᵃ</td>
</tr>
<tr>
<td>ΔΔtHb (180 s) (µmol L⁻¹)</td>
<td>2.42 ± 9.38ᵇ</td>
<td>6.05 ± 3.65ᵃ</td>
</tr>
<tr>
<td>HF in [µmol (100 ml)⁻¹ min⁻¹]</td>
<td>0.44 ± 0.99</td>
<td>0.70 ± 0.60ᵃ</td>
</tr>
<tr>
<td>BF in [ml (100 g)⁻¹ min⁻¹]</td>
<td>0.18 ± 0.44</td>
<td>0.29 ± 0.26ᵃ</td>
</tr>
<tr>
<td>VO₂ in [ml 100 g⁻¹ min⁻¹]</td>
<td>0.013 ± 0.022ᵇ</td>
<td>0.011 ± 0.009ᵃ</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>−1.8 ± 13.7</td>
<td>−3.9 ± 4.7ᵇ</td>
</tr>
</tbody>
</table>

ᵃP < 0.005; ᵇP < 0.05; ns = not significant.

NIRS offers the unique capability of simultaneously measuring hemodynamics and oxygenation in small blood vessels, such as arterioles, capillaries and venules, several centimetres deep in the tissue. This feature makes this method complementary to other diagnostic and monitoring methods. By employing a frequency-domain instrument and a special source–detector geometry mapping of these parameters in the muscle tissue becomes feasible.

11.6.4 Tissue oxygenation

As we mention in several parts of this chapter, the near-infrared method measures the contribution of all the hemoglobin compartments (arterial, venous, and capillary). The separation between the compartments is achieved by the different way the arterial, venous and tissue compartment respond to external manipulation. As we have discussed, the arterial compartment is characterized by the pulse, while the venous compartment can be distinguished using venous occlusion. For determining the contribution of the tissue, the signal is first filtered from the pulse and then the average value is taken, integrating on slow changes occurring during several seconds. In general, unless the venous part is specifically evaluated, the measurement of the optical parameters provides an average value of the tissue and the venous part. The values reported in the literature, unless otherwise stated, refer to the average of these two contributions.

11.6.5 Chromophore concentration measurements

One of the early motivations for the development of the methods of diffuse reflectance was for the determination of the concentration of chromophores for photodynamic therapy. There is vast literature concerning the calculation of the light dose delivered at various tissue depths. For large tissue depths, over one centimetre, the methods we have described in this chapter based on illuminating one point of the tissue and collecting the light at a distant points are the most
accurate. However, this methodology cannot be used for superficial layers. In this case the general methods of diffuse reflectance are used.

11.7 Optical mammography

The human breast is an ideal sample for near-infrared studies because of its accessibility and relatively low optical absorption, which allows for light transmission through the whole breast. Continuous-wave imaging approaches to optical mammography have a long history, being originally proposed in the 1920s [1]. More recent developments (time-resolved instrumentation and theoretical modeling of light propagation in breast tissue), dating back to the late 1980s to early 1990s, have determined a renewed enthusiasm and spurred a new wave of research efforts in optical mammography. The optical detection of breast cancer in the near-infrared is based on the local structural and functional modifications associated with cancer, and not necessarily on optical signatures associated with cancer cells. These modifications arise from cancer-induced effects such as angiogenesis [103,104], alterations to the blood flow and oxygenation [103] and fibroblast proliferation [104], which in turn affect the optical scattering and absorption properties of breast tissue. However, it is not yet demonstrated that these cancer-induced optical perturbations can lead to an effective optical approach to breast cancer detection on the basis of intrinsic contrast. As a result, it has been proposed to introduce extrinsic dyes as optical contrast agents in optical mammography [105–107].

11.7.1 Continuous-wave optical mammography

Transillumination of the breast for diagnostic purposes was first proposed by Cutler in 1929 [1]. This original approach to the optical study of the human breast was soon abandoned because of the poor clinical performance of the method. In the 1970s and 1980s, technical advances led to two optical techniques for breast imaging called diaphanography [108] and lightscanning [109], which brought renewed interest for optical mammography, and even led to commercially available optical imagers. These approaches employed a broad beam of visible and near-infrared CW light that illuminated one side of the breast. On the opposite side of the breast, the examiner visually inspected the light transmission pattern and used a video camera for image recording. Despite some encouraging initial results [110], a number of clinical studies conducted in the late 1980s showed that diaphanography and lightscanning are inferior to X-ray mammography both as a screening and as a clinical tool [111,112]. As a result, medical acceptance of diaphanography and lightscanning has been subdued.

Despite the limited success of diaphanography and lightscanning, continuous-wave approaches have been further refined to develop new, more advanced, and more effective instruments for breast imaging. For example, in the early 1990s, Yamashita and Kaneko developed a two-wavelength CW instrument based on a 2D
raster scan of two optical fibers, one for illumination and one for light collection, on opposite sides of the breast [113]. The effort of solving the inverse imaging problem, to obtain a spatial reconstruction of the breast optical properties, led the Philips Research Laboratories to develop a CW instrument [114] that performs tomographic imaging by combining multiple optical projections [115]. Absorption and scattering cross-sectional images of the breast have also been reported by Jiang et al., who used a finite element method approach to solving the diffusion equation (direct problem) and a regularized Newton iterative method to determine the spatial distribution of the optical properties (inverse problem) [116].

Recently, Barbour and coworkers have developed a CW tomographic imager especially designed to monitor the temporal evolution of the breast optical properties [117]. This latter approach introduces the novel idea that breast cancer may be detected, and possibly discriminated from benign breast lesions, on the basis of its unique temporal dynamics or response to external perturbations. All of these CW instruments of the latest generation have produced encouraging preliminary data on human subjects.

11.7.2 Time-domain optical mammography

The rich information content of time-domain data and the wide range of possibilities offered for data analysis have prompted the development of several time-domain instruments for optical mammography. Some of these instruments are designed to perform a tomographic reconstruction of the breast optical properties by collecting data either around the pendulous breast [118] or over the planes defined by two glass plates used to slightly compress the breast [107,119]. Other approaches are based on analyzing the temporal distribution of the photons transmitted through the breast by considering different time ranges (time gating) [50,51,120]. The light sources are typically pulsed laser diodes, even if more bulky lasers such as dye lasers and titanium:sapphire lasers have been used to collect spectral data [120].

11.7.3 Frequency-domain optical mammography

A number of frequency-domain approaches for the spectroscopic and imaging study of the breast have recently been developed [67,121–128]. Some of these studies focus on understanding the optical contrast provided by tumors, and the optical properties of breast tissue in general [67,124], or aim at developing new approaches to optical tomography of the breast [123,126]. Frequency-domain optical approaches to breast imaging include a planar compression geometry [121,122,125], a circular array of source and detector optical fibers around the pendulous breast [127], or the use of optically matching fluids where the breast is immersed [126]. Carl Zeiss (Oberkochen, Germany) [121] and Siemens AG, Medical Engineering (Erlangen, Germany) [122] have recently designed and clinically tested frequency-domain prototypes for optical mammography. These prototypes have similar design features. Figure 9 shows a block diagram of the
Figure 9. (a) Schematic diagram of the prototype for frequency-domain optical mammography developed by Siemens AG, Medical Engineering [122]. The slightly compressed breast is optically scanned to obtain 2-D projection images at four wavelengths (690, 750, 788, and 856 nm). The optical detector is a photomultiplier tube (PMT).

Siemens prototype. The Zeiss prototype differs in the number of wavelengths (two instead of four) and in the modulation frequency of the laser diode intensity (110 MHz instead of 70 MHz). Figure 10 reports 2D projection images obtained by scanning two collinear optical fibers (one for illumination, one for light collection) placed on the opposite sides of the slightly compressed breast. The total time required to scan the breast is 2–3 min. To generate the mammograms of Figure 10, the frequency-domain optical data have been processed using three algorithms of data analysis, (1) a correction of breast thickness variability and geometrical effects across the image combining amplitude and phase data [129], (2) a second-derivative spatial filter to enhance the display of regions of higher local absorbance [130], and (3) a multi-wavelength data analysis to estimate the oxygenation of detected breast lesions [131]. In particular, the mammograms of Figure 10 report a color-coded representation of hypoxic areas that are superimposed to gray-level representations of regions of negative second-derivative (i.e. local maxima in optical absorbance). As in X-ray mammography, the breast is imaged in two projections, namely craniocaudal (cc) and oblique (ob). Figure 10 reports the craniocaudal (right-hand panel) and oblique (left-hand panel) views of the left breast of a patient with a 3-cm invasive ductal carcinoma. The cancer is readily identified...
in the optical mammograms, and corresponds to a low value of oxygenation. A preliminary analysis, based on the criterion that an optical mammogram is positive if it shows a region of abnormal absorbance in both the craniocaudal and oblique views, has lead to a sensitivity (fraction of cancerous breasts successfully detected) of 72% and a specificity (fraction of non-cancerous breasts correctly evaluated as negative) of 52% on a clinical population of 131 patients [132]. This result is consistent with the sensitivity of 73% obtained on 69 patients with the Zeiss prototype [133].

11.8 Near-infrared imaging of the brain

There is a great interest in applying the near-infrared method to detect and characterize aspects of brain circulation. Firstly, circulation problems in the brain are responsible for a number of diseases, including strokes. Secondly, during activation of the brain activity, there is a local change of the concentration of the oxy- and deoxy-hemoglobin due to regional changes in blood flow.

Non-invasive optical studies of the brain can be broadly classified as either “structural” or “functional”. Structural information is mainly used to detect tumors or hematomas. Gopinath et al., in 1993 [134], applied a continuous wave near-infrared probe (wavelength of 760 nm) to the frontal, parietal, occipital, parasagittal, and suboccipital regions of the skull to detect intracranial hematomas. Functional information derives from slow and fast optical signals, observed during brain stimulation. Several investigators have performed functional measurements on the motor cortex during motor stimulation [135–138], on the visual cortex during visual stimulation [139–142], and on the frontal region during mental work
and on the monitoring of cerebral hemodynamics during sleep [145, 146]. All these studies found a significant correlation between the optical signals and cerebral activity.

Brain activity is associated with changes in optical parameters of the tissue, namely the absorption and the reduced scattering coefficients. It is coupled to changes in regional blood flow, blood oxygenation, and metabolism. Hemoglobin and cytochrome-c-oxidase are the only biological compounds in the brain to exhibit variable absorption of near-infrared (NIR) light in response to changes in oxygen variability. NIRS determines changes in cerebral tissue oxygenation and blood volume by measuring fluctuations in concentration of oxy-hemoglobin, deoxy-hemoglobin and reduction–oxidation state of cellular mitochondrial cytochrome aa3 [5, 147–149].

The transillumination of the adult brain, using safe illumination power levels, does not appear to be feasible. In a reflection geometry, the average photon penetration depth in a homogeneous tissue having an absorption coefficient \( \mu_a \) and a reduced scattering coefficient \( \mu'_s \) is given by \( 0.5[r/(3\mu_a\mu'_s)^0.5]^{0.5} \), where \( r \) is the distance between the optodes [150]. For a typical tissue \( \mu_a = 0.1 \text{ cm}^{-1}, \mu'_s = 8 \text{ cm}^{-1} \), \( r = 4 \text{ cm} \), and the average optical penetration is about 0.8 cm. This optical penetration depth, which can be increased by increasing \( r \) at the expense of signal-to-noise, indicates that optical methods are limited to probing the outer brain cortex. The optical penetration depth can be significantly affected by the presence of a layered structure such as the one present in the head (skin/scalp-skull-cerebrospinal fluid (CSF)-cerebral tunics-brain). In particular, it has been suggested that the clear layer of CSF may cause a light channeling effect that could reduce (but not prevent) the optical penetration into the brain cortex [151]. While the presence of CSF may have an effect on light propagation in the brain, the light channeling effect in the head is much less significant than the one caused by a transparent layer sandwiched between two scattering materials using a phantom [62]. Additional studies [152] indicated that the presence of CSF layer improves the sensitivity of NIRS signal to absorption changes in the adult brain.

Near-infrared spectroscopy offers the advantage of performing transcranial measurements of changes in cerebral hemodynamics and oxygenation. It is a non-invasive, non-ionizing, portable, bedside method, which can provide real-time measurements of these changes. These characteristics make NIRS the ideal tool to study physiological and pathological processes of the brain, concerning adults and infants, in settings ranging from research and diagnostic laboratories to intensive care units and operating rooms [143, 153–155].

Generally, measurement of scattering and absorption coefficients of a particular area of the body can be done non-invasively by placing a light source (emitting light in the near-infrared range—between 700 and 1300 nm) and a detector on two points on the surface of the skin, at a distance of a few cm. Most tissues in the human body are highly scattering.

Although head tissues also absorb light, the absorption coefficient is estimated to be much smaller than the scattering coefficient: the typical value of \( \mu_a \) in animal tissues is of the order of 0.1 cm\(^{-1} \) [156]. These basic optical properties of head tissues indicate that the Boltzmann’s transport equation for photons inside the head
can be solved in the diffusion approximation [13,42,157] and that the propagation of photons through the tissue can be described as a diffusion process [27]. This has several consequences of practical importance.

The individual photon trajectories from the source to the detector are highly variable and they make up an extended volume. Modifications in the optical properties occurring within this volume influence the light intensity and the time-of-flight and the size of the volume determines the spatial resolution of the technique (on the order of one cm). Due to the diffusive nature of light propagation in tissues, only photons traveling deeply into the medium are likely to reach the detector. The volume explored by a source–detector pair located on the surface of the medium (head) is a curved spindle with an average depth of about \( \frac{1}{2} \) the distance between the source and the detector [52]. Therefore, it is possible to study properties of relatively deep structures (such as the cortex) by placing a source and a detector on the surface of the head. The number of photons reaching the detector (i.e., the attenuation) decreases more than exponentially with the source–detector distance (Equation (17)), whereas the phase delay of the photon density wave is linear with distance (Equation (16)). With adult head tissues, the large attenuation of the light determined by the source–detector distance limits the maximum useful source–detector distance to less than 5–6 cm, and effectively limits the penetration of the technique to less than 1–2 cm from the surface.

11.8.1 CW

As seen in previous sections, in general CW methods do not allow the full determination of the optical parameters of the medium. However, they can be used to detect relative changes. In connection to brain studies, the CW measurements are the most common and they have been extensively used to monitor blood flow, the formation of hematomas and in general to detect brain activity [144]. The reason for using such instrumentation is the low cost, the portability and the possibility to monitor parts of the brain for extended period of times. The limitation is that changes in optical parameters cannot be obtained in absolute terms and that it is difficult to separate changes occurring at superficial layers from effects occurring at the cortex.

In the current clinical practice in the USA, the only commercially available instrument using NIR light for the non-invasive functional study of the brain is the INVOS 3100 systems (Somanetics, Troy MI). Several reports question the validity of the information on brain oxygenation that these instruments provide, as compared with the other near-infrared oximetre used in research [158–160].

11.8.2 Time domain

Time domain studies have also been used to measure the brain optical parameters and to monitor brain oxygenation in infants. One of the first successful monitoring of brain oxygenation was obtained using time-resolved methods by Benaron’s group [50]. However, time-resolved methods are in general less sensitive than
frequency-domain methods and are also slower. Therefore, most current studies in brain circulation dynamics are performed using frequency-domain instrumentation.

11.8.3 Frequency domain

This section presents relevant examples in two areas of brain circulation research, namely for the assessment of cardiovascular damage in subjects affected by sleep apnea and for the measurements of brain activity following visual stimulation.

Obstructive sleep apnea syndrome (OSAS) has increasingly been recognized as a cause of poor health. In the middle-aged work force, 2% of women and 4% of men meet the minimal diagnostic criteria for the sleep apnea syndrome (apnea/hypopnea score of 5 or higher and daytime hypersomnolence) [161]. In the last two years the national media alone has variously alerted the public on the deleterious effects of OSAS. OSAS is described as a potentially lethal disease because the resulting hypoxia and hypoxemia lead to functional and structural circulation deficits. Altered quality of life, daytime sleepiness, neuropsychological dysfunction and cognitive deficits have been associated with OSAS as well as cardiovascular disease, including systemic and pulmonary hypertension, arrhythmias and ischemic heart disease [162–166]. Since the brain is very sensitive to hypoxia, it has been suggested that the cerebrovascular morbidity is the result of the chronic, cumulative effects of hypoxia and hypoxemia caused by sleep apnea. Cerebrovascular accidents, ranging from transient ischemic attacks to fatal strokes, are closely associated with this syndrome [167,168].

Polysomnography, the standard multi-instrument overnight recordings, can detect sleep apneas and their degree of severity [169,170]. The use of pulse oximetry determines the arterial saturation (SaO₂) and concomitant electroencephalography determines the sleep stage. However, these data do not provide the clinician with the information on cerebral oxygenation and hemodynamics, which are the main parameters one wishes to determine.

Currently, using NIRS it has become possible to continuously and non-invasively measure tissue oxygenation and cerebral blood volume with high-time resolution. NIRS measures at the level of arterioles, capillaries and venules [92]. It has been used in sleep research, so far, to identify changes in brain oxygenation and circulation, during sleep states, in healthy newborn infants [171,172] and healthy adult volunteers [145,173]. It has been also employed to detect hemodynamic and metabolic changes in the brain of preterm infants with sleep apnea [174] and adults with OSA [175,176] to assess changes in cerebral oxygenation and blood volume. It is a novel, non-invasive, bedside method that has a promises potential to be a very accurate and comparatively inexpensive diagnostic tool for brain function.

The NIRS parameters, such as oxy-(O₂Hb), deoxy-(HHb), and total hemoglobin (tHb) concentrations, and cerebral tissue hemoglobin oxygen saturation (SO₂) were monitored simultaneously by a frequency-domain, dual channel, two-wavelength (690 and 830 nm) tissue oximeter (OxiplexTS; ISS Inc, Champaign, IL). A near-infrared (NIR) dual sensor probe, having a two-channel configuration, was specially designed for these measurements. The optical source fibers were arranged in pairs, such that each pair contained one fiber connected to a source emitting at
each wavelength. The optical signals detected at the tissue surface were guided to the photomultipliers (one per channel) of the oximeter by optical fiber bundles of 3 mm internal diameter each. The output ends of the paired light source fibers were arranged at increasing distances from the input ends of the detector fiber bundles. The four source-detector distances ranged from 1.98 to 4.08 cm (Figure 6).

We applied NIRS during daytime napping and during voluntary breath holding at functional residual capacity (FRC). Different dynamics of $O_2$Hb, HHb, and tHb concentrations ([O$_2$Hb], [HHb] and [tHb]) and $SO_2$ were detected for control and OSA groups during daytime napping and voluntary hypoxia (Figure 11). We recorded reduced cerebral hemodynamic response during breath holding in OSA subjects (Figure 11d) as compared to control non-snorers (Figure 11b). In the OSA subjects, this smaller increase in $[tHb] = [O_2Hb] + [HHb]$ is proportional to the cerebral blood volume and corresponds to the dilatory ability of the cerebral vasculature. A smaller increase, or even a decrease in $[O_2Hb]$ followed by an increase in $[HHb]$ during breath holding, indicates a reduced change in cerebral blood flow that is insufficient to compensate for arterial blood deoxygenation during hypoxia. Hypoxia-induced changes in $[O_2Hb]$, $[HHb]$ (Figure 11c), $[tHb]$, and $SO_2$ due to sleep apnea were comparable with changes due to breath holding (Figure 11d). These changes were not observed in control subjects (Figure 11a).

Figure 11. Examples of respiratory signals and changes in cerebral tissue $[O_2Hb]$ and $[HHb]$ with respect to baseline levels. Shaded areas correspond to apnea episodes. (a) Control non-snorer (28-years-old) during daytime napping; (b) Control non-snorer (29-years-old) during breath holding; (c) OSA subject (73-years-old) during daytime napping; (d) Same OSA (obstructive sleep apnea) subject during breath holding.
In recent years near-infrared spectroscopy (NIRS) has been proposed as a method to study brain hemodynamics, which is simple and inexpensive compared with such “heavy-duty” methods as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Many studies about applying NIRS to monitor functional cerebral hemodynamics have been reviewed in Ref. [70].

For NIRS measurements we use a two-wavelength (758 and 830 nm) frequency-domain (110 MHz modulation frequency) Oximeter (ISS, Champaign, IL), which has sixteen laser diodes (eight per each wavelength) and two photomultiplier tube detectors. At a wavelength of 758 nm, light absorption by the deoxy-hemoglobin (HHb) substantially exceeds absorption by the oxy-hemoglobin (O$_2$Hb), while at 830 nm the O$_2$Hb absorption prevails over the HHb absorption. The laser diodes operate in a sequential multiplexing mode with 10 ms “on” time per each diode [138,177]. Light emitted by these laser diodes is guided to the tissue through 10 m long multi-mode silica optical fibers. Two 10 m long glass fiber bundles collect the scattered light and bring it to the detectors. The paired (758 and 830 nm wavelength) source fibers are attached to the probe at 8 positions. Together with two detectors, they provide ten bi-wavelength source–detector channels with a source–detector distance of 3 cm. The probe is centered at the measured C3 position according to the International 10–20 System.

Each exercise run consisted of a 30 s pre-exercise epoch, ten 20 s stimulation epochs separated by ten 20 s control epochs, and a 50 s after-exercise epoch. During stimulation epochs subjects performed light palm squeezing with the right hand following the rhythm presented by a sound system.

Figure 12 shows a map of folding average [HHb] and [O$_2$Hb] traces. For the light channels situated above the activated area (left part of Figure 12), the characteristic feature was a significant decrease of [HHb] during the stimulation, which was concurrent with a significant increase of the oxy-hemoglobin concentration. Typically rapid changes in [HHb] and [O$_2$Hb] began 2–3 s after the stimulation onset and continued during the next 7–15 s. The [HHb] then fluctuated near its low level for the rest of the stimulation epoch and the beginning of the resting epoch. During stimulation [O$_2$Hb] either fluctuated at its high level or exhibited a slight decrease. A rapid recovery toward the baseline level begins 4–6 s after the onset of the rest epoch in both [HHb] and [O$_2$Hb]. Such behavior of [HHb] and [O$_2$Hb] in the activated area was qualitatively the same in all six subjects of this study.

No significant decrease in the folding average [HHb] traces concurrent with the significant [O$_2$Hb] increase was observed during stimulations in the light channels outside the activated area. Usually [HHb] in such channels fluctuated without correlation with the paradigm function, in some cases [O$_2$Hb], but without significant [HHb] change.

The decrease of [HHb] concurrent with the increase in [O$_2$Hb] agrees with the results of the previous simultaneous fMRI-NIRS study [178], BOLD signal theory and with the basic knowledge of brain physiology [179]. This indicates that the task-related hemodynamic changes measured by NIRS are not due to artifacts but have intracranial origin.

Brain activity is associated with physiological changes in the optical parameters of the tissue that can be assessed by NIRS. Two major types of signals are reported
in the literature: (a) a slow signal in the range of seconds, mainly due to light absorption related in particular to changes in hemoglobin concentration [70,180]; (b) a fast signal in the range of milliseconds which was suggested to be associated with changes in light scattering due to changes in the refractive index at neuronal membranes. This signal has much smaller amplitude than the slow signal and has been described by only a few authors [139].

11.9 Future directions

Near-infrared spectroscopy and imaging are making rapid inroads in clinical and physiological studies. The intrinsic non-invasive character of the technique, the portability and the relatively low cost makes this methodology very attractive in the clinic and for laboratory studies. The information content of the technique is very large. The temporal characteristics are excellent for studies of fast (in the millisecond range) and slow phenomena [137]. As discussed in this chapter, new methods are being proposed that will allow measuring a continuous range of wavelength, thereby vastly increasing the capability to distinguish different chromophores in tissues and to measure the wavelength dependence of the scattering coefficient. The limitation of the method is in the penetration depth in tissue and in the spatial resolution that can be achieved. Penetration and resolution are limited by the physics of light propagation in tissues. In general there is a relationship between penetration and resolution. Superficial tissue layers can be studied with better resolution than deeper layers.

We have mainly discussed mechanisms of contrast provided by naturally occurring substances. There is a rapid developing area in contrast agents for optical
tissue imaging, both as absorption and as fluorescence specific contrast agents. In the field of imaging, instruments are becoming more sophisticated and with many source detector pairs. In brain imaging, we are going in the direction of a full-head scanner that can detect brain hemodynamics on the entire brain surface. From the computational point of view, reconstruction algorithms have become faster and more accurate. We can foresee that maps of optical parameters will become common. Perhaps the major progress is in the understanding of the physiological signals. Until recently, it was unclear if diseased tissue will provide any form of optical contrast. Today, due to better in vivo studies, improved measuring modalities and better understanding of the physiology it is clear that many physiological conditions and processes can be distinguished and monitored.

11.9.1 Potential clinical applications

The areas of potential clinical applications are numerous and are growing every day. Near-in spectroscopy provides a unique tool for the measurements of physiological parameters of tissues. There are two major fields of application of NIRS in the clinic, to obtain functional maps and to monitor changes. Both areas have vast applications in many clinical situations, including neonatal care, brain surgery, organ viability in transplants, monitoring of brain circulation, aging, sport medicine, cancer detection and many others.

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References


17. S. Prahl (of the Oregon Medical Laser Center, Portland, OR) has tabulated the molar extinction coefficients for oxy-hemoglobin and deoxy-hemoglobin using data from W.B. Gratzer and N. Kollias. These tabulated data are available at http://omlc.org.edu/spectra/hemoglobin/summary.html.


