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### Quantitative and localized spectroscopy for non-invasive bilirubinometry in neonates

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# CHAPTER 1

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## General introduction to the thesis

In this introductory Chapter, we describe the shortcomings of the available optical spectroscopic techniques for the medical application of non-invasive measurements of bilirubin and hemoglobin concentrations in blood. We emphasize the need for a spectroscopic technique that can both *quantitatively* and *locally* measure absorption coefficients in tissue. Since such a technique is not readily available, we propose the development of a new spectroscopic technique called low-coherence spectroscopy (LCS). Furthermore, we introduce the problem of non-invasive bilirubinometry in neonates, which will be the primary application for the development of LCS.

## 1.1 Tissue spectroscopy

Light-tissue interactions are the basis of many experimental and routinely used diagnostic procedures in medicine. One of the most extensively examined interactions of light with tissue involves the absorption of light, since this can provide valuable information on the presence of chromophores (i.e. light absorbing molecules such as bilirubin, hemoglobin and melanin), which can be related to the physiological condition of the tissue and the overall condition of the body. Examples of light absorption based techniques that are frequently used in the practice of medical diagnostics are pulse oximetry [1], transcutaneous bilirubinometry [2] and near-infrared brain monitoring [3]. The existence of these non-invasive techniques substantially reduces the need for alternative, often invasive and time consuming diagnostic procedures and provides a unique insight into otherwise unknown body processes.

Although numerous techniques based on light tissue interactions are available for solving medical diagnostic problems, for some problems the existing techniques are not appropriate. One of these problems is the non-invasive measurement of bilirubin and hemoglobin concentrations in blood, for which the alternative is invasive blood sampling. This section explains the reasons for the shortcomings of existing optical techniques for this purpose.

### 1.1.1 Quantitative spectroscopy

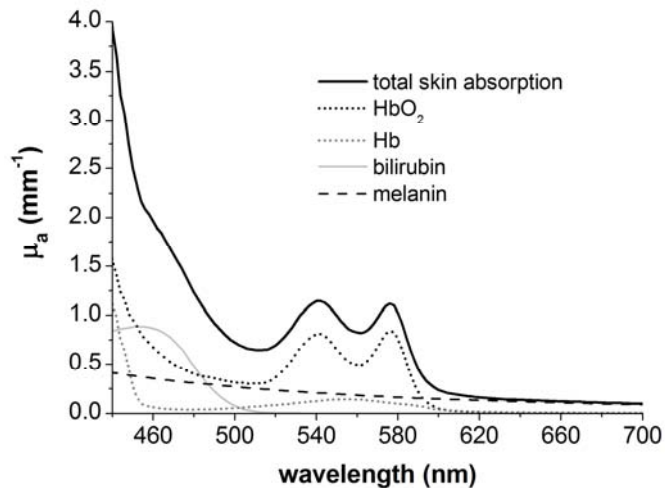
For the measurement of bilirubin and hemoglobin concentrations, *quantification* of tissue absorption is needed, since the amount of tissue absorption is directly related to chromophore concentrations. To quantify absorption, the absorption coefficient is used, for which a detailed definition can be found in Section 1.3. In tissue, the absorption coefficient is wavelength dependent, as has been illustrated for the skin in Figure 1.1.

Wavelength dependent absorption coefficients are generally determined using optical spectroscopy, which measures the wavelength resolved changes in the intensity of light that is reflected by, or transmitted through the tissue. Since the absorption coefficient describes the amount of absorption along a certain photon path (Section 1.3), knowledge of the photon path is required to derive this optical property from the measured spectrum.

The determination of photon paths is one of the main challenges in tissue spectroscopy, since it is difficult to predict the amount of tissue scattering that influences both the length and direction of these paths. Most spectroscopic techniques solve this problem by making model-based assumptions on the photon path length [4-6]. Unfortunately, this introduces an inevitable error in the determination of the absorption coefficient, since model and reality rarely agree completely.

In contrast to making assumptions on the photon path, *measuring* the photon path gives a more exact determination of the absorption coefficient. Several spectroscopic techniques (i.e. time, and phase resolved spectroscopy [7,8]) have been developed for measuring photon paths, but in general these techniques require relatively long photon paths (several mm to cm), which hampers the possibility to measure the absorption coefficient within a small or thin tissue volume such as the microcirculation in the skin for the purpose of non-invasive blood analysis. Although low-coherence interferometry

techniques exist for measuring short photon path lengths ( $< 1$  mm), the application of these techniques is mostly in imaging (i.e. OCT: optical coherence tomography [9]) or other areas [10,11], and does not, or only marginally investigate the possibility for the quantitative and *wavelength resolved* measurement of absorption coefficients.



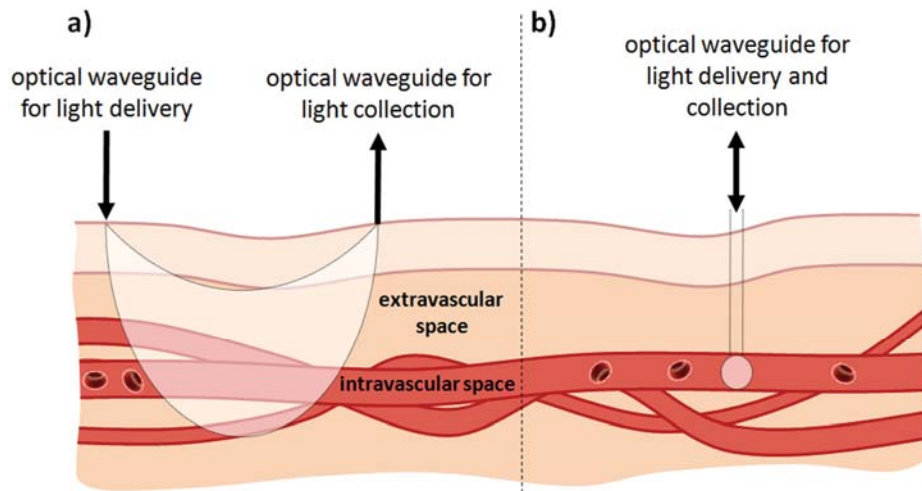
**Figure 1.1** Absorption coefficient spectra of the most important skin chromophores in the visible wavelength range [14], for biologically occurring skin concentrations of hemoglobin (5 g/L, 85% oxygen saturation), bilirubin (70  $\mu$ mol/L) and melanin (0.4%).

### 1.1.2 Localized spectroscopy

Most tissues are optically inhomogeneous media, since they consist of numerous structures with distinct optical properties. As Figure 1.2a schematically illustrates for the case of a 'conventional' spectroscopic measurement on the skin, the light that probes the tissue passes many different tissue structures and layers before it is detected. As a consequence, the optical properties that are derived from the measurement will be (path length weighted) averaged over the probed volume, neglecting the differences in optical properties between the different structures.

For some diagnostic applications, the spatial averaging of optical properties induces an uncertainty in the parameter of interest. This is the case for the determination of bilirubin and hemoglobin concentrations in blood, since the skin cells surrounding the blood vessels in the probing volume (with lower, or absent chromophore concentrations) induce an underestimation of the true concentration. Therefore, besides measuring photon paths, it is also desired to *control* the photon path length in order to confine the probed volume to the tissue structure of interest such as a blood vessel (Figure 1.2b).

Unfortunately, the existing spectroscopic techniques that have the ability to measure the photon path length, lack the ability to precisely control it, due to the random process of tissue scattering [7,8]. However, the previously mentioned low-coherence interferometry based techniques do have the ability to precisely control the photon path lengths of the detected light. Hence, we will investigate the use of low-coherence interferometry for spectroscopic purposes.



**Figure 1.2** Schematic illustration of the skin volume that is probed by a.) 'conventional' spectroscopic techniques, which probe a variety of tissue structures (cell layers, blood vessels etc.); b.) the technique suggested in Section 1.2.1 (LCS), which has the ability to confine the probed volume to a structure of interest, e.g. a blood vessel.

## 1.2 This thesis

### 1.2.1 Low-coherence spectroscopy (LCS)

The objective of this thesis is to develop a method to non-invasively measure bilirubin and hemoglobin concentrations in blood. Therefore, a spectroscopic technique is needed that 1) quantifies the absorption coefficient and 2) measures it locally within a blood vessel in the skin, without the influence of surrounding tissue (Figure 1.2b). Since no existing spectroscopic technique meets with these requirements, a new spectroscopic technique will need to be designed and developed. As mentioned in Section 1.1, low-coherence interferometry offers both precise control over, and knowledge of the photon path. Therefore, we will investigate the possibility to do low-coherence interferometry based spectroscopy, which we will call low-coherence spectroscopy (LCS). This thesis will describe the essential first steps in the design, development and validation of this new technique.

### 1.2.2 Non-invasive bilirubinometry

Within the frame of non-invasive bilirubin and hemoglobin concentration measurements in blood, we specifically focus on non-invasive bilirubin measurements (bilirubinometry) in neonates who are admitted to the neonatal intensive care. Due to a relatively small total blood volume, required measurement frequency and often presence of other clinical complications, these patients have the highest need for a non-invasive alternative to invasive blood sampling.

Although devices based on optical spectroscopy for measuring bilirubin levels in neonates exist for over 30 years, the clinical utility of the technique is limited to a

screening method for hyperbilirubinemia, rather than a replacement for invasive blood sampling. The main reason for the limited clinical value of these devices is the fact that cutaneous bilirubin concentration measurements by spectroscopy (within a volume consisting of both intravascular and extravascular space, see Figure 1.2a) are compared to the gold standard of total serum bilirubin concentration measurements by invasive blood sampling (within a volume of whole blood, i.e. the intravascular space). Since these are two different concentrations, the current spectroscopic devices cannot equal the performances of invasive blood sampling.

If we can use LCS for the measurement of bilirubin concentrations within a single blood vessel (Figure 1.2b), a one to one comparison with total serum bilirubin concentration measurements will be achieved. Therefore, we expect that the use of LCS can significantly improve the clinical value of non-invasive bilirubinometry and lead to less pain and complications for preterm neonates.

### **1.2.3 Outline of the thesis**

This general introduction to the thesis explained the current shortcomings of available optical spectroscopic techniques for the non-invasive measurement of bilirubin and hemoglobin concentrations in blood. In the remaining part of the thesis, we will describe the development of a new spectroscopic technique (LCS) that may aid in solving this problem. Since we primarily aim on using this technique for non-invasive bilirubinometry in neonates, the current status of non-invasive bilirubinometry, its limitations and opportunities are described in Chapter 2.

When designing a new optical technique for measurements on neonatal skin, knowledge of the optical properties of neonatal skin is required. Therefore, we measured the bulk optical properties of neonatal skin in a large patient population, as will be described in Chapter 3. A detailed definition of the optical properties of importance for this thesis will be given in the next section (Section 1.3).

Chapters 4 to 7 describe the development of LCS. First, the possibility of using LCS for the quantitative measurement of absorption coefficient spectra in tissue simulating media will be demonstrated in Chapter 4. Since LCS measures the total attenuation coefficient spectrum, which is the sum of the absorption and scattering coefficient spectra (Section 1.3), knowledge of the contribution of scattering to the LCS signal is important for accurate absorption measurements. Therefore, scattering contributions to the LCS signal will be investigated in Chapter 5. In Chapter 6, the exact control over the size and depth of the measurement volume in LCS will be demonstrated and the first *in vivo* results with this technique will be shown. To further enhance the clinical value of the technique, Chapter 7 addresses possibilities for enhancing the speed and sensitivity of LCS by introducing a new method for spectroscopic detection. Finally, Chapter 8 reflects on the current status of LCS for transcutaneous bilirubinometry and describes the necessary steps for further development of the technique.

## **1.3 Definition of optical properties**

When light interacts with tissue, it will be attenuated by both scattering and absorption processes [12]. Light absorption occurs when photon energy is transferred to light

absorbing molecules, i.e. chromophores. The amount of absorption is quantified by the absorption coefficient  $\mu_a$  in units of reciprocal distance ( $m^{-1}$ ). Note that all wavelength dependent parameters in this thesis will be denoted by a bold-faced character. The reciprocal of the  $\mu_a$  describes the average path length that a photon can travel in a medium before it is absorbed. For a medium with only one type of independently absorbing particles, the  $\mu_a$  is defined by the cross section of the particle  $\pi r^2$  (units:  $m^2$ ) and the absorption efficiency  $Q_a$  (dimensionless) [12]:

$$\mu_a = N_a \cdot \pi r^2 \cdot Q_a \quad (1.1)$$

in which  $N_a$  denotes the number of absorbing particles per units of volume ( $m^{-3}$ ). Figure 1.1 shows the absorption coefficient spectra of the most important skin chromophores in the visible wavelength range. When more than one type of chromophore is present in a medium, the total, or bulk absorption coefficient of the medium is the sum of all the individual chromophore contributions per unit of concentration:  $\mu_{a,skin} = \sum_i (c_i \cdot \mu_{a,i})$ , with  $c_i$  the relative contribution of chromophore  $i$ , assuming independent absorption events.

Light scattering by tissue occurs when photons encounter structures or particles with a higher or lower refractive index than the surrounding medium, such as cell membranes, collagen fibers and mitochondria. In this thesis, only elastic scattering processes are considered, which involve a change in the direction of photon propagation without any loss of photon energy. Similar to the absorption coefficient, tissue scattering is quantified by the scattering coefficient  $\mu_s$ , from which the reciprocal describes the average path length that a photon can travel in a medium before it is scattered. For a medium with only one type of independently scattering particles it is defined as [12]:

$$\mu_s = N_s \cdot \pi r^2 \cdot Q_s \quad (1.2)$$

in which  $N_s$  is the number of scattering particles per units of volume and  $Q_s$  is the scattering efficiency of the particle. Similar to the absorption coefficient, the total, or bulk scattering coefficient of the medium is the sum of the (independent) contributions of all scattering particle types. In tissue, the bulk scattering coefficient has a power dependency on wavelength, since shorter wavelengths have a higher probability to be scattered [12]:

$$\mu_s \propto a \cdot \lambda^{-b} \quad (1.3)$$

in which  $a$  is a scaling factor and  $b$  denotes a parameter defined as the scatter power. Both absorption and scattering processes contribute to the total attenuation of light along a certain path. Hence, the attenuation coefficient  $\mu_t$  is defined as the sum of  $\mu_a$  and  $\mu_s$  [12]:

$$\mu_t = \mu_a + \mu_s \quad (1.4)$$

The probability of a photon to be scattered under a certain angle is described by the scattering phase function  $\mathbf{p}(\theta)$  [12]:

$$\int_0^{4\pi} \mathbf{p}(\Omega) \cdot d\Omega = 2\pi \int_0^\pi \sin(\theta) \mathbf{p}(\theta) \cdot d\theta = 1 \quad (1.5)$$

in which  $d\Omega = d\phi \cdot d\theta$  denotes integration over the solid angle, and  $\theta$  the angle in the plane of incidence.

When the scattering angle  $\theta = \pi$  (or  $180^\circ$ ), light is scattered back in the direction of incidence. In that case, we can define the back scattering coefficient  $\mu_b$ :

$$\mu_b = \mu_s \cdot \mathbf{p}(\theta = \pi) \quad (1.6)$$

Note that the formal definition of  $\mu_b$  by Van der Hulst includes an additional factor  $4\pi$  [12]. For the LCS measurements described in Chapter 5, we define the  $\mu_{b,NA}$  as the  $\mu_b$  integrated over detection numerical aperture (NA) of the system ( $\theta$  from  $\pi-NA$  to  $\pi$ ). Hence, the  $\mu_{b,NA}$  is a measure for the amount of light that is detected after being back scattered by the sample, with a dependence on the properties of both the system and the medium.

Another parameter that is important in LCS, is the group refractive index. The group refractive index  $n_g$  depends on all wavelengths  $\lambda$  within the spectral bandwidth that is regarded, and is defined as:

$$n_g = n + \lambda \frac{\partial n}{\partial \lambda} \quad (1.7)$$

in which  $n$  is the wavelength dependent, or phase refractive index of the medium.

Scattering is isotropic when  $\mathbf{p}(\theta)$  is independent on  $\theta$ , otherwise it is anisotropic. The latter is often the case for tissue, which is why we define the scattering anisotropy  $\mathbf{g}$  as the average of the cosine of the scattering angle [13]:

$$\mathbf{g} = \langle \cos(\theta) \rangle \quad (1.8)$$

Hence,  $\mathbf{g} = 1$  describes the case of purely forward scattering,  $\mathbf{g} = -1$  the case of purely backward scattering, and  $\mathbf{g} = 0$  the case of isotropic scattering.

When photons are scattered many times before they are detected, e.g. for diffuse photon distributions, the scattering anisotropy affects the amount of photons that are captured within the detection NA of the system. As a consequence, the 'effective' attenuation of light by scattering is lower than predicted by  $\mu_s$  in forward scattering media such as tissue ( $0 < \mathbf{g} < 1$ ). Therefore, the reduced scattering coefficient  $\mu_s'$  is defined as [13]:

$$\mu_s' = \mu_s (1 - \mathbf{g}) \quad (1.9)$$

Similarly, the effective attenuation coefficient  $\mu_{\text{eff}}$  becomes [13]:

$$\mu_{\text{eff}} = \sqrt{3 \cdot \mu_a (\mu_a + \mu_s')} \quad (1.10)$$



Equations 1.3 and 1.8 to 1.10 are particularly important for the measurements described in Chapter 3; equations 1.3 to 1.8 are of importance for the measurements described in Chapters 4 to 7.

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