Oxygen measurements in connection to PDT using time-resolved diffuse reflectance spectroscopy -development and testing of a new system

Master's thesis by
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ABSTRACT

Oxygen measurements in connection to photodynamic therapy (PDT), have been performed, using time-resolved diffuse reflectance spectroscopy. PDT is a treatment method mainly used for cancer tumours. The treatment relies on three important parameters; a photosensitizer, light and oxygen.

Clinical measurements have been performed on patients with *basal cell carcinoma*, *squamous cell carcinoma* and *cutaneous lymphoma lesions*. The aim was to measure the oxygen content and to see if there were any changes in the oxygen content in the tumours during PDT. This has been done by using the difference in absorption coefficients for haemoglobin and oxyhaemoglobin at the wavelengths 660 nm and 786 nm. The absorption coefficients have been measured with time-resolved diffuse reflectance spectroscopy. By using the relation between the absorption coefficient and the concentration, the concentrations of haemoglobin and oxyhaemoglobin were determined.

The results are inconclusive. In some measurements the concentration of oxyhaemoglobin was increasing during the treatment and in some it was decreasing. These observations could be caused by several properties of the tissue other than the oxygen consumption during the treatment, such as variation in the perfusion and how much of the blood in the tumour was arterial and how much was venous. Also the limited robustness of the time-resolved instrument and the evaluation program make the interpretation difficult.

Although the results are varying, the concentration of oxyhaemoglobin never drastically decreases. This could be due to the fact that there is enough oxygen in the tumours or that the treatment was interrupted every time a measurement was performed and during this break some re-oxygenation could have occurred.

There are big potentials for assessing the oxygen levels in tissue by using time-resolved measurements, but before the instrument and the evaluation program in this study are used again, they need further testing and calibration. Also the instrument needs to be more clinically adapted.

Contents

1 AIMS

Photodynamic therapy (PDT) is an investigational local treatment method mainly used for cancer tumours. In PDT, light is used to excite a light sensitive agent called a photosensitizer. When the photosensitizer is excited a chemical reaction involving molecular oxygen is started. The oxygen is transferred to a reactive state, which causes cell death. It is important that there is enough oxygen that can be excited by the sensitizer in the tumour since this is what is causing the cell death.

The aim of this Master's Thesis is to investigate if there is enough oxygen in the tumours during PDT or if the oxygen is consumed to such a high extent that it hampers the treatment.

To be able to do this, the difference in absorption spectra for haemoglobin and oxyhaemoglobin at certain wavelengths has been used. By using the relation between the absorption coefficient and the concentration, the concentrations of haemoglobin and oxyhaemoglobin could be determined. The absorption coefficients are determined by using time-resolved diffuse reflectance spectroscopy.

2 PHOTODYNAMIC THERAPY

Light has been used for treatment purposes for many years. The Greeks used heliotherapy 3000 years ago, which was a whole body exposure to the sun. The treatment with light is divided into two main groups, phototherapy and photochemotherapy.

In phototherapy the light, which can be of various wavelengths and intensity, is interacting directly with the tissue. Every cell in the alighted area is affected in the same way. How the tissue responds to the light may vary depending on the application and is today not fully understood. This form of treatment is used mostly in dermatology, e.g. in the treatment of psoriasis, but nowadays it is also used in psychiatry where it is possible to treat sleeping disorders and some types of depression[1].

In photochemotherapy the light is used to excite a light sensitive agent. The agent is either naturally existing in the body or it has been e.g. injected intravenously or applied with a cream. The agent, called a photosensitizer, is illuminated thus initiating a chemical reaction, leading to cell death. The treatment is selective partly because only the cells exposed to both the photosensitizer and light are affected, further the photosensitizers are often accumulated to a higher extent in diseased than in healthy tissue.

Photodynamic therapy (PDT) is a form of photochemotherapy, where the chemical reactions involve molecular oxygen. Photodynamic therapy is mostly used for treatment of cancer tumours, but it is also used in ophtalmology, e.g. in the treatment of age-related macular degeneration, which is an eye-decease. In this study, PDT is going to be used to treat *basal cell carcinoma* (BCC), which is the most common type of skin cancer. This type has an indolent behaviour but can cause big problems due to its local invasive growth. The photosensitizer used in the treatment of BCC is often δ-aminolevulinic acid, ALA.

The advantages of PDT compared to other oncological treatment modalities are that the treatment generally does not require any anaesthesia and can be repeated unlimitedly, due to the comparably harmless nature of the treatment. The healing is also relatively fast and provides good cosmetic results.

2.1 MECHANISMS OF PDT

The three important components in photodynamic therapy are, as mentioned above, light, a photosensitizer and oxygen. There are two different types of reactions during PDT, Type I and Type II. They can act at the same time or one at a time and both of them rely on the presence of oxygen. In both cases the light is absorbed by the photosensitizer, which is excited from its ground state, S_0, to the first excited singlet state, S_1. From this state the molecules are rapidly transferred to the lowest triplet state T_1 through intersystem crossing. The transition is forbidden as a pure dipole-dipole transition but can happen because of the small energy separation between the two states. The transition from the triplet state to the ground state is "forbidden" for the same reason, and due to this the probability for this is very low. This leads to a relatively long lifetime for the triplet state T_1, in

the order of 100 μs. There is thus a high probability that the molecule interacts with other molecules in the surrounding.

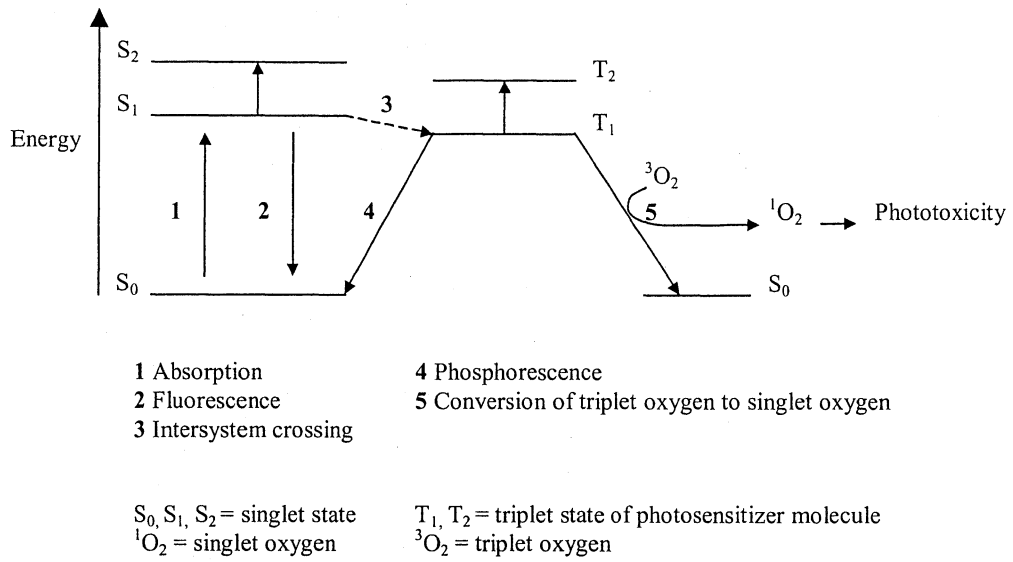

Figure 1 The process of PDT[2].

In the Type I reaction the energy of the excited photosensitizer is transferred to another molecule via hydrogen abstraction or electron transfer.

In the Type II reaction the excited photosensitizer is deexcited and the energy is transferred to a nearby oxygen molecule in its ground state, triplet state, $^3O_2$, which is then excited to its singlet state, $^1O_2$. A triplet state means that two electrons have their spins aligned in parallel, see Figure 2, which gives the molecule a magnetic angular momentum. This means that molecular oxygen is paramagnetic in its ground state. In a singlet state all the electrons have their spin paired, see Figure 2, leading to a zero magnetic angular momentum for the molecule.

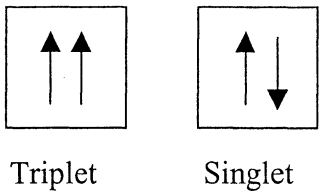

Figure 2 In the triplet state two electrons have their spins aligned in parallel. In the singlet state all electrons have their spins paired, which means that the two electrons in a pair have opposite spin directions.

The singlet state has a relatively long lifetime because it is inefficient for the molecule to change back to the triplet state[3]. Singlet oxygen is very reactive and reacts with proteins, unsaturated lipids, nucleic acids etc. This means that it

destroys cancer tissue and this process is the desired one in PDT. Even though it is inefficient for the singlet oxygen to go back to its ground state there is a small possibility for this as well. The transition happens after collision with another particle and the energy that is released from the singlet oxygen is in the form of radiation at 1270 nm, see Figure 4.

After the photosensitizer has been deexcited, it is back in the ground state and can be used again. This means that no more photosensitizer needs to be added during the treatment.

2.2 PHOTOSENSITIZER

There are some important requirements on the photosensitizer. One is that the efficiency must be high, meaning that it must produce a lot of radicals, such as singlet oxygen from a small amount of photosensitizer. If the efficiency of the photosensitizer is high, a smaller amount of the photosensitizer is sufficient. This is positive because it limits the amount of photosensitizer required, minimising any side effects and shortens the time when the patient is sensitive to light.

Another important property is high selectivity, which means that the photosensitizer is more easily accumulated in cancer cells than in normal tissue. This is important because it is only the diseased cells that are going to be treated, the healthy tissue should ideally remain unaffected.

One of the most important properties of the photosensitizers is high absorption in the wavelength range where the tissue has low absorption. The tissue has low absorption in the near-infrared wavelength region. Light in this wavelength band can propagate deeper into the tissue and can be used to treat tissue further into the body.

2.2.1 δ-AMINOLEVULINIC ACID (ALA)

As mentioned earlier, the photosensitizer used for the treatment of BCC is δ-aminolevulinic acid (ALA). ALA is naturally occurring in the body and it is not photodynamically active. It is normally produced in the haem-cycle, which is a chain of biochemical reactions where haem is produced. Haem is a non-protein pigment that is needed in the formation of haemoglobin. Each haemoglobin molecule needs four haems. Haemoglobin is needed in the formation of red blood cells, in fact there are 280 million haemoglobin molecules in one red blood cell[4].

After having applied ALA topically to the skin, it enters the haem-cycle, in the cells that have been exposed to the ALA. In this cycle, ALA will be converted to protoporphyrin IX (PpIX), which is a fluorescent and photodynamically active compound. The last step in the formation of haem in the cycle is when an iron-ion is incorporated in PpIX, see Figure 3.

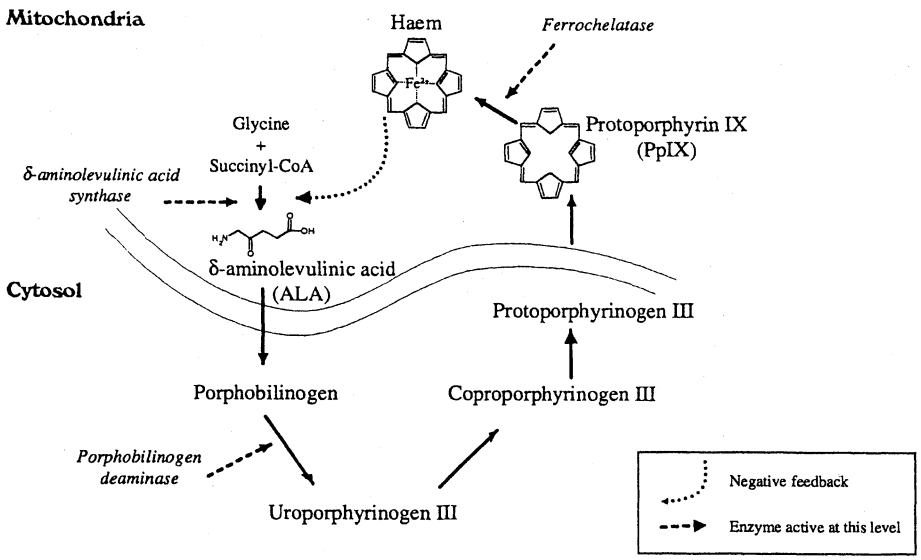

Figure 3 In the haem-cycle ALA is after some steps converted to PpIX, which is a photodynamically active compound[1].

Cells in more proliferate states produce haem more actively, yielding a more rapid build-up of PpIX in those cells. Also, in the condition of excess amount of ALA, the formation of PpIX from ALA is significantly quicker than the formation of haem from PpIX. This means that it is possible to get a surplus of PpIX, which is the photodynamically active compound.

ALA is a hydrophilic molecule and the keratin layer of the skin acts as a barrier when ALA is applied topically with a cream. This keratin layer is often damaged in BCC lesions and the ALA can penetrate more easily, yielding a further enhanced selective effect. The ALA-cream is usually applied 4 to 6 hours before the treatment, giving it time to penetrate as much as possible into the lesion. Another advantage of ALA is that its accumulating time is rather short and it only stays in the body for one or two days.

The excitation wavelength for PpIX is 635 nm and this sets some limitation to ALA-PDT, as light of this wavelength only penetrates a few millimetres in tissue, which means that it is not possible to treat thicker lesions by superficial illumination.

2.3 FRACTIONATION

Fractionation is one attempt to improve the efficiency of PDT. Fractionation means that the treatment light is switched off for one or many periods during the treatment to avoid oxygen deprivation during the therapy. During this time the tissue is supposed to be re-oxygenated, which means the levels of oxygen is recovering and the effect of the treatment during the rest of the treatment might increase. Other advantages of fractionation could be a better transportation of the photosensitizer molecules to the treatment area and also there is the possibility of redistributing the sensitizer while the light is switched off. The scientists' opinions disagree with how long time the light should be switched off and if fractionation really makes any difference at all.

The group by Pech et al.[5] has after investigations concluded that they could not give any recommendations if fractionation should be used or not. Also van den Boogert et al.[6] have shown that there is no observable increase in PDT efficiency if fractionation is used. Other groups have however shown that there is a positive effect from fractionation. For example Messmann et al.[7] present results indicating that the number of interruptions is not important as long as there is at least one. They get the best result when the break lasts for 150 s.

Another group, Curnow et al.[8], also prefers a fractionation time of 150 s. A longer pause does not give better results. They refer to Foster who says that the re-oxygenation takes 45 s and that a longer time than this is actually unnecessary. Other results were obtained by others[9] who have shown that at least 10 minutes are needed to achieve better results with fractionation. Only two minutes yielded no effect.

2.3 PROBLEMS WITH THE DOSIMETRY

As mentioned above, there are some limitations in the treatment with PDT. One of the most important limitations is the penetration depth of the treatment light. For light at about 635 nm, which is used in the ALA-PDT treatment, the penetration depth is about 2-5 mm. This means that it is impossible to treat lesions thicker than this. Another difficulty is that it is hard to know how much ALA is needed and how much healthy tissue in the vicinity of the tumour is affected by the treatment. Also, it is important to know for how long the ALA should be applied, before the treatment starts. Wang[1] says that the limitation of the treatment is the penetration depth of ALA rather than the light. Today usually fixed values of the ALA amount, application time and wavelength of the light are used. This is not ideal, because there are big differences between different lesions. For example they have different thickness, the penetration time for ALA is different and temperature variations are affecting both the diffusion of ALA and the transformation rate of ALA to PpIX. Also the amount of oxygen in different tissues varies.

To optimise the ALA-PDT treatment it is necessary to be able to measure the treatment efficiency during the treatment. This has been done in different ways as will be seen in the next sections.

2.3.1 SINGLET OXYGEN LUMINESCENCE MONITORING

The best way of measuring the PDT efficiency would be to measure the concentration of the singlet oxygen that takes part in the cell destroying process, see process 1) in Figure 4. This would be a direct measurement of the efficiency since this is the cytotoxic agent in PDT. The problem is, though, that there are no ways of measuring this part of the singlet oxygen. What one could do is to measure the phosphorescence that occurs when the singlet oxygen undergoes a collisionally induced transition to the ground state. The emission appears at 1270 nm, see process 2) in Figure 4, and as mentioned in section 2.1 this process is not very likely to happen. If this phosphorescence is measured and the relationship between how much of the singlet oxygen is taking part in each of the processes 1) and 2), then one would know the concentration of the singlet oxygen that participates in reaction 1).

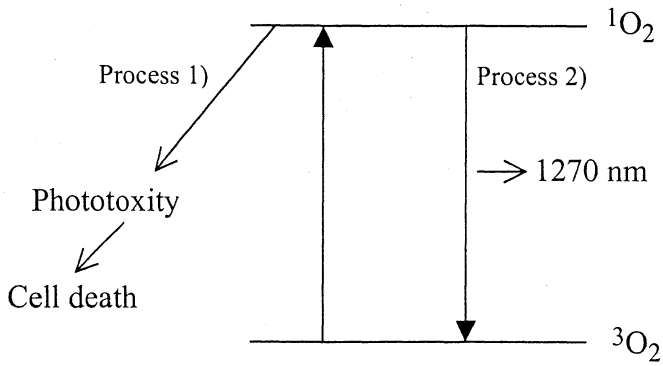

Figure 4 The processes of singlet oxygen. Process 1) is the cell destroying process and in process 2) phosphorescence occurs and the emitted light is at 1270 nm.

It is though not very easy to measure the phosphorescence in process 2). The difficulty of measuring the signal is due to that the signal is very weak and hard to detect. One group who has done it is Parker et al.[3] The central difficulty in that study was to separate the emission of singlet oxygen from the photosensitizer's infrared fluorescence. This problem was solved by looking at the delay in time between the two. The fluorescence from the sensitizer occurs at the same time as the exciting light, while the emission of singlet oxygen is delayed in time. The delay is due to the fact that formation of singlet oxygen is not directly coupled to the optical excitation. Parker et al.[3] states that they got good results and that it is possible to measure the concentration of singlet oxygen by this optical method.

The problem is that Patterson et al.[10] have tried to redo the same measurements and they did not observe any $^1O_2$ emission under any conditions in tissue, although it was possible in an aqueous solution. They believed that something was wrong with the measurements done by Parker et al[3]. The main explanation for the bad result is that singlet oxygen has a reduction in lifetime in the cellular environment and Patterson et al. think that the measurements could be possible only if the detectors are technically improved. Recently it has been shown that the measurements are feasible with newly developed IR detectors[11].

2.3.2 PHOTOBLEACHING

As mentioned before, the photosensitizer goes back to its ground state in the energy transfer to oxygen and because of that, the photosensitizer can be used again. Another scenario might also occur for the sensitizer, it can be oxidized by the singlet oxygen, which is produced in the surrounding[12]. This process is called photobleaching or photodegradation. This can be observed as a reduction in the PpIX fluorescence intensity and at the same time a new fluorescence peak arise. This peak is observed at 670 nm when PpIX is photobleached during PDT. An indirect way of measuring the singlet oxygen and the PDT damage could be to monitor the PpIX photobleaching. It is hard to know the connection between the photobleaching and the treatment efficiency, though. One group who has tried this is Robinson et al.[13] They have so far shown that the rate of photobleaching of PpIX is increased with decreasing irradiance and short periods of light fractionation.

2.3.3 MEASUREMENTS OF PPIX, TRIPLET OXYGEN AND LIGHT

Another way of getting an indirect measurement of the treatment efficiency seems to be to measure the concentration of photosensitizer, triplet oxygen and light, which means the three most important parameters in PDT. Potter et al.[14] have investigated the concentration of photosensitizer as a function of the light dose and watched the effects on tissue while varying these parameters.

The triplet oxygen exists in the blood in two forms. Some of it is bound to the iron atom in the haemoglobin and in that case there are two oxygen atoms bound, so the oxygen is carried as molecular oxygen. This form is called oxyhaemoglobin, HbO_2. The other form is the oxygen that is dissolved in the blood and not connected to the haemoglobin. This oxygen is also in molecular form and not ionic oxygen[15]. In the process of PDT it is the dissolved molecular oxygen that participates in the reaction. There should however be a connection between the two forms of triplet oxygen. If there is a lot of dissolved oxygen in the blood, the haemoglobin should also be saturated with oxygen. In the PDT process it is important that there is enough oxygen during the treatment and there are different approaches of measuring the concentration of triplet oxygen in tissue. Some of these methods are briefly described in the next section.

2.3.3.1 Triplet oxygen measurements

Some groups have developed mathematical models of the oxygenation in tissue, e.g. the Kroghs cylinder model[16,17]. This model is used to give an approximate description of the effects of increased oxygen consumption on the spatial distribution of oxygen close to a capillary. The capillaries are assumed to be cylindrical and have the same diameter.

Other groups have tried to measure the oxygenation directly. Some have done it with a pO_2-micro electrode[18]. They have performed oxygen measurements during PDT with Photofrin as a photosensitizer, by using a pO_2-probe inserted into the tumour, see Figure 5.

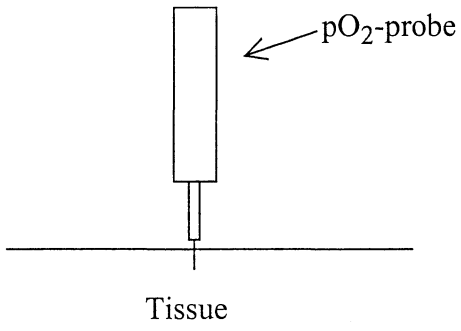

Figure 5 A schematic illustration of a pO_2-probe inserted into the tissue.

This probe measures the dissolved oxygen in the blood, which means the oxygen that takes part in the PDT process. This is a good way of investigating if there is sufficient

oxygen in the tissue. The drawback with this method is that it is invasive and it would be better to perform it non-invasively to avoid any possible side effects.

One way of measuring the oxygenation non-invasively is to do it spectroscopically. A group who has done this is Pham et al.[19] They have measured the concentration of oxyhaemoglobin and the concentration of haemoglobin to see how much of the haemoglobin that is saturated with oxygen. This means they are not measuring the oxygen that is participating in the PDT process. But as mentioned in section 2.3.3 there is a connection between the two forms of triplet oxygen in the blood.

The measurements were performed on tumour implants in mice and the photosensitizer they used was Benzoporphyrin-derivative (BPD). They have used frequency-domain photon migration (FDPM) to get the absorption and reduced scattering coefficient, respectively. FDPM uses sinuosidally intensity-modulated near infra-red light, which propagates in tissue as highly damped diffuse photon-density waves, whose characteristics depend on the optical properties of the tissue. The group measured the frequency-dependent phase shift and the decay of the amplitude from the diffusely reflected waves. The absorption and scattering coefficients were derived after having fitted the measurement to analytically derived model functions. The measurements were done at four different wavelengths, 674, 811, 849 and 956 nm. The wavelength dependent absorption coefficients were used to determine the tissue concentration of Hb, HbO_2, BPD and H_2O. This was possible due to the relation between the molar extinction coefficient ε and the concentration and the absorption coefficient, see section 6. They measured the concentrations in normal tissue and in cancer tissue prior and during PDT. They did not investigate how the oxygenation was affected by fractionation.

2.4 THE INTENTIONS OF THIS STUDY

In this study the concentration of triplet oxygen was going to be investigated during PDT in tumours of the type *basal cell carcinoma* with the photosensitizer ALA. Also the effects of fractionation were going to be studied.

In the studies with fractionation, there were going to be one break with a duration of 180 s. This length was chosen because it was long enough for making measurements and hopefully some re-oxygenation should have occurred.

The measurements was going be performed in a similar manner as Pham et al.[19] did, but instead of using FDPM, time-resolved spectroscopy was going to be used. Only the concentrations of Hb and HbO_2 were going to be investigated in this study and due to that, it was only necessary to conduct measurements at two wavelengths and the ones chosen were 660 and 786 nm. At the same time as the time resolved measurements were going to be performed, reference measurements were simultaneously to be done with a pO_2-probe. Half of the population of patients were going to be treated with fractionation and the other half without.

3 LIGHT AND TISSUE

The interaction of light in tissue is a complicated process. It is an important task in the development of using light within medicine to understand this process. To optimise different kinds of treatments, mathematical models of light propagation in tissue have been developed, see section 4, and they are being continuously improved. Tissue consists of different sorts of cells, which all have different properties and functions. Light transport in tissue is governed by different processes, such as absorption, scattering and reflection.

3.1 ABSORPTION

The absorbing components in tissue are called chromophores. Absorption of light in tissue occurs when the energy of a photon is the same as or higher than the energy between the electronic states of a chromophore in the tissue. The chromophore may then be excited to a higher energy state and the surplus of energy is then most likely to be transferred to heat motion to other molecules in the surroundings. This occurs due to collisions.

This means that the probability for absorption is wavelength dependent. The absorption is described by the absorption coefficient, μ_a, which is defined as the probability for absorption per unit length and its unit is inverse centimetres, [cm^{-1}]. The most important chromophores in tissue in the visible and near infra-red wavelength region are melanin, haemoglobin and water[1,12]. In the region between 630 and 1300 nm, the absorption of the chromophores is very low. This region is called *the tissue optical window* and because of the low absorption the penetration of light is relatively high in this range. Above 1300 nm water is dominating the absorption and below 500 nm, melanin and haemoglobin are dominating. In Figure 6 the absorption spectra of tissue chromophores are shown.

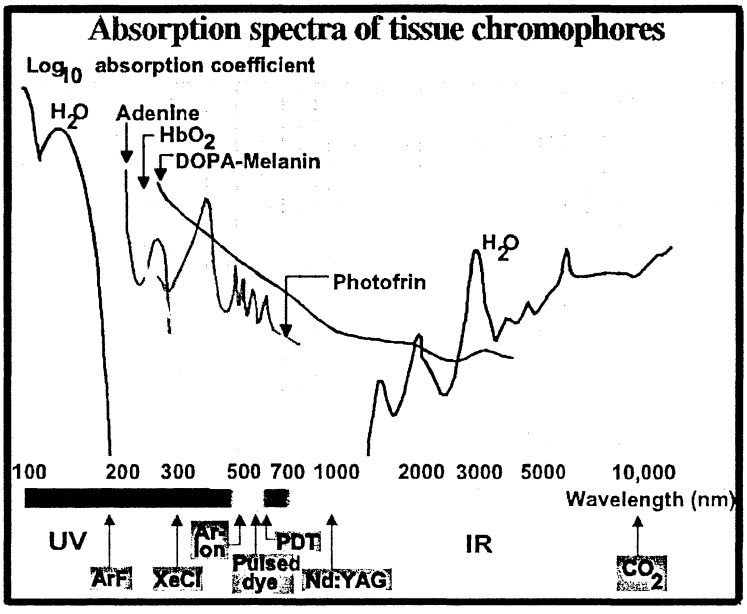

Figure 6 Absorption spectra of tissue chromophores[20].

3.1.1 WATER

The most common molecule in the body is water. The amount of water is different in different types of tissue. For example in muscle tissue the water content can be as high as 75%, while in adipose tissue it is only 20%. According to Figure 6 it looks like the absorption of water in the visible region is negligible, but above 1300 nm and below 200 nm it is one of the main absorbing chromophores. This indicates that the absorption spectra in Figure 6 is from tissue with rather low water content because according to an absorption spectrum of pure water the absorption starts to rise above 600 nm, see Figure 7. In most tissue, water is one of the dominating absorbing chromophores for light with a wavelength of 900 nm or more.

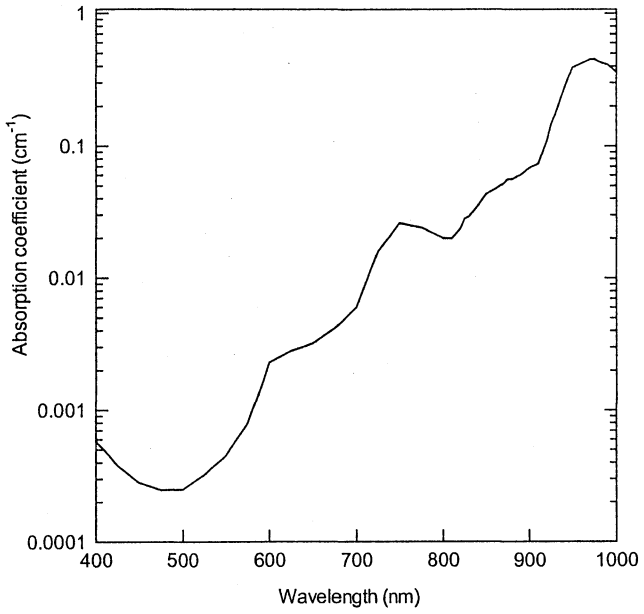

Figure 7 Absorption coefficient of pure water[21].

3.1.2 HAEMOGLOBIN

One of the important proteins in blood is haemoglobin, which is a haem protein. The role of this protein is to transport oxygen to the cells. To the protein, four haem groups are attached and in the middle of each group there is an iron atom. It is due to this atom that it is possible for oxygen to be connected to the protein[21].

The blood content in tissue is only a few percent. Despite this, the absorption in the visible is dominated by blood. One important thing is that the absorption of blood depends on the oxygenation. Haemoglobin and oxyhaemoglobin have different absorption spectra, see Figure 8. This will be used to determine the oxygenation in tissue in this study.

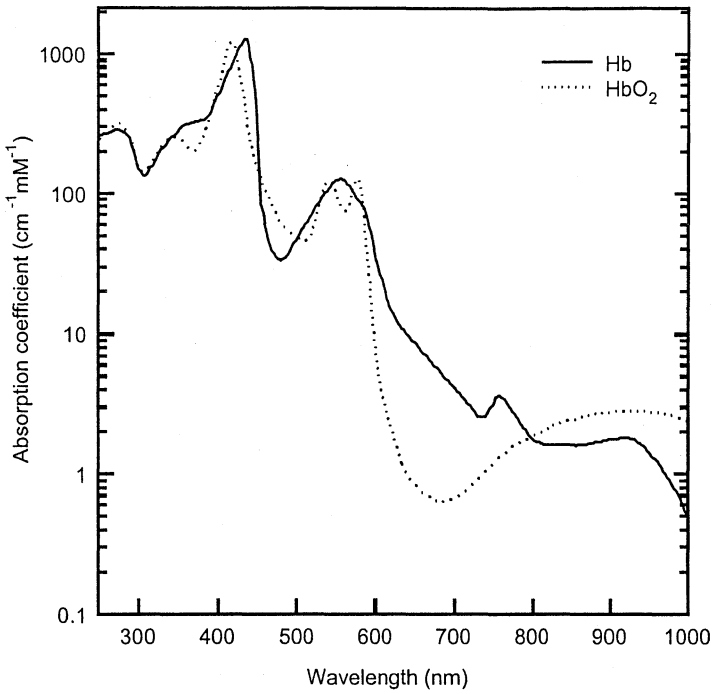

Figure 8 Absorption coefficient of human haemoglobin, Hb, and oxyhaemoglobin, HbO_2 [21].

3.1.3 MELANIN

In the skin there is a dark pigment, called melanin. It is also present in hair and in the iris of the eye. Melanin is the greatest absorber in these tissues and it is always present as a thin layer. This layer acts like an attenuation filter because of the absorption, when for example the skin is alighted. Due to it being a thin layer, the light passing the skin is only reduced and not affected in other ways. When performing measurements on people with light skin it is usually no problem to get enough light through the skin, but dark skin consists of more melanin and when measuring on people with darker skin this might be a problem[21]. It is not possible to show the absorption spectra for pure melanin because there is no way of extracting melanin in its pure form. Instead the absorption has been approximated by the empirical formula below[21]:

$$\mu_a = 1.70 \cdot 10^{12} \lambda^{-3.48} \quad cm^{-1}$$

The spectrum of this is shown in Figure 9.

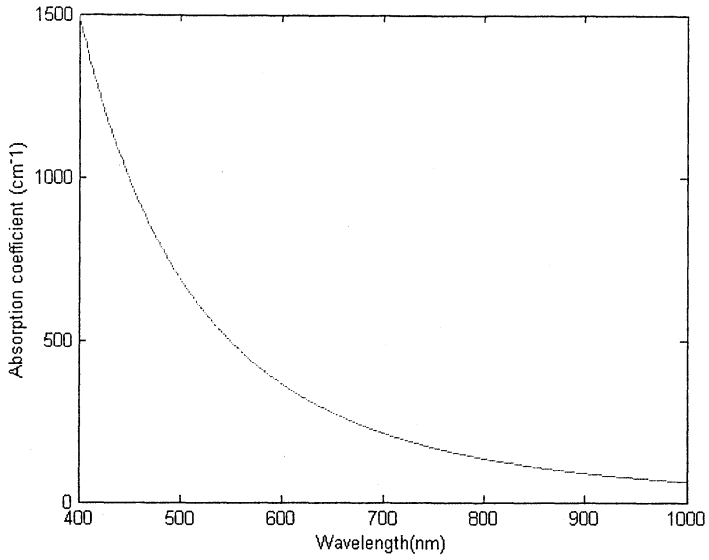

Figure 9 Absorption coefficient of melanin

3.2 SCATTERING

In tissue, photons are scattered many times. Scattering occurs when the incoming light has another energy than the energy of the resonant frequencies of the scattering media. For example Rayleigh scattering occurs when the energy of the incoming photons is less than the energy of the resonant frequencies. What happens then is that the incoming light starts an oscillation of the chromophore's electron cloud, with the same frequency as the incoming light. This makes the atom behave as a dipole and it starts to radiate at the same frequency. A photon is sent out in some direction and its energy is the same as the energy of the incoming photon. This means that Rayleigh scattering is elastic.

Another scattering process, which is not elastic, is Raman scattering. In this scattering process the chromophore is excited to a virtual energy level and from this level it goes back to the ground state immediately. The emitted light will then have another or the same wavelength as the incoming light[12,22].

When the scattering is elastic the photon only changes direction, but when it is non-elastic it loses energy as well. The scattering properties of tissue are not as wavelength dependent as those for absorption. The scattering is described by the scattering coefficient, μ_s, which is defined as the probability for scattering per unit length and its unit is inverse centimetres, [cm^{-1}]. In the optical window, where the absorption is low, the scattering is relatively high. The absorption coefficient, μ_a, is about 0.1 cm^{-1}, while the scattering coefficient, μ_s, is about 10 cm^{-1}.

The scattering in tissue is strongly directed in the forward direction, which means it is not isotropic. A factor g, has been introduced in order to describe the degree of anisotropy. It is measured as the mean cosine of the scattering angle. This means that if the scattering was directed totally backward the g factor would be -1 and if it was directed totally forward g would be 1. If it had been completely isotropic g would have been 0, see Figure 10. In tissue the factor g is between 0.7 and 0.99 in the visible and near-infrared region[23].

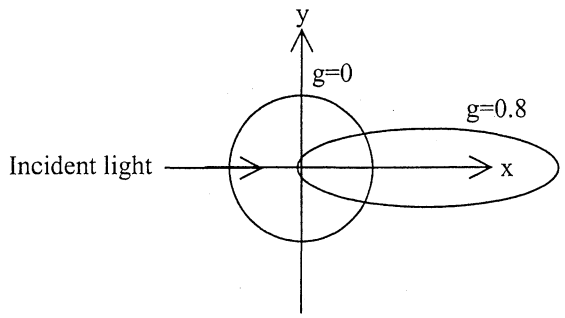

Figure 10 The illustration shows the scattering probability for different scattering directions. When g=0 the light is scattered isotropically, which means that it is scattered to the same extent in all directions. When g=0.8 most photons are scattered in the forward direction.

In tissue optics the scattering is often described as the reduced scattering coefficient, $\mu_s' = \mu_s(1-g)$. The reduced scattering coefficient converts the anisotropic conditions to isotropic ones. If the scattering is strongly backward directed g is negative, and this makes the reduced scattering coefficient high, which it should be because the incoming light is strongly affected by the scattering. If the scattering is in the forward direction, as in tissue when g is high, the reduced scattering coefficient is low and this agrees with the fact that scattering in the forward direction does not affect the photon path very much.

The scattering depends on the variations in refractive index in the tissue structures. The most possible scattering centres in tissue are the cell nucleus, the mitochondria and the cell membrane. It has though been shown that most of the scattering occurs within the cells and not in the outer membranes[24].

3.3 REFLECTION

When light passes from one media to another with different refraction index, reflection takes place. For normal incidence of the light the reflectance, R, is defined as:

$$R = \left(\frac{n_2 - n_1}{n_2 + n_1} \right)^2$$

Where the light is passing from a media with refractive index n_1 to a media with refractive index n_2, see Figure 11.

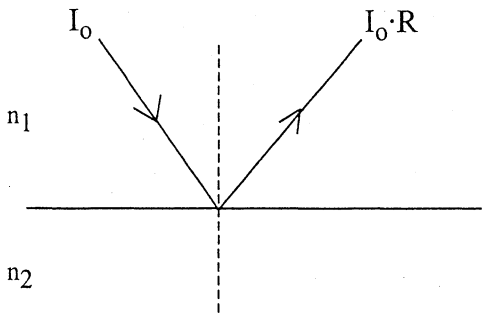

Figure 11 Reflection of light in a boundary between two different media with refractive index n_1 and n_2, respectively.

Tissue has a refraction index of about 1.4 and air has a refraction index of 1. The reflection when light is passing from air to tissue is then about 2-4 %[25].

4 MATHEMATICAL MODELS

4.1 THE TRANSPORT EQUATION

The transport equation is a description of the energy transportation of photons in turbid media. The equation is derived by using the energy conservation on the photons in a small volume dV. The conservation means that the photons only can be added or subtracted from the photon distribution function in specific interactions. The photon distribution function N(**r**,**s**,t) is the number of photons in the volume dV at the point **r**, with the direction **s** at time t and its unit is given in [photons/(m^3sr)]. In the following text each term of the equation will be presented leading to the complete equation in the end of this section. In Figure 12 the volume dV is shown and also the different terms in the equation are illustrated.

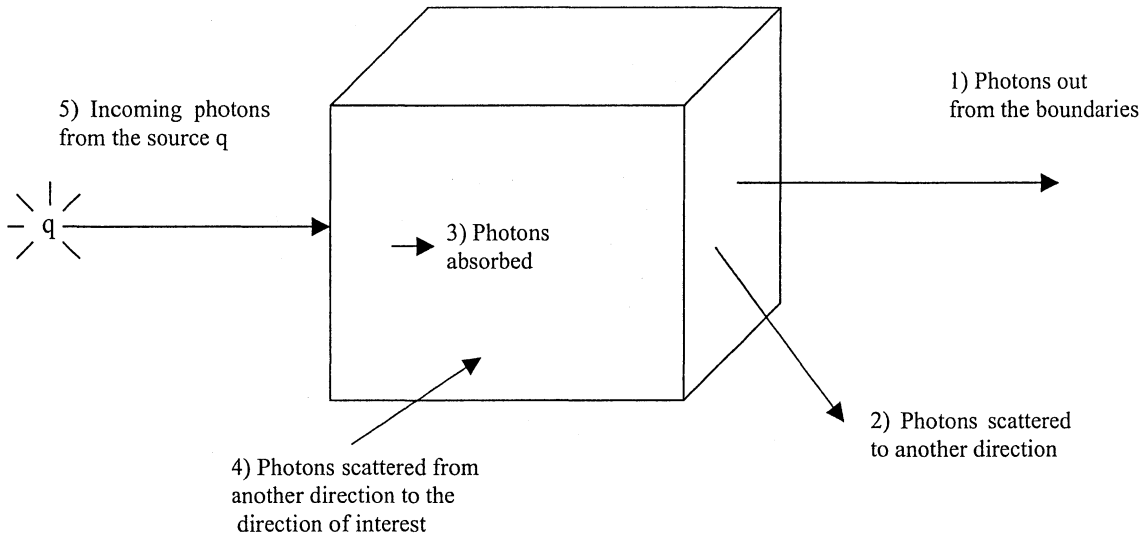

Figure 12 The five different terms in the transport equation.

The first term describes the loss of photons at the boundaries of the small volume dV. This loss is expressed as a surface integral.

$$1) \qquad -\int_S cN(\mathbf{r},\mathbf{s},t)\mathbf{s}\cdot dS$$

By using Gauss' theorem this can be expressed as a volume integral, which is to prefer when the other terms are in this form. It then looks like:

$$-\int_V c\mathbf{s}\cdot\nabla N(\mathbf{r},\mathbf{s},t)dV$$

The second and third term of the transport equation describes the loss of photons in the volume by scattering from one direction to another and by absorbing an incoming photon.

2) $$-\int_V c\mu_s(\mathbf{r})N(\mathbf{r},\mathbf{s},t)dV$$

3) $$-\int_V c\mu_a(\mathbf{r})N(\mathbf{r},\mathbf{s},t)dV$$

Term number four describes the gain of photons which are scattered within the volume form one direction **s'** into the direction **s**, in the volume and this term looks like:

4) $$+\int_V c\mu_s(\mathbf{r})\int_{4\pi} p(\mathbf{s}',\mathbf{s})N(\mathbf{r},\mathbf{s}',t)d\omega' dV$$

In this term p(**s'**,**s**) is the probability function for the scattering, which means that it describes the probability for a photon in the direction **s'** to get scattered into the direction **s**.
The last term in the transport equation is due to the photons from a light source q.

5) $$+\int_V q(\mathbf{r},\mathbf{s},t)dV$$

All these terms should equal the total change of photons in the media.
The complete equation is then expressed as:

$$\int_V \frac{\partial N(\mathbf{r},\mathbf{s},t)}{\partial t}dV = -\int_V c\mathbf{s}\cdot\nabla N(\mathbf{r},\mathbf{s},t)dV - \int_V c\mu_s(\mathbf{r})N(\mathbf{r},\mathbf{s},t)dV - \int_V c\mu_a(\mathbf{r})N(\mathbf{r},\mathbf{s},t)dV +$$
$$+\int_V c\mu_s(\mathbf{r})\int_{4\pi} p(\mathbf{s}',\mathbf{s})N(\mathbf{r},\mathbf{s}',t)d\omega' dV + \int_V q(\mathbf{r},\mathbf{s},t)dV$$

Usually the transport equation is expressed with the radiance L(**r**,**s**,t) instead of the photon distribution function N(**r**,**s**,t). The relation between them is L(**r**,**s**,t)= N(**r**,**s**,t)hvc and the unit of the radiance is [W/(m^{2}sr)]. If the functions are continuous the volume integrals can be dropped and this makes the transport equation look like this:

$$\frac{1}{c}\frac{\partial L(\mathbf{r},\mathbf{s},t)}{\partial t} = -\mathbf{s}\cdot\nabla L(\mathbf{r},\mathbf{s},t) - (\mu_s+\mu_a)L(\mathbf{r},\mathbf{s},t) + \mu_s\int_{4\pi} L(\mathbf{r},\mathbf{s}',t)p(\mathbf{s},\mathbf{s}')d\omega' + q(\mathbf{r},\mathbf{s},t)$$

The transport equation is, as mentioned above, only valid for the assumption that the photons, which are not absorbed, do not lose their energy when they are interacting with the surrounding media. More precisely expressed, no energy is lost or gained by interactions. Another important thing to know is that the light source must be monochromatic. The equation can be solved both analytically and numerically but in order to do so some simplifications are needed. It can also be used to simulate the

transport of photons in scattering media, by using Monte Carlo simulations. The simplification used to solve the equation analytically is the diffusion approximation.

4.2 THE DIFFUSION EQUATION

The diffusion equation is an approximation of the transport equation. The approximation is done by assuming spherical symmetry for the scattering particles. This means that the light is scattered isotropically, the same amount in all directions. Due to this, the functions in the transport equation can be expanded into the lowest order of spherical harmonics. After many steps[26] the diffusion equation is expressed like:

$$\frac{1}{c'}\frac{\partial}{\partial t}\phi(\mathbf{r},t) - D\nabla^2\phi(\mathbf{r},t) + \mu_a\phi(\mathbf{r},t) = S(\mathbf{r},t)$$

where D is the diffusion constant and is dependent on the absorption and scattering according to:

$$D = \frac{1}{3(\mu_a + \mu_s')}$$

c' is the speed of light in tissue, $\phi(\mathbf{r},t)$ is the energy fluence rate at point **r** at time t. The relation between the energy fluence rate and the radiance is

$$\phi(\mathbf{r},t) = \int_{4\pi} L(\mathbf{r},\mathbf{s},t)d\omega(\mathbf{s}) = \int_{4\pi} N(r,s,t)h\nu c d\omega(s)$$

Finally S(**r**,t) is the light source.
The advantage of the diffusion equation is that it can be solved analytically for simple geometries, like an infinite or semi-infinite homogenous space or a homogenous slab. When the geometry gets more difficult a numerical solution is necessary. The diffusion equation is only valid when the reduced scattering coefficient is much larger than the absorption coefficient ($\mu_s' >> \mu_a$), which means that the light propagating in the media is diffuse. To guarantee that the light reaching the detector is diffuse, the source and detector must be separated in space and time. One rule of thumb is that the light has to be scattered at least 10 times.

In this study it is the diffuse reflectance from the tissue that is actually measured. The diffuse reflectance can be derived by

$$R(\mathbf{r},t) = -D\frac{\partial}{\partial z}\phi(\mathbf{r},z,t)$$

at z=0, which is in the boundary between the air and tissue.
The diffuse reflectance, for a semi-infinite medium, is given by

$$R(\mathbf{r},t) = (4\pi Dc')^{-3/2} z_0 t^{-5/2} \exp(-\mu_a c' t)\exp\left[-\frac{(\mathbf{r}^2 + z_0^2)}{4Dc't}\right] \qquad (1)$$

where z_0 is $1/\ \mu_s'$. Often the time dispersion curves are plotted in a logarithmic scale and the logarithm of the reflectance R is given by:

$$\ln[R(r,t)] = -\frac{3}{2}\ln(4\pi Dc') + \ln(z_0) - \frac{5}{2}\ln(t) - \mu_a c' t - \frac{r^2 + z_0^2}{4Dct}$$

If this function is derivated in time it will look like:

$$\frac{d\ln[R(r,t)]}{dt} = -\mu_a c - \frac{5}{2t} + \frac{r^2 + z_0^2}{4Dct^2}$$

and in this form it is possible to read out that for later times of t, the slope of the curve is given by - $\mu_a c$, which is a linear function of the absorption. The absorption is then given by

$$\mu_a = \left(\frac{-1}{c'}\right)\lim_{t\to\infty}\frac{\partial \ln R(r,t)}{\partial t}$$

which means that the absorption coefficient is given by the final logarithmic slope of the reflectance curve. The final part of the curve is invariant to μ_s' and the earlier part of the curve are invariant to μ_a and due to this it is possible to derive the reduced scattering coefficient from the time to maximum of the reflectance curve.

These two ways of getting the absorption and reduced scattering coefficient will not be used in this study. Instead the entire analytical solution will be fitted to the measured time dispersion curve and it is possible to change some parameters until the analytical solution fits the measured curve well. In this way it is possible to derive the two coefficients μ_a and μ_s'. The program which is used for this is made by the Italian group at Polytecnico in Milan and the method they have used is called the Levenberg-Marquardt method and it is a non-linear curve fitting algorithm[27].

5 TIME RESOLVED SPECTROSCOPY

As mentioned above time resolved spectroscopy is going to be used in this study and this is one way of getting to know the absorption coefficient, μ_a, and the reduced scattering coefficient, μ_s'. The measurements are performed by sending short laser pulses into the tissue in one fibre and collecting the diffuse reflected light with another fibre at some fixed distance from the first fibre. When a light pulse is sent into tissue it will be broadened in time because of the multiple scattering in tissue. The photons arriving first to the detector have not been scattered as many times as the ones arriving later. This can be seen in Figure 13, where the photon in path 1 will reach the detection fibre much quicker than the photon in path 2 because the photon in path 2 has been scattered significantly more times, resulting in a longer optical path length.

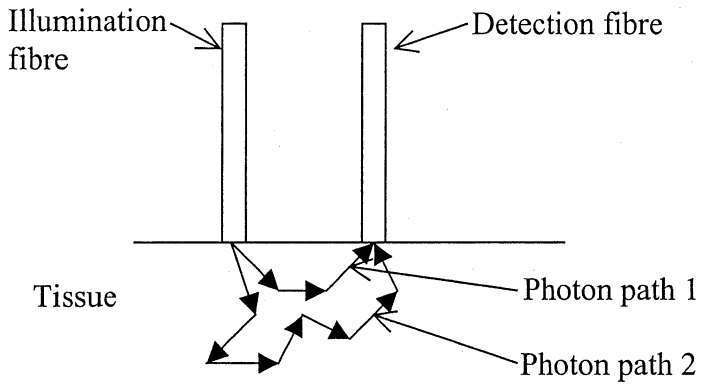

Figure 13 The photon in path 1 has not been scattered as many times as the photon in path 2. Due to this the photon in path 1 will reach the detector before the photon in path 2.

The time, from firing the laser until a photon reaches the detector, is measured and by making a histogram of the photons versus time a temporal point spread function (TPSF) is created. In Figure 14 a schematic picture of the broadening of the injected laser pulse is shown.

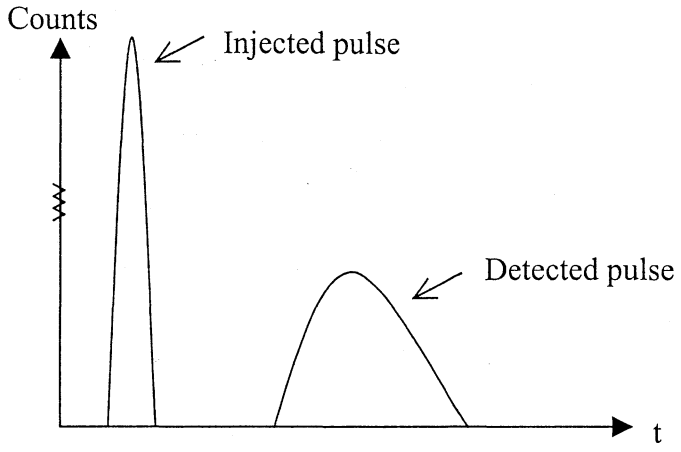

Figure 14 A schematic picture showing how the pulse reaching the detector has been broadened on its way through the tissue. Also the energy of the detected pulse is much lower than the energy of the injected pulse.

By studying this function the two optical properties μ_a and μ_s' can be determined. This is done by using the diffusion equation, which is mentioned in section 4.2. During the measurement it is important that the two fibres are not too close to each other because, as mentioned previously, the diffusion equation is only valid for diffuse light. This means that the photons from the first fibre must have been scattered a sufficient number of times before it reaches the detecting fibre. In this study the distance between the fibres was 1 cm. It is seen in this study, when the diffusion equation is fitted to the TPSF, that it is not fitted very well on the left flank, and this is due to the fact that the left flank comes from the photons that have not been scattered enough times, so the diffusion equation is not really valid there. As mentioned in section 4.2 the earlier part of the curve is more or less independent of μ_a, while the later part is mostly determined by μ_a. In this study it is primarily the absorption coefficient that is of importance. The μ_a coefficient is mainly decided by the right flank of the curve and this means that it does not really matter that the left flank is badly fitted.

The advantages of time resolved spectroscopy compared to other methods, for example spatially resolved measurements, are that it is more insensitive to inhomogenities[21]. The only consequence of inhomogenities, which might absorb some of the photons, is that the intensity of the detected light is reduced. This does not affect the measurements, since only the shape of the curve matters. Another advantage is that it is possible to measure different sizes of volumes. To measure small volumes the two fibres are held as close to each other as the diffusion equation allows. To measure deeper into the tissue the fibres are held further apart.

The disadvantages of this kind of spectroscopy are that the instrumentation is relatively expensive and complicated. The main reasons for this is the expensive detector, see section 7.1.

6 HOW TO DECIDE THE OXYGEN SATURATION

As mentioned above, it is the absorption coefficient which is of importance in this study. This means that it must be a relation between the absorption coefficient and the concentration of the absorbing substances. The relation is that the absorption coefficient is equal to the sum of all contributing absorbing chromophores in the tissue[26]. This can be expressed as:

$$\mu_a = \sum_i \varepsilon_i C_i$$

where ε_i is the extinction coefficient of substance i expressed with the unit $[cm^{-1}/(moles/litre)]$ and C_i is the concentration in [moles/litre] also noted as molar [M].

The question is then, which are the main absorbers in skin? In section 3.1 absorption was discussed, but the figures were only showing the absorption of pure substances and not putting these in relation to the amounts of those substances in the skin. In Figure 15, absorption spectra for the main absorbers in typical skin tissue are shown. These spectra differ from the spectra in section 3.1 in the way that they are all scaled to the typical volume fractions of the main chromophores in tissue.

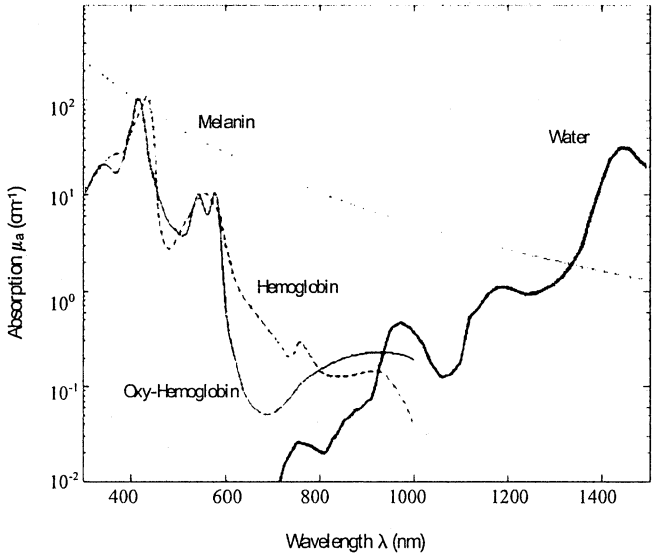

Figure 15 Absorption spectra of the typical skin tissue chromophores water, melanin, haemoglobin and oxyhaemoglobin. The spectra are scaled to the typical volume fractions of the chromophores in tissue[28].

It is shown in the figure that melanin has high absorption for all wavelengths in this region and that water has a higher absorption than haemoglobin and oxyhaemoglobin after about 900 nm. Due to this it is necessary to measure at wavelengths shorter than this because it is the haemoglobin and oxyhaemoglobin which are of interest. For this study it was decided to use two lasers with the wavelengths 660 nm and 786 nm, respectively. When looking at absorption spectra at these wavelengths, one can see

that the dominating substances are haemoglobin, Hb, and oxyhaemoglobin, HbO_2. One exception from this is melanin, which has the highest absorption in the whole optical window. The good thing, though, is that this does not have to be taken into account because the layer of melanin is very thin and it affects the measurements only the way an attenuation filter would have done. This makes the problem much easier to solve and since there are only two unknown concentrations, only two absorption coefficients will be needed.

The relation between the absorption coefficients and the concentration in skin is then concluded in the two equations below:

$$\begin{cases} \mu_{660} = \varepsilon_{660}^{Hb} \cdot [Hb] + \varepsilon_{660}^{HbO_2} \cdot [HbO_2] \\ \mu_{786} = \varepsilon_{786}^{Hb} \cdot [Hb] + \varepsilon_{786}^{HbO_2} \cdot [HbO_2] \end{cases} \qquad (2)$$

Here the extinction coefficients ε are known from tabulated data at different wavelengths, μ_{660} and μ_{786} are the absorption coefficients for 660 nm and 786 nm respectively. [Hb] and $[HbO_2]$ are the unknown concentrations of haemoglobin and oxyhaemoglobin.

7 INSTRUMENTATION

The instrument used for the time resolved measurements, named MAAN 230, is consisting of two main parts, a computer and the apparatus. The most important components in the apparatus are a detector and a laser driver with two laser heads[29]. Two fibres are connected to the box, one for sending the laser pulses and one for detection. They both have a length of 1.5 m because of the regulation in hospitals, which says that the equipment has to be at a distance of at least 1 m from the patient. The fibres are of the type graded-index fibres. Both fibres are connected to a measuring probe, which is held in contact to the tissue. Between the probe and the detector there is a filter wheel, which makes it possible to regulate the amount of light reaching the detector. A schematic picture of the instrument is shown in Figure 16.

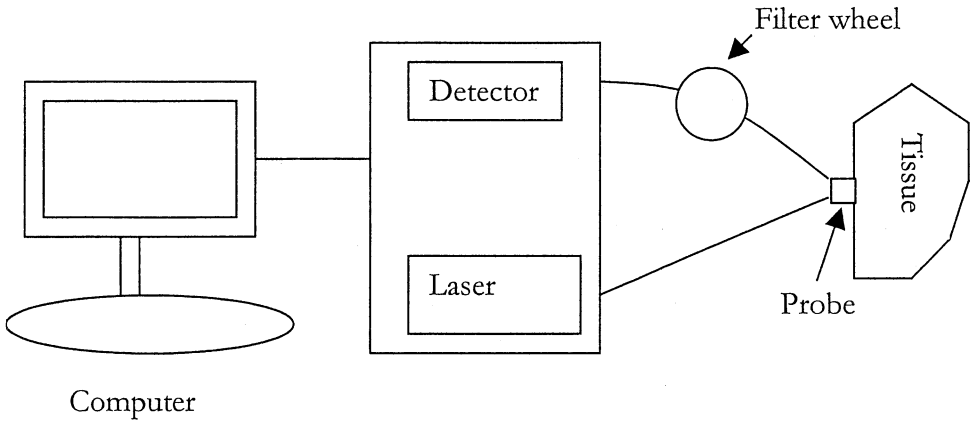

Figure 16 A schematic picture showing the main components of the instrument; a computer, a laser, a detector, a filter wheel and two fibres connecting the probe. The probe is held in contact with the tissue.

The time resolved detection is of the type time-correlated single-photon counting, TCSPC, which is a method based on single photon statistics and it detects single photons.

7.1 THE DETECTOR

The detector is a photomultiplicator, PMT, (Hamamatsu R3809-59 MCP-PMT). The principle of an ordinary PMT is that an incoming photon liberates an electron from a metallic surface in vacuum through the photoelectric effect. The liberated electron is accelerated by an externally applied electric field and soon it strikes another metallic surface, which liberates more electrons. These electrons strike a further metallic surface and liberate even more electrons and so on. In this way the signal is amplified. The first metallic surface is a photosensitive cathode and the other ones are called dynodes. After passing some dynodes, the number of them depends on how big the amplification has to be, the electrons strike the anode where the signal is detected as a current, see Figure 17.

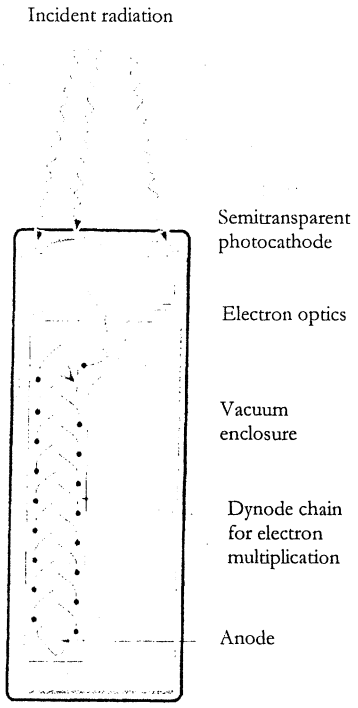

Figure 17 Schematic picture of a photomultiplicator, PMT.[22]

The detector R3809 is the fastest PMT available on the market, but also the most expensive and sensitive one. This sort of detector does not have an ordinary dynode chain. Instead there is a microchannel plate, MCP. The MCP is the most sensitive part of the detector. Inside the channels there is a coating, which decreases slowly by the influence of the signal electrons. This degradation of the channels increases if the detector is exposed to high levels of light, like when it is saturated or if the light in the room is turned on. This coating decides the lifetime of the detector.

The cathode is made of Ag-O-Cs and these types of cathodes are sensitive up to about 1200 nm[30]. This is more than necessary for the measurements in this study, where the highest wavelength is 786 nm.

A problem with a PMT is that it has a rather large dark current at room temperature, which means that there is a small current from the anode even when there are no incoming photons into the detector. The dark current is reduced when the detector is cooled. To cool the detector a Peltier element is used. This element uses a current to make one side of the element cold and one side hot. The cold side of the element is placed next to the detector, while the other side is pointing away from the detector. This makes the detector as cold as -30°C and hence reduces the dark current. The Peltier element only works well if the hot side is chilled and this is done by a water cooling system. When the detector is cooled to such low temperatures there is a risk of condensation in the detector, and to avoid this, the detector should be flushed with nitrogen before the cooling is turned on. A sufficient frequency for this has turned out to be once a week.

7.2 THE LASER

The laser source has got four different laser heads and it is diode lasers named PDL 808 Sepia which are used. The motivation for having four laser heads is to make it possible to measure with four different wavelengths at the same time. In this study only two of the lasers are used and the wavelengths of these are 660 nm and 786 nm.

There are two important requirements for the lasers. The first is that they have to produce a certain amount of pulses every second in order to achieve fast measurements. This is no problem for this laser because it has an output frequency ranging from one single shot to 80 MHz. In this study as many pulses per second as possible is preferred, which makes the choice of 80 MHz easy. A second important factor is that the instrumental function has to be as small as possible, since the machine will never be able to measure a curve that is more narrow than the instrumental function. Ideally the instrumental function should be less than 100 ps, in order to be able to measure time resolved curves with a short inter-fibre distance of 1 cm. This was not very easy to attain with the instruments in this study and it was not possible to get the instrumental function lower than 200 ps. There are several factors that determine the instrumental function such as the laser pulse length and the broadening in the instrument. The latter is e.g. due to mode dispersion in the optical fibres, broadening in the detector, time resolution and jitter in the electronics.

With the PDL 808 Sepia the lasers can be driven either synchronously, delayed or in a user defined sequence. All these modes have been used in this study.

7.3 TIME-CORRELATED SINGLE-PHOTON COUNTING

In the computer there is a module called SPC-300 from Becker & Hickl GmbH. This module contains a complete electronic system for recording fast light signals by time-correlated single-photon counting, TCSPC. The method is, as mentioned above, based on the detection of single photons of a periodical light signal. It might sound strange that it is possible to detect only one photon when the pulse comes from a laser, but the fact is that when light has low power and very high repetition rate, 80MHz in this case, the light intensity is usually very low so the probability for the detector to detect one photon in one signal period is much less than one.

The method of TCSPC is illustrated in Figure 18. When a photon reaches the detector a pulse goes from the detector to the time-to-amplitude-converter. The pulse triggers the converter and starts an internal clock. The clock stops when the next pulse from the laser is fired. This time interval is converted, in the-time-to-amplitude converter, TAC, to an electrical pulse which has an amplitude that is directly proportional to the time interval. After this the pulse is fed into a multichannel analyser that converts the amplitude of the pulse into a channel number. This number is stored in the computers memory. This happens every time a photon is detected and for every photon a count will be added to one of the channels. When the measurement is finished and no more photons reach the detector, a histogram, representing the shape in time of the signals, is formed[31].

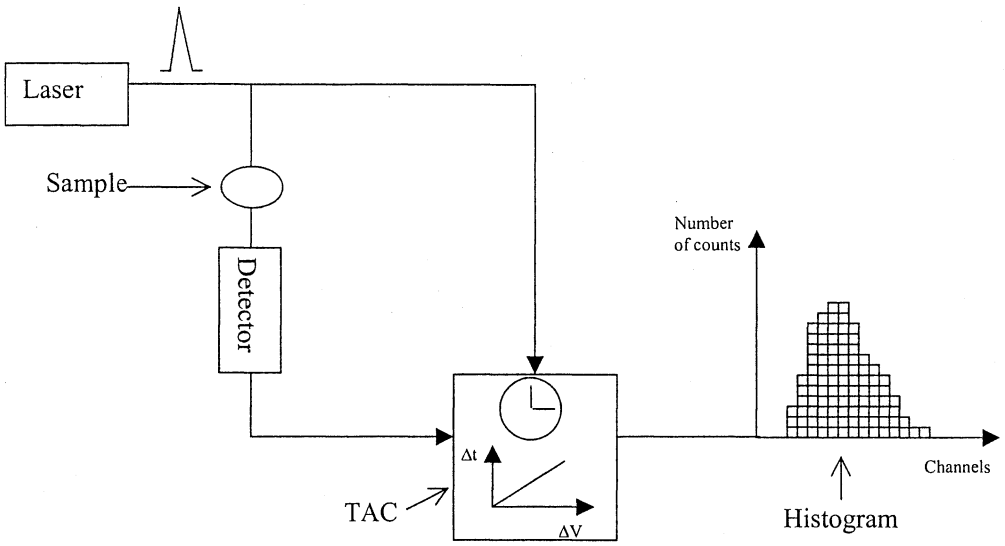

Figure 18 The principle of time-correlated single-photon counting.

A good question now is what would happen if two photons from the same pulse are detected in the detector? The answer is that only the first one would be taken into account, as the clock does not start again until after the stop signal has been sent from the laser. As mentioned above, the probability of detecting one photon per laser pulse is less than one, so it very rarely happens that two photons are detected, which means that this is not a problem. If the probability of detecting more than one photon per laser pulse gets too big, the early photons will be treated with special favour and the distribution in the histogram would be skewed toward early times. This is called the pile-up effect[21]. A guideline of making this effect negligible is to keep the ratio of detecting one photon per laser pulse as low as 1:30.

In this study the two lasers will preferably be used at the same time. This can be done either by using the lasers in sequence which means they will be driven at 40 MHz each or they can be used at the same time, which means both of them will have a frequency of 80 MHz. The last alternative was chosen in this study, because having as many pulses per second as possible is desirable since it reduces the measuring time. When the lasers are used at the same time, they are fired simultaneously. In order to get two histograms, one of the lasers has to be delayed and this was done by using a longer cable to one of them. One could then think that this would not work because the pulses from the laser with the short cable would always reach the detector first, resulting in a situation where no pulses from the other laser would be taken into account. The reason why it does not work like this is that, as mentioned above, the probability for a photon from one pulse to reach the detector is low. Due to this the photons from the laser with the longer cable will be counted some times and the laser with the shorter cable will be counted other times. This means that there will be two different histograms, one for each laser, which is desired.

7.4 THE PROBE

The demands on the probe were that it would have to keep two fibres exactly 1 cm apart from each other and that it must be easy to hold. Also it had to be as small as possible in order not to get in the way of the treatment laser. This was solved by making a probe of steel. It consists of two thin pipes, wide enough to insert a fibre into each one of them, see Figure 19. In the upper part of the pipes there is a plastic screw that makes it possible to fix the fibre in its correct position. The two pipes are held in their right positions by a small block that has two holes drilled 1 cm apart.

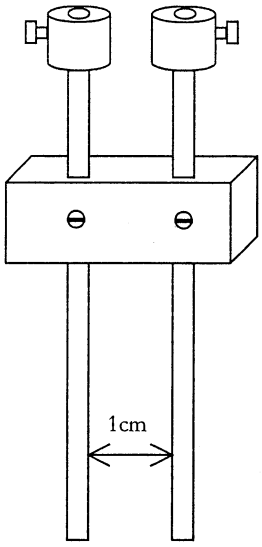

Figure 19 A schematic picture of the probe

7.5 THE FILTER WHEEL

As seen in Figure 16 the filter wheel is situated between the detector and the probe. Its function is to reduce the light before it is reaching the detector in order not to get the detector saturated. The wheel has very low transmittance in one part and then the transmittance is increasing successively until it reaches a level of full transmittance in the opposite part of the wheel. Due to this it is possible to decide how much light one wants to get into the detector by turning the wheel to the desired position. In the stand where the wheel is positioned, it is also possible to put other filters when this is needed.

8 MEASUREMENTS

The aim of this study was to measure the tissue oxygenation during PDT both non-invasively with time resolved spectroscopy and invasively with a pO_2-probe. The advantage of combining both methods is that the time resolved method monitors the oxygen bound to the haemoglobin, HbO_2, and the pO_2-probe measures the dissolved oxygen in the blood. These two types of oxygen are related to each other as mentioned in section 2.3.3, and because of that it would have been interesting to compare the two. Unfortunately this was not possible because we were not able to obtain a probe for clinical use. Due to this, only the time-resolved measurements were performed.

The intention was to investigate tumours of the type *BCC* only, but other skin diseases such as *squamous cell carcinoma* (*SCC*), *lymphoma*, *actinic keratoses* and *hereditary inflammatory skin disease* (*morbus Hailey-Hailey*) were also investigated.

All measurements were conducted at the Oncology Department at Lund University Hospital.

The protocol of the study stated that measurements were to be performed before, during and after the PDT treatment. The intention was also to see how fractionation could affect the treatment. There were though some problems with measuring during treatment so the measurement procedure had to be changed and developed during the different measurements.

For the first measurements it was not possible to measure at the same time as the PDT-laser was turned on, due to that the detector would have been saturated. Later on, this was addressed by using different filters in front of the detector. Another issue during the measurements was that in the beginning it was only possible to measure with one diagnostic laser at a time, but in the later measurements it was possible to use both diagnostic lasers simultaneously.

In the following part, the overall procedure of the performance will be presented and after that each measurement will be presented with both performance and results.

8.1 MEASUREMENT PROCEDURE

In this section a case study of the measurement and the evaluation of the data will be presented thoroughly, making it possible to understand the evaluation procedure. A total of ten measurements were performed on human skin lesions and the date, diagnosis and measurement occasions are presented in Figure 20. The measurements were performed before ALA, before PDT, two times during PDT, immediately post PDT and post PDT. In the table below the times in minutes are presented with the measurement before PDT as starting point.

Patients/Measurements	1	2	3	4	5	6
Diagnosis	*BCC*	*BCC*	*Actinic keratoses*	*BCC*	*BCC*	*SCC*
Date	030312	030312	030228	030305	030318	030404
Before ALA	NA	NA	-240	NA	NA	NA
Before PDT	0	0	0	0	0	0
First interruption	3	3	3	3	3	3
Second interruption	8	8	8	8	8	7
Immediately post PDT	10	10	10	10	10	10
Post PDT	27	20	13	20	20	13

Patients/Measurements	7	8	9	10
Diagnosis	*lymphoma*	*SCC*	*Inflammatory skin disease*	*lymphoma*
Date	030404	030404	030404	030423
Before ALA	NA	NA	NA	NA
Before PDT	0	0	0	0
First interruption	3	3	3	3
Second interruption	9	9	7	7
Immediately post PDT	14	14	10	10
Post PDT	19	19	14	15

Figure 20 Diagnosis, date and measurement times in minutes with the measurement before PDT as starting point for the 10 different patients. NA-not available for measurements.

All the patients, except for those in Measurement 7 and 8, were treated with the PDT-laser for 10 minutes. The patients in Measurement 7 and 8 were treated with a LED lamp-array from Photocure (CureLight 128) instead. The treatment in these measurements lasted for 14 minutes instead of 10 minutes. The total light dose was 60 J and the fluence rate did never exceed 120 mW/cm^2. This was the same throughout all the measurements in this study.

The measurement described in this section was one of the first in this study. At this stage it was not possible to fire the lasers at the same time, because there were side peaks from one laser that went into the main peak of the other laser and vice versa. Due to this, two measurements had to be done at each measuring occasion, first with the 660 nm laser and then with the 786 nm laser.

Another problem with this measurement was that it was not possible to measure at the same time as the PDT-laser was turned on. When trying this in a previous measurement the detector was saturated even when the major part of the backscattered light was filtered out with an optical filter. In order to avoid that this time, the PDT-laser was turned off after 3 and 8 minutes while the measurements were made, approximately 2 minutes each time.

The measurements had to be conducted in a completely dark room, due to the sensitivity of the detector. To achieve this at the hospital four big masonite covers were created and put in front of the four windows in the room.

First, an instrumental function was measured, one for each diagnostic laser wavelength. The instrumental function was measured by directing the laser light from the illumination fibre directly onto the detection fibre. To manage this without saturating the detector, a special instrument, shown in Figure 21, was made. The instrument was

fastened onto one of the rods on the probe and then the other fibre in the other rod was put into the other end of this instrument. In order not to get too much light into the detector, the instrument was squeezed together in two places, so that only a few photons could pass into the detector.

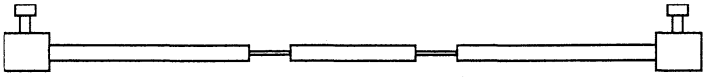

Figure 21 An instrument for measuring the instrumental function. A fibre is inserted into each end of the instrument.

The instrumental function was measured before the treatment and after the treatment. It was important that the instrumental function was as narrow as possible, otherwise the instrumental function would distort the real measurement too much. For the measurement presented in this section, the instrumental function for the 660 nm laser is shown in Figure 22.

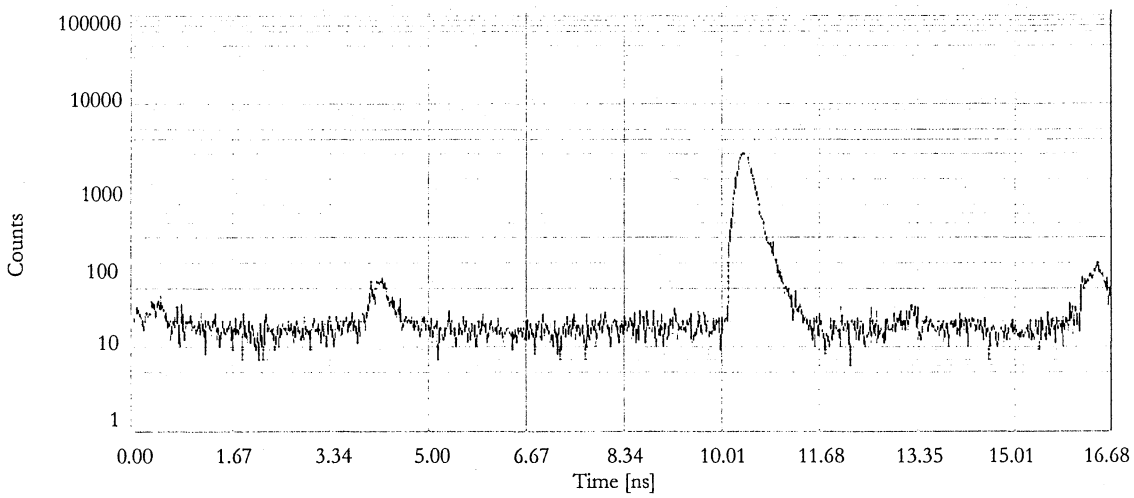

Figure 22 The instrumental function of the 660 nm laser. Number of counts is plotted as a function of time.

The full width half maximum (FWHM) value of this function was 0.28 ns, evaluated at the peak around 10ns. The two smaller peaks seen in the figure were arising from some reflections in the fibres or cables. They did not disturb the measurement when they were so far away from the main peak.

The instrumental function from the 786 nm laser had a FWHM value of 0.21 ns and is shown in Figure 23.

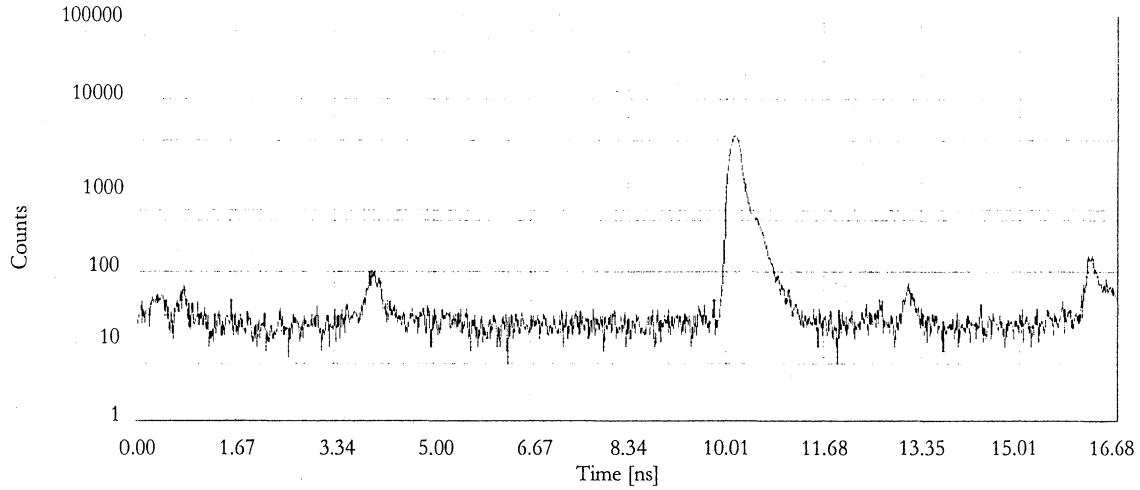

Figure 23 The instrumental function of the 786 nm laser. Number of counts is plotted as a function of time.

The measurements on tissue were performed by holding the probe gently in connection to the tumour. It was important not to press the probe too hard against the tissue, since this would disturb the perfusion. Only tumours bigger than 1 cm in diameter could be measured due to the probe geometry. The measurement done before the PDT treatment started is shown in Figure 24 for the wavelength 660 nm.

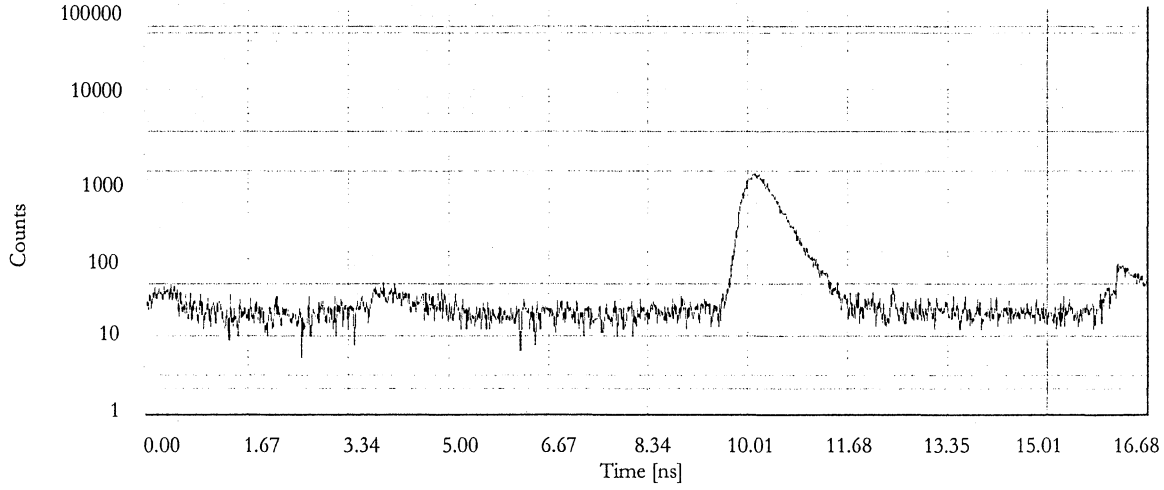

Figure 24 A measured curve on tissue with the 660 nm laser. Number of counts is plotted as a function of time.

The FWHM value of this curve is 0.55 ns. The broadening of this curve is due to both the tissue and the instrument. The measured curve is thus a convolution of the instrumental function and the actual diffuse reflectance curve at the tissue.

The curve for the 786 nm is presented in Figure 25. This curve has a FWHM value of 0.49 ns.

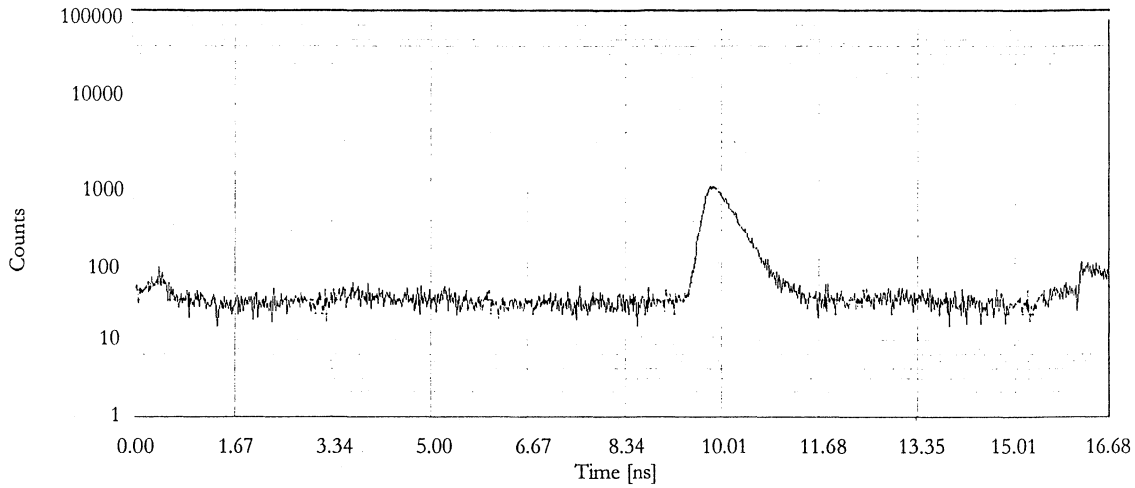

Figure 25 A measured curve on tissue with the 786 nm laser. Number of photon counts is plotted as a function of time.

To evaluate the measured curves, a program called FIT was used. As mentioned in section 4.2, the diffuse reflectance equation was fitted to the measured curve. Before this was done, the instrumental function was convolved with the diffusion equation. This was done in order to take the broadening of the instrument into account because, as mentioned before in this section, the measured curve is a convolution of the instrumental function and the actual diffuse reflectance curve.

To get the diffusion equation to fit the measured curve as well as possible, different parameters in the FIT program can be changed.

As can be seen in Figure 23 there can be more than one peak in a measurement. To only consider the one of interest, this one was cut out before the evaluation was performed. In order to compensate for the new point of origin, a free time-shift t_s is introduced, making the diffuse reflectance equation look like this:

$$R(\boldsymbol{r},t) = (4\pi Dc')^{-3/2} z_0 (t-t_s)^{-5/2} \exp(-\mu_a c'(t-t_s)) \exp\left[-\frac{(\boldsymbol{r}^2 + z_0)}{4Dc'(t-t_s)}\right]$$

The fit can be improved when the diffuse reflectance equation is allowed to be translated a little in time. The shift is also used when the signal is delayed in fibres etc. In this study, it was mainly this parameter that was changed.

Other parameters that had to be defined in the program were the ones that set the area of interest. This was done by using two markers, one on each side of the curve. According to an Italian group[27] the best results are often achieved if the left marker is set to 80% of the peak value and the right marker to 1% of the peak value, see Figure 26. When these markers are set, only the area in between will be taken into account.

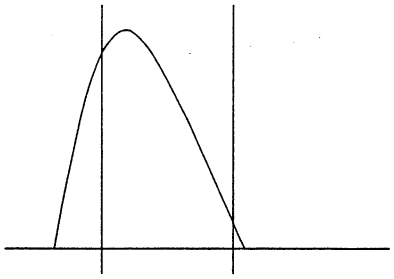

Figure 26 A schematic picture showing the two markers that define the area that will be taken into account in the evaluation. Ideally the left marker is set to 80 % of the peak value, while the right marker is set to 1 % of the peak value.

Figure 27 shows a plot from the evaluation program FIT. The curve to the left is the instrumental function, the one to the right is the measured curve and the last one, which is lying over the measured curve, is the one that has been fitted to the measured one. Even if it is not very easy to see in this figure, the fit is always better on the right hand side of the curve than it is on the left. This is due to the fact that the diffusion equation is not valid for the earliest photons, because these have not been scattered a sufficient number of times.

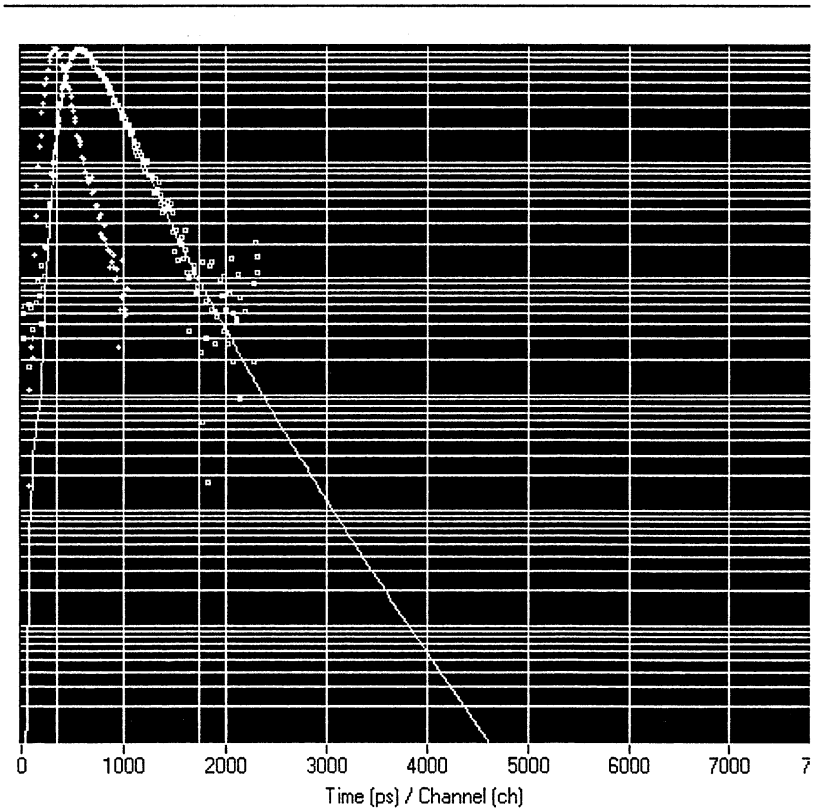

Figure 27 A figure showing the fitting. The left curve is the instrumental function, the right curve is the real measurement and on top of that one is the fitted curve.

When the fit was performed the values of μ_a and μ_s' were displayed. In order to know how good the fit was, there is a reduced χ^2 value, which describes the deviation of the measured curve from the theoretical one. The fit routine iteratively altered the fit parameters, until the lowest least square value was reached. The χ^2 value should thus be as low as possible.

8.1.1 Results for Measurement 1

The values derived when all the five measurements where analysed are presented in Table 1 for the 660 nm laser.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.11	11.8	164.90	1.00
After 3 minutes	0.11	11.1	152.50	0.52
After 8 minutes	0.12	12.6	177.90	0.48
Immediately after PDT	0.12	11.4	201.90	0.67
17 minutes after PDT	0.08	8.0	201.60	0.56

Table 1 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

In this table the χ^2 values are rather low, which should indicate that it was possible to do good fittings. The values of μ_s' is quite stable, varying from 8.0 cm^{-1}to 12.55 cm^{-1} and this seems reasonable since the scattering should not be very affected by the treatment. The values of μ_a will be used later in this section.

In Table 2 the evaluated values for the 786 nm laser are presented.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.13	12.7	30.40	0.74
After 3 minutes	0.13	11.5	6.10	0.90
After 8 minutes	0.12	8.9	5.20	0.40
Immediately after PDT	0.11	7.6	6.20	0.62
17 minutes after PDT	0.09	8.6	6.20	0.76

Table 2 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

Also here the μ_s' values are quite stable and the values of χ^2 are low.

As mentioned in section 6 the concentration of haemoglobin and oxyhaemoglobin can be calculated from the absorption coefficients, due to the relation in equation (2). The values of the extinction coefficients[32] are presented in Table 3.

Extinction coefficient	$[cm^{-1}/M]$
ε_{660}^{Hb}	3226.56
$\varepsilon_{660}^{HbO_2}$	319.6
ε_{786}^{Hb}	957.36
$\varepsilon_{786}^{HbO_2}$	740.0

Table 3 The extinction coefficients for haemoglobin and oxyhaemoglobin at 660 and 786 nm.

By solving equation (2) the concentrations of haemoglobin and oxyhaemoglobin were determined and the results are presented in Table 4.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.020	0.15
After 3 minutes	0.018	0.15
After 8 minutes	0.023	0.14
Immediately after PDT	0.025	0.12
17 minutes after PDT	0.013	0.10

Table 4 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

In Figure 28 these values are plotted versus time. The treatment time goes from 0 to 10 minutes in the figure and the measurement just before the treatment is at –1 minute.

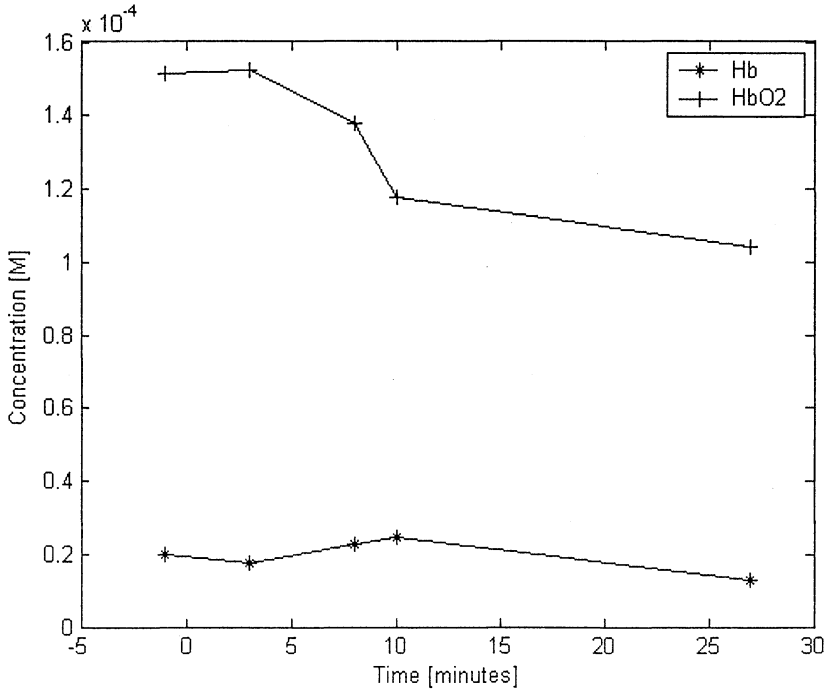

Figure 28 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

In Figure 28 one can see that the concentration of oxyhaemoglobin is decreasing during and after the treatment. The concentration of haemoglobin is not varying very much.

8.2 MEASUREMENT 2

This measurement was performed in the same way as the case study in section 8.1.

8.2.1 RESULTS FOR MEASUREMENT 2

The calculated values of the 660 nm and 786 nm laser are presented in Table 5 and Table 6, respectively.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.06	3.5	237.90	0.58
After 3 minutes	0.09	0.1	-19.90	0.81
After 8 minutes	0.07	3.0	54.30	0.56
Immediately after PDT	0.08	3.3	78.90	0.76
10 minutes after PDT	0.10	5.1	-18.00	1.20

Table 5 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.07	0.1	-40.70	0.78
After 3 minutes	0.08	3.3	-101.99	1.03
After 8 minutes	0.08	3.1	-54.90	0.85
Immediately after PDT	0.08	3.7	-66.40	0.79
10 minutes after PDT	0.08	2.7	-43.30	0.99

Table 6 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

It seems like the values of μ_s' measured with the 660 nm laser 3 minutes after the PDT started and the value measured with the 786 nm laser before the PDT are unreasonably low. This can be seen because they are both almost as low as the absorption coefficients. If this was the case one would nearly not have gotten any signal at all because so much would have been absorbed compared to the amount of photons that would have been scattered. However this was not the case because the signal was just as large as in all the other measurements.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 7.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.011	0.076
After 3 minutes	0.019	0.082
After 8 minutes	0.011	0.092
Immediately after PDT	0.016	0.088
10 minutes after PDT	0.024	0.082

Table 7 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

The values are plotted in Figure 29.

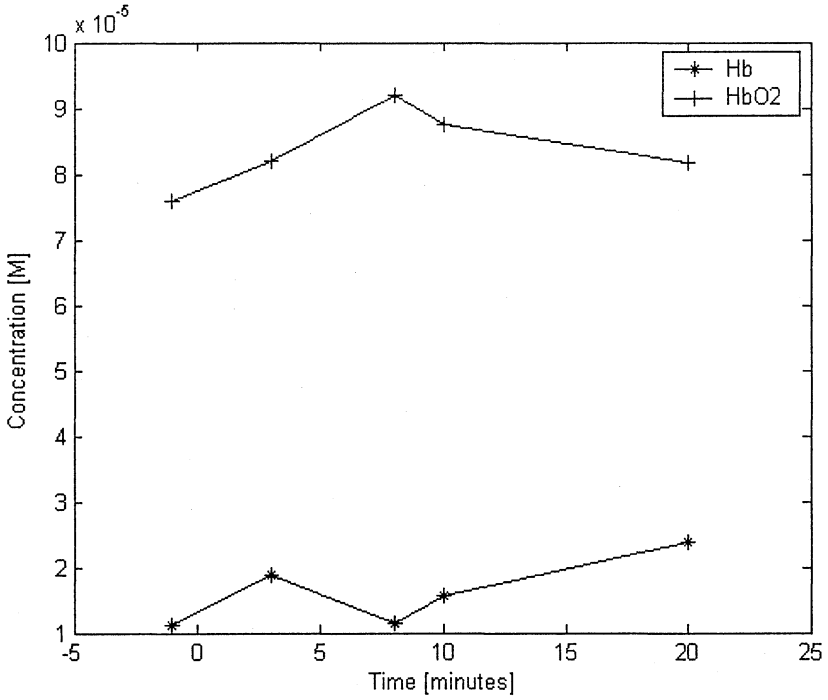

Figure 29 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

As seen in Figure 29 the concentration of oxyhaemoglobin increases during the first 8 minutes of the treatment and after that it decreases. The concentration of haemoglobin increases all the time except between 3 and 8 minutes in the treatment.

The first two values in both of the curves have a high degree of uncertainty due to the low values of the reduced scattering coefficients.

8.3 MEASUREMENT 3

The very first measurement was performed in a completely dark room with no windows. The aim was to carry out the measurements without turning off the PDT-laser. In order not to saturate the detector, five Schott filters were inserted in the mount of the filter wheel. Three of the filters were reducing the light below 660 nm and two of them were reducing the light below 645 nm. This was thought not sufficient for blocking out the PDT-laser, which had a wavelength of 635 nm. One possible problem was that the 660

filters were going to block out not only the PDT-laser, but also the light from the measuring laser at 660 nm. This seemed to be true, but enough light was passing, although the signal was not very strong. The real problem was instead that the detector was saturated even with the filters, which meant that the PDT-laser had to be turned off twice during the treatment in order to be able to perform the measurements at 3 and 8 minutes after the treatment had started.

In this measurement a measurement before ALA was applied was also performed. This was done 4 hours before the treatment started.

8.3.1 Results for Measurement 3

The calculated values of the measurement with the 660 nm and 786 nm laser are presented in Table 8 and Table 9, respectively.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before ALA	0.17	4.7	18.40	0.47
Before PDT	0.26	15.7	-67.70	0.63
After 3 minutes	0.53	68.5	-263.10	0.66
After 8 minutes	0.36	32.9	140.20	1.06
Immediately after PDT	0.22	13.7	91.70	0.71
3 minutes after PDT	0.30	29.9	53.90	0.86

Table 8 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before ALA	0.17	14.9	-127.90	0.72
Before PDT	0.33	36.0	-334.20	0.73
After 3 minutes	0.16	3.7	-209.00	2.56
After 8 minutes	0.19	11.6	140.20	0.64
Immediately after PDT	0.12	5.0	-140.00	0.58
3 minutes after PDT	0.20	15.9	-205.00	0.81

Table 9 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

Analysing the values of the reduced scattering coefficient μ_s', they seem to vary unreasonably much. It seems like something has gone wrong with these measurements. The reason is probably that there were too many filters, leading to a too low total count of photons. The fact that the detector was saturated for a short while might also have affected the measurements in a negative way. The saturated detector could explain the high value of μ_s' in the measurement with the 660 nm laser at 3 minutes after the treatment had started. The high value of μ_s' measured with the 786 nm laser before the PDT treatment started can though not be explained by that.

The concentrations of haemoglobin and oxyhaemoglobin were calculated and presented in Table 10.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before ALA	0.04	0.18
Before PDT	0.04	0.40
After 3 minutes	0.16	0.00
After 8 minutes	0.10	0.14
Immediately post PDT	0.06	0.09
10 minutes post PDT	0.08	0.17

Table 10 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

The values are plotted in Figure 30.

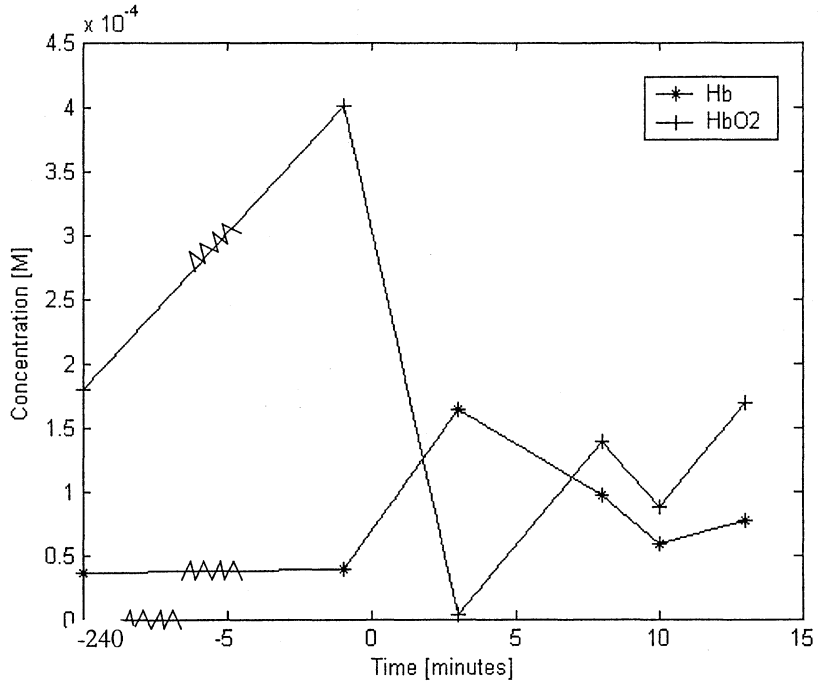

Figure 30 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

In this figure, the value at –240 minutes is the measurement made before the ALA was applied.

The high values obtained for the reduced scattering coefficients before PDT and 3 minutes after the PDT started, indicate that something went wrong in these measurements. It is thus difficult to conclude anything about the concentrations at these points.

8.4 MEASUREMENT 4

This measurement was performed in the same way as the case study in section 8.1.

8.4.1 RESULTS FOR MEASUREMENT 4

The calculated values of the measurement with the 660 nm and 786 nm laser are presented in Table 11 and Table 12 respectively.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.23	9.5	128.90	0.67
After 3 minutes	0.25	5.4	-197.90	0.76
After 8 minutes	0.20	3.6	79.90	0.83
Immediately after PDT	0.24	3.5	165.10	0.57
10 minutes after PDT	0.19	3.4	199.90	0.39

Table 11 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.22	4.2	104.20	0.48
After 3 minutes	0.23	3.9	101.10	0.48
After 8 minutes	0.25	3.5	-67.70	0.54
Immediately after PDT	0.29	8.7	-42.60	1.27
10 minutes after PDT	0.27	7.4	-44.20	0.78

Table 12 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

From these values the concentration of haemoglobin and oxyhaemoglobin were derived. The values of the concentrations are presented in Table 13

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.049	0.24
After 3 minutes	0.052	0.25
After 8 minutes	0.030	0.31
Immediately after PDT	0.042	0.34
10 minutes after PDT	0.027	0.33

Table 13 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 31.

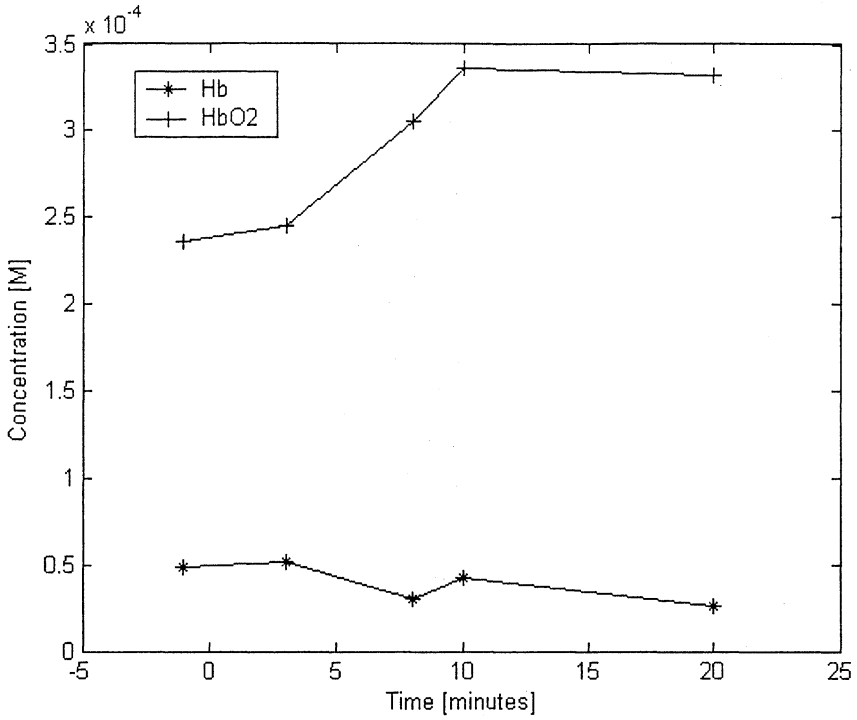

Figure 31 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

In this figure it seems as if the concentration of oxyhaemoglobin is increasing during the treatment, but after that it is decreasing slightly. The concentration of haemoglobin is fairly constant.

8.5 MEASUREMENT 5

In the measurement in section 8.3, measuring was tried at the same time as the PDT-laser was turned on. Even though five filters were inserted in order to block out the PDT-laser, it did not work. The reason for this was assumed to be that the PDT-laser was emitting light not only at exactly 635 nm but also a range of wavelengths around this one. The five filters did not block these wavelengths around the 635 nm sufficiently.

A solution to this problem was to insert a band-pass filter in front of the PDT-laser beam. This filter was supposed to transmit light only at 635 nm. Since the five Schott filters were supposed to block light at 635 nm out this should work. When this was tested in the lab it was shown that two of the 660 nm Schott filters and only one of the 645 nm Schott filters were sufficient, in order to not get the detector saturated.

At this stage it was also possible to measure with the two diagnostic lasers turned on at the same time. This was tested in the lab, but unfortunately it was not possible to do this at the same time as the PDT-laser was on. The reason for this was that when the PDT-laser was on, the Schott filters had to be placed in the mount of the filter wheel. The problem with firing the diagnostic lasers at the same time as the Schott filters were used was that the Schott filters reduced the light from the 660 nm laser substantially but it did not reduce the light from the 786 nm laser accordingly. When one laser at a time was fired it was possible to change the transmittance to the detector with the filter

wheel. So when the 660 nm laser was fired the filter wheel was positioned so that it had a very high transmittance in order of getting enough light into the detector, although the Schott filters were reducing the light to such a high extent. In the same way, when the 786 nm laser was fired, the filter wheel was positioned so that it had a much lower transmittance, because the Schott filters were not reducing the 786 nm light as mentioned before. When the two lasers were fired simultaneously it was not possible to change the filter wheel individually for the two laser beams. Instead the wheel had to be in the right position for the 786 nm laser, because otherwise the detector would have been saturated. With the wheel in this position, the light from the 660 nm laser is reduced far too much, leading to an unacceptably long measurement time. A long measurement time is possible in a laboratory setting, but not at the hospital. Thus the measurement made at the same time as the PDT-laser was turned on, was made with the diagnostic lasers fired one at a time.

In this measurement the measurement at 3 minutes with the 786 nm laser after the PDT-laser was turned on could unfortunately not be performed due to medical reasons.

8.5.1 Results for Measurement 5

The calculated values of the measurement with the 660 nm and 786 nm laser are presented in Table 14 and Table 15, respectively.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.34	23.3	-176.70	1.27
After 3 minutes	0.22	11.2	-140.70	0.85
After 8 minutes	0.35	20.1	-154.30	1.46
Immediately after PDT	0.29	12.4	-139.80	0.78
10 minutes after PDT	0.22	7.3	-140.00	0.76

Table 14 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.34	24.7	-42.50	0.68
After 3 minutes	-	-		-
After 8 minutes	0.22	3.9	103.00	0.52
Immediately after PDT	0.21	3.8	79.90	0.45
10 minutes after PDT	0.23	5.6	30.90	0.57

Table 15 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

There was a problem with the measurement done with the 660 nm laser and that was that even though the detector was not saturated, the filters and the PDT-laser made the peak very small. The background from the PDT-laser was too high making the evaluation of these curves very difficult.

The calculated values of the concentration of haemoglobin and oxyhaemoglobin are presented in Table 16. Note that no calculation of concentration could be done at 3 minutes after the treatment had started.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.070	0.37
After 3 minutes	-	-
After 8 minutes	0.091	0.18
Immediately after PDT	0.071	0.20
10 minutes after PDT	0.042	0.26

Table 16 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

The values of the concentrations are plotted in Figure 32.

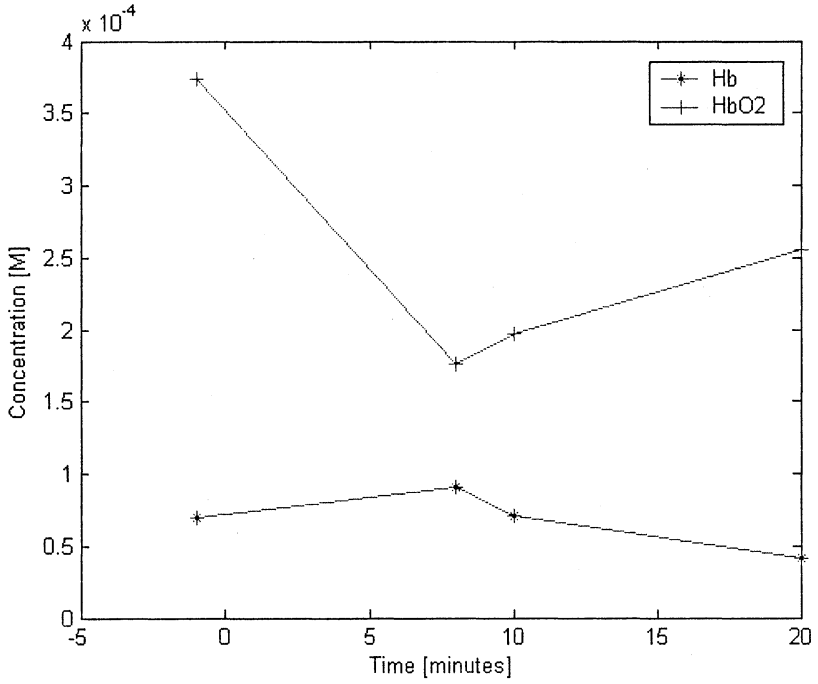

Figure 32 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

In the figure it is seen that the concentration of oxyhaemoglobin decreases considerably until 8 minutes after the treatment had started.

8.6 MEASUREMENT 6, 7, 8, 9

The following four measurements were performed on the same date, and all the measurements were made in exactly the same way. Due to this they will all be presented in this section. As seen in section 8.5 it was not very good to measure at the same time as the PDT-laser was turned on. Thus, for the measurements in this section the laser was

turned off every time the measurements were performed. This made it possible to measure with both diagnostic lasers at the same time.

The four different measurements in this section are named Measurement 6, 7, 8 and 9, respectively.

8.6.1 Results for Measurement 6

The patient in this measurement was treated for 10 minutes with the PDT-laser. The evaluated measurements are presented in Table 17 and Table 18. The last measurement was performed 3 minutes after PDT.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.28	10.2	299.50	0.78
After 3 minutes	0.26	9.6	-67.50	0.58
After 7 minutes	0.21	4.6	42.20	0.62
Immediately after PDT	0.26	11.9	8.00	0.58
3 minutes after PDT	0.26	12.0	7.00	0.53

Table 17 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.16	3.6	421.50	0.63
After 3 minutes	0.19	7.4	-79.20	1.02
After 7 minutes	0.18	7.5	-41.90	0.70
Immediately after PDT	0.16	6.1	17.90	0.50
3 minutes after PDT	0.16	4.0	67.00	0.89

Table 18 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 19.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.076	0.12
After 3 minutes	0.064	0.17
After 7 minutes	0.047	0.18
Immediately after PDT	0.068	0.13
3 minutes after PDT	0.067	0.13

Table 19 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 33.

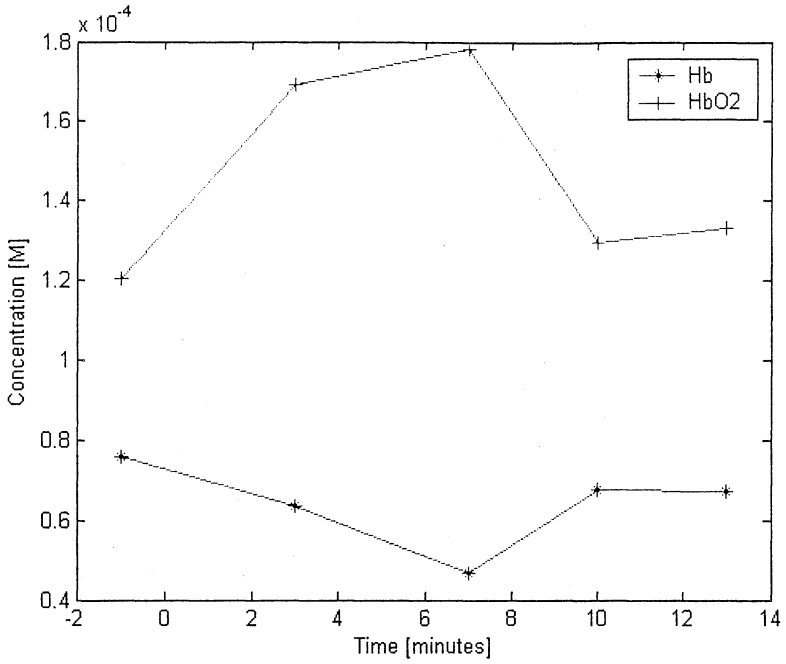

Figure 33 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

In this plot the concentration of oxyhaemoglobin is increasing during the first 7 minutes of the treatment and then it is decreasing significantly.

8.6.2 Results for Measurement 7

The patient in Measurement 7, was treated with a LED lamp-array from Photocure (CureLight 128), instead of the PDT-laser. This did not change the measurement conditions except that the treatment time was 14 minutes instead of 10 minutes. Due to this the second interruption during the PDT was made 9 minutes after the treatment started. The evaluated measurements are presented in Table 20 and Table 21.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.20	8.1	-54.60	1.03
After 3 minutes	0.19	5.3	-18.60	0.80
After 9 minutes	0.39	31.9	-237.90	0.93
Immediately after PDT	0.18	2.9	42.30	1.09
5 minutes after PDT	0.31	9.9	-54.10	1.04

Table 20 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.11	5.2	-56.40	0.76
After 3 minutes	0.26	18.5	-164.10	2.20
After 9 minutes	0.18	6.7	-118.00	1.71
Immediately after PDT	0.17	6.1	-79.00	1.63
5 minutes after PDT	0.18	5.8	-66.20	1.63

Table 21 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

In these tables the values of the reduced scattering coefficients vary considerably. The values at 9 minutes after the treatment started in Table 20 and after 3 minutes in Table 21 are unreasonably high.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 22.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.05	0.09
After 3 minutes	0.03	0.31
After 9 minutes	0.11	0.10
Immediately after PDT	0.04	0.18
5 minutes after PDT	0.08	0.14

Table 22 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 34.

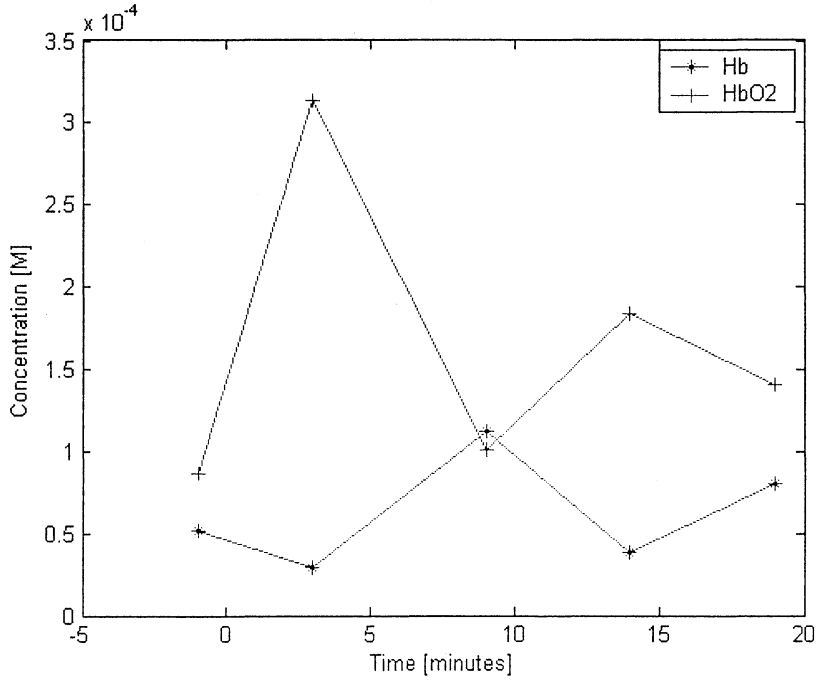

Figure 34 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

The concentration of oxyhaemoglobin varies considerably in this measurement.

8.6.3 Results for Measurement 8

This patient was treated with the CureLight 128 for 14 minutes. The evaluated measurements are presented in Table 23 and Table 24.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.48	11.2	42.30	0.76
After 3 minutes	0.72	97.3	-240.00	1.22
After 9 minutes	0.33	7.0	5.00	0.63
Immediately after PDT	0.38	5.0	19.10	0.50
5 minutes after PDT	0.44	5.6	79.80	0.91

Table 23 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.21	4.8	55.00	0.85
After 3 minutes	0.26	11.5	5.90	0.81
After 9 minutes	0.23	6.0	-6.20	0.73
Immediately after PDT	0.31	8.2	-42.50	1.10
5 minutes after PDT	0.28	4.1	79.50	0.71

Table 24 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

All the values in these tables seem to be reasonable except for the one taken 3 minutes after the treatment started with the 660 nm laser. In that measurement the reduced scattering coefficient is far too high compared to the other ones. Also the shift t_s at this point is much lower than the others. This indicates that something might have gone wrong in that measurement.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 25.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.14	0.10
After 3 minutes	0.22	0.07
After 9 minutes	0.08	0.21
Immediately after PDT	0.09	0.31
5 minutes after PDT	0.11	0.23

Table 25 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 35.

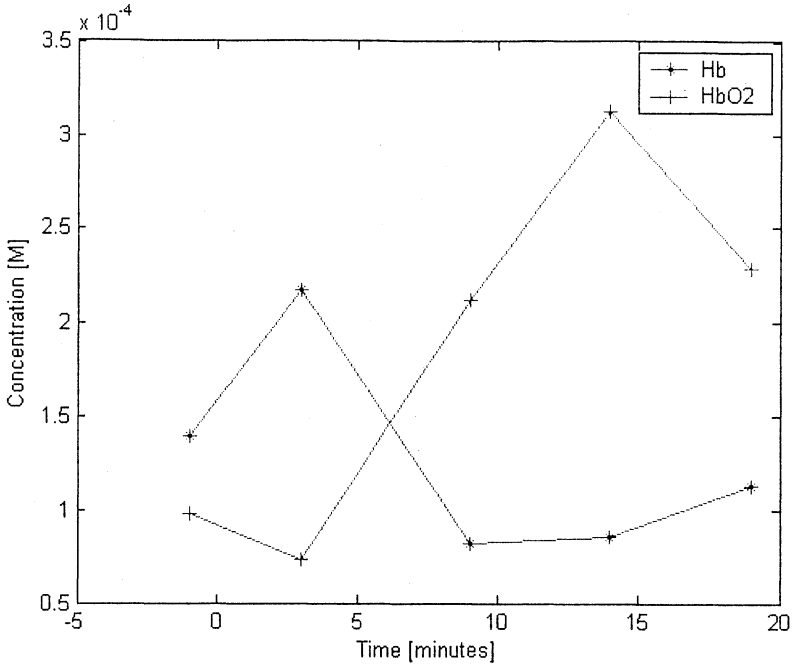

Figure 35 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

The concentration of oxyhaemoglobin started to increase after 3 minutes and lasted until the treatment was finished, after which it decreased.

8.6.4 Results for Measurement 9

This patient was treated with the PDT-laser for 10 minutes. The evaluated measurements are given in Table 26 and Table 27.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.15	4.5	29.80	0.77
After 3 minutes	0.16	3.7	90.80	0.60
After 7 minutes	0.16	5.6	55.10	0.58
Immediately after PDT	0.12	3.6	105.00	0.53
4 minutes after PDT	0.16	3.4	128.10	0.57

Table 26 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.10	3.5	44.10	0.53
After 3 minutes	0.14	0.1	-79.30	1.83
After 7 minutes	0.13	5.9	-5.90	1.13
Immediately after PDT	0.14	5.7	16.30	1.08
4 minutes after PDT	0.17	5.8	30.50	0.80

Table 27 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

It seems like the value of the reduced scattering coefficient is too low for the measurement with the 786 nm laser at 3 minutes after the PDT started. This indicates some error in this measurement.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 28.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	0.038	0.08
After 3 minutes	0.036	0.14
After 7 minutes	0.037	0.13
Immediately after PDT	0.021	0.17
4 minutes after PDT	0.029	0.20

Table 28 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 36.

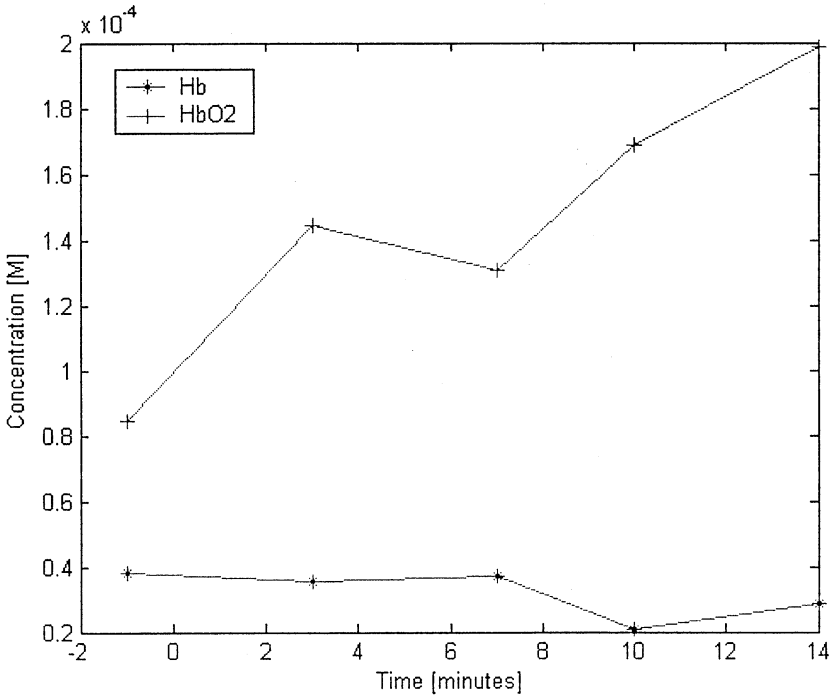

Figure 36 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time.

This figure shows that the concentration of the oxyhaemoglobin is increasing while the concentration of haemoglobin is quite constant.

8.7 MEASUREMENT 10

This patient was treated with the PDT-laser for 10 minutes. This was the last measurement made in this study and at this stage the filter wheel was placed inside the machine. Except from this, the measurements were performed in the same way as the previous measurements in section 8.6. One difference, though, was that the cooling of the detector did not work as well as before. It took three hours to get the detector cool enough and even after this the dark current was bigger than usual. Due to this it was not very easy to evaluate the measurements with the FIT-program. The right marker seen in Figure 26 had to be moved so that 8% of the right flank was not taken into account instead of only 1%.

8.7.1 RESULTS FOR MEASUREMENT 10

The results of the measurement are presented in Table 29 and Table 30.

Measurement, 660 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.13	0.2	131.8	0.75
After 3 minutes	0.29	7.9	31.30	0.39
After 7 minutes	0.27	7.6	112.90	0.77
Immediately after PDT	0.32	8.8	127.70	0.86
5 minutes after PDT	0.31	4.3	104.50	0.72

Table 29 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 660 nm laser.

Measurement, 786 nm	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	t_s [ps]	χ^2
Before PDT	0.34	9.0	-16.90	0.66
After 3 minutes	0.27	4.9	-15.00	0.96
After 7 minutes	0.30	7.4	30.40	0.75
Immediately after PDT	0.50	22.4	-33.10	1.00
5 minutes after PDT	0.31	7.0	-30.60	0.50

Table 30 The values of μ_a, μ_s', t_s and χ^2 for the measurements with the 786 nm laser.

Some of the values are a bit suspicious, e.g. the reduced scattering coefficient before the PDT started for the 660 nm measurement, which has a value of 0.2 cm^{-1}. This value is unreasonably low, because it is only about twice as big as the absorption coefficient. The reduced scattering value for the 786 nm laser taken immediately after the treatment seems too high.

The concentrations of haemoglobin and oxyhaemoglobin are presented in Table 31.

Measurement	Hb-concentration [mM]	HbO_2-concentration [mM]
Before PDT	-0.005	0.46
After 3 minutes	0.060	0.28
After 7 minutes	0.050	0.34
Immediately after PDT	0.036	0.63
5 minutes after PDT	0.064	0.33

Table 31 The concentrations of haemoglobin and oxyhaemoglobin for the different measurements.

These values are plotted in Figure 37.

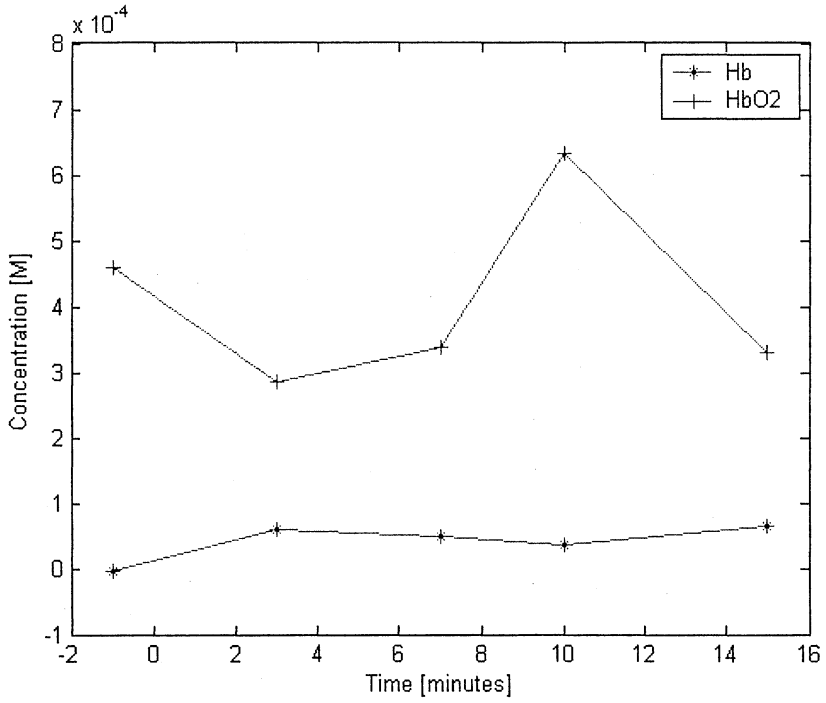

Figure 37 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function time.

Concerning the concentrations it seems strange that the first value of the haemoglobin is negative but it can probably be explained by the strange value of the reduced scattering coefficient, which indicates that something has gone wrong in this measurement.

9 EVALUATIONS

To evaluate these measurements was not the easiest thing to do. The measurements have been performed under different conditions and the results are highly varying. There are several different aspects to take into account, one of them being the reliability of the different measurements.

Other aspects that have to be considered in the evaluation are, for example, how the perfusion changed during and after the treatment, how much of the blood in the tumours was arterial and how much was veneous? Another question is if some of the oxyhaemoglobin was converted into haemoglobin. In that case the level of haemoglobin should increase when the level of oxyhaemoglobin decreases.

9.1 THE RELIABILITY OF THE MEASUREMENTS

When looking at the results in section 8, they are quite varying. There are some measurements that seem to be good, for both the reduced scattering coefficient and the time shift t_s. Then there are others that have some strange values both when it comes to μ_s' and the time shift t_s. One problem is that it is not known how much a strange value for the scattering coefficient is influencing the absorption coefficient. What is known is that, as mentioned in section 4.2, the shape of the earlier part of the curve is mostly determined by the μ_s' while the later part is mostly determined by the μ_a. Also it is mentioned in section 8.1 that the earlier part of the curve is not fitted very well to the diffusion equation. This could mean that even if a μ_s' value seems far too high or low it could be due to a bad fit on the left flank but a correct one on the right flank. But the most likely thing is that when a μ_s' value is very high or low something has gone wrong with that measurement.

In some of the measurements there are some values of t_s that differ very much from the others, even within the same measurement and with the same laser. This seems a bit strange because the markers, which define the curves, have been set to the same values throughout the measurement with the same laser. When the values are varying very much within one measurement one could suspect that something is wrong.

The following section is divided into three subsections. The first is the evaluation of the measurements with reasonable values of μ_s', the second deals with the measurements with one odd value and the last one is for the measurements that seem to have two or more strange values of μ_s'.

9.1.1 MEASUREMENTS WITH STABLE μ_s'

There are a few of the measurements that seem to have reasonable values of μ_s' and these are Measurement 1, 4 and 6. Out of these three, Measurement 1 is the best because it also has stable values for t_s. In Measurement 1, the oxyhaemoglobin is decreasing both during the treatment and after. The concentration of haemoglobin is fairly constant. This seems to support the fact that in the process of PDT, the free oxygen is consumed. This should also lead to a decrease in the oxyhaemoglobin contents.

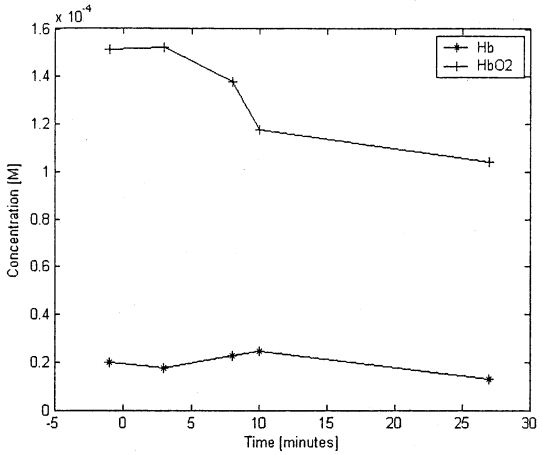

Figure 38 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time for Measurement 1.

In Measurement 4 the μ_s' values are reasonable, but there are some variations in the t_s values. These measurements show an increase in the concentration of oxyhaemoglobin during the treatment and after the treatment it is nearly constant.

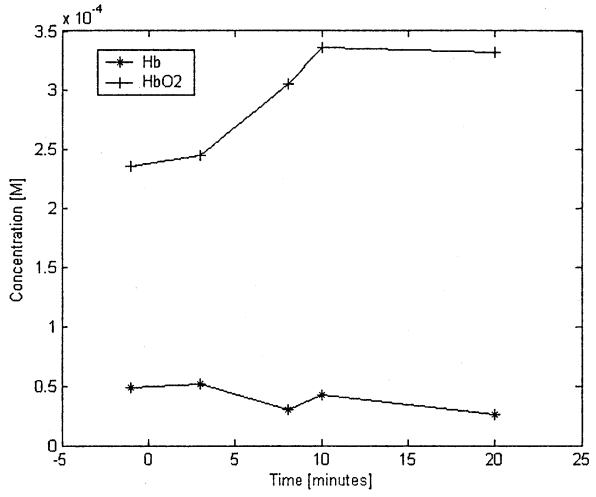

Figure 39 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time for Measurement 4.

For Measurement 6 there are also some variations in t_s but the μ_s' values are not varying too much. Here the oxyhaemoglobin is rising until it is decreasing at the end of the treatment. After the treatment it is increasing slightly again. The increase after the treatment does agree with the fact that after the treatment the levels of oxygen should start to rise again.

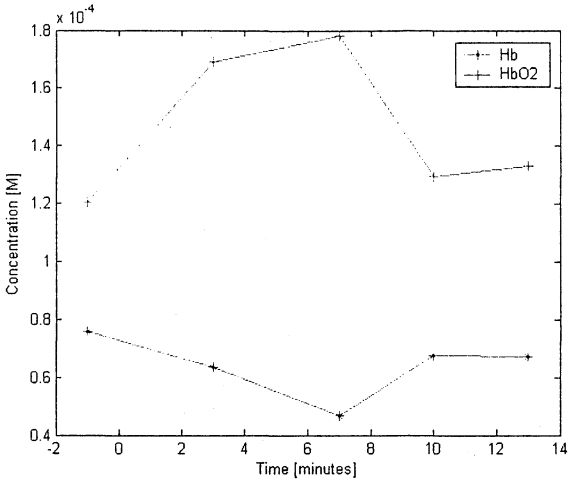

Figure 40 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time for Measurement 6.

9.1.2 MEASUREMENTS WITH ONE STRANGE VALUE OF μ_s'

In this part, measurements with one strange μ_s' value is presented. The first one with one strange value is Measurement 8. In this case it is the measurement at 3 minutes after the treatment started that is not valid. When looking at the remaining points, see Figure 41, it seems as if the oxyhaemoglobin is rising all the time during the treatment and after that it is decreasing. For Measurement 8 the concentration of haemoglobin is higher than it has been for all the other measurements.

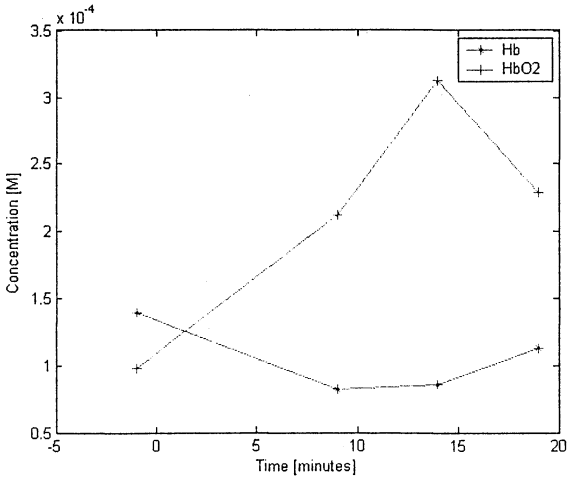

Figure 41 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time for Measurement 8.

The second measurement with one strange value of μ_s' is Measurement 9. Also here it is the measurement at 3 minutes after treatment start that is not valid. When this point is

ignored it seems like the oxyhaemoglobin is increasing all the time, both during the treatment and after, see Figure 42.

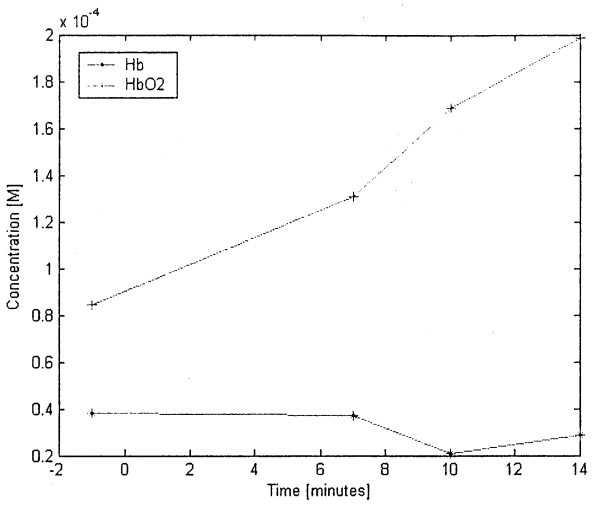

Figure 42 The concentrations of haemoglobin and oxyhaemoglobin plotted as a function of time for Measurement 9.

9.1.3 MEASUREMENTS WITH TWO OR MORE STRANGE VALUES OF μ_s'

For Measurement 3 there are three points that cannot be taken into account due to strange values of μ_s'. Due to this Measurement 3 will not be analysed more than already has been done in section 5.3.

Measurements 2, 7 and 10 had two strange values of μ_s' and it is not meaningful to analyse the remaining 3 points in the plots.

Measurement 5 is not analysed because this measurement was done at the same time as the PDT-laser was on. This made the evaluation very difficult and the results are not reliable.

9.2 PERFUSION

There have been studies made on how the perfusion changes during and after PDT[33]. In that study the perfusion has been investigated with a laser-Doppler perfusion imaging device. The conclusion of this study was that the perfusion in the tumour was the same immediately after the treatment as it was before. An hour after PDT the perfusion in the lesion had increased with 50% compared to before the PDT. In the surrounding of the tumour the perfusion had doubled immediately after PDT and was still increasing one hour after the treatment. One disadvantage with these laser-Doppler measurements is that they could not be performed at the same time as the therapeutic laser was turned on. This means that it is impossible to monitor the perfusion changes during the treatment with this method.

The perfusion is connected to the oxygenation in the way that when the perfusion increases more oxygenated blood flows to the tumour. This means that after the

treatment, when the perfusion is increasing, also the oxygenation should increase. But this would in fact happen even if the perfusion was not increasing, because after the treatment no more oxygen is consumed, so the oxygen levels should rise anyway.

When looking at the valid measurements, two of them have an increasing concentration of oxyhaemoglobin, two of them is decreasing and one is fairly stable. During PDT it seems like the oxyhaemoglobin is increasing in all the measurements, at least in the beginning, except for Measurement 1.

It would have been interesting to know if the perfusion was increasing during the treatment because that would mean that the oxygenation could rise during the treatment even if oxygen is consumed. It is not very likely that the perfusion is rising during the treatment because, as mentioned above, the perfusion in the tumour was the same after PDT as it had been before. So in that case it would first increase and then decrease. If this was the case it could mean that the concentration of oxyhaemoglobin is increasing in the beginning of the treatment and decreasing at the end of the treatment, which is the case for Measurement 6.

9.3 Arterial and Veneous Blood

When it comes to how much of the blood that is arterial and how much is veneous it is hard to find an exact number, although it is clear that in the kind of measurements done in this study, both forms are involved. In measurements done with pulse oximetry[34] the contribution from the veneous blood is subtracted so that the results from these measurements are only from arterial blood.

Probably the amount of arterial and veneous blood can vary a lot from one tumour to another. If a tumour has got much arterial blood then the levels of oxygenation should not decrease, but if a tumour has got much veneous blood then the oxygenation should be more likely to decrease.

In the valid measurements in this study this could for example be interpreted as Measurement 1 had a lot of veneous blood and Measurement 9 had a lot of arterial blood. Unfortunately this was not possible to verify by other methods in this study.

9.4 Oxyhaemoglobin converted into haemoglobin

If the concentration of oxyhaemoglobin was decreasing this would mean that the oxygen molecule is leaving the haemoglobin. This should mean that the concentration of haemoglobin would increase. It should also be the other way around, when the oxyhaemoglobin is increasing, free oxygen is bound to the haemoglobin so that the haemoglobin should decrease.

When looking at the measurements in this study this seems to be correct, in almost all the measurements the concentration of haemoglobin is increasing when the concentration of oxyhaemoglobin is decreasing and vice versa. This could be an effect of the theory described above, but it could also be due to that when the concentrations are calculated they are dependent on each other. This can be shown by solving equation (2) analytically.

$$\begin{pmatrix}[Hb]\\ [HbO_2]\end{pmatrix}=\begin{pmatrix}\varepsilon_{660}^{Hb} & \varepsilon_{660}^{HbO_2}\\ \varepsilon_{786}^{Hb} & \varepsilon_{786}^{HbO_2}\end{pmatrix}^{-1}\begin{pmatrix}\mu_{660}\\ \mu_{786}\end{pmatrix}=\frac{1}{\varepsilon_{660}^{Hb}\varepsilon_{786}^{HbO_2}-\varepsilon_{660}^{HbO_2}\varepsilon_{786}^{Hb}}\begin{pmatrix}\varepsilon_{786}^{HbO_2} & -\varepsilon_{660}^{HbO_2}\\ -\varepsilon_{786}^{Hb} & \varepsilon_{660}^{Hb}\end{pmatrix}\begin{pmatrix}\mu_{660}\\ \mu_{786}\end{pmatrix}$$

Since all the extinction coefficients are positive a change in the absorption coefficients have opposite effects on Hb and HbO_2 respectively.

9.5 FRACTIONATION

In the beginning of this study one of the aims was to see how fractionation affects the treatment. Unfortunately it was not possible to measure during the treatment. Instead the treatment had to be interrupted every time a measurement was done. This means that during each treatment two short fractionations were done. In the measurements made in the beginning, when the diagnostic lasers were fired one at a time, the measurements had a duration of about 2 minutes, one for each laser. In the last measurements, when the two lasers were fired simultaneously, the break only lasted about 1 minute. During this time some re-oxygenation has probably occurred, see section 2.3. This could be the reason why the levels of oxyhaemoglobin are increasing during the treatment in most of the valid measurements.

The levels of oxygen should have recovered more in the measurements with a duration of 2 minutes than in the ones with 1 minute. By looking at the plots it is however impossible to come to any conclusion about this. It is hard to say what would have happened if no fractionation had been done.

9.6 BLOOD VESSELS

Different tumours have probably different amounts and sorts of blood vessels. When the probe is held onto the tumour there is a chance that it is placed immediately above a large vessel, or in contrary on some small vessels with veneous blood. This would make quite a big difference for the measurements. This might be one of the reasons why the measurements are quite varying.

9.7 THE INSTRUMENT

One of the most important things to take into account in the evaluations is how stable the instrument is. The instrument MAAN 230 has never been used before in clinical applications and the instrument is not evaluated in the form it has been now. Before the instrument was constructed it had been used with all the parts separately, which means that the detector, the laser unit and the cooling system e.g. were not placed together. In those measurements also another detector than the one used for these studies was employed. In this form it has been evaluated[35] and is considered to be reliable. This evaluation should have been redone when the instrument was rebuilt. Unfortunately this study had been postponed 3 months already during the time the instrument was rebuilt and when it finally was working, there was no time to evaluate the new system in detail

before the real measurements had to be started. Some phantom measurements were however made after the real measurements, see section 9.7.1.

When the system was designed, it was supposed to have an instrumental function with a FWHM value of 0.15 ns. This was not possible to achieve in reality where the instrumental function had a width between 0.2 and 0.28 ns in all the measurements. An instrumental function as wide as this, when the measurements had a FWHM value of about 0.5 to 0.6 ns, is not ideal. This could be one of the reasons why some values are looking odd in the measurements that are considered not to be valid in this study. One solution to avoid this problem could have been to separate the rods in the probe so that the distance between them would have been more than 1 cm. This would have made a wider measurement curve which would have been less affected by such a wide instrumental function. The reason why this was not done was that when the measuring fibre and detecting fibre are more separated, this leads to a larger measurement depth. This was not desired in this study where thin BCC were investigated. Also the detected signal would have been very weak resulting in a long measurement time, which is not desirable in a clinical setting.

Another problem with the instrument was that the cooling was not working very well all the time. This made the dark current higher than it probably would have had to be. The reason why the cooling was not working as it should was probably that the water hoses inside the instrument were sharply bent, not allowing enough water to flow through.

9.7.1 PHANTOM MEASUREMENTS

After all the clinical measurements were performed, some new measurements were performed on a phantom with known values of μ_a and μ_s'. These measurements were conducted in order to establish how reliable the instrument was and also if the instrument used to measure the instrumental function, see Figure 21, was influencing the measurements. The misgiving about the instrument in Figure 21, was that it might influence the instrumental function due to that the photons are scattered inside the rod before they are reaching the detector. In that case all the measurements could be wrong. To get to know the truth, one instrumental function was taken with the instrument and one was taken by letting the detection fibre and illumination fibre be placed in holders separated from each other. This means that the laser beam was passing free in the air from one fibre to another. This had not been allowed at the hospital, but it is no problem in the lab. After the two instrumental functions were taken two measurements were performed with the probe on a phantom with a μ_a value of 0.05-0.06 cm^{-1} and a μ_s' value of 12.5 cm^{-1}. The results from the measurements are presented in Table 32.

Instrumental function in air	μ_a [cm^{-1}]	μ_s' [cm^{-1}]	χ^2
Measurement 1	0.12	6.2	1.48
Measurement 2	0.13	6.4	1.45
Instrumental function taken with the instrument			
Measurement 1	0.11	6.6	1.58
Measurement 2	0.13	6.4	1.48

Table 32 Values of μ_a, μ_s'and χ^2 for the measurement made with an instrumental function taken in air and with an instrumental function taken with the instrument in Figure 21, respectively.

As can be seen in this table, the values of μ_a and μ_s' do not differ very much whether the instrumental function is measured in air or with the instrument. The conclusion is that the instrument should not have affected the measurements negatively. A problem is, though, that the values do not agree with the values of the phantom. This means that the absolute values in the measurements in this study could be wrong, but the trends in the haemoglobin and oxyhaemoglobin curves should probably be correct.

9.9 THE EVALUATION PROGRAM FIT

The evaluation program FIT developed by Alessandro Torricelli and Antonio Pifferi et al. at the Politectnico in Milan[27] is very powerful but not very user-friendly, especially when there is limited documentation and no real user guide. By varying different parameters, e.g. the area of interest or the shift t_s, completely different results can be achieved. Also, it is possible to keep the scattering coefficient to a fix value if one thinks this parameter is not varying a lot in the measurements. If this is done, other results are achieved. What is right and wrong is hard to say, but at least all the measurements are evaluated in the same way in this study.

When it comes to the χ^2 value, this should indicate if the fit is good or bad. This is not the case all the time, though. For example in Measurement 2, where there is an unreasonably low μ_s' value, the χ^2 value is as low as for all the other values which have normal values of μ_s'.

10 CONCLUSIONS

The aim of this study was to see if the levels of oxyhaemoglobin were sufficient in the tumours during PDT. To do this with time-resolved spectroscopy using the present instrumentation is not an easy task. There were a lot of difficulties out of which the main one was that the wavelength of the PDT-laser was so close to the wavelength of one of the measurement lasers. This made it hard to block the PDT-laser from effecting the measurements. Due to this difficulty the PDT-laser had to be switched off during the measurements, leading to two short fractionations. The measurements made during the same time as the PDT-laser was turned on were too bad to be evaluated.

In the valid measurements, no clear trend could be seen in the concentration of oxyhaemoglobin. Instead the concentration was increasing after PDT in some of the measurements and decreasing after some. One could however see that in most of the measurements the oxygenation was increasing during PDT. It is not very surprising that the measurements do not show a uniform trend, because there are many aspects to take into account that can vary from one tumour to another, e.g. perfusion, the amount of arterial and capillary blood and also the locations of blood vessels. A conclusion that could be drawn, though, is that the oxygenation is not drastically decreasing at the times measured during the treatment. This could be due to the fact that the treatment in those measurements was fractionated. Because of this it is hard to say if there is enough oxygen during a normal treatment done without fractionation.

It is, however, hard to know how reliable the measurements are because the instrument and evaluation program have never been thoroughly evaluated.

In the future these measurements could be improved. By using a special interference-filter it would be possible to totally block the PDT-laser out and it would then be possible to perform the measurements during the treatment.

There are big potentials in time-resolved measurements but before the instrument MAAN 230 is used again it should be thoroughly tested and evaluated. Also evaluation and development of the FIT program should be performed.

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