Blood interference in fluorescence spectrum – Experiment, analysis and comparison with intra-operative measurements on brain tumor

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Blood Interference in Fluorescence Spectrum

Experiment, analysis and comparison with intraoperative measurements on brain tumor

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Bachelor Thesis

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Abstract

The optical touch pointer (OTP), a fluorescence spectroscopy based system, assists brain surgeons during guided brain tumor resection in patients with glioblastoma multiforme (GBM). After recording and analyzing the autofluorescence spectrum of the tissue, it is possible to distinguish malignant from healthy brain tissue. A challenge during the intraoperative measurements is the interference of blood. If it gets in contact with the laser pointer, the blood blocks the light transmission to and from the tissue. The purposes of the project were to study and categorize patterns of blood interference and to present possible solutions to avoid signal blocking by blood.

To measure fluorescence and reflection two devices were used respectively, the OTP which has a spectrometer and a blue laser, and the diffused reflection spectroscopy system (DRS) which has a spectrometer and a white light source. Both operate independently from each other and are connected to a fiber optical probe. A similar scenario to the one in the operation theater was simulated in the lab. Fluorescence and diffuse reflection measurements with and without blood were realized on skin and on two different plastic fluorescent standards. The results were analyzed with the aid of MatLAB, and compared with data collected in the hospital during brain tumor resection.

The highest autofluorescence of brain tissue and skin is reached at approximately 506 nm. Although skin and both plastic standards have different optical properties regarding color or rather fluorescence, all of them presented very similar curves when blood on them blocked partially or completely the light transmission. A blood layer of more than 0.1 mm thickness blocks the blue laser light. Blood absorption happens at 541 and 577 nm due to oxy-hemoglobin (HbO_2) in both liquid and dried blood. When the fluorescence spectrum is available but weak, the reflection spectrum contains two dips (traces of HbO_2 at 541 and 577 nm). In brain there were cases in which light absorption occurred additionally at other wavelengths than the absorption peaks of deoxy-hemoglobin (Hb) and HbO_2. Blood interference during the OP can be prevented if the probe rests in a saline solution after every measurement. In this way the fresh blood sticking on the probe dissolves in the solution. For dried or coagulated blood, additional manual cleansing is needed.

Keywords: autofluorescence, blood absorption spectra, diffuse reflection spectroscopy, optical touch pointer, intraoperative optical measurements

Zusammenfassung

Der Optical Touch Pointer (OTP), ein auf Fluoreszenzspektroskopie basierendes System, unterstützt Hirnchirurgen während geführten Entfernungen von Hirntumoren bei Patienten mit Glioblastoma Multiforme (GBM). Nach der Speicherung und Analyse des Autofluoreszenzspektrums des Gewebes kann bösartiges von gutartigem Hirngewebe unterschieden werden. Eine Herausforderung während der intraoperativen Messungen stellt die Blutinterferenz dar. Falls Blut in Kontakt mit dem Laser Pointer kommt, blockiert es die Lichtüberführung zum und vom Gewebe. Die Projektziele bestanden in der Analyse, dem Studium und der Kategorisierung von Blutinterferenzmustern, sowie dem Aufzeigen möglicher Lösungen und Vorschläge zur Verhinderung einer Signalblockade.

Um Fluoreszenz und Reflektion messen zu können, wurden zwei Geräte verwendet. Der OTP besitzt ein Spektrometer und einen Laser mit blauem Licht, während das Diffuse Reflection Spectroscopy System (DRS) aus einem Spektrometer und einer Weisslicht-Quelle besteht. Beide Systeme funktionieren unabhängig voneinander und sind über eine optische Faser verbunden. Ein ähnliches Szenario wie im Operationssaal wurde im Labor nachgestellt. Fluoreszenz und diffuse Reflektionsmessungen mit und ohne Blut wurden auf der Haut, sowie auf zwei verschiedenen Fluoreszenz Kunststoffstandards durchgeführt. Die Ergebnisse wurden einerseits mithilfe von MatLAB analysiert und anderseits mit gesammelten Daten des Spitals aus früheren Entfernungen von Hirntumoren verglichen.

Die höchste Autofluoreszenz sowohl beim Hirntumor als auch bei der Haut wird bei ca. 506 nm erreicht. Obwohl die Haut und beide verwendeten Kunststoffstandards aufgrund ihrer Farbe oder Fluoreszenz unterschiedliche optische Eigenschaften besitzen, weisen alle sehr ähnliche Fluoreszenzkurven auf wenn sie mit Blut verschmiert sind, welches teilweise oder ganz die Lichtübertragung blockiert. Eine Blutschicht von nur 0.1 mm Dicke ist bereits ausreichend um das blaue Laserlicht zu blockieren. Aufgrund von Oxyhämoglobin (HbO_2) findet die Lichtabsorption von Blut bei Wellenlängen von 541 und 577 nm statt, unabhängig davon, ob das Blut flüssig oder trocken ist. Wenn das Fluoreszenzspektrum schwach aber vorhanden ist, zeigt das Reflektionsspektrum zwei tiefen (Spuren von HbO_2 auf 541 und 577 nm). Beim Gehirn traten Fälle auf bei denen Lichtabsorption auf anderer Wellenlänge als auf den Absorptions- Maxima von Deoxyhämoglobin (Hb) und HbO_2 beobachtet werden konnte. Im Labor wurde nachgewiesen, dass Blutinterferenz während der OP verhindert werden kann wenn die Messsonde nach jeder Messung in einer Salinen Lösung liegt. So geht frisches Blut von der Sonde in die Lösung über und trocknet nicht darauf. Falls das Blut bereits geronnen oder trocken ist, ist zusätzliche manuelle Reinigung erforderlich.

Acknowledgment

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1. Introduction

The aim of this project is to measure the autofluorescence of blood and skin with a prototype fluorescence spectroscopy system called optical touch pointer (OTP) developed at the Department of Biomedical Engineering at the Linköping University. The OTP is being clinically evaluated at the Linköping University Hospital to assist brain surgeons during guided brain tumor resection using fluorescence in patients with glioblastoma multiforme (GBM). This is a common and aggressive malignant tumor which looks very similar to the surrounding healthy tissue. Based on fluorescence spectroscopy, the OTP helps to distinguish malignant from healthy brain tissue during the operation by recording the fluorescence spectrum. The fluorescence spectrum is different in the malignant brain tissue due to the photosensitizer which only breaks and accumulates in the tumor. The signal is then analyzed to quantify the fluorescence intensity which might be an indication of the tumor malignancy. A challenge during the intraoperative measurements is the interference of blood which is available in good portions in the surgical site. If blood comes in contact with the laser pointer, it attenuates the excitation and emission of light. If a certain quantity of blood is surpassed, the blood blocks the light transmission to and from the tissue, making analysis of the signal unreliable. This effect is disturbing during the surgery when the source of artifact is of doubt and rinsing the probe with saline does not always succeed. It is thus of importance to study and categorize patterns of blood interference on the recorded fluorescence signals.

In this thesis, measurements are conducted under different controlled circumstances on skin and fluorescence standards with and without blood. The reference standard data are compared with the blood sample data to determine how the reference spectrum is affected if the probe contains blood. The results are analyzed with the aid of MatLAB.

2. Theoretical Background

2.1. Basic Principles of Anatomy and Physiology

2.1.1. Skin

With an approximate area of 2 m^2 and a weight of 10 kg, the skin is the biggest organ in the human body. Some of its functions are protection, sensation, temperature regulation, the production of vitamin D and excretion. The skin is roughly divided in epidermis, dermis and hypodermis (Figure 2.1). The *epidermis* is the superficial part of the skin and acts as a barrier against microorganisms, chemicals, abrasion, UV-light and to prevent water loss. It also produces vitamin D, contains melanin and gives rise to hair, nails and glands. The *dermis* is below the epidermis and is responsible for the flexibility and structural strength of the skin. It also contains many lymph vessels and blood vessels because this is where a great part of the exchange with nutrients, gases and waste products [1] (microcirculation, see section 2.1.2) takes place. Beside collagen, elastic fibers, nerve fibers, oil and sweat glands and hair follicles, the dermis also has melanin. The lowermost skin layer, the *hypodermis*, is mainly used for fat storage (adipose tissue) and has loosely dispersed elastic fibers. It also contains blood vessels, nerve fibers, and small amounts of melanin. Its main function is to avoid or slow heat loss.

The structure of the skin varies along the body; for example the sole of the foot has an up to 2 mm thick layer, while some other parts are hairy and some others thin and hairless. Between the dermis and the epidermis there is a layer called papillary region, which is composed of elastic fibers, connective tissue, collagen and adipose tissue [2].

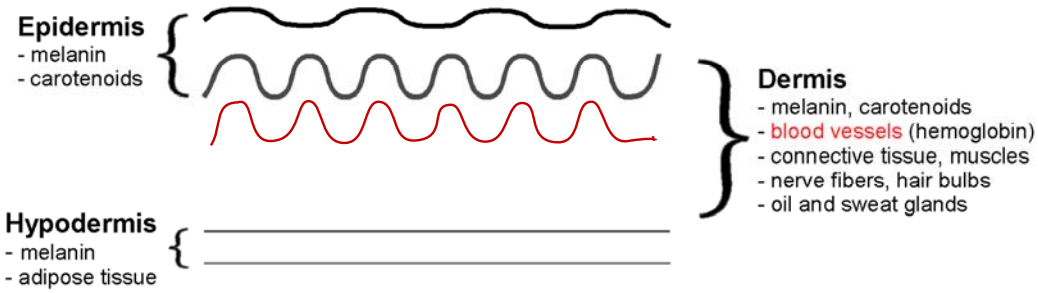


Figure 2.1 Structure and layers of the skin

Melanin, hemoglobin and carotene are the three pigments which contribute to skin color. All of them have a strong light absorption in the UV and blue wavelength. Only *melanin* is produced in the skin by melanocytes, and its color ranges from yellow to reddish-brown to black [3]. The largest amounts of melanin are located in the epidermis, 50-100 µm below the skin surface [4]. Melanin is a polymer that provides us protection from the sun because of its light absorption properties, and its synthesis is stimulated when skin is exposed to sunlight. Its concentration is variable ranging from very low in light Caucasian skin (type I) to very high in black African skin (type VI). The skin types are mentioned according to the Fitzpatrick Skin-Type Chart [5].

The dark red color of oxygenated *hemoglobin* circulating through the microvascular network of the dermis (50-500 µm below skin surface) gives a pinkish hue to skin [4]. This can be clearly seen on Caucasian skin because it contains only small amounts of melanin and is therefore nearly transparent, exposing the color of the hemoglobin underneath it [3].

Carotenes are a large group of molecules usually found in carrots and tomatoes. If we consume carotene-rich products, some carotene molecules accumulate in the epidermis and dermis, while the remaining ones circulate in the blood stream. Their absorption peak in normal skin is at a wavelength of 482 nm. In order to develop a yellowish tint on account of the carotenes, an individual has to have a high intake of carotenoid rich food [6]. Their color is more intense on hand palms and feet soles, as here the epidermis is much thicker than in the rest of the body, and carotenoids accumulate mostly in the epidermis. In this thesis only the absorption values of hemoglobin are relevant for the blood interference analysis in the autofluorescence spectrum of skin within the visible range.

2.1.2. Blood

The circulating blood in the human body is a suspension of blood cells in liquid. The blood cells consist of red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes). Erythrocytes constitute about 45% of the blood volume, while leukocytes and platelets make up less than 1% of it [3]. Blood cells are surrounded by blood plasma which is a solution of water (90%), electrolytes and water-soluble proteins [7]. Plasma takes about 55% of the total blood volume [3]. The function of blood includes formation of clots in order to prevent blood loss, defense against microorganisms, oxygen and nutrients delivery to all body cells, transport of metabolic waste and hormones, maintenance of a normal blood pH of 7.4 and of a body temperature of about 37ºC, and regulation of electrolyte concentration and fluid volume.

Many blood vessels, like arteries and veins, compose the complex circulatory system. Its principal responsibility is to control and regulate the blood flow, so that blood can fulfill the functions mentioned in the paragraph before. Arteries distribute oxygenated blood from the heart through the whole body. On the contrary, veins transport deoxygenated blood from the body to the heart, which will be oxygenated in the lungs thereafter. Blood flows also through venules (Ø ~50 µm), arteriovenous shunts (Ø ~40 µm), arterioles (Ø 20–50 µm), metarterioles (Ø 10–15 µm), and capillary loops (Ø 4–10 µm); they are the smallest vessels and are part of the body's microcirculation [6]. Their number depends on the nutritional needs of a particular tissue. The tasks of the microcirculatory network are to provide every single cell with oxygen and nutrients, as well as to transport metabolic byproducts from the cells, so that they can function properly [6].

The normal erythrocytes are biconcave discs and have a diameter of about 7-8 µm and a thickness of 2 µm [8]. They squeeze slowly through the tiny capillary loops in order to facilitate nutritional exchange at the cellular level. The cytoplasm of the red cells contains hemoglobin (Hb), a protein that is responsible for gas transport. It carries oxygen from the lungs to the body cells and likewise binds CO_2 from the body cells to the lungs. Venous blood contains less than 53% oxy-hemoglobin, in contrast arterial blood contains 90-95% [6]. Figure 2.6 in section 2.5.2 shows the absorption curves of oxy- and deoxy-hemoglobin.

2.2. Light-Tissue Interaction

Light is composed of photons (light units) and is characterized by frequency and wavelength. Visible light has wavelengths ranging from 400 to 700 nm. The lower range outside of it (100–400 nm) is referred to as ultra violet light (UV), and the range between 700 nm–100 µm is called infrared (IR). These two regions are not visible for the human eye.

The *incident light* is swayed and modified by the capacity and inherent structure of the sample (e.g. tissue) to absorb, reflect, transmit or scatter light, as shown in Figure 2.2. Knowing the amount of reflected light and the incident light intensity, the transmittance value can be easily calculated. Since light intensity *I* [cd] is the perceived power of a source emitting light in a certain direction, its value is wavelength dependent. If a sample has a high reflection intensity, it means that its light absorbance is low. On the other side, direct reflection is the difference in the refractive index[a] between air and a medium. Owing to this fact, light can be reflected at the surface into a single outgoing direction when tissue is illuminated rather than being diffusely scattered [10].

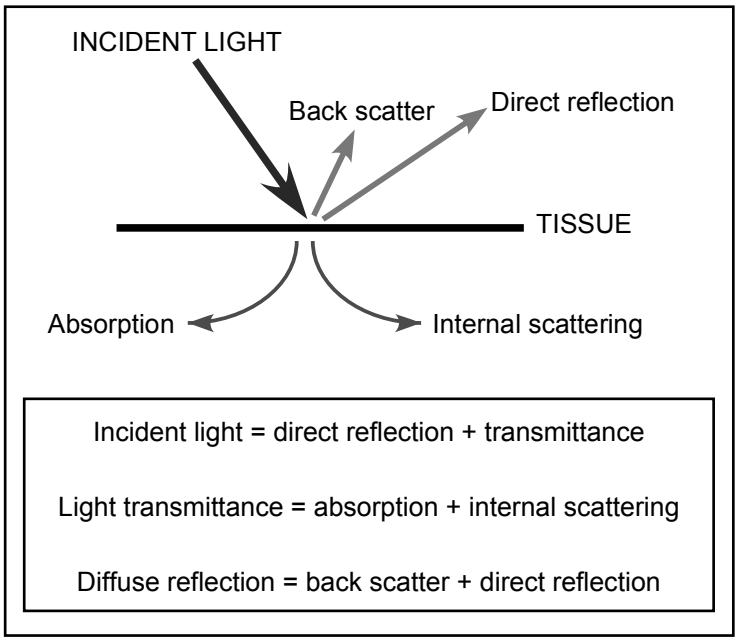


Figure 2.2 Light tissue interaction

The incident light intensity I_o decreases exponentially with the molar absorptivity of the absorber in dependence of the wavelength, $\varepsilon(\lambda)$ [ml/(g·mm)], concentration of the absorbers in the material, C [g/ml], and the light travel distance through the material, l [mm]. This proportionality is stated by the Beer–Lambert law, defined in Eq. 2.1. I represents the recorded remitted intensity. If I is less than I_o, then the sample has absorbed some of the light [9]. However, the Beer–Lambert law is only valid if at least four conditions are fulfilled: light should be monochromatic (one color), the absorbing medium should not scatter light, the incident light should have parallel rays traversing the same length, and if two or more absorbers are present their absorption process should

[a] Measure of the angle at which light is refracted (bent) when passing through a particular material compared to vacuum.

be independent [6]. Despite these limitations, the Beer's law is the basis for spectroscopy measurements.

$$\textbf{Eq. 2.1} \quad I(\lambda) = I_o(\lambda) \cdot e^{-\varepsilon(\lambda)\cdot C \cdot l}$$

The knowledge about the optical properties μ_a, μ_s and g of human blood and tissue provide important information for therapeutic and diagnostic applications in laser medicine and medical diagnostics [8]. In addition, they are dependent on physiological parameters like osmolarity, oxygen saturation, hematocrit, flow conditions, etc. During *light transmittance* two phenomena of scattering or absorption may happen to the photons. Scattering results from a forced change in the light direction, due to the internal structure, differences in refractive index and composition of the tissue (e.g. mitochondria and collagen fibers can scatter light). The scattering coefficient μ_s [cm^{-1}] describes how many times per unit length a photon will change its direction [9]. In a homogeneous medium free from particles no scattering exists. Eq. 2.2 gives the relationship between scattering coefficient and wavelength, where A and b are constants:

$$\textbf{Eq. 2.2} \quad \mu_s = A \cdot \lambda^{-b}$$

In contrast, absorption occurs when the light energy is absorbed by molecules in the tissue, for example by hemoglobin. The absorption coefficient μ_a [cm^{-1}] describes the probability per unit distance for an absorption event to occur and is strongly wavelength dependent [9]. In other words, it indicates how easily a medium can be penetrated and takes values between 0 and 1. The higher the absorption coefficient, the smaller the reflection. As denoted in Eq. 2.3, μ_a is the sum of the product of the extinction coefficient ε [$M^{-1}cm^{-1}$] and the concentration *c* [M] of the absorber. Depending on the molecule, the absorbed energy is partly or completely converted into heat, resulting in a temperature increase in the tissue. In case of fluorophores, part of the absorbed energy is re-emitted in form of fluorescence. Melanins, water, lipids, deoxy- and oxy-hemoglobin are the principal absorbers in the human body [10]. Since the overall absorption in tissue is low in the wavelength region 650-1300 nm, this interval is called tissue optical window [11]. When the absorption is low, light can penetrate deep into the tissue.

$$\textbf{Eq. 2.3} \quad u_a(\lambda) = \sum_k \varepsilon_k(\lambda) \cdot c_k(\lambda)$$

To determine the approximate depth at which light absorption happens, the reversal function of μ_a is calculated (Eq. 2.4). It indicates the depth which is reached by most of the photons. The energy of the photons is wavelength dependent, so that is why light with large wavelengths (~600 nm) penetrate deeper in tissue than light with shorter wavelengths.

$$\textbf{Eq. 2.4} \quad d_a = 1/\mu_a$$

The anisotropy factor g describes the amount of forward direction retained after a single scattering event [12]. Anisotropy means that light will not be scattered with the same probabilities in all directions. g is the mean value of cos (θ), where θ is the angle between the incoming light and the scattered light (Eq. 2.5). If the scattering is equal in all directions (isotropic), g is close to zero. A value of g close to one means that the photons are rather scattered in the forward direction, and a value of g = -1 means total

backscattering [9]. In tissue g has values between 0.7 and 0.9 and the light is mostly scattered in a forward direction [10].

$$g = mean(\cos(\theta)) \qquad \textbf{Eq. 2.5}$$

If light is scattered many times in tissue, light will become diffuse and μ_s and the g factor cannot be separated [10]. Therefore the reduced scattering coefficient μ_s' (Eq. 2.6) is used to determine how far the photons must travel before light can be classified as isotropic. Many small steps with a size of $1/\mu_s$' [cm] describe photon movement. Each step has only one partial deflection angle θ [12]. If scattering occurs before absorption, the value of μ_a will be much smaller than μ_s'.

$$\mu_s' = \mu_s(1 - g) \qquad \textbf{Eq. 2.6}$$

The total attenuation coefficient μ_t [cm^{-1}] is the sum of the absorption coefficient μ_a and the scattering coefficient μ_s, thus μ_t describes light transmittance (Eq. 2.7).

$$\mu_t = \mu_a + \mu_s \qquad \textbf{Eq. 2.7}$$

Diffuse reflection means that the incident light is spread at a number of angles, and it occurs mostly on uneven surfaces. If the reflection is direct (mirror-like, on a high polished flat surface), then the angle of the reflection is the same as the angle of incidence. Backscattering takes place when the light, which entered the tissue initially, goes back to the direction it came from with another angle as a result of scattering in the tissue.

2.3. Fluorescence Spectroscopy

Fluorescence spectroscopy can detect or trace quantities of inorganic, organic, mutagenic, toxic, or carcinogenic fluorophores[b] from a sample [13]. Besides, spectroscopy is a highly sensitive, safe and quick method. Molecules can vibrate and rotate, causing the energy to split into several sub-levels. If the energy of an incoming photon matches the difference between two energy levels in the molecule, the photon can be absorbed [10]. When the molecule releases excess of energy, heat and/or light emission result. The emitted photons have different frequencies which can be analyzed by a spectrometer. This device measures and records the intensity per frequency, wavenumber or wavelength [6]. The emission spectrum is of a lower energy and a longer wavelength than the absorbed light, due to the previous loss of energy. Hence, the emitted light has a different color as the absorbed light. The fluorescence process can be summarized in 3 steps (see Figure 2.3):

1. Excitation: a beam of light stimulates electrons in a fluorophore.
2. Energy loss: until the fluorophore loses some energy, it remains in an unstable transient status, adopting the lowest excited state for a very short time.
3. Emission: The fluorophore goes back to the ground state. The energy excess is released as light and the substance appears luminous ($\lambda_{em} > \lambda_{ex}$).

[b] Molecule capable of fluorescing upon absorbing light energy from an external source

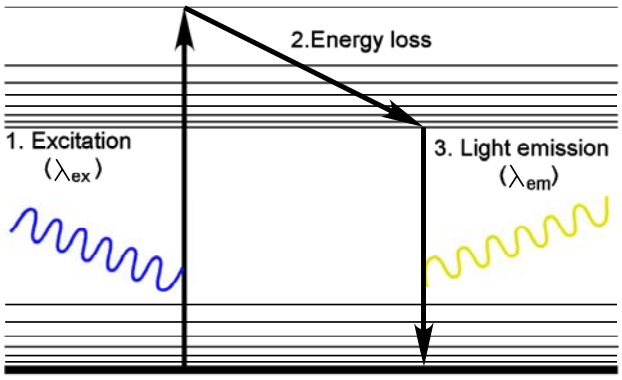


Figure 2.3 Fluorescence process

Almost all fluorophores can undergo the fluorescence process for indefinite times and can thus generate a signal many times. Others turn into instable structures after several illuminations and they can no longer fluoresce [13]. This process is known as photobleaching. Details about it will not be explained in this report.

2.3.1. Fluorescence excitation and emission spectra of fluorophores

As already mentioned before, a fluorophore is a substance capable of emitting light after having absorbed energy. Endogenous fluorophores originate from an organism, tissue or cell, giving rise to autofluorescence. In contrast, exogenous fluorophores have their origin outside the organism and they have to be added to a target molecule in order to obtain fluorescent signals from the molecule, cell or tissue.

Usually, spectroscopic measurements for tissue diagnostics are preformed with a wavelength between the UV and IR spectra [10]. To perform fluorescence spectroscopy on a specific fluorophore, its maximum absorption and emission peak should be considered. Each fluorophore has a specific wavelength at which most of its molecules absorb a maximum of energy. Outside of the energy absorption range, there is no excitation or emission.

2.4. Autofluorescence

Autofluorescence is the fluorescence of a tissue due to its endogenous fluorophores. Normally, it occurs upon excitation with blue or UV light. It can help to recognize the structures of interest (e.g. cancerous tissue from healthy tissue) or it can interfere during detection of other fluorophores. Tissue autofluorescence is usually excited by light in the blue-green region [10].

Most cells contain endogenous fluorophores in their mitochondria and lysosomes. They become excited by UV/blue light of suitable wavelength. The most common fluorophores in the human body are flavin (FAD) and pyridinic (NADH) coenzymes, aromatic amino acids and lipopigments [14]. Normally, the emission spectra of these substances are broad as a result of the interaction between neighboring molecules and the large number of vibration levels [11]. If there is a malignant transformation in the tissue its endogenous fluorophores undergo a change. Also the often increased vascularization in tumors can lead to a lower fluorescence level, since the blood flow can absorb a large amount of light. These modifications can be detected in the intensity and spectral profile

of autofluorescence. Examples of endogenous fluorophores in the human skin and brain are listed in Table 2.1.

Table 2.1 Excitation and emission from endogenous fluorophores [15, 16]

Fluorophore	Tissue			$\lambda_{Excitation}$ [nm]	$\lambda_{Emission}$ [nm]
	Skin	Brain	Blood		
NADH	x	x	x	350	460
FAD	x	x	x	410	510-530
Elastin	x	x	-	420, 460, 360, 425	500, 540, 410, 490
Collagen	x	x	-	325, 333, 370	380, 400, 460
Tryptophan	x	x	x	275	350
Endogenous	x	x	x	260, 400	610, 630, 675
Lipopigments	x	x	-	340 – 395	450, 600
Keratin	x	-	-	370	460

2.4.1. Autofluorescence of the skin

Biological tissues, like the skin, have various endogenous fluorophores such as tryptophan (an aromatic amino acid), NAD (nicotinamide adenine dinucleotide) and structural proteins like elastin and collagen. Their optical properties depend on their actual metabolic status and the environment, resulting on modifications in the distribution and amount of the fluorophores [17]. Hence, autofluorescence can provide information about the physiological and morphological state of cells and tissues in some cases without the need of staining or fixing the samples [14]. According to different studies, peaks of excitation and emission were obtained for skin at 380 nm and 470 nm respectively [15].

2.4.2. Autofluorescence of the blood

According to one study by Masilamani et al. [18], the best blood autofluorescence results are given at an excitation wavelength of 400 nm. Owing to the porphyrin fluorophores, healthy blood shows two weak peaks at 590 nm and 630 nm (Figure 2.4).

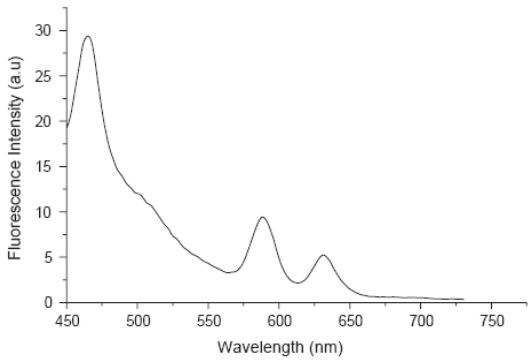


Figure 2.4 Blood autofluorescence of a healthy person [18]

The strong band present at 465 nm is due to acetone and saline water, which were used to extract the blood cells prior to the measurement. A weak band at 495 nm is also observable due to acetone fluorescence. Both bands are seen independent of the instrument parameters for the autofluorescence measurements as well as of the individuals' sex and age [18]. It should be mentioned that the blood autofluorescence spectrum may change in cancer patients by showing peaks due to porphyrin.

2.5. Absorption Spectra

In this section some examples of the absorption spectra of skin and blood are shown. The molar extinction coefficient ε(λ) [$cm^{-1}M^{-1}$] on the y-axis (log) represents how strong the respective chromophore absorbs light at the given wavelengths.

2.5.1. Absorption spectrum of skin

Melanin is a pigment not only in skin, but also in hair and in the iris of the eye. Together with hemoglobin, both are the most dominant chromophores[c] in skin and other tissues. They absorb light in the visible (400 – 700 nm) and UV (100 – 400 nm) regions, but the best absorption takes place within the blue region (450 – 475 nm) [17]. Thus, melanin protects the lower skin layers from the UV-rays emitted by the sun. Lipids are also absorbers, but rather in the infrared region (700-1000 nm). In Figure 2.5 the curves for melanin and water are shown. Since melanin is composed of eumelanin and pheomelanin, both curves are plotted. Water, in contrast, is found in the whole body, intracellular as well as extracellular. It absorbs weakly in the visible wavelength range, but stronger in both UV and in the infrared region [6].The data were obtained from the Oregon Medical Laser Center [19, 20].

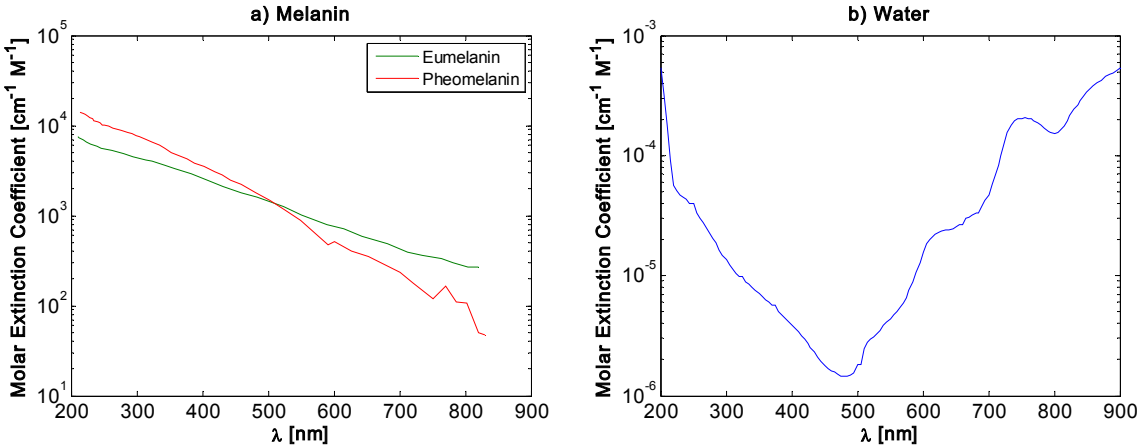


Figure 2.5 Absorption curves for melanin and water

2.5.2. Absorption spectra of blood

One of the most important components of blood is hemoglobin. This pigment transports 65 times more oxygen in the erythrocytes than plasma does, and it can be found in two forms, deoxygenated and oxygenated [10, 12]. The light absorption spectrum varies in these two types due to the different molecular structure of oxygenated hemoglobin.

[c] Pigment. Functional group

With data from the Oregon Medical Laser Center [21], the absorbance curves of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (Hb) were plotted in MatLAB (Figure 2.6): HbO_2 has two maximal absorption peaks at 541 nm and 577 nm within the light visible range while Hb has only one peak at 555 nm within this window.

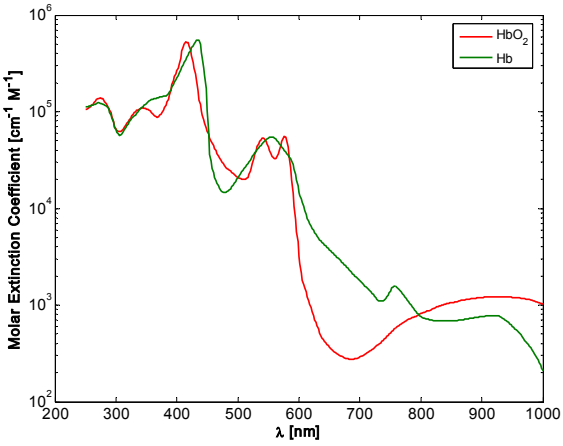


Figure 2.6 Absorption spectra of hemoglobin

3. Materials and Methods

3.1. Devices

In order to measure fluorescence and reflection an optical touch pointer and a reflection spectroscopy system were used. Both devices operate independently from each other. The fiber optical probe has four junctions, two for the light emission and two for the light collection, which are connected to the appropriate devices. Their mode of operation is described in sections 3.1.1 and 3.1.2. It should be mentioned that the terms probe and (laser) pointer are used equivalently.

3.1.1. Optical Touch Pointer

At the Department of Biomedical Engineering (Linköping University), an optical touch pointer (OTP) [22] based on fluorescence spectroscopy has been developed. The OTP measures fluorescence with either a continuous or a pulsed blue laser source with a maximal excitation light at a wavelength of 405 nm. In this project the OTP was deployed to measure the autofluorescence of blood and skin with the pulsed modulation under different conditions and parameters (see also Figure 3.1) :

1. A blue laser with a maximal power of 50 mW.
2. A spectrometer operating between 240-850 nm wavelengths with about 3 nm resolution. It measures the light intensity in arbitrary units [a.u.].
3. A long pass yellow cutoff filter of 450 nm, which is placed in front of the detector slit of the spectrometer, eliminates the back-reflected light at 405 nm.
4. The data acquisition card (DAQ) together with a software developed in LabVIEW®, control the laser effect, pulse generation and its synchronization.
5. On the back part there are two interfaces, one to connect the OTP to the computer and another for the power supply.

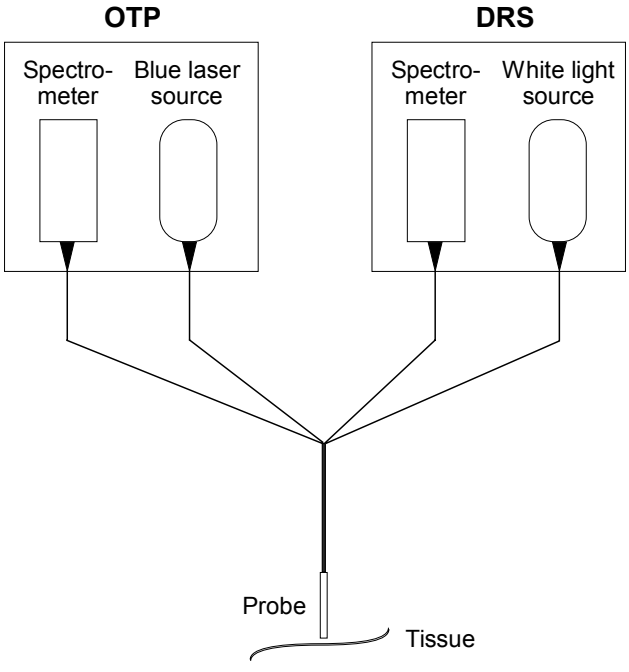


Figure 3.1 Hardware design of OTP [22] and diffuse reflection spectrometer [23]

3.1.2. Diffuse Reflection Spectroscopy (DRS)

A previously developed system based on spectroscopy was used to measure diffuse reflection (DRS) [23]. A white light halogen lamp (AvaLight-HAL-S[d], Avantes BV, Netherlands) was used as the source. The white light was sent to the tissue through an optical fiber and the diffuse reflected light was in turn collected and transmitted to the spectrometer (AvaSpec 2048-2[e], Avantes BV, Netherlands) by another optical fiber (Figure 3.2, Figure 3.3). For each measurement series a calibration measurement was performed on a shielded white reference surface (WS-2, Avantes BV, Netherlands) to normalize the intensity level of the data (see Figure 3.7). The data was collected with a LabVIEW® program. This program also allows the real-time display of data. An interface on the back of the white light source device connects it to the power supply, and another interface behind the spectrometer connects it to the computer.

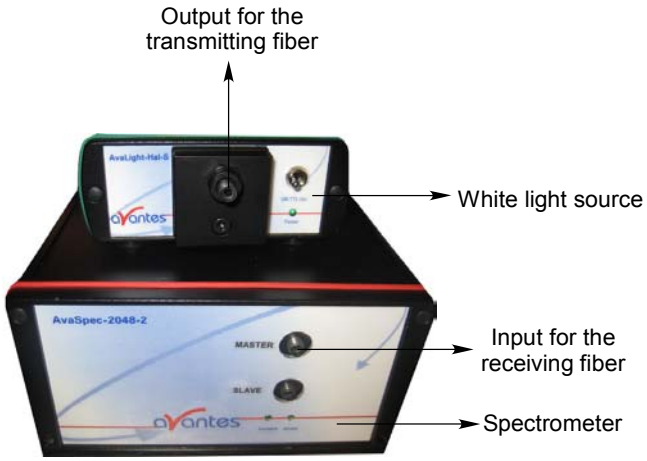


Figure 3.2 Devices for white light transmission and collection

3.1.3. Fiber Optical Probe

To bring the excitation laser light and white light to the tissue in order to measure autofluorescence and reflection respectively, an optical fiber probe as shown in Figure 3.3 was used (l_{probe} = 12 cm, l_{cable} > 4 m, Ø = 2 mm) [22]. This optical fiber probe is composed of four cable bundles with different fiber diameters and number of fibers. Two of the cable junctions coming from the output of both devices and the remaining two are connected to the device inputs.

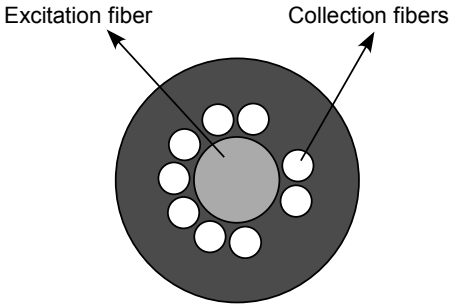


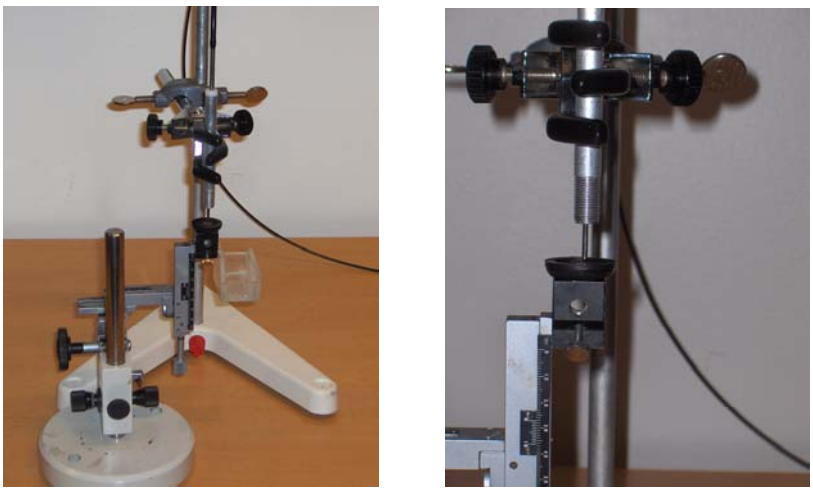
Figure 3.3 Optical fiber probe [22]

[d] Wavelength range: 360-2500nm; Power supply: 24VDC / 1.25A; Optical power 200µm fiber: 0.5mW
[e] Wavelength range: 200-1100nm; Power supply: 12VDC / 350mA; Signal/Noise 200:1

The transmitting fiber in the center ($Ø_{cladding}$ = 640 µm, $Ø_{core}$ = 600 µm) carries excitation light from the blue laser to the probe. There are eleven surrounding fibers ($Ø_{cladding}$ = 240 µm, $Ø_{core}$ = 200 µm). Nine of them collect light from the probe and transfer it back to the spectrometer in OTP. Another one transmits white light to the probe, and the other fiber transmits the diffuse reflected white light to the spectrometer in the diffuse reflection spectroscopy system (these ones are not shown in Figure 3.3).

3.2. Experimental Set Up

Three fluorescent objects were used: two plastic standards A and C, and skin. For a reference measurement a black surface was also employed. The laser pointer was held on a fixed position with the aid of a holder. A metal tube was used to fit the probe and gain more stability, as the laser pointer itself was too thin to be held by the holder alone. A positioner with a scale in mm was the base for plastic standards A and C, and also for a black surface used in one of the measurements (Figure 3.4). For the measurements on skin, the forearm of a volunteer was fixed in a plastic box with a window (Figure 3.6).



a) Laser holder and sample **b) Probe and positioning stage**

Figure 3.4 Expermental setup

3.3. Fluorescent Objects

Two fluorescent plastics, one with a yellow and one with orange color material, as well as skin were used as objects for fluorescence testing when excited with light. Their experimental setup is described below.

3.3.1. Fluorescent standards

For the calibration plastic standards A and C were used. These standards have stable fluorescence properties and a maximum intensity at different wavelengths. Both are depicted on Figure 3.5. Standard A is yellow with its maximal fluorescence peak at 573 nm and standard C instead is orange and has a maximal fluorescence peak at 506 nm.

Standard A has a higher fluorescence and reflection than standard C. To compare the results reference measurements were also calculated on a black surface, where the absorption was almost zero.

Standard A Standard C

Figure 3.5 Fluorescent standards

3.3.2. Skin

Prior to the measurements, the forearm of the test persons (n=3) was fixed in a holder to gain more stability (see Figure 3.6). The optical probe was positioned orthogonally to the forearm, and stayed in contact with the skin without the application of force.

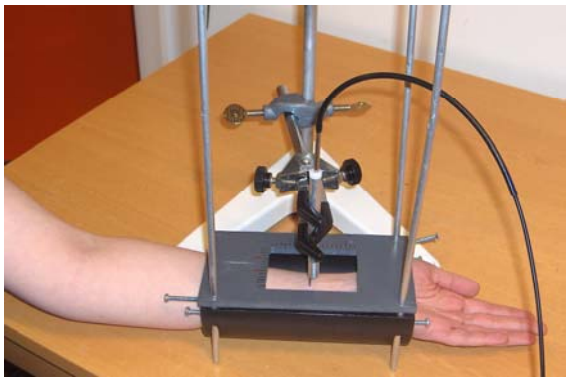


Figure 3.6 Measurement on the skin

3.4. Blood

Blood samples from healthy volunteers (n=3) were taken with the aid of low flow lancets with a puncture depth of 1.8 mm (HTL STREFA S.A. Ozorków, Poland). One of the blood samples was taken with a syringe by a nurse in order to have a bigger blood volume (5 ml) for the experiments. After some drops were placed on the standard reference holder, the optical probe was fixed into a metal stake. Measurements were done with 5 mW as well as with 10 mW laser power at varying distances (d=0, 0.5, 1, 1.5 and 2.5 mm) between the probe and the blood, and both plastic standards to see which one provide better results.

Since this experiment included blood handling, additional hygienic precautions were required. Both lancets and other materials which came in contact with the blood were separately disposed. The plastic references and the optical probe were disinfected with alcohol. Hand washing and disinfection before and after working with the probes were self-evident.

3.5. Measurement Procedure

The software settings allow choosing the number of spectra to be taken before saving the measurement, and to generate the pulses simultaneously with the spectrometer. It is preset that the laser pulse width has to be the same as the integration time of the spectrometer. The spectra are saved and can be analyzed afterwards. There is also an online presentation mode which permits to control the signal quality and make the desired adjustments in the measurement setup before recording the signal. All measurements on skin were performed on the inner forearm. Previous to the measurements with blood, a reference measurement was performed, both with fluorescence and reflection system. The measurements were done in the following order:

1. Fluorescence and reflection reference curves without blood of the two plastic standards A and C, and of skin.
2. Correlation between intensity and power of both plastic standards, of a black reference, and of skin.
3. Fluorescence and reflection curves of the plastic standards and skin with blood. The considered parameters for each series of measurements were:
    a) Different distances between probe and standard (0, 0.5, 1, 1.5, and 2.5 mm. Greater intervals produced no additional relevant results).
    b) Fresh liquid blood, semi-coagulated blood and dried blood. Approximately 10min after the blood was taken it was semi coagulated and viscous. Depending if there were 1 or 3 drops of blood, after 15–20 min the drops were completely dried.
    c) One drop of blood was enough to block the fluorescence and measurements, more drops did not provide additional information.
4. Rinsing the probe with blood in the lab with a saline of NaCl 0.9% (B. Braun Melsungen AG, Melsungen, Germany).

Two points are noteworthy: first of all, measurements should always take place in the same place. There is a significant difference in the autofluorescence intensity if the measurement is realized on the inner forearm or on the back of the hand. Secondly, depending on the skin color, autofluorescence varies considerably. It could be observed that fluorescence of white skins (n=1) of type I reached an intensity maximum of about 780 a.u., and skin of type II (n=1) reached it at 680 nm on the inner forearm, while dark skin (n=1) of type VI had a maximum intensity at approximately 125 a.u. on the same place (see Figure 4.2.e).

For the measurements with blood on skin a couple of blood drops were put on the inner forearm of a test person to see how reflection and fluorescence of blood on skin look like. The laser pointer was either above blood, inside of it or in contact with skin. It was cleaned two times to see if the blood spectra changes and to ensure a reliable result.

3.6. Data Analysis

All the data were analyzed in MatLAB version 7.1.0 (MathWorks™, Inc., Natick, MA, USA). For fluorescence spectroscopy, each presented spectrum is the result of the light spectrum array minus the dark spectrum array. Depending on the measurement, some parameters were additionally calculated. It was not possible to choose the same y-axes scaling for all figures, because of strong deviations between the measured signals. The x-axes of the fluorescence intensity plots show always the range of visible light (450–750 nm). On the contrary, the x-axes of the diffuse reflection plots have a range between 400 and 1000 nm, due to the wider spectrum of white light. Values below 450 nm were filtered, because they were not of interest.

The data spectrum collected by the diffuse reflection spectroscopy system was first normalized (Figure 3.7.b). This was done by dividing the respective spectrum by the calibration spectrum which was collected from a white surface after each measurement series (Figure 3.7.a). When plotting the reflection spectrum on the same graph as the fluorescence spectrum, the reflection spectrum was scaled, so that it was clearly observable on the plots. Its size is scaled with factor 10 on all plots, with exception of plot e in the appendix (Figure 10.2), where it was multiplied by 100.

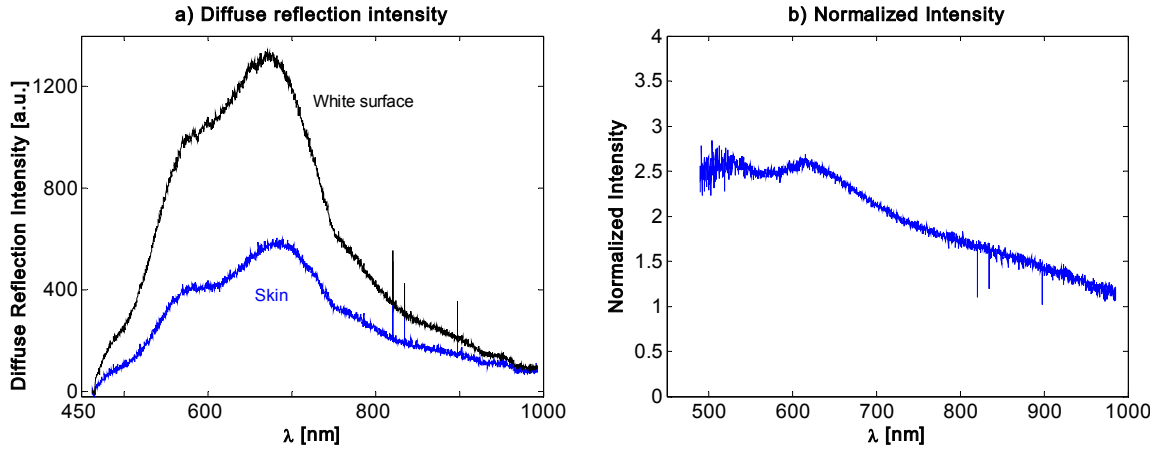


Figure 3.7 Example of a normalized skin diffuse reflection spectrum

4. Results

The parameters of each set of measurements were the same for plastic standards A and C, and for skin to make an objective comparison of the obtained results for the three different objects. Firstly, the relationship between fluorescence intensity and power was plotted and a set of reference measurements on the plain and clean fluorescence objects were performed. The autofluorescence of blood on a black surface was measured to look for any possible autofluorescence. Afterwards, the fluorescence intensity and reflection spectra of the surfaces with blood at different distances from the probe were investigated. Control measurements of layer thickness and dried blood were done to investigate the thickness effect on the blood light absorption pattern. The results were compared with intraoperative in vivo brain fluorescence from the University Hospital Linköping, which was obtained during brain tumor resection. Finally, practical solutions for the probe cleaning process with saline were tested.

4.1. Intensity vs. Power

Two plastic references (standards A and C) as well as the skin of one volunteer were used to measure the autofluorescence against the output power at the laser pointer (see Figure 4.1). The correlation coefficient *r* was calculated to investigate for a possible linearity. Since *r* is bigger than 0.99 in all cases, it means that power and autofluorescence intensity have a linear correlation to each other.

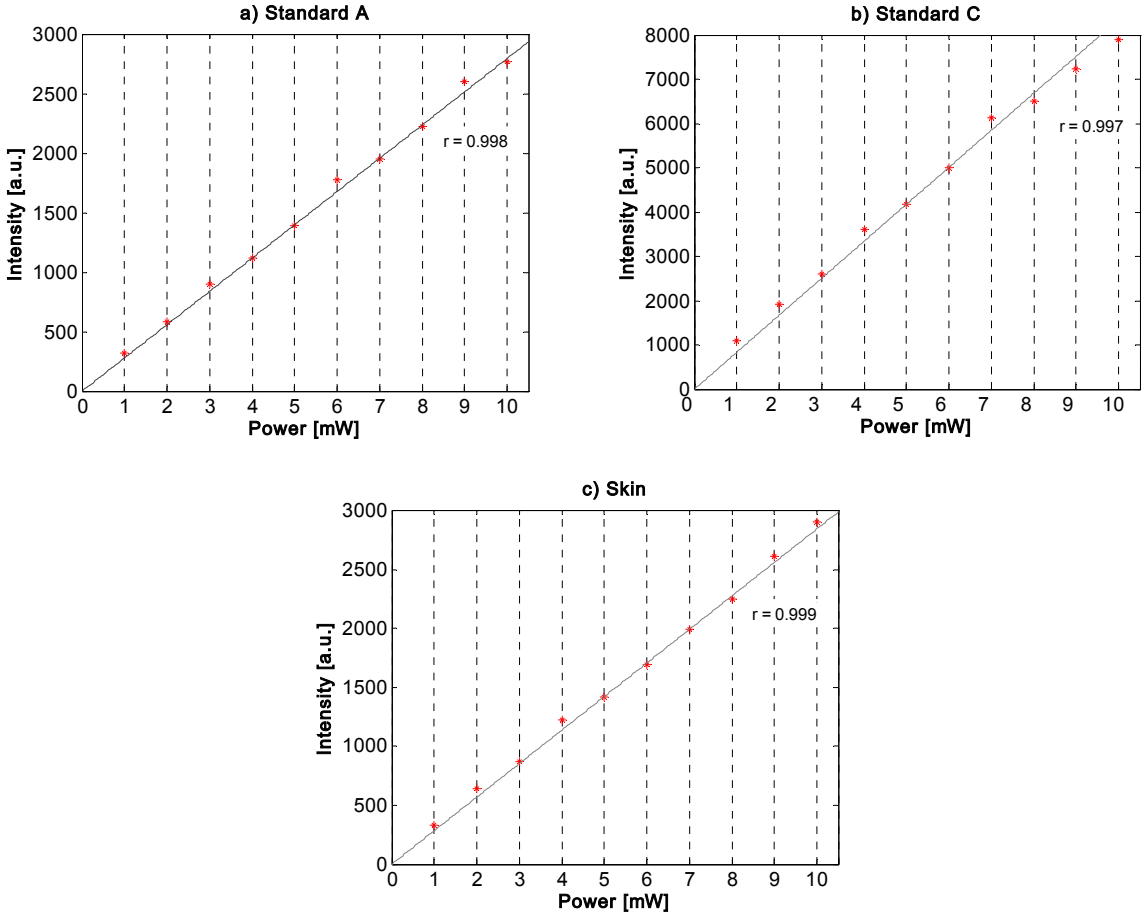


Figure 4.1 Intensity vs. power from the three objects

4.2. Reference Measurements without Blood

For the reference measurements of the fluorescence intensity and the diffuse reflection intensity of both plastic standards (A and C) and skin, a laser power of 10 mW was used. At a distance of d = 1.5mm from standard C, the probe was found to collect a maximal signal. In contrast, standard A and skin have a maximum peak at distance zero when the probe is in contact with the object. To compare the results on skin, the measurements were repeated on three different test persons with skin types I, II and VI.

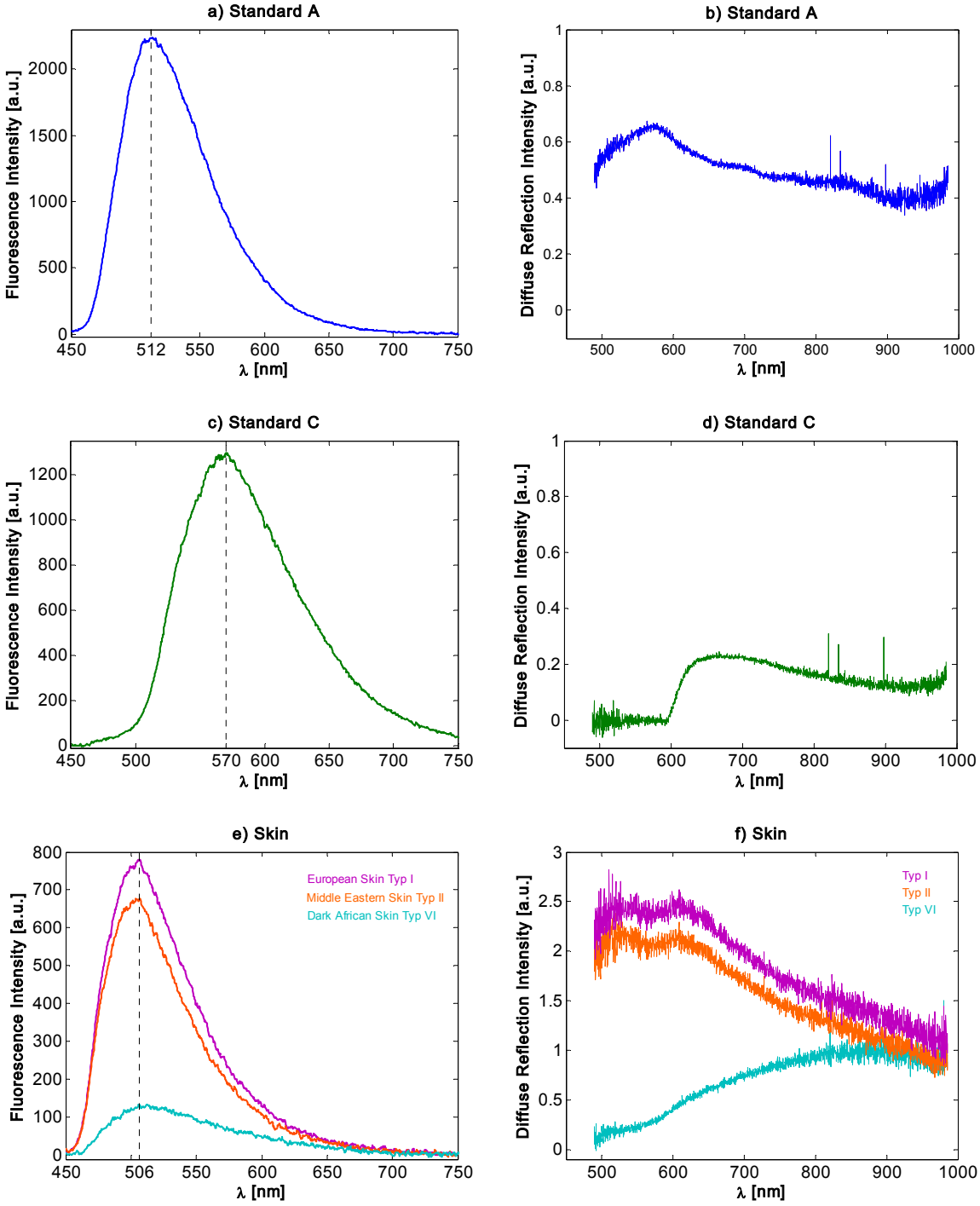


Figure 4.2 Refernce measurements without blood (left) fluorescence and (right) diffuse reflection

According to the measurements realized, standards A and C have a maximum intensity value at 512 and 570 nm respectively and the skin autofluorescence has a maximum at approximately 506 nm, independent of the skin type (Figure 4.2.a, c, e). At the same points the diffuse reflection intensity was measured and the normalized reflection was calculated and plotted (

Figure 4.2 b, d, f). This means that the measured intensity spectrum was divided by the calibration spectrum measured on a white surface (see section 3.6). The reflection peak in

Figure 4.2.b is located in the visible spectrum at a wavelength of about 550-600 nm, where the yellow color actually is, because standard A is yellow. On the other side, the reflection peak in

Figure 4.2.d lies approximately in the range between 620-700 nm, due to the orange-reddish color of standard C. Skin reflection (

Figure 4.2.f) is generally higher in the 500-616 nm range with two dips at about 541 and 577 nm, because of the light reflection of oxy-hemoglobin (see section 2.1.1).

4.3. Blood Autofluorescence

Given that the black surface should not reflect light back, two small drops of blood were placed on a black surface to measure the suspected blood autofluorescence. The highest peak (about 12 a.u.) was reached at a distance of d = 0.5 mm between probe and blood (Figure 4.3). The obtained curve for the blood autofluorescence looks different than the curve plotted by Masilamani et al. (Figure 2.4). The group of Masilamani added acetone and a saline solution to the blood so the fluorophores could separate from the rest of the blood components. Afterwards they measured only the fluorescence of the blood fluorophores (e.g. porphyrins) after extracting them with acetone, whereas in this experiment the autofluorescence of the whole blood was measured. The effect of blood autofluorescence in our measurements is thus supposed to be negligible.

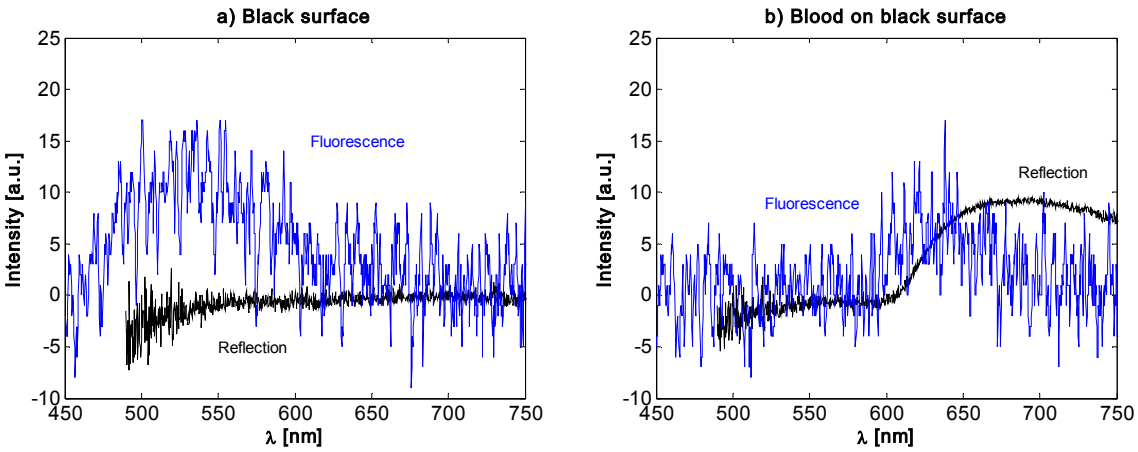


Figure 4.3 Autofluorescence of a black surface with and without blood

4.4. Measurements with Blood

Blood samples were taken and one drop was placed on both plastic standards and on skin (inner forearm). The purpose was to see how strong blood blocks the light and how the fluorescence and the diffuse reflection intensity spectra of the objects change in dependence of the distance between the object and the probe. It was also considered if the blood was liquid or completely dry, and if the probe was in or above the blood. A power of 10 mW was used for the blue laser. Moreover, the absorption peaks of deoxy-hemoglobin (555 nm) and oxy-hemoglobin (541, 577 nm) are also plotted. After about 20 min the blood dried and no other significant measurements could be done thereafter.

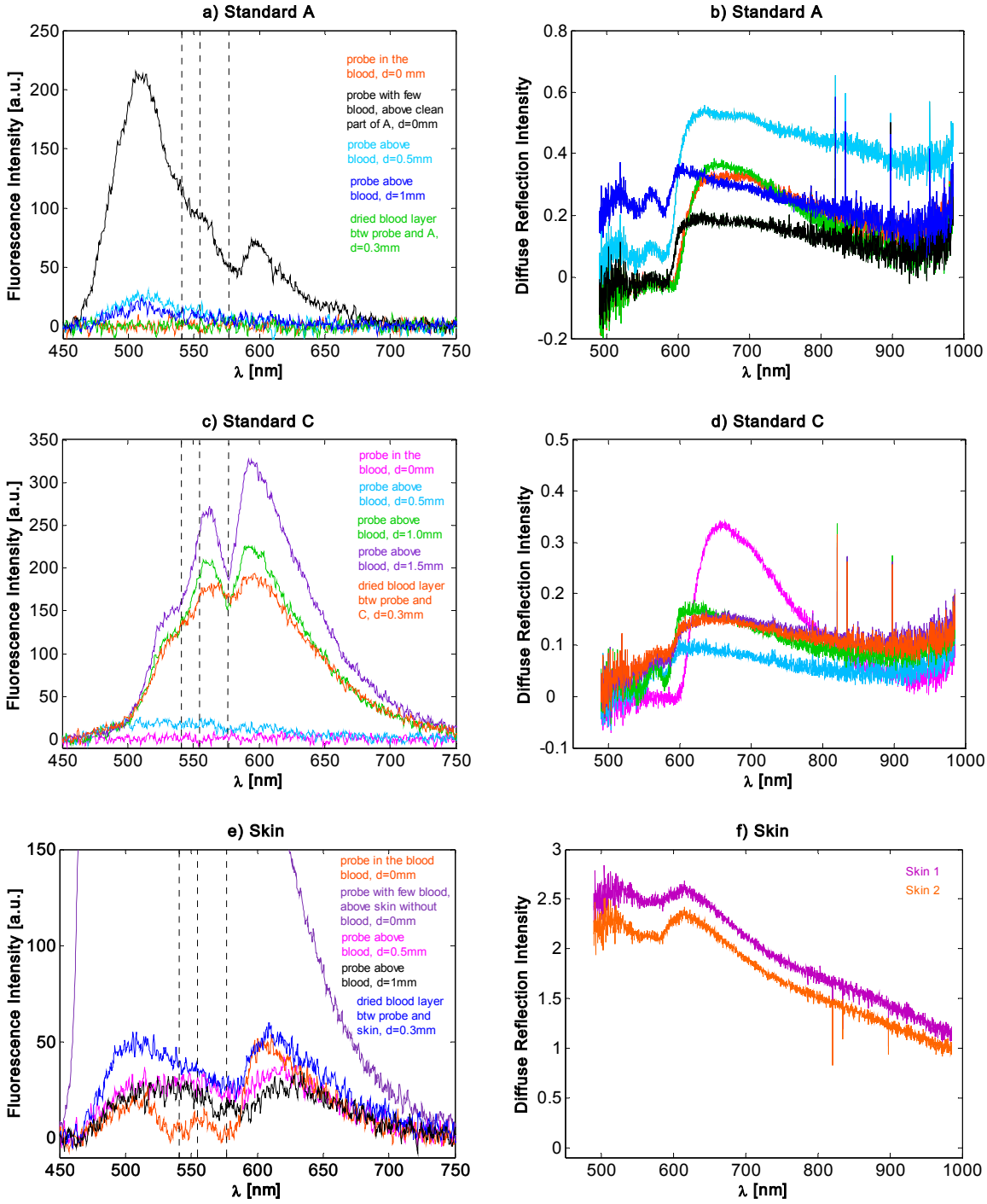


Figure 4.4 Blood measurements on a,b)standard A, c,d) standard C and e,f) skin. Measurements at one point are plotted with the same color.

Comparing the corresponding fluorescence and reflection plots with each other, as illustrated in Figure 4.4, in cases where the fluorescence signal is very weak or blocked (see in Figure 4.4 a-b, c-d, and e-f) the diffuse reflection spectrum is available and shows a peak in the red region. The slope in the red region tail was higher in this case. Where a fluorescence signal is available, the reflection signal is available in the whole visible region with dips at 541 and 577 nm where the oxy-hemoglobin in blood has its peak of absorption. Thus, although the relationship between the fluorescence and the diffuse reflection does not follow an obvious pattern because of the different light source and fiber configuration used, further investigation on this issue might be informative. The white light covers the whole spectra between 450 and 1000 nm, where longer wavelengths have more penetration depth. Therefore the diffuse reflection spectroscopy system has a broader light range and is more sensitive than the fluorescence spectroscopy system which uses a blue laser (405 nm). Individual measurements with the respective fluorescence and reflection spectra on skin are included in the appendix.

4.5. Blood Thickness and Volume

Without the aid of special microscopes capable of measuring micro-order layer thickness, like the scanning electron microscope, it is very difficult to measure the thickness of the blood layer and to exactly reproduce it for further experiments. Since a blood layer of about 0.1 mm (see Table 4.1) is sufficient to block the fluorescence signal, all measurements with a thicker layer show a blocked signal.

In the same way it is complicated to define and adjust the blood volume of one or three drops of blood. Measurements with more than one big blood drop coming out from a syringe and with more than two small drops coming from a punctured finger blocked the signal. It was not relevant to exactly define neither the blood layer thickness nor the blood volume, since the experiments were done under situations resembling the operation theater where blood is available in random volumes.

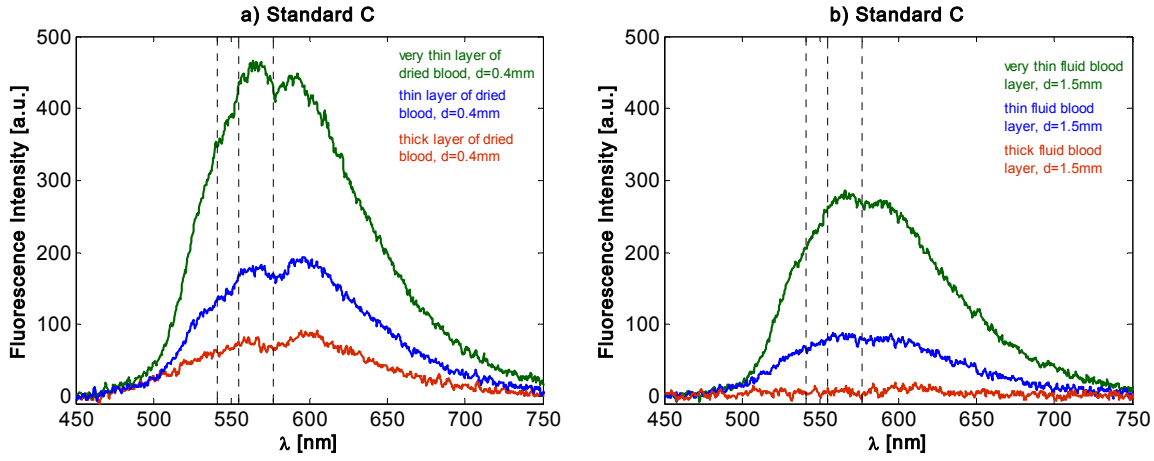


Figure 4.5 Autofluorescence of (a) dried and (b) liquid blood at random volumes

Figure 4.5.a shows fluorescence curves for different thicknesses of dried blood. It is not possible to tell exactly how thick the layers were. To start, a very thin layer of blood was extended with the aid of a needle upon standard C. After approximately 7 min the blood dried and measurement number one was done (green curve). Then, a second

layer of blood was added to the first one and spread in a similar fashion. 10 min later two measurements were realized, one on a middle thick part of the blood surface (blue curve) and another one on a thicker part (red curve). On the thicker part the light is assumed to be almost blocked. On the other side, Figure 4.5.b shows the fluorescence curves for different volumes of blood. As before, the liquid blood was expanded with a needle over standard C and immediately thereafter a measurement was realized. Twice more, a little blood was added with a syringe, expanded, and the fluorescence of the plastic reference was recorded. In the last measurement, the fluorescence signal was completely blocked by the blood. Standard C shows similar curves for both dried and liquid blood. The strongest blood absorption appears at 577 nm, where oxy-hemoglobin absorbs light.

In order to have a more precise numerical estimation of the minimal blood thickness that blocks light, the calculation of missing data was done with help of the parameters mentioned in section 2.2 and some values presented by Roggan et al. [8]. To show how the absorption changes with the wavelength, four values were chosen: 400, 500, 600 and 700 nm; the coefficients correspond to the oxy-hemoglobin. (Table 4.1).

Table 4.1 Light depth penetration in blood

Optical properties of blood	Symbol / Equation	λ = 400 nm	λ = 500 nm	λ = 600 nm	λ = 700 nm
Absorption coefficient	μ_a	10 mm^{-1}	1.6 mm^{-1}	1 mm^{-1}	0.1 mm^{-1}
Scattering coefficient	μ_s	18 mm^{-1}	48 mm^{-1}	32 mm^{-1}	30 mm^{-1}
Anisotropy factor	g	0.98	0.99	0.98	0.98
Approximate penetration depth	$d_a = 1/\mu_a$	0.1 mm	0.6 mm	1 mm	10 mm

The results resemble the expectations. As noted in sections 2.2 and 2.3.1, light waves with longer wavelengths have lower frequency and lower energy. This means that the corresponding absorption is low and therefore light can penetrate deeper into the tissue. For this reason light can travel much deeper at 700 nm as at 500 nm before it is completely absorbed. It should also be mentioned that the lowest light absorption in blood within the visible spectrum takes place at 700 nm.

4.6. Comparison with Brain Data

In Figure 4.6 the typical fluorescence spectra collected during brain tumor surgery are shown. The data comes from a group of test persons with GBM who were operated on the University Hospital Linköping in 2009. Before proceeding with the brain tumor resection, the patients had to take a dose (5 mg/kg body weight) of 5-aminolevulinic acid (5-ALA) [22]. 5-ALA is an exogenous substance that provokes the accumulation of porphyrins in cancerous brain cells, and therefore can be used as intraoperative fluorescence guidance during the operation [22].

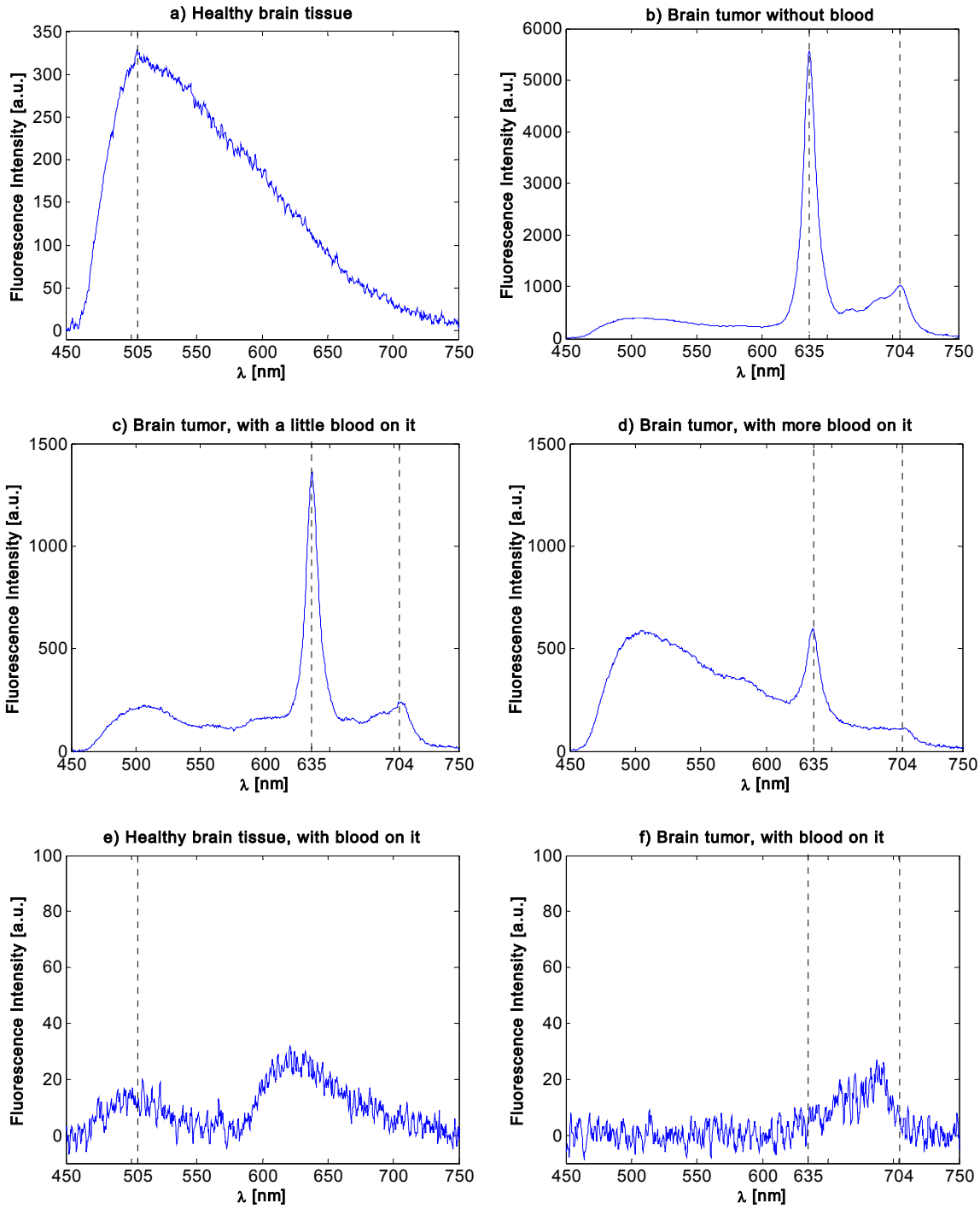


Figure 4.6 Fluorescence of healthy and cancerous brain tissue

Figure 4.6.a shows the autofluorescence of healthy brain tissue without blood on it with a maximum intensity at 506 nm, which is exactly the same as for skin. Figure 4.6.b, c, and d show the fluorescence spectrum of tumor tissue (without blood in b, with blood in c and d) where the peaks at 635 and 704 nm are the fluorescence of 5-ALA induced protoporphyrins [22]. The small peak at 510 nm in b, c and d is due to the pathological changes of the cancerous brain tissue. The blood interference on different measurement points is shown in Figure 4.6.c-f. The last two plots (Figure 4.6.e, f) show no significant peaks, because blood is blocking the light absorption in the tissue.

These data can be compared with the patterns seen in section 4.4. Likewise, when a large amount of blood comes between the pointer and the target tissue, all the fluorescence signal is blocked. Even the strong fluorescence of the porphyrins (~5000 a.u.)

does not come through the blood except for a weak red light (Figure 4.6.f). But with less blood volume it is possible to get a weak fluorescence signal with dips of blood absorption at 635 and 704 nm (Figure 4.6.c, d). As mentioned in previous sections, it is difficult to measure the amount of blood on the tissue. The statements “little blood” or “more blood” are absolutely subjective.

4.7. Rinsing and Practical Solutions

As observed in the reproduced lab measurements, the blood can stick on the probe and cause light blockage. During the actual operation the surgeon uses a saline solution to rinse superficially the tissue and the instruments. However, in between the optical measurements, the probe rests in a plastic bag while being exposed to air. One solution can be to leave the probe tip in saline solution instead. To reproduce the situation, a saline solution of 0.9% NaCl was poured in a plastic box (6 x 4 x 3 cm), to see how easy or difficult it is to rinse a bloody probe. If the fresh liquid blood was covering the laser pointer, practically all the blood dissolved in the solution, and the probe was ready to be used again. If dried or coagulated blood was sticking on the laser pointer, it was necessary to clean it manually with a piece of flannel as dried blood did not dissolve quickly by itself.

5. Discussion

A practical problem that may arise during surgery is that the interference of blood with the probe during intraoperative measurements disturbs the autofluorescence signal collected from the tissue. In order to categorize the different curves obtained in the operation theater, a similar scenario was simulated in the lab. Instead of measuring the autofluorescence of brain tissue with and without blood, two plastic standards (A and C), and the skin and blood of three volunteers, respectively, were used. The highest autofluorescence of brain tissue and skin is reached at 506 nm. Although skin and both plastic standards have different optical properties regarding color and fluorescence, all of them presented very similar curves when blood on their surface blocked partially or completely the light absorption. After reproducing the experiments under the same conditions, the results followed practically the same pattern and the categorization of the curves could be performed. One challenge was to use an equal amount of blood every time. However, since the experiments should resemble the situation at the operation theater, where random blood volumes are present, it was not relevant to realize the measurements with a defined volume of blood.

In section 4.1 the relationship between fluorescence intensity of both plastic standards (A and C), and skin against the laser power is presented. This was performed to investigate how power correlates with the fluorescence intensity and to choose an appropriate power setting. As a result, all the following measurements were done with a laser power of 10 mW, to get the highest possible signal.

Reference measurements of the used objects without blood were compared to the ones with blood. Looking back at Figure 4.2, only by seeing the peaks of diffuse reflection (Figure 4.2.b, d, f) and their corresponding wavelengths, it is possible to trace the color of the object. It would have been interesting to also measure fluorescence and diffuse reflection intensity on a person with dark skin (type VI). According to the results obtained, the maximum of fluorescence is reached at a wavelength of about 506 nm, but the fluorescence intensity is much lower due to the high melanin concentration and hence absorption. Another experiment to consider in future is to calculate the autofluorescence from the reflection spectrum. White light also includes blue light and other wavelengths which excite the fluorophores of the objects.

The yellow plastic cutoff filter (450 nm) in the OTP device (see section 3.1.1) has some autofluorescence because of its material. After sending the blue laser light to the black surface, a low fluorescence was observed due to some blue light reflection on the black surface. In principle, a black surface is not supposed to cause reflection, because it absorbs the light totally. Looking at Figure 4.3.a there is some fluorescence between 520 and 560 nm, also in the yellow range of light, as the filter color. The fluorescence intensity is considered negligible relative to the intensity levels of the fluorescence signals collected from the fluorescent standards and the skin. The collected reflection signal with DRS was zero.

Figure 4.3.b shows the signals collected from the blood on the black surface. The black surface was chosen to have the minimal reflection from the background surface. The yellow fluorescence of the filter is not seen anymore, as the blood highly absorbs the blue light. At approximately 640 nm, i.e. in the red region, there is a peak in both fluorescence and reflection signals due to the backscattered reflection from blood. Blood has a low absorption in the red region which makes it appear red.

Figure 4.4 shows the fluorescence intensity signals (plots a, c, e) and the diffuse reflection signals (b, d, f). In general, when the fluorescence signal is blocked, the reflection light is high in the red region and near to zero in the lower wavelengths, given that white light covers the whole visible light spectra and blue laser only the blue region.

When the autofluorescence signal is available with the traces of blood absorption at 541 and 577 nm, the reflection signal is available in all the visible light wavelengths, with dips at 541 as well as 577 nm. The slope of the tail in the red region appears to be less in the later case. However, these observations are general and to find the exact relationship between these two measurement methods, it is suggested to realize more investigations on that topic.

An experiment to see if there is a relationship between the aggregate state (liquid or dried) and the amount of blood was also realized. The blood autofluorescence signal was measured at different thicknesses of dried blood layers, as well as at different volumes of liquid blood. In doing so neither the volume of blood nor the thickness of the blood layers could be measured properly, because special devices for layer thickness measurements are needed. However, the obtained curves for the same standard look very similar to each other (Figure 4.5). This shows that light absorption occurs at the same wavelengths, principally where oxy-hemoglobin absorbs light, independently of the aggregate state or quantity of blood. In both cases, when there was plenty of blood between the probe and the object, no signal was obtained.

With collected data from intraoperative measurements realized at the hospital during brain tumor resection, a comparison between those curves and the curves obtained in the lab was done. Diffuse reflection curves could not be compared, because during the operation no refection measurements were done. The patterns of blood absorption on skin as well as on healthy and cancerous brain tissue are not exactly the same, because the brain has also other chromophores which are only present there (see Figure 4.4.e, Figure 4.6). On cancerous brain tissue also exogenous fluorophore of porphyrin is present. Porphyrin has a peak at 635 and 704 nm.

When the probe was held in saline solution, the blood dissolved and the pointer was blood free (see section 4.7). This however only happened when the blood was still liquid. Additional mechanical cleaning of the probe with a cleaning rag was necessary if the coagulation process was fairly advanced, so that blood was quite viscous and sticking on the probe, or if the blood was already dried and also sticking on the laser pointer.

6. Conclusion

In conclusion, the blood creates traces on the fluorescence spectrum at 541 and 577 nm due to oxy-hemoglobin's absorption spectrum which has two peaks at these wavelengths. The fluorescence signal which is excited by a 405 nm laser is easily blocked with a 0.1 mm thick blood layer. Diffuse reflection spectroscopy shows to have more sensitivity for detecting the existence/amount of blood in front of the laser pointer. However, as the light source and the fiber connections are different from the ones in the fluorescence spectroscopy system, the correlation between these two measurement types should be further investigated. The combination of the two systems might help to calculate the blood interference from the fluorescence signals post operatively and allow a correct quantification of the fluorescence signal even if some traces of the blood are apparent in the signal.

The fluorescence intensity curves of brain tissue showed not only absorption peaks at the oxy-hemoglobin wavelengths, but also at other wavelengths due to additional chromophores which are only present in the brain. During the operation it is strongly recommended to keep the probe in saline solution directly after each measurement for the time it is not being used. In this way, sticking of the blood on the probe will be avoided and the pointer will be cleaned and ready for the next intraoperative measurements. Time will also be saved, because it is faster to put the probe after every measurement in the saline solution instead of cleaning it manually.

7. List of Figures

8. List of Tables

9. Abbreviations

a.u.	Arbitrary units
DRS	Diffuse reflection spectroscopy
FAD	Flavin adenine dinucleotide
FHNW	Fachhochschule Nordwestschweiz
GBM	Glioblastoma multiforme
Hb	Deoxygenated hemoglobin
HbO_2	Oxygenated hemoglobin
IR	Infrared
NAD^+	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced form of NAD^+
OTP	Optical touch pointer
UV	Ultraviolet

10. Appendix

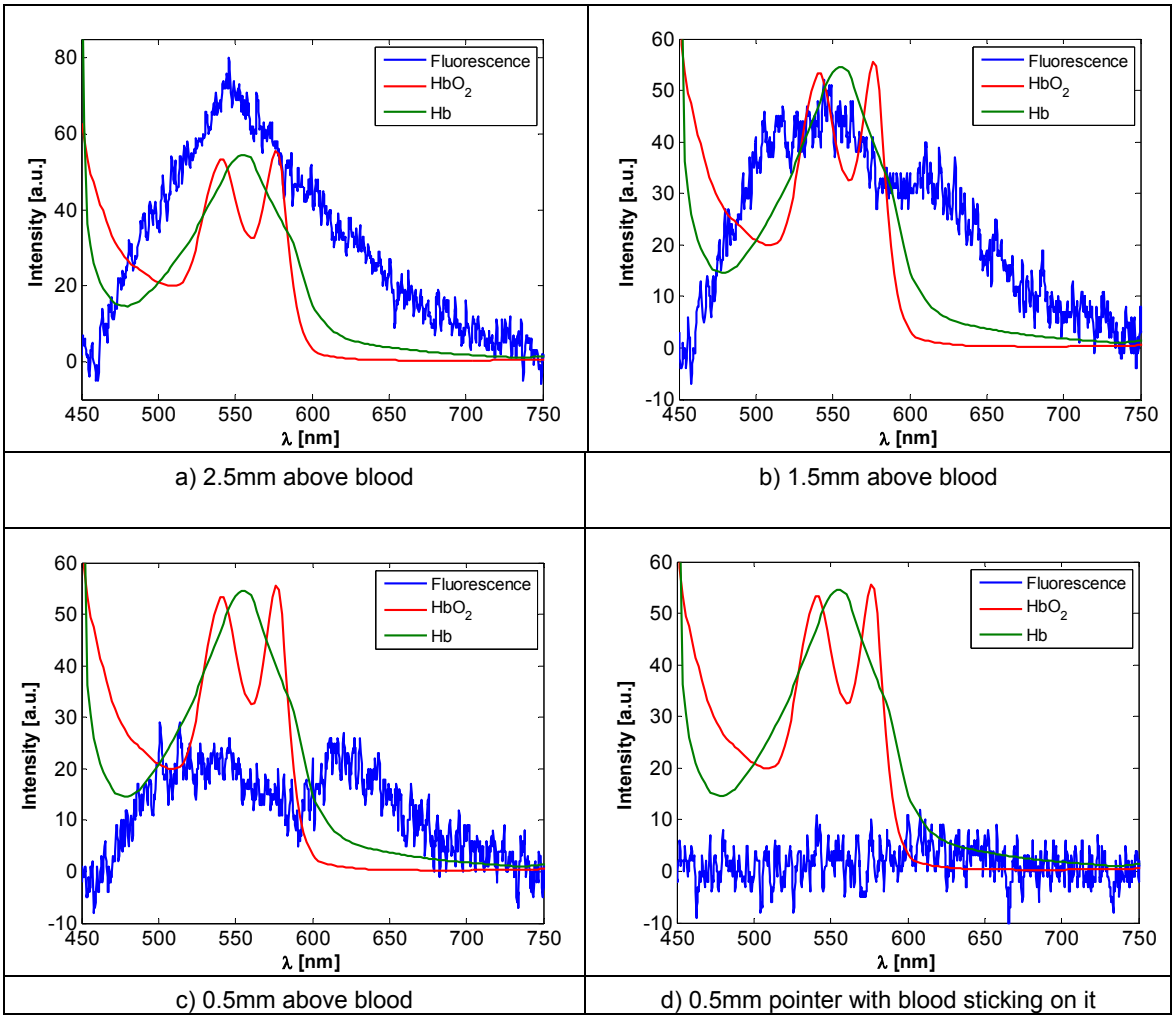
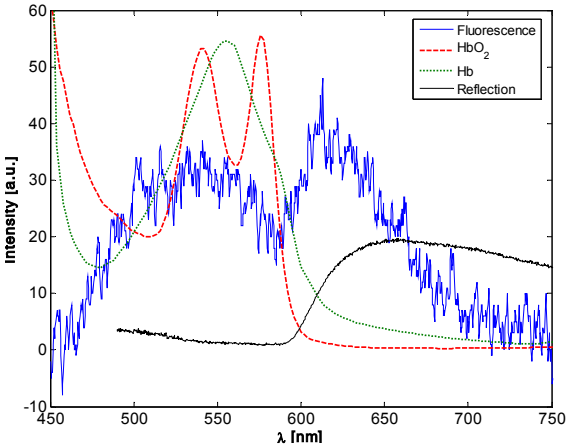
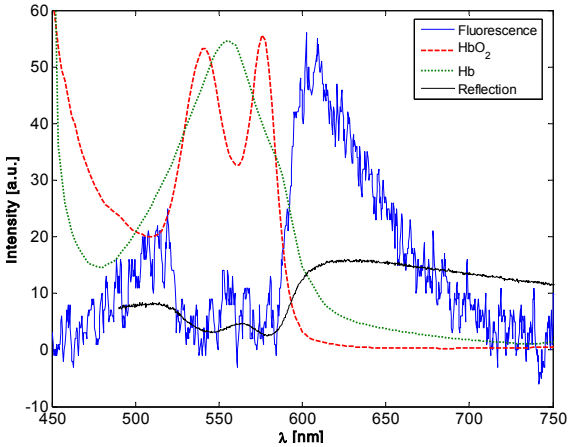


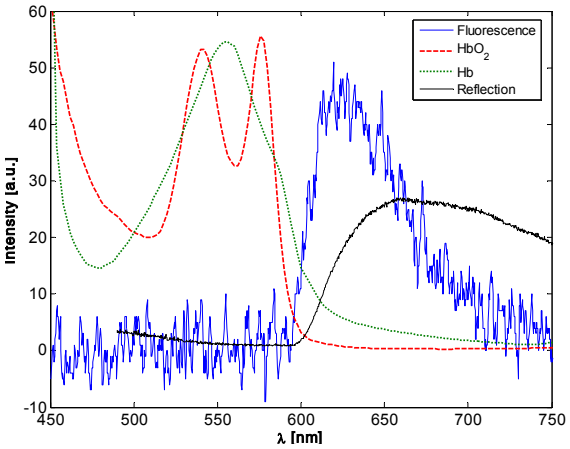
Figure 10.1 Blood and hemoglobin fluorescence spectra on standard C



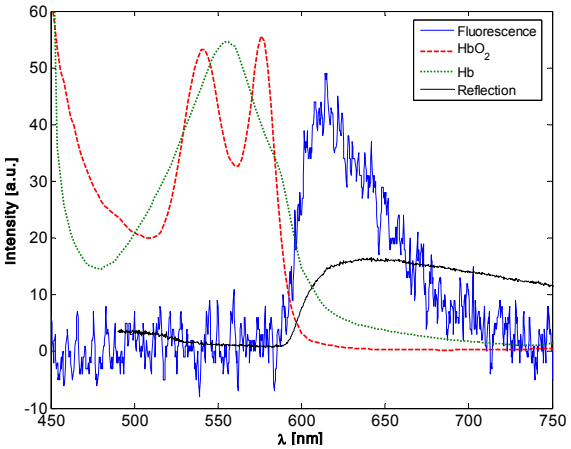
a) 0.5mm above blood



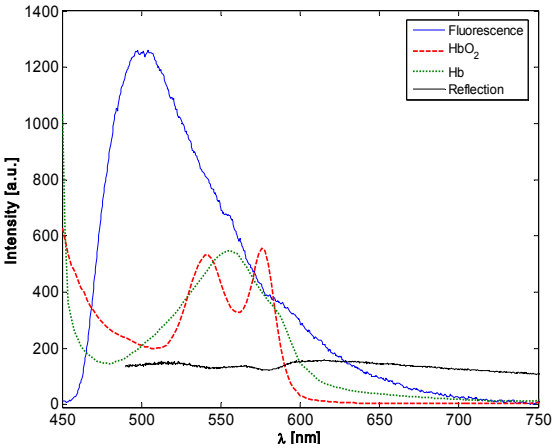
b) in the blood, skin contact



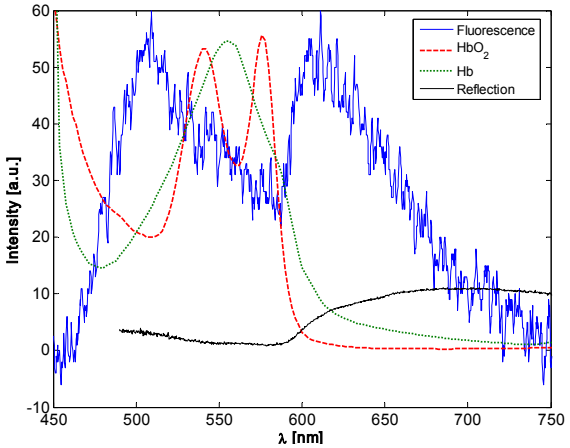
c) 1mm above skin, dried blood on pointer



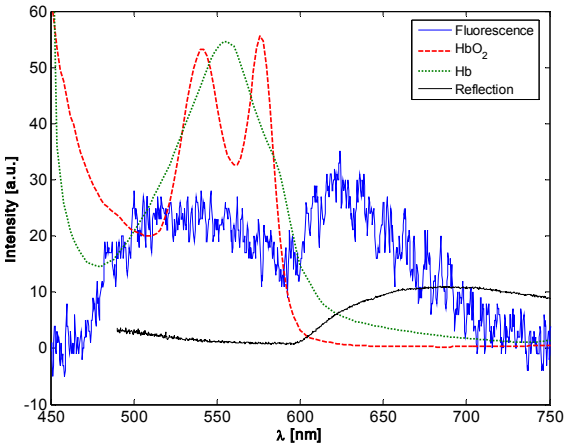
d) Pointer on skin, in another blood spot



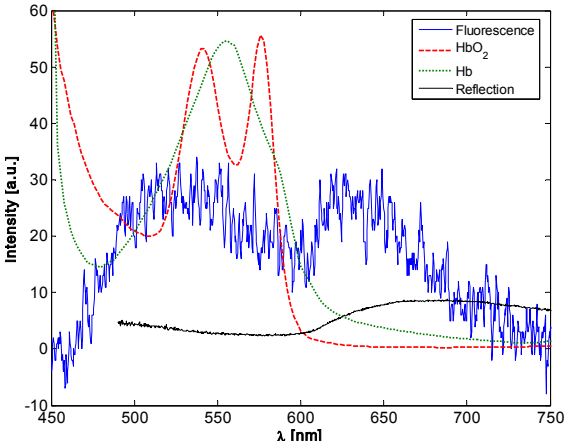
e) Blood on pointer, no blood on skin.
Pointer in contact with skin



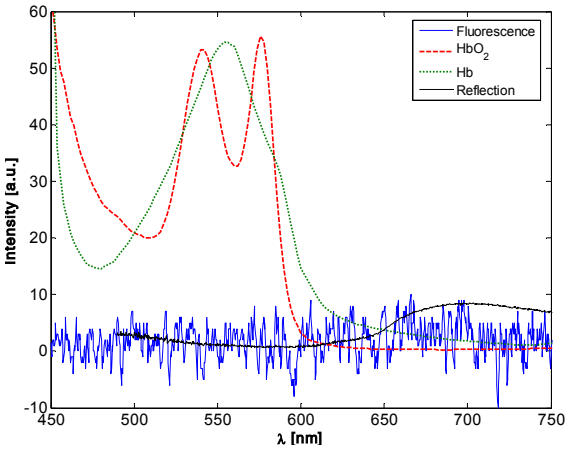
f) Laser pointer was cleaned.
Thin layer of dried blood on skin



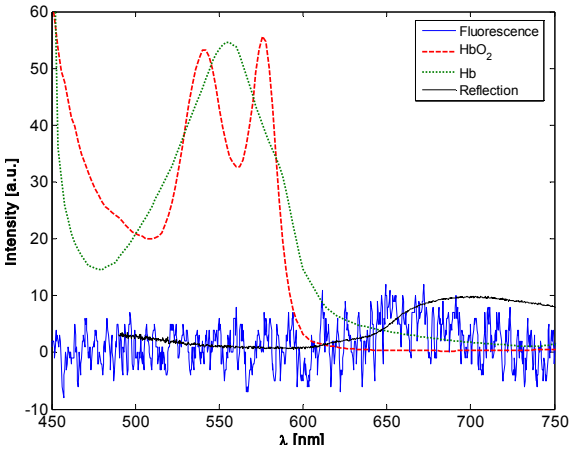
g) Pointer was cleaned again. In touch with dried blood on another spot



h) Thin layer of coagulated blood between skin and probe



i) 0.5mm above skin, dried blood layer on pointer



j) On skin, dried blood layer on pointer

Figure 10.2 Blood and hemoglobin fluorescence spectra on skin

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