

# An in vitro method for continuous haematocrit monitoring with simultaneous haemolysis detection

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## *Abstract*

Due to the relationship between blood volume and hematocrit in a haemodialysis patient Gambro's Blood Volume Sensor (BVS) continuously monitors the hematocrit (the quota of red blood cells in full blood) to determine any changes in the blood volume. In its current state the BVS continuously monitors infrared light transmission from a single light emitting diode through the bloodlines of one of their haemolysis machines, the AK200.

The purpose of this thesis was to improve on the BVS. The aim was to measure the hematocrit and to detect haemolysis of bovine full blood by monitoring the light transmission at multiple wavelengths in the infrared spectra.

Different levels of hematocrit were measured by adding certain amounts of a 0.7% NaCl solution between measurements. Haemolysis levels from 15 % were detected by identifying relative transmission signal strength of multiple wavelengths. Three laser diode modules at 809nm, 930nm and 980 nm were used. Each laser measured on a separate amount of blood taken from the same batch.

Full blood, bovine or human, scatters light very much. In the BVS an aluminum holder that surrounds the illuminated part of the blood line is worn out over time and the reflecting properties of its surfaces change. To reduce the signal's dependence on reflected light the polished aluminum holder was replaced by an absorbing holder made of a black polyvinyl.

A model of the light propagating through the BVS optoholder was also made in the optical simulation program FRED. The purpose of the model was to see how different parameters like scattering coefficients, absorption coefficients, refractive indexes and reflections affected the transmitted light so that the experimental results could be validated.

The haemolysis detection results were validated in a spectrometer at haemolysis levels from 2 %.



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# 1. INTRODUCTION

## 1.1. Haemodialysis treatment in general

When a patient suffers from kidney renal failure haemodialysis is a common method for removing unwanted blood contents like potassium, urea and surplus water. It is one of three available medical techniques for treating patients with kidney failures, the other two being peritoneal dialysis and kidney transplantation (1).

Haemodialysis is performed by connecting a patient's blood circuit to a haemodialysis machine that ultrafiltrates the patient's blood. While performing a haemodialysis treatment the machine continuously keeps track of a number of parameters that are important for the treatment. This is done because the concentrations of the blood contents are shifting a lot during a haemodialysis session and these values imply what state the patient is in. One such parameter is the haemoglobin concentration which is used to approximate the patient's blood volume. The blood volume, or BV, is, as the name implies, the total volume of a person's blood plasma and blood cells.

If the ultrafiltration exceeds the refilling rate of the blood compartment the patient's blood volume may sink to the point that the patient enters a serious medical condition called hypovolaemia, or in this case dialysis-induced hypovolaemia. If this happens the patient may suffer from multiple organ failure, brain damage, coma or even death. It is therefore relevant to continuously monitor blood volume changes during a haemodialysis session (2).

## 1.2. The Gambro Blood Volume Sensor

Gambro's haemodialysis machine, the AK200 S, utilizes the Gambro Blood Volume Sensor, BVS in short, to monitor changes in a patient's blood volume by continuously measuring the haemoglobin concentration in the blood. This is accomplished by measuring the infrared, or IR, light that is transmitted through a part of the blood lines used to connect the machine to the patient's blood circulation. This part of the blood line will be referred to as *the cuvette* in rest of the text. The light is emitted by a LED, or Light Emitting Diode, and is detected by a photodiode.

To measure changes in the blood volume of a patient, the BVS continuously measures the transmitted light. This signal is unfortunately dependant on a wide variety of parameters, but is intended to measure the amount of attenuating elements in the blood. Attenuating elements are particles or molecules that absorb or scatter the light so that it doesn't reach the detector. Pure undiluted blood, or full blood as it is called in medicine, interacts with light in quite complicated ways which will be explained more carefully in section 2.3 "*Light Propagation and Interaction in Full Blood*".

For better or worse the transmitted signal is interpreted as a function simply of the haemoglobin concentration. The haemoglobin concentration in turn is approximated to be inversely proportional



to the blood volume, so knowing the changes in haemoglobin concentration over a period of time gives a good estimate of the blood volume change.

There are several derivatives of haemoglobin, all with different optical characteristics. To avoid treating the derivatives as individual parameters the wavelength of the light is chosen to a specific isobestic point where the optical characteristics of the derivatives are identical. Thus the concentrations of various types of haemoglobin can be treated as one parameter.

### **1.3. Modifications of the Blood Volume Sensor**

In this thesis a number of changes were made to the BVS to improve its performance. Multiple wavelengths were used instead of a single isobestic wavelength. The light source was changed from LED to laser, and the holder for the cuvette was made absorbing instead of reflecting.

#### **1.3.1. Absorbing Cuvette Holder**

Each BVS unit must be calibrated to be able to measure the blood volume changes. The optical part of the BVS is wired to a separate IC board that stores calibration data and measuring algorithms. However, the cuvette is held in a holder of polished aluminum. The state of the polish deteriorates over time, especially if the machine is in frequent use. Since blood has a scattering effect on light it is assumed that a portion of the emitted light exits the cuvette and reflects off the polished aluminum back into the cuvette and into the detector. As the polish of the aluminum deteriorates its reflectiveness is assumed to decrease which causes the initial calibration values that were assigned to a unit to become inaccurate over time.

A part of this thesis describes an attempt to make the BVS independent of the light scattered out of the cuvette by replacing the reflective aluminum holder with a holder made out of a black plastic material called delrin. This material absorbs the IR wavelengths instead of reflecting them back into the cuvette. The downside of this change is of course that less overall signal is detected resulting in a reduced Signal-to-Noise Ratio, or SNR.

#### **1.3.2. Haemolysis Detection**

In the haemodialysis machine AK200 S the flow in the blood lines is created by so called blood pumps. This is done by squeezing the lines in sequences which in effect creates a valve pump. As the blood is very delicate the pumping must be performed as gently as possible to protect the blood from getting destroyed. If the lines get bent the red blood cells' membranes can break open and release the contents. When the membrane is destroyed and the contents are released to flow freely in the blood the cell is said to haemolyse. Mostly the lines only haemolyse a small fraction of the blood, the treatment can be disastrous otherwise. A way to detect any haemolysis during a haemodialysis session would therefore be interesting.

### **1.3.3. The Laser as a Light Source**

To improve on the signal strength the LED was replaced by a diode laser module. There are two main advantages of using a laser instead of a LED. First of all, the light is collimated which has a positive impact on SNR. Laser light is also a lot more monochromatic (3) which is very good if a specific wavelength is desired, like in this case where the isobestic wavelength is used to eliminate the dependence of different haemoglobin types.

## **1.4. The Goals**

The main goal was to construct a sensor that could monitor the haematocrit in full blood and simultaneously scan for any signs of haemolysis. To achieve this, a theoretical model was created in the optics simulation program FRED to get a good understanding of how the light is distributed in the BVS. The sensor was supposed to be a modification build on the BVS, using the same blood lines, detector and cuvette geometry.



## 2. THEORY

### 2.1. Dialysis – Cause, Effect and Anatomy

#### 2.1.1. Renal Failure

Renal failure, or uremic poisoning, is a serious medical condition. It occurs when a person's kidneys fail to function adequately. Physiologically this means that the glomerular filtration rate is decreased i.e. that the kidneys can no longer remove the waste products and excess fluids from the blood. The urine production is reduced and its components accumulate in the body which causes a lot of body systems to deteriorate. Common symptoms can be fatigue, anorexia, itching and nausea.

There are two forms of renal failure acute renal failure and chronic kidney disease. Acute renal failure means as the name suggests a quick decrease in renal function. There are many causes for acute renal failure and the treatment is highly individualized. Sometimes dialysis is required as treatments can take a long time. Chronic kidney disease, or CKD, can have several stages. Stage 1 CKD is the mildest stage and has few overt symptoms while stage 5 CKD is a very serious medical condition which requires either a kidney transplant or regular dialysis. Stage 5 CKD can be developed over a period of time or be the result of a long term acute irreversible disease (4).

#### 2.1.2. Dialysis

Dialysis is a treatment used to compensate for the lost kidney function of a patient by cleaning the patient's blood from waste products and excess water. There are two main types of dialysis treatment haemodialysis and peritoneal dialysis. Peritoneal dialysis won't be treated here since the BVS is used solely for haemodialysis.

##### *Haemodialysis*

In haemodialysis access to the patient's blood is required. The blood is let out of the patient through catheters that are connected to the patient's circulatory system. To get a higher blood flow an arteriovenous fistula is often created in the patient. This means that a surgeon connects an artery and a vein in some part of the body, bypassing the capillaries, to create a very high blood flow. Two catheters are connected to the fistula in order to draw and return the blood.

The blood is led through a line that connects to the dialyzer. The dialyzer consists of hollow fibers made of a semi-permeable material. The blood flow is led through these fibers. Around the fibers is a space through which a dialysis fluid is led, but in the opposite direction. Osmosis causes the unwanted waste products in the blood to pass the semi-permeable fibers into the solution. To remove the surplus water from the blood the hydrostatic pressure is increased on the blood side which causes the blood to pass the semi-permeable filter. The process of removing excess fluid is called ultrafiltration (5).

### *Problems Caused by Haemodialysis*

During a dialysis session the fluid removal rate can be faster than the body's ability to refill the blood plasma. This causes the intravascular blood volume to decrease and the patient enters a condition called hypovolaemia. This fluid imbalance can cause hypotension, multiple organ failure, cramps, dizziness and nausea (6).

Hypotension in itself is a major problem in dialysis since more than half of the patients experience reduced blood pressure following a haemodialysis treatment (7). If the dry weight estimation of the patient is erroneous the haemodialysis machine can draw too much water from the patient and cause hypovolaemia. The dry weight is even defined as the lowest weight a patient can tolerate without developing the symptoms of hypotension (8). The monitoring of blood volume changes in the patient during a haemodialysis session is therefore relevant.

#### **2.1.3. Blood**

The contents of blood can be divided into blood plasma and the different types of blood cells. The blood plasma is composed mostly of water, approximately 90% of the volume. The rest of the blood plasma is a mix of various proteins, nutrients, electrolytes and waste products (5). The main electrolytes affecting the shape of blood cells (see 2.2.3) are sodium and potassium. Table 2.1 shows a table of experimentally measured concentrations of electrolytes in bovine blood along with other data from (9). Table 2.2 shows data on various bovine blood properties obtained from paper (10).

	Hb [g/l]	O <sub>2sat</sub> [%]	pH	pCO <sub>2</sub> [mm Hg]	Na [mmol/l]	K [mmol/l]
<b>Min</b>	80	96	7.21	56	141	2.25
<b>Max</b>	180	100	7.33	73	148	3.0

Table 1:

Experimentally acquired haematologic data of bovine blood. Sodium has a much higher concentration than potassium and is the electrolyte that affects the osmolality of the blood the most.

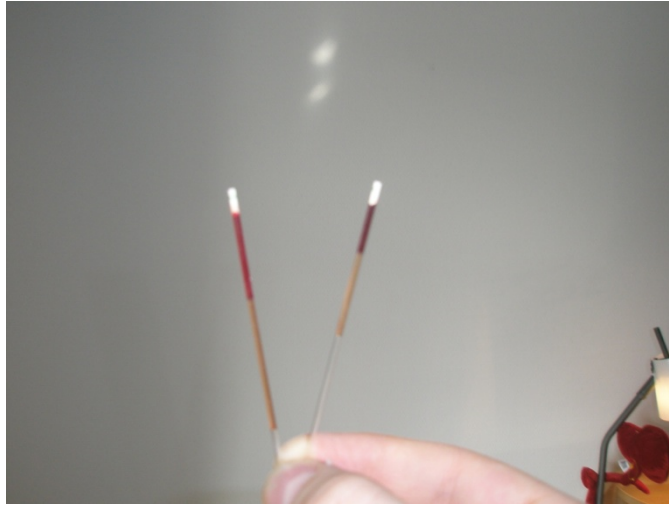
	Field Range	“Normal” (Target) Range
<b>Haemoglobin [g/dl]</b>	12.7 – 14.0	13.4 (10.4 – 16.4)
<b>Mean Corpuscular Hb[pg]</b>	14.9 – 14.9	14.9 (13.9 – 15.9)
<b>Mean Corpuscular Hb Concentration [g/dl]</b>	10.9 – 63.0	37.0 (35.0 – 39.0)
<b>Mean Corpuscular Volume [fl]</b>	13.0 – 63.7	40.4 (35.4 – 45.4)
<b>Packed Cell Volume [l/l]</b>	0.106 – 0.614	0.360 (0.30 – 0.42)
<b>Platelets [1/l]</b>	275 – 1300	812 (750 - 875)
<b>Red Blood Cell Concentration [10<sup>12</sup>/l]</b>	8.5 – 9.5	9.0 (8.0 – 10.0)
<b>White Blood Cell Concentration [10<sup>9</sup>/l]</b>	6.5 – 25.5	7.2 (6.2 – 8.2)

Table 2:

The data shows haematologic data of cattle. “Field range” values are typical haematologic values obtained from field tests and “normal range” values are values that are considered to lie within the normal range of healthy cattle. It can be seen that the red blood cells vastly outnumber the white ones, the mean cell values can therefore be assigned to erythrocytes with a small margin of error.

The blood cells can be separated into thrombocytes, erythrocytes and leukocytes. The thrombocytes are responsible for blood clotting and the leukocytes are a part of the body’s immune system – they are also known as the white blood cells. The erythrocytes, or the red blood cells, are the one transporting oxygen in the blood. The volume concentration of blood that consists of red blood cells is called the haematocrit (5).

The haematocrit can easily be measured by using a centrifuge. If a small cuvette containing blood is placed along the radial direction and subjected to the centrifugal acceleration the heavy blood cells will push past the plasma and end up stacked upon each other in one side of the cuvette. The rest of the cuvette will be filled with a slightly yellow colored transparent liquid that is the blood plasma. By simply measuring the amount of blood cells and plasma with a ruler or micrometer the haematocrit can be determined. Figure 2.1 shows a centrifuged cuvette.



*Figure 2.1: Two centrifuged cuvettes containing blood. The left cuvette contains haemolysed blood which can be seen as it is a little brighter.*

#### **2.1.4. Haemoglobin and its Derivatives**

The red blood cells are responsible for the transportation of oxygen in the blood and thus they contain the molecule that is common for nearly all vertebrates, haemoglobin. An unbound haemoglobin molecule, the reduced haemoglobin HbR, has the ability to bind oxygen to itself and become oxygenated haemoglobin HbO (11). This causes the optical properties of the molecule to change as described in section 2.2.4.

Other types of haemoglobin can also occur if the HbR molecule is exposed to molecules that are compatible with the bindings of the haeme group. Smokers for example have an elevated concentration of haemoglobin bound to carbon monoxide called carboxyhaemoglobin or COHb. This is the reason why carbon monoxide is toxic. If the haemoglobin molecules are taken by carbon monoxide there is no oxygen transport which results in suffocation. A typical value on the fraction of CO bound haemoglobin can be 1-1.5% in a non smoker while smokers can have up to 13%. The work presented in this thesis is performed on bovine blood, so any influence of COHb has been ignored.

Haemoglobin derivatives such as methaemoglobin (MetHb) and sulphaemoglobin (SHb) can also occur but their concentrations are so small that their influence can be omitted. Excessive use of drug abuse can increase MetHb concentration. The influence of MetHb and SHb has been omitted in this work for the same reason as with COHb (12).

#### **2.1.5. Bovine Blood as Substitution**

The experiments described in this thesis were made on bovine blood. Experimenting with human blood is expensive and requires special handling since the blood can be infected with diseases.

Both human and bovine blood is made up of red blood cells containing haemoglobin but they have different cell sizes, haematocrit and intracellular haemoglobin concentrations. The bovine blood cell has a mean corpuscular volume, or MCV, somewhere around  $40 \mu\text{m}^3$  (10) while the human blood cell

has  $80\text{-}100\ \mu\text{m}^3$ . The MCV is calculated by dividing the packed cell volume of centrifuged blood with the red blood cell count.

The mean corpuscular haemoglobin concentration, MCHC, is 37 g/dl (10) in bovine cattle while humans have 34 g/dl on average. The MCHC is calculated by dividing the total Hb mass from a centrifuged blood sample by the packed cell volume.

Even though the same haematocrit values for both blood types will have slightly different amounts of haemoglobin the possibility to detect haemolysis while measuring haematocrit should be comparable. The high concentration of haemoglobin in the bovine blood can be seen as a worst case scenario for human blood.

## **2.2. Blood Properties that Affect the Light Transmission**

### **2.2.1. Haematocrit**

There are numerous factors that affect the transmission of light through a sample of full blood. The volume concentration of erythrocytes in the blood is a very important one since they contain haemoglobin molecules, which are responsible for most of the light absorption. As can be seen in Figure 2.2 the various haemoglobin molecules absorb strongly in the visible spectrum, but as the spectrum approaches the IR the absorption coefficient begins to decline. This is the reason why blood is red and why IR light was chosen for the transmission measurements.

Also a difference in refractive index between the cell membrane and the surrounding cell plasma causes the blood cells to scatter incoming light. The total attenuation of light in a certain direction is thus dependant on both absorption and scattering by erythrocytes.

The erythrocyte ratio in blood is roughly equal to the haematocrit, since the majority of the blood cells are actually erythrocytes. This makes the haematocrit a good estimation of the scattering and absorption properties of the blood.

### **2.2.2. Haemolysis**

When the cell membrane of a red blood cell is destroyed the content of the cell is released into the cell plasma where it can flow freely. This process is called haemolysis and it can affect the light transmission significantly.

It was assumed that the destruction of the cell membranes would reduce the scattering coefficient of the blood while the concentration of absorbing molecules still remained constant. This would result in an increase of transmitted light. The experiments performed in this work proved that assumption to be wrong though. The light transmission decreased significantly with haemolysis as can be seen in the results. This will be discussed further in the discussion in section 5.3.2.



### 2.2.3. Osmolality

In a solution that contains a semipermeable membrane gets affected by a hydrostatic pressure that arises from different concentrations of solute across the membrane. The osmolality is a measure of the number of molecules of a solvent that affect the hydrostatic pressure per kilogram of solvent.

Normally in blood the osmolality inside and outside a cell is equivalent. If the osmolality on the inside or the outside of the cells changes, the hydrostatic pressure increases on one side so that water flows into or out of the cell to equalize the osmolalities.

In blood the osmolality is strongly dependant on the concentrations of electrolytes in the blood, mainly sodium and chloride. If the concentration of sodium in the blood plasma is increased, the osmolality of the plasma will be higher outside the cells. Since variations of the osmolality across the cell membranes cause the cells to change shape, as the cell sucks up or releases water, the scattering of light will be affected (see effects of Mie equations in section 2.3.1) and thus the transmission (13).

During haemodialysis the only main parameters that affect the osmolality of the cell plasma is the urea and sodium. Urea doesn't affect the cell shape since the molecules responsible for the hydrostatic pressure can penetrate the semipermeable cell membrane. This leaves the sodium as a single parameter that affects the osmolality (14). A table of electrolyte concentrations can be seen in *table 2.1*.

### 2.2.4. Haemoglobin Derivatives

The various haemoglobin derivatives described in section 2.1.4 all have different absorption spectra. The two major derivatives that are accounted for are oxygenated haemoglobin, HbO, and reduced haemoglobin, HbR. As seen in Figure 2.2 these molecules have different absorption coefficients, so the grade of oxygenation in the blood will affect the transmission of light except in the isobestic points like 805 nm, where the absorption coefficients of the HbO and HbR coincide. This means that the ratio between the two haemoglobin derivatives can be ignored and they can be treated as a single haemoglobin concentration. The BVS in its current state utilizes this, using a single 805 nm LED.

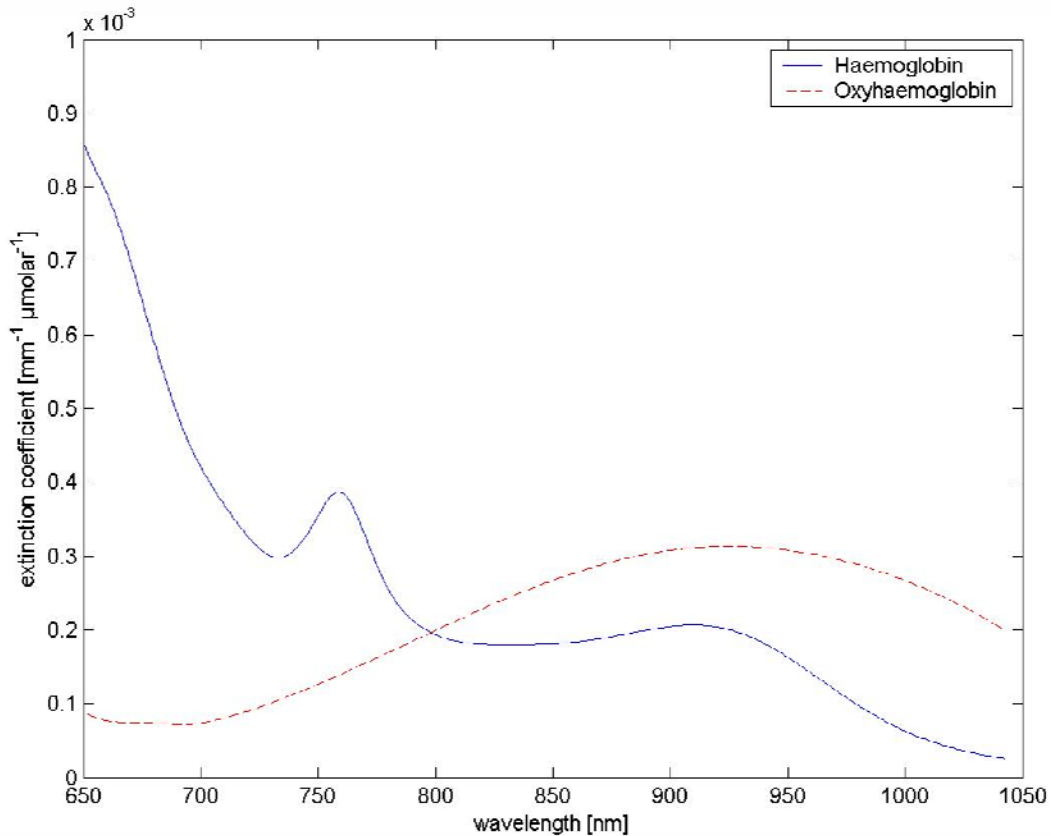


Figure 2.2: The table shows the extinction coefficients of reduced haemoglobin HbR and oxygenated haemoglobin HbO. The isobestic point is located around 800 nm.

### 2.2.5. Blood Flow

The blood flow was a possible parameter that could affect the distribution of red blood cells during the experiments. A laminar flow might cause the blood cells to gather in the center of the line due to the parabolic flow profile. This might also cause the blood cells to align themselves in some way, so that their flow resistance is reduced. In the case of turbulent flow the blood cells will have a distribution and alignment that is determined by the flow.

If the blood has a low flow or no flow at all, the cells might form a so called rouleaux which basically consists of a lot of red blood cells stapled on top of each other. This might reduce the absorption caused by the blood since the absorbing elements are concentrated in certain areas.

Since the haemodialysis lines used in the experiments will have a diameter of at least 4mm, the pumps will be working at 200ml/min and the viscosity of blood is 3-4 cP (15) the Reynolds number is approximately 0.3 which suggests a laminar blood flow.

## 2.3. Light Propagation and Interaction in Full Blood

The scattering of light is complex and a single model can't describe all types of scattering. The intensity of a single scattering event at a certain angle of observation depends on a lot of parameters: the wavelength of the incoming light, the refractive index of the medium, the refractive index of the scattering particle and the size and shape of the particle. In human tissue things can get further complicated since the scattering objects can be aligned in a certain direction, making the material anisotropic. Because of this there exists a wide variety of models, some of which are restricted to special types of scattering, others that are usable in most cases.

When describing light propagation in a scattering medium there are two theory branches to choose from: The Radiative Transport (RT) equation or Maxwell's equations. Maxwell's equations should be used when looking at the light distribution when wave properties are important, for example in a glass slab, where the interference of the reflected waves determines how much light is transmitted. Maxwell's equations are used to derive Mie's equations that describe the scattered light from spheres that are equal to, or greater in size than the wavelength of the light (16). Even though Mie's equations won't be used to model the light distribution in the work presented here some conclusions can be drawn by studying them.

### 2.3.1. Cell Shape and Mie Scattering

When light at a wavelength equal to or smaller than the diameter of spherical particles it is interacting with is scattered, the scattering can be described using Mie theory. Since the work described in this thesis only included wavelengths between 0.8-1 $\mu\text{m}$  and the size of a typical human erythrocyte is 6-8  $\mu\text{m}$  (4), Mie scattering was considered as the dominant type. Mie scattering is a solution of the Maxwell equations for spherical particles (17) and since the blood cells are more like flat discs the solution can't be used in this case. However, it can be observed that the angular distribution of the scattering is heavily dependent on the size and shape of the scattering particle, so care must be taken as the form of the blood cells is highly dependant on the solution they are suspended in.

### 2.3.2. Radiative Transport Equation

The RT equation treats the light as a stream of photon particles, each carrying a certain amount of energy. It completely omits wave aspects of light like interference, diffraction and polarization, and describes instead at the "photon economy" of a volume i.e. the incoming, outgoing and created photons. This equation is very good for when looking at the light energy deposition in scattering materials. The equation is valid for a medium where the inhomogenities are small and uniformly distributed.

The material is described by three parameters: the absorption coefficient  $\mu_a$ , the scattering coefficient  $\mu_s$  and the anisotropy factor  $g$ . The names of the absorption and scattering coefficients explain pretty well what they do. They can be interpreted as the probability of absorption or scattering per unit of length, and thus they are expressed in units of [ $\text{cm}^{-1}$ ]. Since the absorption and

scattering in a certain material happens with equal probability along the entire material the RT model can be seen as a material with randomly placed absorbing and scattering areas.

The anisotropy factor can be seen as the average cosine of the angle between the scattered and incoming light of a single scattering event. If  $-1 < g < 0$  the light is scattered backwards in average and if  $0 < g < 1$  the light is mostly scattered forward. The two extreme cases  $g = -1$  and  $g = 1$  are for complete backwards and forwards scattering and completely isotropic scattering is represented by  $g = 0$ . Human tissue has generally a  $g$ -factor of  $0.8 - 0.95$  i.e. it is mainly forward scattering.

Often the scattering coefficient and the anisotropy factor are combined into a single reduced or effective scattering coefficient  $\mu_s' = \mu_s * (1-g)$  (17). The reduced scattering coefficient is used in optics to see if the medium supports single scattering. If multiple scattering can be neglected, i.e. the probability that a single photon is scattered more than once, the modeling of the light becomes much easier.

### *The Phase Function*

The  $g$ -factor is only a measure of the average scattering angle. The function that models the actual distribution of angles is called the phase function  $p$ . It is a probability density function of the cosine of the zenith deflection angle and as such it is normalized to unity. The most commonly used phase function for dense tissue scattering is the Henyey-Greenstein function, even though it was originally derived to describe the scattering of starlight in interstellar matter. Equation 1 shows the Henyey-Greenstein phase function.

*Equation 1*

$$p(\cos\theta) = \frac{1 - g^2}{2(1 + g^2 - 2g\cos\theta)^{3/2}}$$

### **2.3.3. Monte Carlo Simulation of Light**

The RT equation has no solution by itself, and thus it must be solved by using approximations and applying boundary conditions. This approach would require a program or script written specifically to solve the RT equation for the geometry of the BVS cuvette, which can be quite complicated. A license of the optical simulation program FRED was available at Gambro. This program uses a probabilistic approach based on the RT equation to find the distribution of light in a volume. Since the theoretical model wasn't a central part of the work the author chose to use a commercial simulation program: FRED, instead of creating his own program to solve the RT equation.

The Monte Carlo simulation is a probabilistic approach to solving the light distribution in a scattering material. This approach doesn't solve the RT equation directly but it simulates the light transport in a statistical approach. The scattering and absorption of the material is done by randomly placed areas whose densities are expressed by  $\mu_s$  and  $\mu_a$ . The anisotropy factor and the phase function still describe the direction of the scattering.

In the Monte Carlo simulation the paths of the photons are simulated by taking random steps along the photon path. The step size as well as the azimuthal and zenithal angle is randomly chosen after each interaction of the photon. The step size distribution function of the photon can be derived from

the probabilistic definitions of the absorption and scattering coefficients, and can be seen in *equation 2*.

*Equation 2*

$$p(s) = (\mu_a + \mu_s)e^{-(\mu_a + \mu_s)s}$$

The probability distribution of the zenithal angle is given by the phase function while the azimuthal angle is assumed to have a rectangular distribution of  $1/2\pi$ .

The photons are created in the location of the light source and given a propagation vector and a weight set to unity at start. Absorption is accounted for by reducing a certain amount, seen in *equation 3*, of the photon's weight at every interaction between the photon and the material.

*Equation 3*

$$w' = w\left(\frac{\mu_a}{\mu_a + \mu_s}\right)$$

A new propagation vector is calculated from the zenithal and azimuthal angular distribution equations as well as the new step size from *equation 2*. The photon then travels the step length in the new direction and new rolls are made and so forth.

When the photon reaches a boundary it can either be reflected, transmitter or absorbed, depending on the boundary's properties. The photon's propagation can also end if the photon's weight falls below a certain threshold. To account for the energy lost in this event the photon has one chance out of  $m$  to get its photon weight multiplied by  $m$ . By repeating the process for enough photons a distribution of the deposited energy can be obtained.

#### **2.3.4. Models of Transmission Signal Dependency of Absorber / Scatterer Concentration**

##### ***The Beer-Lambert Law***

The Beer-Lambert Law is the simplest of models for light propagation in a medium. The law can be derived by assuming that the change of intensity over an infinitesimal distance is proportional to the intensity. Figure 2.3 shows the exponential behavior of the Beer-Lambert equation (Equation 4).

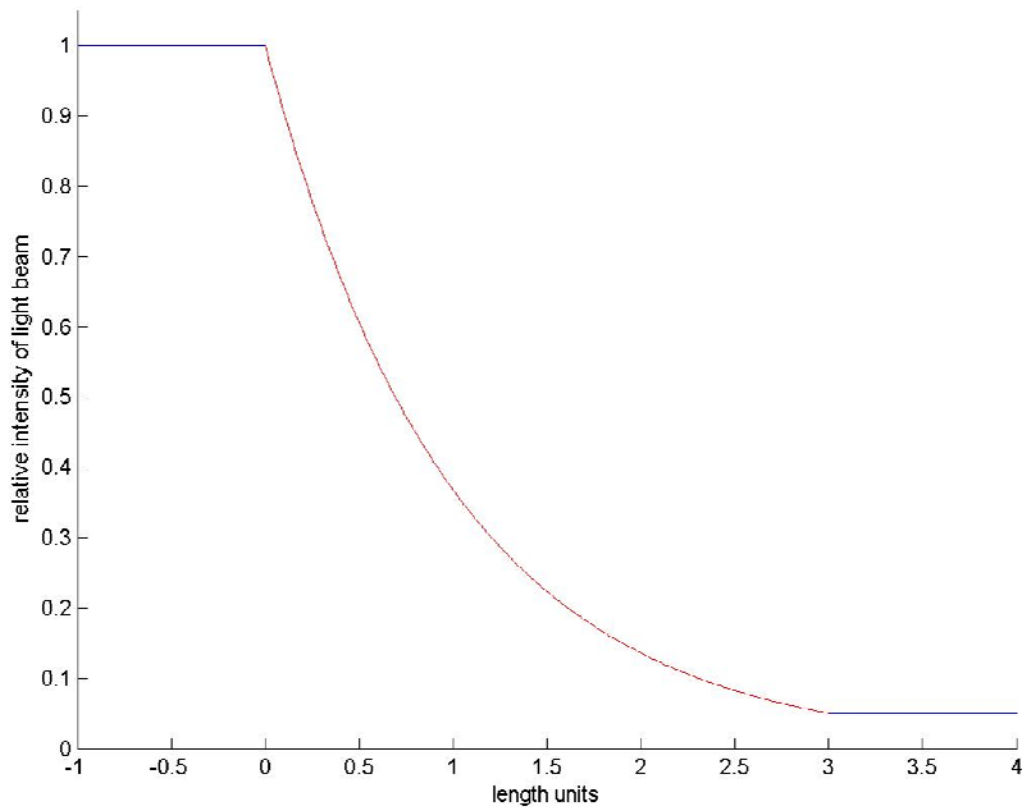


Figure 2.3: The graph shows how the intensity of a beam is reduced as a function of penetration depth for a material. The total attenuation coefficient  $\mu_t$  is set to 1 per length unit.

Equation 4

$$I(x) = I_0 e^{-\mu_t x}$$

Under certain circumstances, where light that is scattered away from the direction of propagation never scatters back into the detector, and where some photons go through the medium unaffected, this model works well. A moderately thick sample of blood, however, is very scattering and virtually none of the transmitted photons are unaffected by the material. The attenuation coefficient  $\mu_t$  consists of both absorption and scattering.

A quick estimation of how much of the light that remains unscattered through a blood sample of 4 mm thickness can be calculated with the Beer-Lambert Law. Assuming that the absorption coefficient is 1.95 and the reduced scattering coefficient is 0.75 in units of  $[\text{mm}^{-1}]$ , the transmission reaches a value of approximately 0.002%. The scattering and absorption coefficients for blood were found in (18) and are actual calculated values for blood at 805 nm. This shows that the Beer-Lambert law is not a good model for light transmission through thick full blood samples as it doesn't account for the light that scatters multiple times and still hits the detector.

### ***A Modified Beer-Lambert Law***

Previous works done with BVS-like cuvettes and wavelengths, (9) & (14), that treat blood volume change detection by light transmission through bovine blood present a modified model of the transmission signal, which was obtained empirically.

*Equation 5*

$$I = I_0 e^{\alpha x^2 + \beta x + \gamma}$$

The coefficients  $\alpha$  and  $\beta$  depend on the geometry of the sample and  $\gamma$  depends on the intensity and distribution of light from the source. Since the model has only been tried for thin blood samples in (9) & (14), (max 0.75 mm) it may not fit the results obtained from the experiments described in this work.

### **2.3.5. Reflection and Polarization**

Snell's Law is common in all wave affiliated physics, particularly optics. It describes the angles of reflection and refraction when a wave passes a boundary between two materials in which the wave has different propagation velocities. Total reflection occurs at the boundary when the incoming wave has an angle of incidence that is greater than the critical angle This is called a specular reflection while a reflection where the light has entered and been scattered from inside the material is called a diffuse reflection.

The Brewster angle is an optical phenomenon in which the reflection of a light beam only contains one type of polarization. If the angle of reflections is perpendicular to the refracted beam light that has a polarization parallel to the plane of propagation cannot be reflected (3).

## 3. MONTE CARLO SIMULATION EXPERIMENT

### 3.1. FRED Simulation Software

The commercial simulation software FRED was used to create a model of the light distribution in the improved BVS. It is an increasingly popular tool to model light propagation a course treating FRED are even available for PhD at Lund University.

The program is mainly intended for ray tracing, that is modeling the light as beams and seeing how the beam interacts with the it's environment through reflection and refraction. But there are multiple options for more advanced effects affiliated with light, like impact of polarization on reflection, reflectance angle distributions caused by unevenness on surfaces (surface scattering) or small mirrors (refraction), isotropic scattering caused inside a volume and a lot of other effects.

The program defines all geometric volumes by their boundaries, which means that the beams change their "rules of propagation" each time they pass a "traceable" boundary. By defining the walls a room is created. For each wall the program requires two materials to be defined, one for each side of the wall. The program has a long list of materials commonly used in optics, but it also allows the user to define new materials.

The volume scattering effects are modeled by Monte-Carlo simulation, so to simulate volume scattering in a certain material the absorption and scattering coefficients must be defined in the program as well as the phase function and g- factor.

The light source is defined by the user and can vary from the simplest point source to polarized Gaussian beam of different modes. The total output power of the light source can be set to any value, but it doesn't affect the relative light distribution in the geometry. The number of traceable beams produced by the light source can be chosen to virtually any number; the higher the number the better the result, but it takes time.

Detection surfaces can be placed where the user wishes to analyze the power deposition or power flux.

### 3.2. The BVS Model

#### 3.2.1. The Geometry

The BVS model was created by looking at the blueprints of the original model. The only real changes to the model were the holder and the light source. The light source was outside the BVS holder so it was easily changed, and the delrin holder had the same shape, only different reflecting properties.

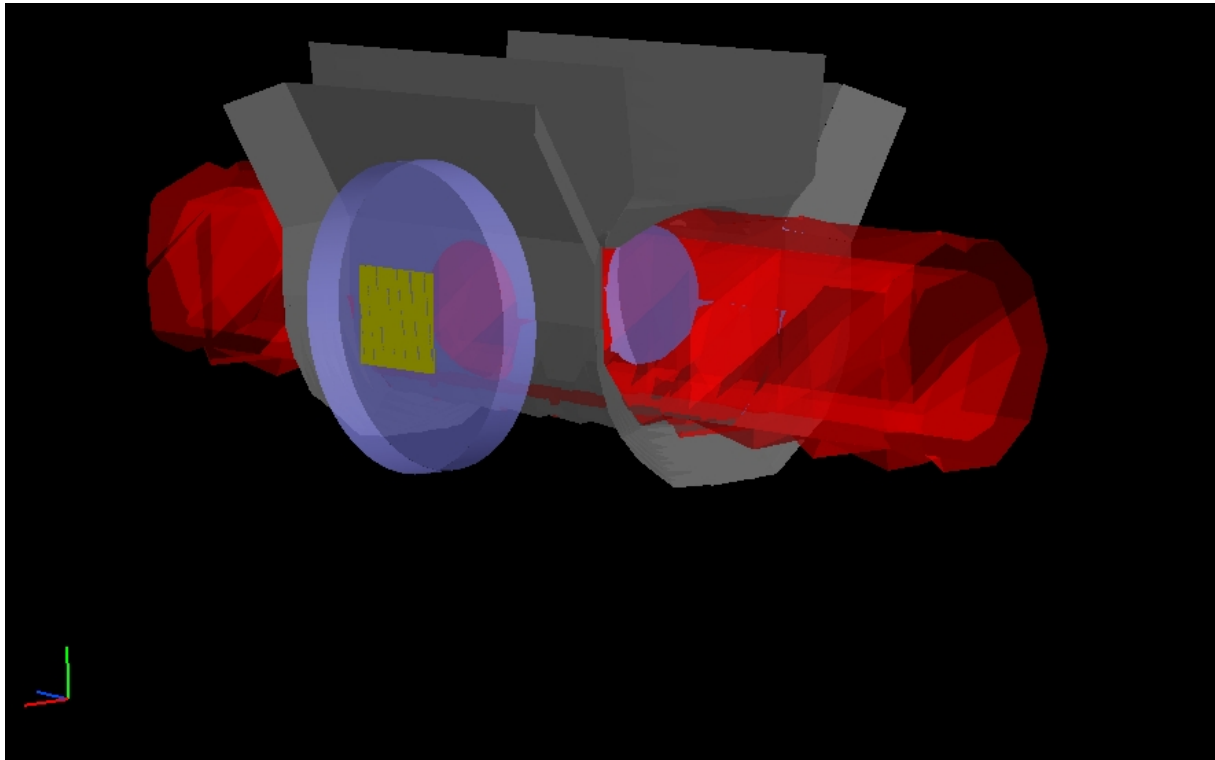
The resulting BVS model can be seen in Figure 3.1. It contains the holder and the lid which are shown in black, the cuvette which has a blue color and the glass protection lenses also shown in blue. The detector is shown in tan and the light source is invisible. The model is only 18 mm wide, but



increasing the width didn't give any signal changes, and the cuvette isn't longer than 15mm in any case.

The holder of the model was set to either total reflectance or total absorption to simulate the aluminum and delrin holder respectively. The aluminum isn't all that polished of course, but it should at least give a picture of how much of the light is reflected of the surfaces.

The distance between the cuvette and the bottom of the holder was set to 85  $\mu\text{m}$  but varying that value didn't change the result very much.



*Figure 3.1: The figure depicts the BVS model with all parts. The gray part is the holder/lid, the red is the cuvette, and the blue parts are dust protection windows. The tan part is the detector.*

### **3.2.2. The optical parameters**

The optical parameters for blood were obtained from (18). The only wavelength that was modeled was the isobestic 805 nm. The absorption coefficient was set to  $1.95 \text{ cm}^{-1}$  and the reduced scattering coefficient to  $0.75 \text{ cm}^{-1}$ .

### **3.2.3. The Light Sources**

Two different light sources were used for modeling the BVS. The LED was modeled as a point source with a  $50^\circ$  illumination angle. A LED has an angular distribution, but the point source is a sufficient approximation. The laser light was modeled by a uniformly distributed, collimated and coherent light source which is quite close to the truth.

The only wavelength used was the 805 nm since data of the optical characteristics of blood were easy to obtain for that wavelength.



## 4. THE EXPERIMENTS

### 4.1. Experiment Introduction

The goal of the experiments was to determine if it is possible to detect haemolysis while measuring the haematocrit by modifying the Gambro BVS in the following way:

- The cuvette holder made of polished aluminum was replaced by one made of black delrin.
- The LED of the BVS emitting at 805 nm was replaced by three diode laser modules emitting at 805 nm, 930 nm and 980 nm.
- The data analysis software/hardware was replaced in order to fit the multiple wavelengths measurements.

All experiments were performed on bovine blood since experiments involving human blood require very high safety precautions and must be conducted in special laboratories in order to prevent the spread of diseases.

### 4.2. Haemolysis Detection and Haematocrit Monitoring

The idea was to be able to measure both the haematocrit and to detect haemolysis during haemodialysis by doing transmission measurements with multiple wavelengths. Since the absorption and scattering coefficients are affected in a different way from haemolysis than from haematocrit it should be possible to tell the difference between the two cases by looking at how the transmission of two or more wavelengths is affected.

For example, if both the absorption and scattering coefficients increase if the haematocrit is increased the signal should decrease for all wavelengths. If the blood gets haemolysed instead the absorption would increase because the cells' contents are released while the scattering would remain more or less unchanged since the cell membranes are still present. This would result in a slightly weaker decrease for the wavelengths that have a high scattering coefficient. Thus the ratio between the two wavelengths' signals would look different than in the case of a haematocrit increase.

The idea of looking at a ratio between two wavelengths' signals is common in spectroscopy. It has the advantage of eliminating any errors that are common for both signals as well as the need for absolute calibration for the signals.

The light transmission dependence on haemoglobin derivatives makes isobestic points like 805 nm the only suitable wavelength for quantitative measurements of haematocrit. Since the goal of this work was to construct a sensor that detects haemolysis while it is actually measuring the haematocrit the other wavelengths are solely used for detecting haemolysis. The intention was that even a low degree of haemolysis in the blood would give a very different ratio between the wavelength signals than any haematocrit and oxygenation variations.

Three wavelengths were chosen at 805 nm, 930 nm and 980 nm, respectively. All chosen wavelengths are in the Near Infrared light spectrum, or NIR, because the absorption of the haemoglobin molecules is significantly lower there. This can be seen in Figure 2.1. Two wavelengths might have sufficed, but the third would allow the measurement of a third parameter besides haemolysis and haematocrit.

### **4.3. The Equipment**

#### **4.3.1. The Light Sources**

The BVS ordinarily uses a LED that emits 805 nm IR light at a power of 5 mW. The half intensity illumination angle of this LED is approx. 50°, which makes the light quite dependant on reflections of the cuvette holder. By replacing the LED with a laser diode module the light is beam shaped until it enters the scattering blood. This reduces the dependence of the holder's reflection, but it also reduces the reflection from the first glass lens caused by high angle of incidence.

Since full blood has very high light attenuation, transmission measurements are usually done on very thin samples. In the blood line set used with the BVS the cuvette has an inner diameter of 4 mm which is quite a lot for transmission measurements through a medium that is so scattering. A laser beam should give a much better signal. The spectral purity of a laser also comes in handy when using the isobestic point at 805 nm to measure the haematocrit.

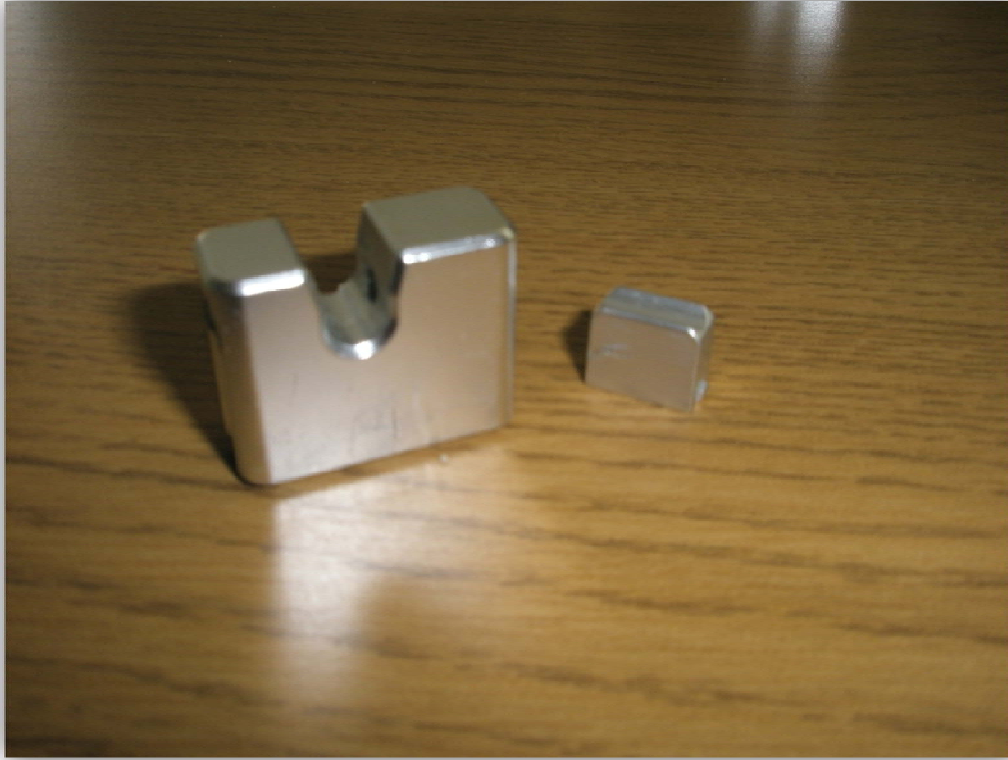
Besides the isobestic wavelength two other wavelengths were used for transmission measurements, 930 and 980 nm. The extinction coefficient curves in Figure 2.2 show that the IR region has a much lower attenuation coefficient than the visible spectrum and since there are no more isobestic points further up the NIR spectrum the wavelengths were chosen so that they were insensitive to wavelength variations.

The ordered lasers were single mode continuous wave and had an output power of 5 mW and they were all driven by a DC voltage. Since the detector would be less than 30 cm from the light source the lasers were ordered with a focal distance of 1 m.

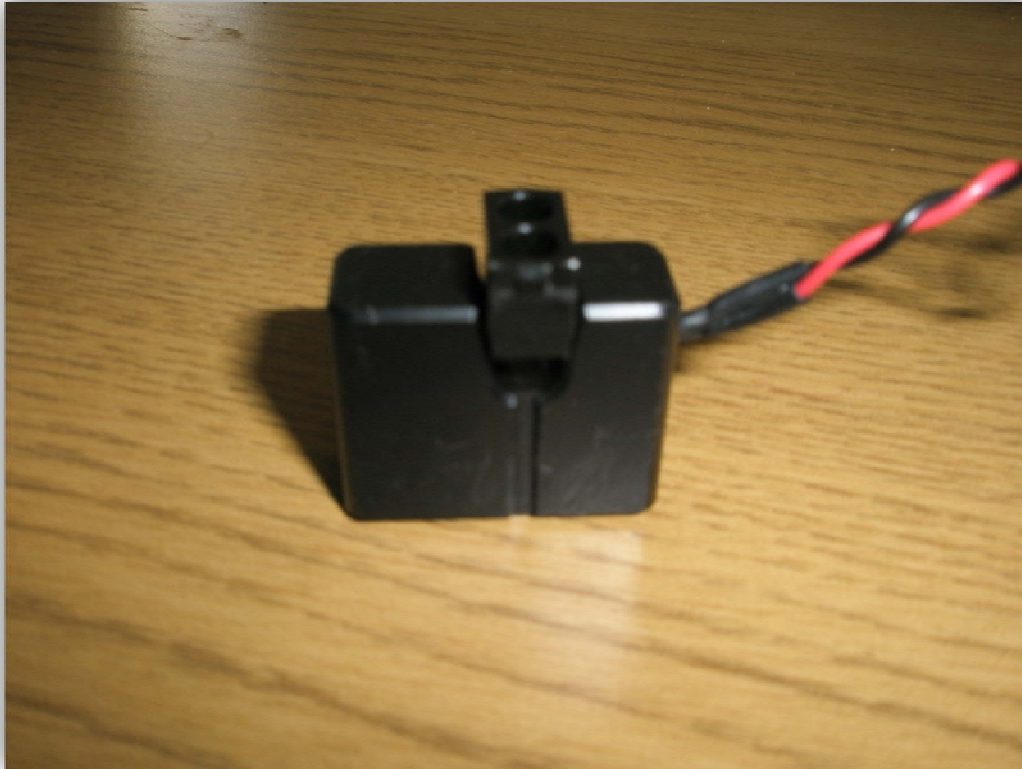
#### **4.3.2. The Holder**

Figure 4.1 depicts the BVS cuvette holder with its lid. The cuvette is placed between the holder and the lid. Since the laser diode module is too big to fit into the hole for the LED marked on the left side it had to be mounted outside the holder. Two optical glass windows can be seen along the light propagation path, one on each side of the cuvette. They are simple glass lenses with no curvature since their only purpose is to keep the dust away from the more sensitive equipment like the detector and the laser. Since the windows sit inside sockets in the holder they often gather a lot of dust and dirt which can cause unwanted light absorption. Because of this the windows were cleaned before the measurements. The attenuation of light caused by the lenses for each wavelength must be taken into account to get the true relative signal strength.

As previously stated the original BVS holder is made out of polished aluminum. It can be seen in Figure 4.1 and the delrin copy of can be seen in Figure 4.2.



*Figure 4.1: The reflective BVS holder made out of polished aluminum. The lid can be seen to the right.*



*Figure 4.2: The absorbing BVS holder made out of black delrin. It has a geometry that is identical to the original reflective one.*

#### **4.3.3. The Photodiode**

The detector used in the BVS consists of a simple photodiode that is sensitive in the IR. To obtain the correct relative transmission values of the blood the signal from each light source must be multiplied by an efficiency factor, but this won't affect the sensors ability to detect and measure blood properties. The same photodiode was used for all the experiments so the efficiency wouldn't vary.

The photodiode is connected to a plastic stick which in turn is fixed to the holder by a screw. If the screw was set too tight the plastic stick with the detector would bend, which affected detection efficiency. Some early experimental data was considered invalid because of this.

#### **4.3.4. The Blood Pump and the Blood Lines**

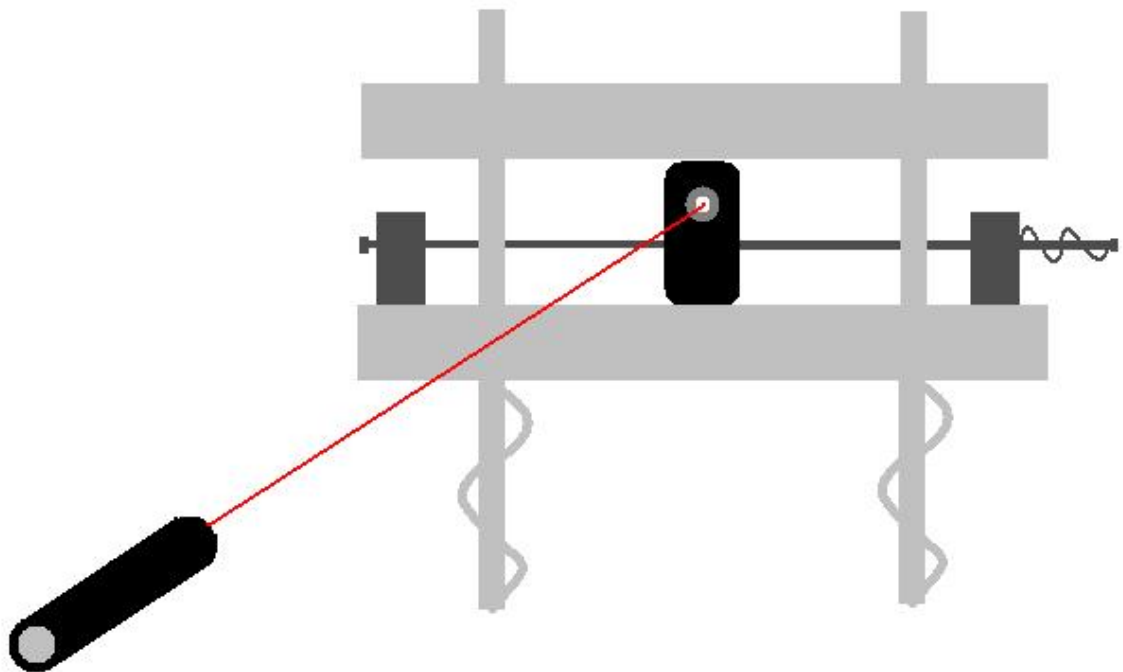
To induce a flow in the blood lines a Gambro Blood Pump was used. This is the same type of pump used in the AK200 S machine. The blood flow was set to a constant value of 200 ml/min which is a common flow during a haemodialysis treatment.

#### 4.3.5. The Fixation

For the measurements to be consistent the sensor needed a fixation to keep the light source, cuvette and holder in place. This turned out to be quite complicated, since there were many different movable parts that all had to be in a fixed position at the time of the measurement.

Many attempts were made to create a stable fixation and trial and error resulted in the arrangement shown in Figure 4.3. To keep the sensor as similar to the original design as possible only one laser diode module could be used at a time. Since the laser diode modules weren't perfectly collimated, variations in astigmatism and focusing length between the different laser units demanded that the position of the detector was adjustable in order to maximize the signal after each laser change.

The holder, cuvette and lid were squeezed between a base and a lid plate. These plates were made of plastic and were connected to the optics plate with springs so that the holder could be adjusted in the vertical direction as seen in Figure 4.3. It would have been easier to fixate the holder by two screws from the sides, but the sides of the holder had to remain open to give access to the blood line and the top lid plate was necessary to keep the lid of the holder in place. For vertical adjustments the holder was pinched between a metal bar attached to a spring and screws as seen in Figure 4.3.



*Figure 4.3: The BVS holder fixation as seen from the front. The bright grey bottom and lid were adjustable in the vertical direction and the dark grey screws and bar adjusted the holder in the horizontal direction.*



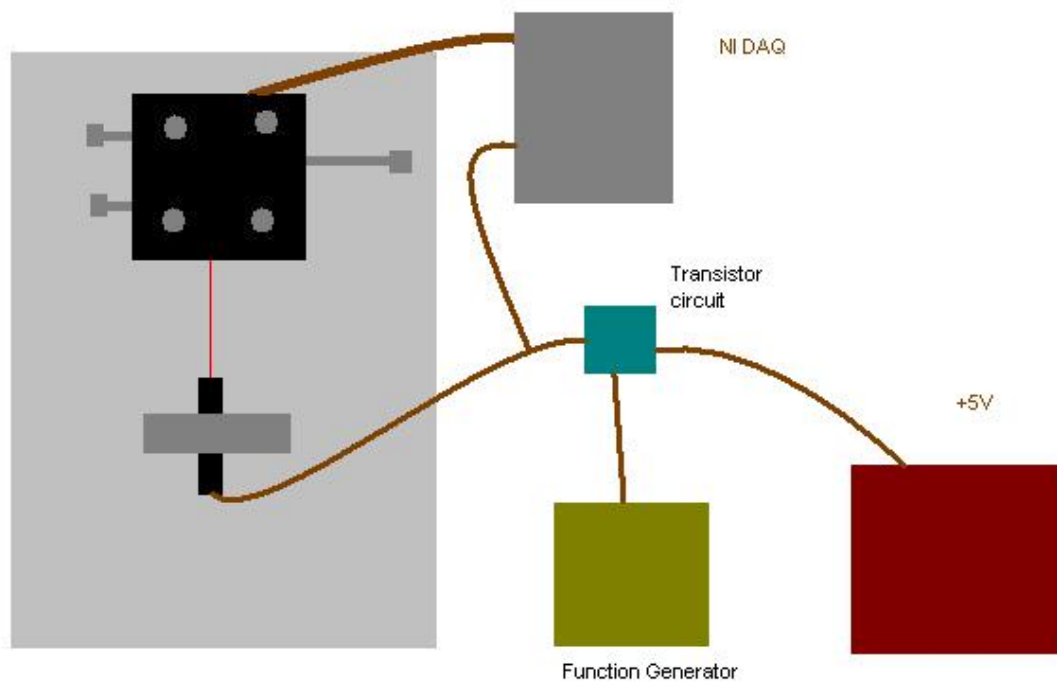


Figure 4.4: A schematic picture of the setup. The function generator causes the transistor circuit to periodically apply voltage to the laser which causes it to shoot pulses. The transmitted signal is detected by the DAQ card which also monitors the laser voltage.

#### 4.3.6. The Data Acquisition

The lasers were modulated at 25 Hz so that the signal from the fluorescent light could be subtracted. Since light from the fluorescent tubes in the laboratory can affect the detected signal the lasers were modulated at 25 Hz. The pulse generator couldn't deliver the 50 mA needed for operating the laser while modulating the voltage so a custom circuit had to be used. Figure 4.5 shows the coupling scheme that uses a transistor and a pulse generator to modulate a +5 V DC voltage. This gave a slight capacitive effect on the pulsed voltage as seen in Figure 4.4 but this could be neglected since the laser started operating at 2 V.

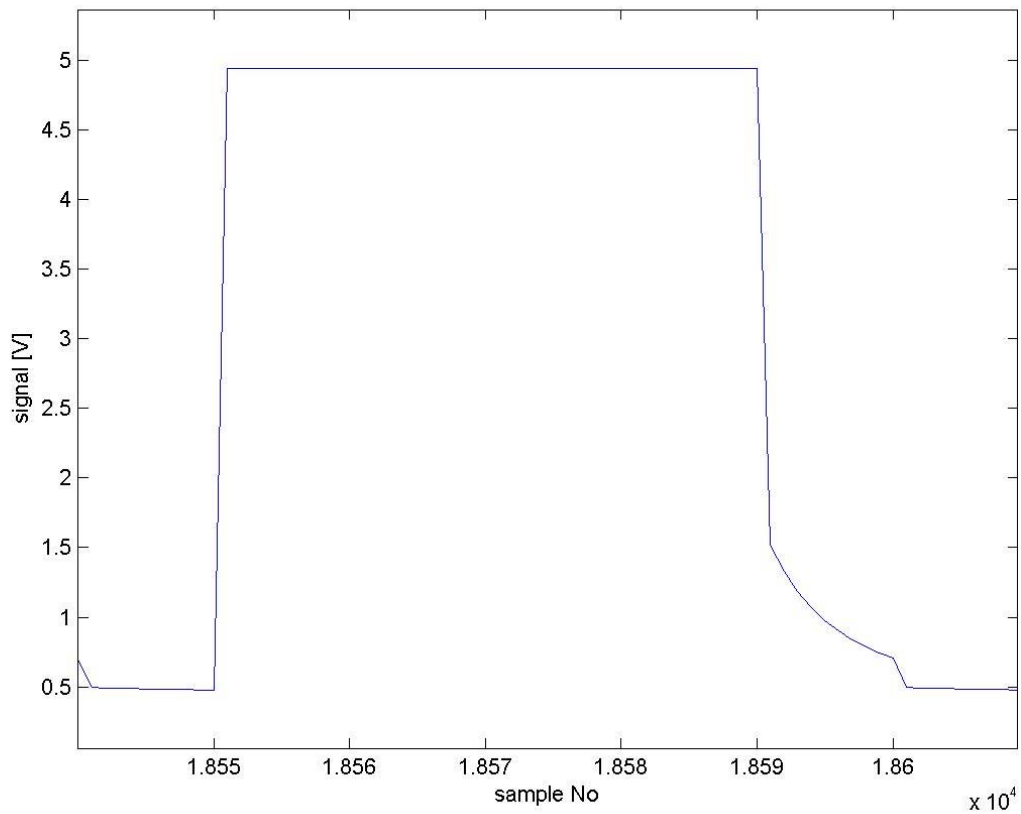


Figure 4.5: A typical voltage pulse. The capacitive effect didn't affect the light source since the laser had a threshold voltage of +2 V.

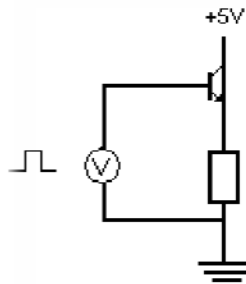


Figure 4.6: The coupling scheme of the laser drive. A transistor and a function generator were used to modulate a constant voltage into pulses.

The data acquisition was done by a National Instruments 6024 DAQ card connected to a PC with the DAQ program LabVIEW. Two channels were used, one to monitor the current from the photodiode

and one to monitor the voltage to the lasers. The NI-6024 DAQ card has a 12-bit resolution over an interval of +10 V to -10 V which proved to be sufficient.

The script written in LabVIEW was set to measure for 30 seconds, during which it collected a single pulse, or 80 data samples, at 2000 Hz. Each data set was triggered by the laser voltage when it increased past 0.2 V. The script included the option of adding a 50 Hz notch filter or an LP filter to the signal which proved to be unnecessary for analyzing the data, as will be seen in the results, but it was very good for optimizing the position of the detector after a laser module switch.

#### 4.3.7. Data Analysis

The data acquired from the measurements was stored as raw data files and analyzed in Matlab. The script in LabVIEW had an option of filtering the signal by either a low pass filter or a notch filter, but they were only used after a laser module change to maximize the signal.

The transmitted signal was pulse shaped as seen in Figure 5.6. To obtain a value proportional to the transmitted light of each pulse the median value of the baseline before the pulse, marked with an O in the figure, was subtracted from the median value of a pulse, which is marked by an X in the figure. The median of the new baseline-independent values was then used to represent the signal proportional to the light transmission.

The best fit to the data points obtained from the haematocrit measurements proved to be an exponential with a second order polynomial as seen in *equation 5*. The data points from the haemolysis measurements didn't seem to follow a simple function so a cubic-spline interpolation was used to interpolate between the data points. (Cubic spline interpolation uses third order polynomials, so it can't be directly compared to the exponential polynomial behavior of haematocrit changes, see section 5.)

Each wavelength could be multiplied by a weight factor calculated from the absorption in glass and cuvette, and the detection efficiency, to get the real transmittance, but this is not interesting since the measurement and detection of haematocrit and haemolysis are still possible. The glass attenuation is probably quite complex because of interference, so the easiest way to determine it would be to make a simple absorption measurement in a spectrophotometer. The detection efficiency of the PD also affects the signal and can be found on its datasheet.

### 4.4. The Experiment Procedures

#### 4.4.1. Maximizing the Signal

Since the fixation only held one measuring laser at a time the laser modules had to be used in sequence. The beams of the laser modules had slightly varying alignments, so each switch caused a shift in the direction of the beam.

Since the polarization of the laser had a major impact on the transmission through the cuvette, the laser module had to be rotated until maximum signal was obtained. The astigmatism of the modules

caused the focus of the beam to rotate as well. To point the beam directly at the detector and maximize the signal the position of the holder had to be adjusted both vertically and horizontally each time.

#### **4.4.2. The Haematocrit Measurements**

To see if the sensor could measure haematocrit levels in blood with the laser modules the transmission through blood samples with varying haematocrit was measured from all wavelengths.

Before each test the haematocrit of the undiluted blood batch was measured by using a centrifuge as described in section 2.2.1 also each liter of blood was mixed with 1-2 grams of heparin to prevent the blood from coagulating.

For each wavelength a certain amount of undiluted blood was taken from the original batch. This blood was poured into a flask and pumped through the blood lines which were connected to the sensor. After each measurement the blood was diluted with a 0.7% NaCl solution to lower the haematocrit by a few percent, and a new measurement was made when the transmission signal stabilized. Usually the blood was diluted six times, which gave a total of seven different haematocrit measurements.

#### **4.4.3. The Haemolysis Measurements**

To detect haemolysis, haemolysed blood was needed. By mixing bovine blood with a common kitchen mixer for 20 minutes the blood was clearly haemolysed, but to an unknown degree. It turned from dark red to a very bright red color. By centrifuging a sample of the haemolysed blood as described in section 2.1.3 the blood plasma got a slightly pink nuance, while the packed cells got a lot brighter. This is an indication that the contents of the blood cells are floating in the blood plasma.

The haemolysis was measured similarly to the haematocrit. Two batches of blood were taken from the original batch and one was haemolysed. A part of the pristine batch was poured into the blood lines and a measurement was performed. A small amount of haemolysed blood was then mixed into the blood circuit to “increase the haemolysis level” and a new measurement was done when the transmission signal stabilized. The level of haemolysis was only increased three times since the goal was to detect haemolysis, not measure it.

## 5. RESULTS AND DISCUSSION

### 5.1. Pre-Measurements

In order to see if the changes made to the BVS were any better some pre-measurements were performed before the real experiments started. These pre measurements included comparing the signal strength of the lasers to the LED, determining the impact of the reflectiveness of the holder, and empirically finding the right concentration of sodium in the buffer used for suspending the blood cells.

#### 5.1.1. Absorptive/Reflective Holder

The change of holder material from reflecting to absorbing was one of the proposed improvements to the BVS, but the loss of the dependence on the aluminum polish came at a price of signal strength. A quick comparison was made to see how much of the signal was lost due to the absorption of the cuvette holder. The transmission was measured from the original LED at 805 nm, using a calibration stick i.e. a cuvette filled with a material which, at the wavelength 805 nm, has similar optical properties to full blood with 70-80 Hb/l haemoglobin concentration. The light was modulated at 25 Hz to so that the 50 Hz fluorescent tubes could be subtracted from the signal.

The signal strength turned out to be twice as strong in the reflective holder, but the signal was so weak in both cases, it had to be averaged multiple times to be detectable. A low pass filter (50 Hz) was soldered from passive electronic components and used to improve the signal slightly.

#### 5.1.2. LED/Laser Measurements in Absorptive Holder

The original LED has a half intensity angle of 50° which means that a big part of the light reflects of the holder before it enters the cuvette. When the reflective holder was switched for an absorptive one the LED gave a very weak signal, so a laser diode module was tested to see if the signal strength improved. A calibration stick that was equivalent to 70-80 Hb g/l stick was used instead of blood.

The average signal strength from the laser module was nearly 8 times stronger than that from the LED, even though their output power was the same. The improvement could mostly be credited to the collimation of the light.

Another problem that might have been circumvented by using a laser was the light absorption caused by dust that gathers at the around the glass windows. The collimated beam of the lasers had a beam diameter of approximately 1 mm which is much smaller than the diameter of a window.

#### 5.1.3. Transmission Variations caused by Osmolality

Since the haematocrit measurements would require blood samples with different levels of haematocrit an alternative for blood plasma was needed to suspend the blood cells. In section 2.2.3

it is stated how the osmolality of the suspension fluid impacts the blood cell size and shape. Sodium concentration and urea have the strongest influence on osmolality, but sodium can't pass the semipermeable membrane and causes water to flow in or out of the cell to equalize the hydrostatic pressure.

In early measurements a 0.9% NaCl buffer was used to suspend the cells, the results can be seen in Figure 5.1. The measurement on the undiluted blood for some wavelengths resulted in a higher transmittance than the first diluted measurement, which shows that the osmolality of the solution was different than that of the blood plasma.

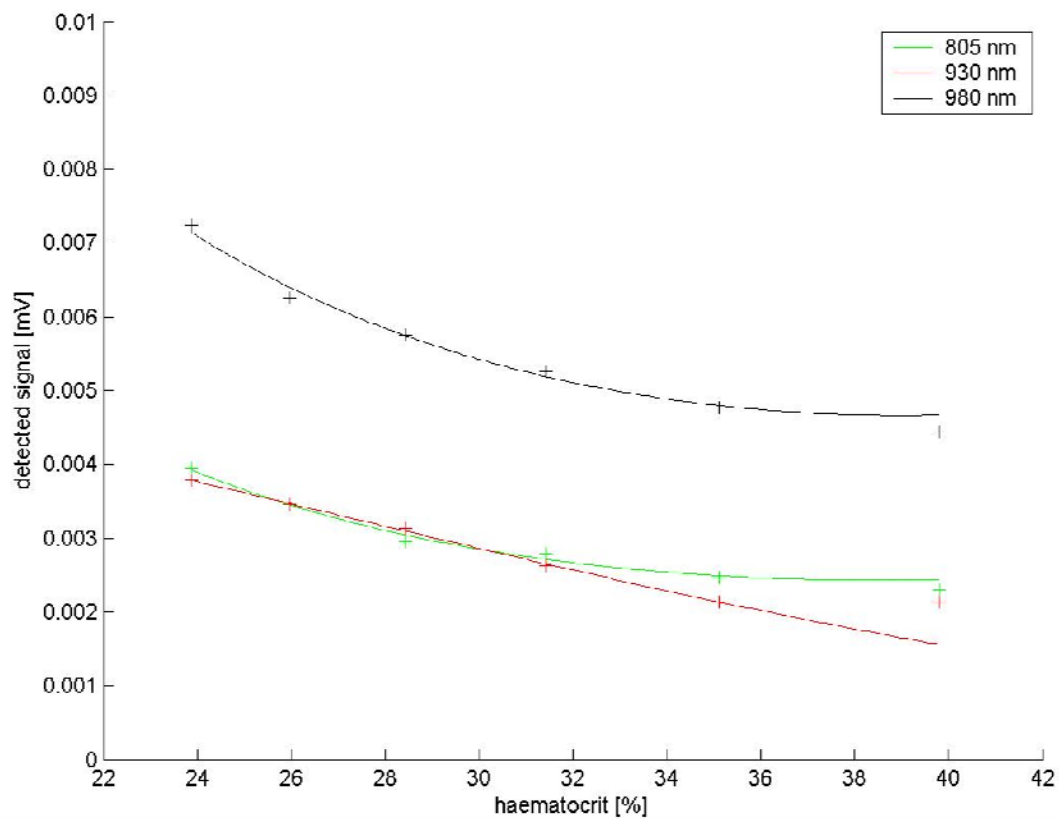


Figure 5.1: The detected signal from an early haematocrit measurement showing an irregularity in the first undiluted point of measurement (40%). This was later corrected by changing the sodium concentration in the NaCl solution.

Conductivity measurements done contemporary at Gambro indicated that a 0.7% NaCl solution affected the blood cells in a manner more similar to blood plasma, so the new experiments were performed with a sodium concentration of 0.7% in the solution. The undiluted measurements seem to follow the same curve as the rest of the measurements as seen in the results.

#### 5.1.4. Initial Fixation Problems

The first results from the haematocrit measurements showed very inconsistent results. After each laser module switch the signal changed very much. The holder was sandwiched to the optics table by a lid which could be moved vertically along bars, very similar to the one in *figure 4.3*. The holder's position was thus not adjustable in any direction after fixation, which limited the measurements to one laser, since the inconsistency of the data became too great after a laser switch.

The method of detecting haemoglobin (which is directly related to haematocrit, see theory section 2.2.1) changes by looking at IR light transmission was already established, so the fixation of the holder was replaced by the adjustable one described in section 4.3.5., which improved the data consistency drastically.

## 5.2. Haematocrit Measurements

### 5.2.1. Haematocrit Measurements Results

The haematocrit measurements show good consistency with previous works (9) & (14), even though a lot of the equipment was changed. To each set of data points a function based on the modified Beer-Lambert Law described in section 2.3.4 was fitted. The following figures show both the data points and the fitted function.

The relative transmission intensities for all three wavelengths can be seen as functions of the level of haematocrit in Figure 5.2.

Figure 5.3 shows a measurement conducted on another batch of blood, and Figure 5.4 shows the combined graphs. The signals of the isobestic wavelength 805 nm are nearly identical, which shows that the laser modules are working very well for haematocrit measurements. The other wavelengths are varying slightly as was predicted since the different blood batches probably have quite different amount of oxygen.

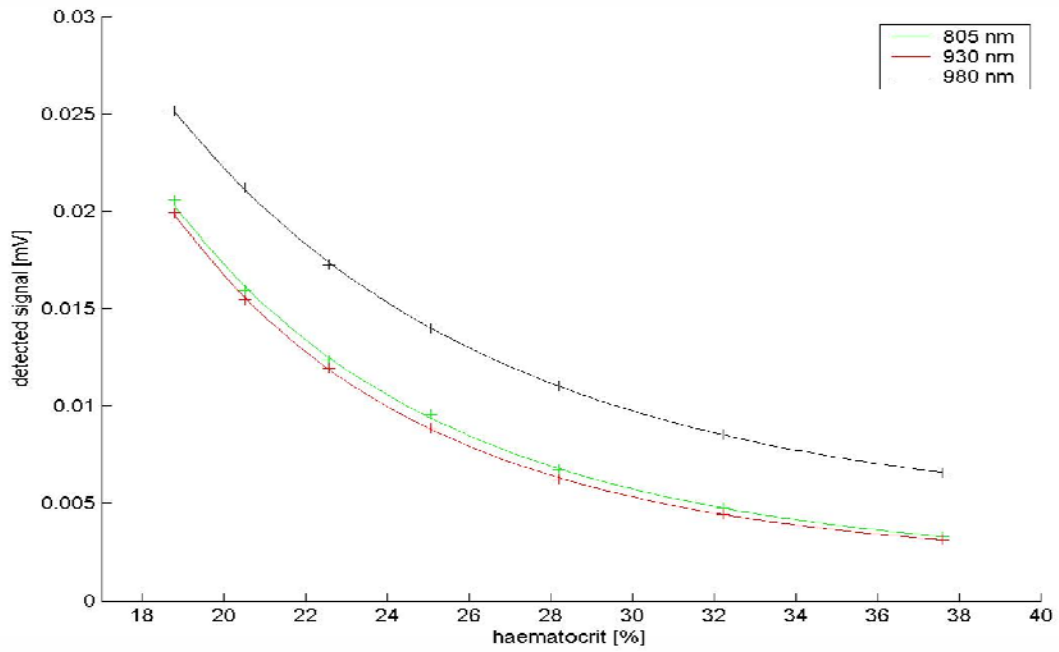


Figure 5.2: Haematocrit measurement 1. The polynomial exponential fit is very good for all signals.

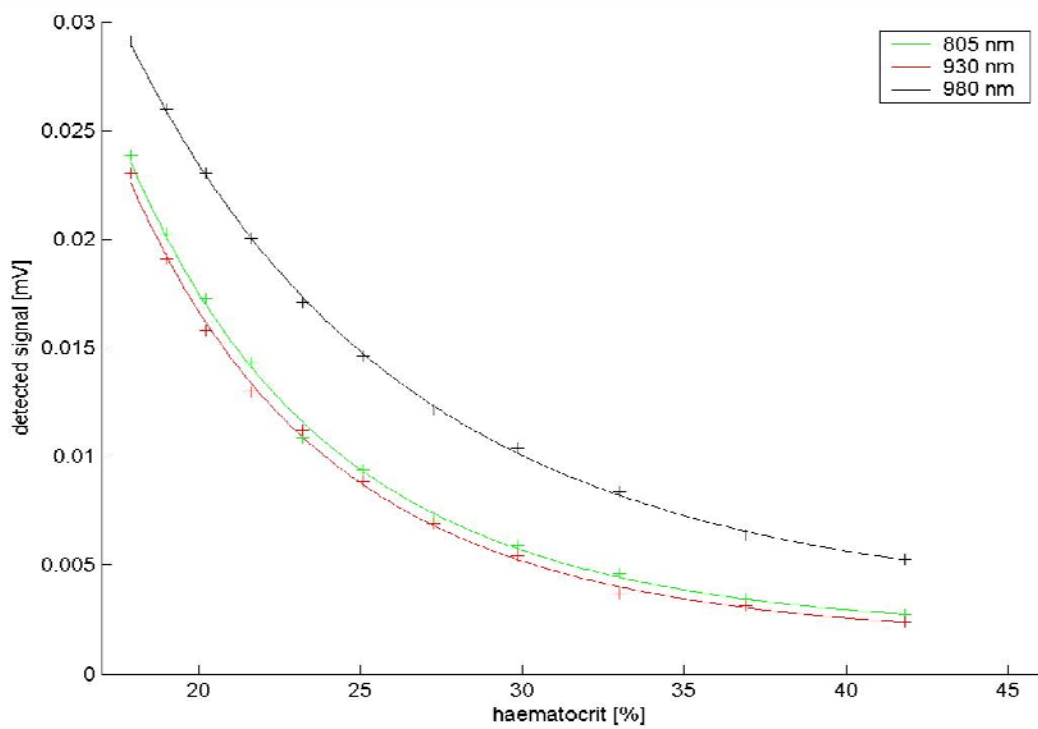


Figure 5.3: Haematocrit measurement 2. The transmission signal from a second batch of blood. The measurement was done to check the consistency of the measurements.



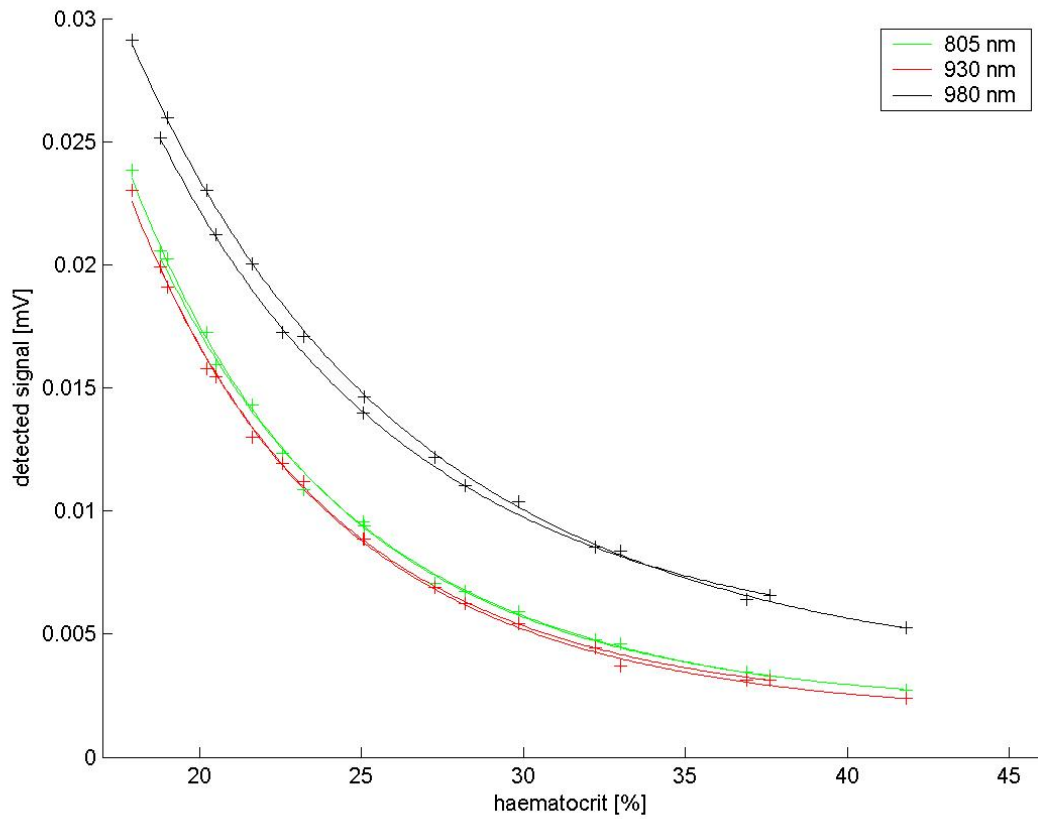


Figure 5.4: Measurement 1 and 2. The signals of the two measurements look very similar. The 805 nm wavelength is almost exactly the same.

The values of the coefficients from the modified Beer-Lambert Law can be seen in table 3 from both measurements. The coefficients of the different measurements seem to correspond well to each other.

	$\alpha$	$\beta$	$\gamma$
<b>805 nm meas.1</b>	0.0021	-0.2163	-0.5804
<b>930 nm meas.1</b>	0.0025	-0.2395	-0.3023
<b>980 nm meas.1</b>	0.0018	-0.1708	-1.0980
<b>805 nm meas.2</b>	0.0024	-0.2310	-0.3698
<b>930 nm meas.2</b>	0.0023	-0.2335	-0.3553
<b>980 nm meas.2</b>	0.0014	-0.1531	-1.2372

Table 3: The table shows the coefficients of a polynomial exponential fit as described in 2.3.4. (Equation 5) from the two haematocrit measurements.

The relative light transmission ratios from both measurements are shown in Figure 5.5. There are slight differences in the ratios from the two measurements since the oxygenation level was different. No exact information can be extracted from the ratios about changes in the haematocrit, only how they behave on average if the haematocrit changes.

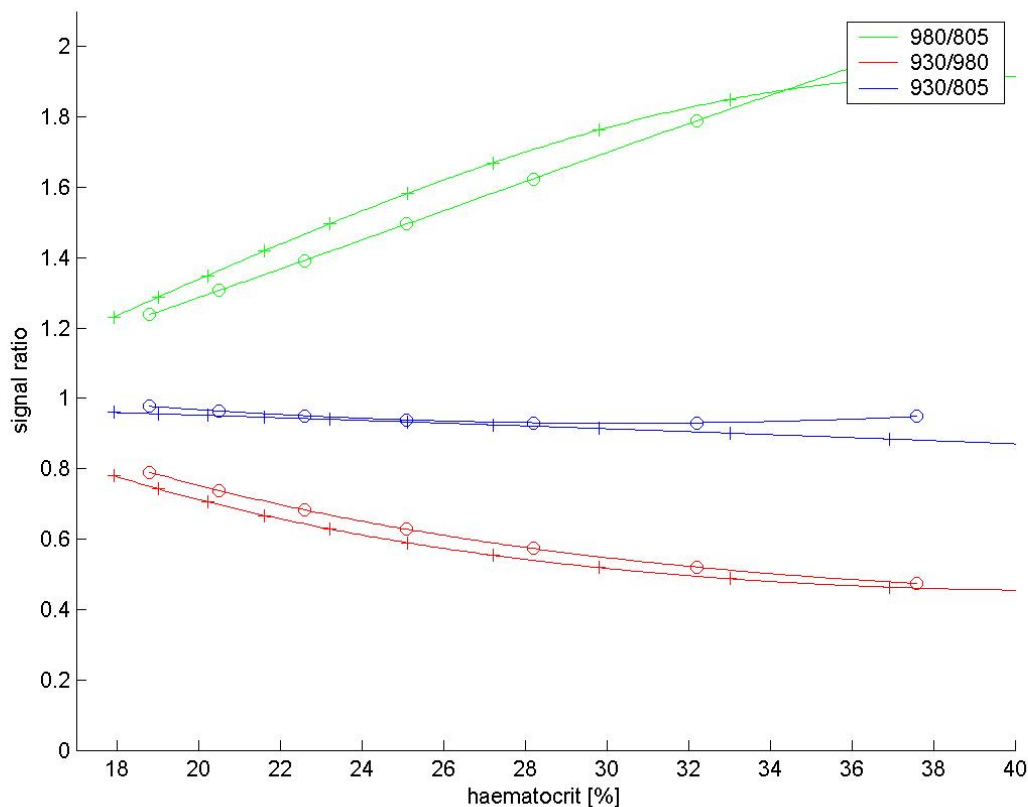


Figure 5.5: The ratio of the detected signals from measurements 1 and 2. Even though they differ a little they still are very alike. Much information can't be obtained here, but the resulting ratio of haemolysed blood is much different

The flow of blood was constantly set to 200 ml/min during the haematocrit measurements, but no changes in transmission were detected when varying the flow between 100 ml/min and 500 ml/min. When the blood pump was turned off for an hour the transmission values increased slightly during that time.

The signal strength was strongly dependent on the orientation of the polarization of the laser. Turning the laser in its holder caused the signal to vary somewhere around 40-70 %. This can of course partly be caused by the imperfect collimation of the laser modules, but the polarization was assumed to be the highest factor of influence. The light emitted from lasers is polarized. In most materials this affects the reflection and interference of the light, see section 2.3.5, but in some materials, typically polymers or other plastic materials, the polarization of the light can cause big variances in absorption properties. This is because the long molecules in the polymers are oriented in a certain way. This makes the material anisotropic, and the polarization has a great impact on how the light interacts with the molecules.

The cuvette of the blood lines is made of a polymer material, so that explains why the polarization had a big impact on the signal strength. To optimize the signal the laser had to be turned and the

detector position adjusted repeatedly. When all changes to either detector position or polarization orientation resulted in a lower transmittance the signal was considered to be optimized.

### 5.2.2. Haematocrit Measurement Discussion

The change to laser diode modules gave a great improvement in signal strength as explained in section 5.3.1. Figure 5.6 shows a typical set of samples obtained by the DAQ-card. This improved signal quality can have many explanations:

- The collimated beam has more light going in “the right direction” at start. Even though the light scatters it doesn’t change the direction completely.
- The collimated beam has no angle of incidence
- The collimated beam is not influenced by the dirt that gathers around the edges of the optical windows.

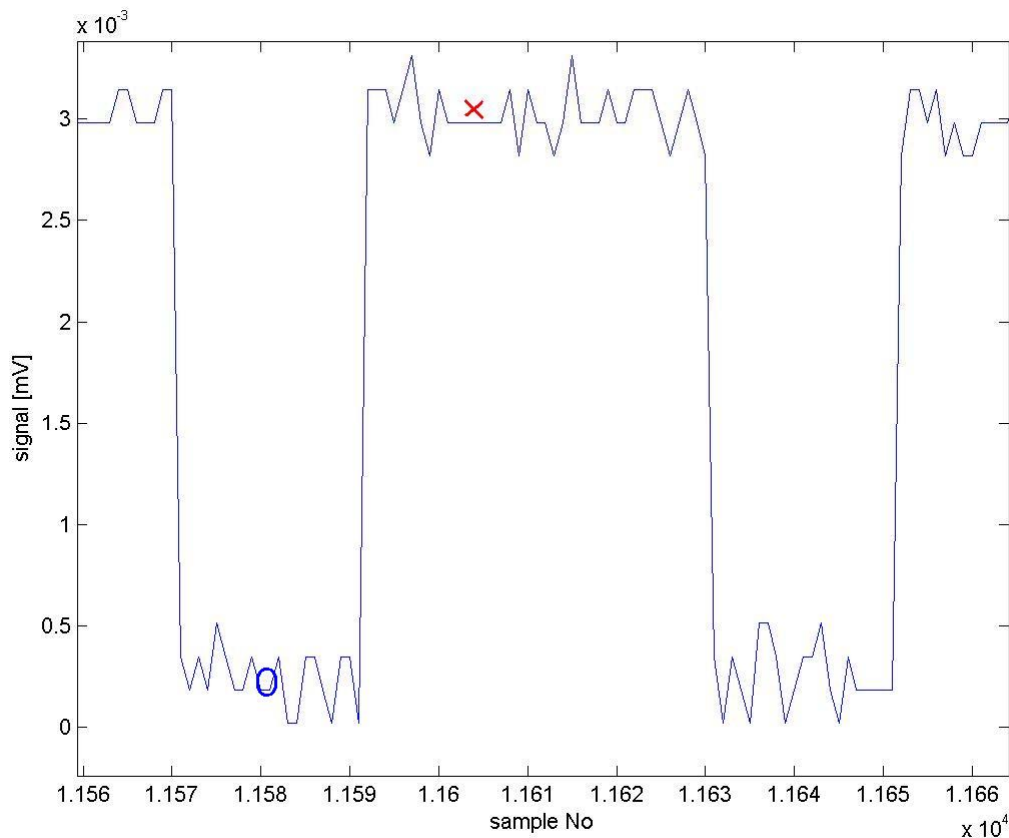


Figure 5.6: The typical signal sample obtained from a laser module.

The laser also has an improved degree of monochromaticity, which was important since an isobestic point was used to measure the haematocrit irrespective of the oxygenation ratio of haemoglobin in the cells.

The laser diode modules used in these experiments were of course more expensive than the LED’s that are used in the original BVS, but the advantages are numerous. The laser diode modules contain

built in electronics for the power supply, a lens system and the laser diode. The assembling of the components could be done on site at a reduced cost.

Since the BVS's dependence on the reflectiveness was a way of preserving signal strength it makes good sense out of a technical perspective to change the LED to a laser if the reflectiveness is unwanted. The signal strength is obviously enough. It may even be possible to do the same measurements with a 1 mW laser instead of a 5 mW.

### 5.3. Haemolysis Measurements

#### 5.3.1. Haemolysis Measurements Results

A simple glance at the haemolysed batch of blood showed that it was haemolysed, so haemolysis detection seemed quite easy in the visible region of the spectra.

Figure 5.7 shows the transmission signals as functions of the degree of haemolysis for each laser used. It can be seen that the transmission of most wavelengths was reduced when the whole blood was mixed with the haemolysed blood.

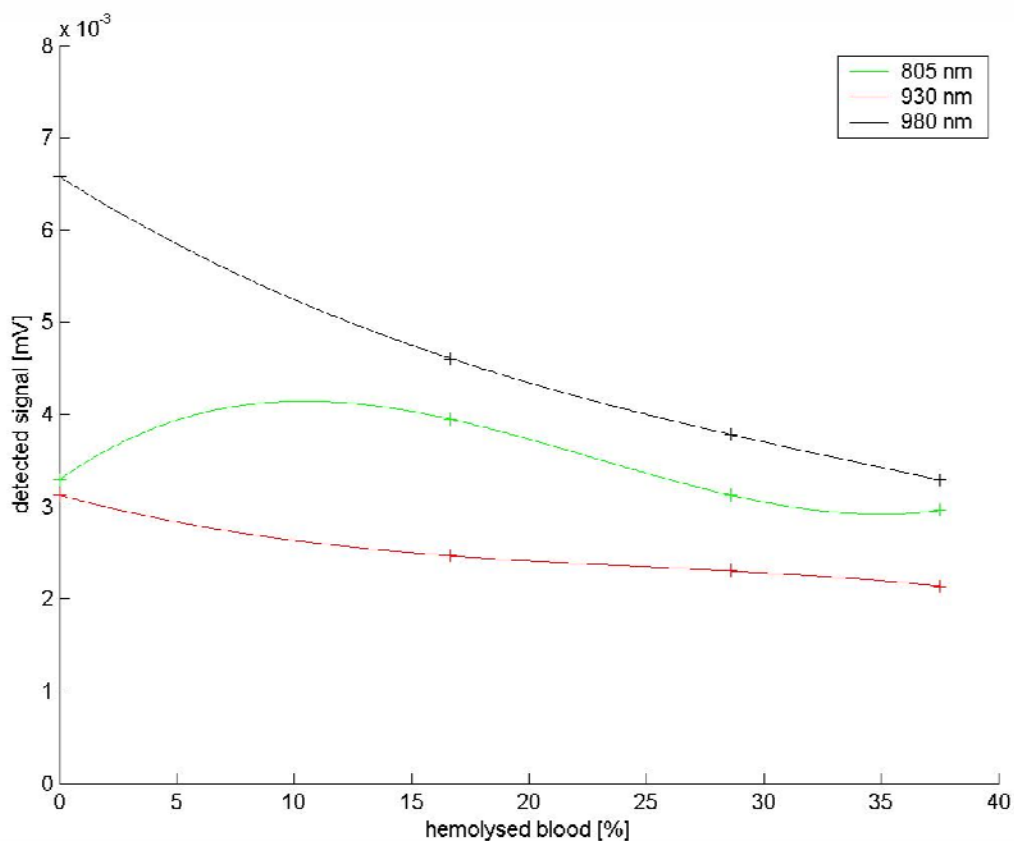


Figure 5.7: The detected signal as a function of the amount of haemolysed blood added to the undiluted sample. The degree of haemolysis in this fraction of added blood is unknown but believed to be high.

The 805 nm transmission is at first increased and is later reduced by increased degree of haemolysis, which can be explained by shape changes of the blood cells due to the change in osmolality. Since the electrolytes inside the cells flow freely when the cell is haemolysed the osmolality of the surrounding cell plasma may change. This in turn may cause the blood cells to shift in shape and size, which may cause big differences in their scattering properties.

The haemoglobin molecules of a haemolysed cell flow freely in the cell plasma once the cell membrane opens. This is probably the main reason of the reduction in light transmission as the haemolysis level increases.

The signal ratios shown in Figure 5.8 are showing a completely different pattern than the ones from haematocrit measurements in Figure 5.5. The effects of haematocrit changes are much different than those of haemolysis increase, which was the intended goal of the experiments.

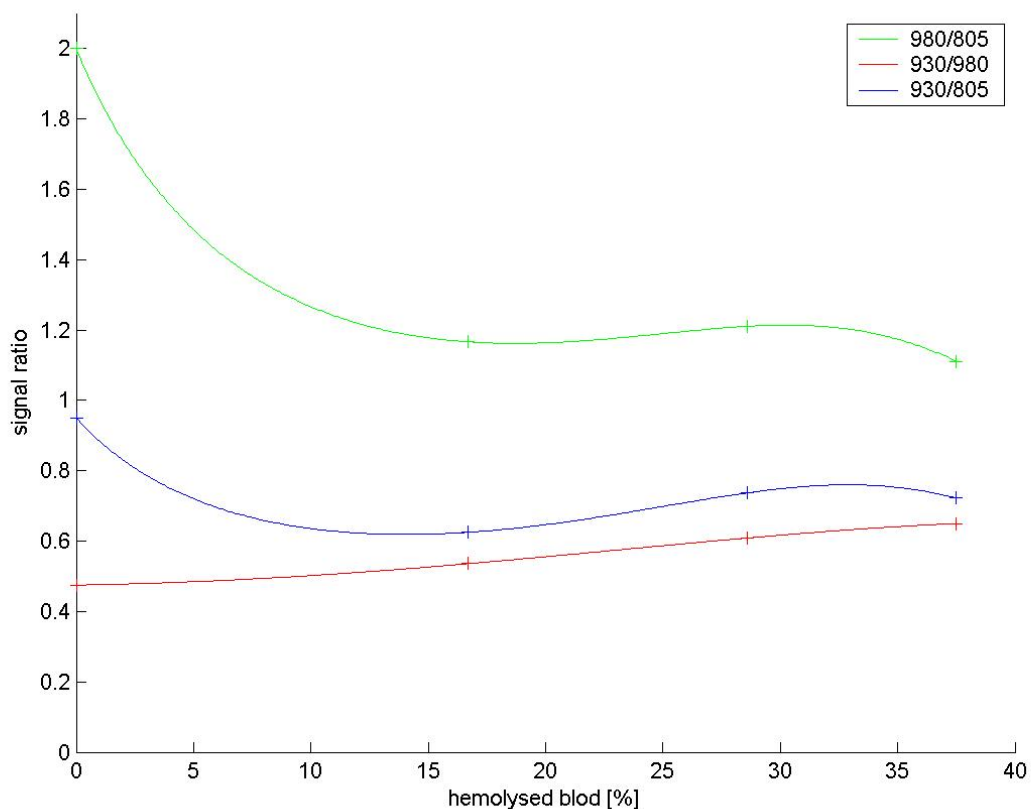


Figure 5.8: The ratio of the signals as a function of the amount of haemolysed blood. It looks very different than the ones in Figure 5.5.

### 5.3.2. Haemolysis Discussion

The haemolysis experiments proved that detection of haemolysis is possible while measuring haematocrit. The effects of haemolysis on the optical properties of the blood are complicated due to the sensitivity of the blood cells to osmolality changes. Unlike the haematocrit data the haemolysis data didn't fit to a single function, which is an indication that the transmission depends on many

different processes like osmolality, haemoglobin concentration in the blood plasma, the state of the remaining blood cell membranes, etc.

The haemolysis did, however, create a large shift in the relative transmittance for the wavelengths which was different from the effects of haematocrit changes. If the multiple light sources could be integrated into the BVS in some manner that wouldn't require manual changes of the light source, for example by having multiple detectors, or letting the light sources illuminate the blood at different angles from the detector, a sensor that could measure both haematocrit and haemolysis continuously during haemodialysis would be possible.

Another interesting possibility would be to use both isobestic points at 805 and 548 nm to have a sensor that is totally independent of the ratio of HbO to HbR. Even though the blood is highly absorbing for shorter wavelengths a simple diode laser might have sufficient power to give a decent signal in a thinner cuvette.

#### **5.4. Possible Haemolysis Detection Algorithms**

A preliminary algorithm for detection of haemolysis during a haematocrit measurement is presented here. Figure 5.9 shows all of the results of the previously mentioned experiments. The 930/980 signal ratio behaves almost the same during both haemolysis and haematocrit change, while a strong decline can be seen in both the 980/805 and 930/805 haemolysis ratios. This is due to the strong increase of the 805 nm signal which seems to respond differently than the other wavelengths. Thus it can be said that if the 805 nm signal increases while the 980 nm or the 930 nm signals decrease haemolysis might be present. Another possibility is to assume that if the 930/805 signal falls below 80% of its initial value haemolysis is present.

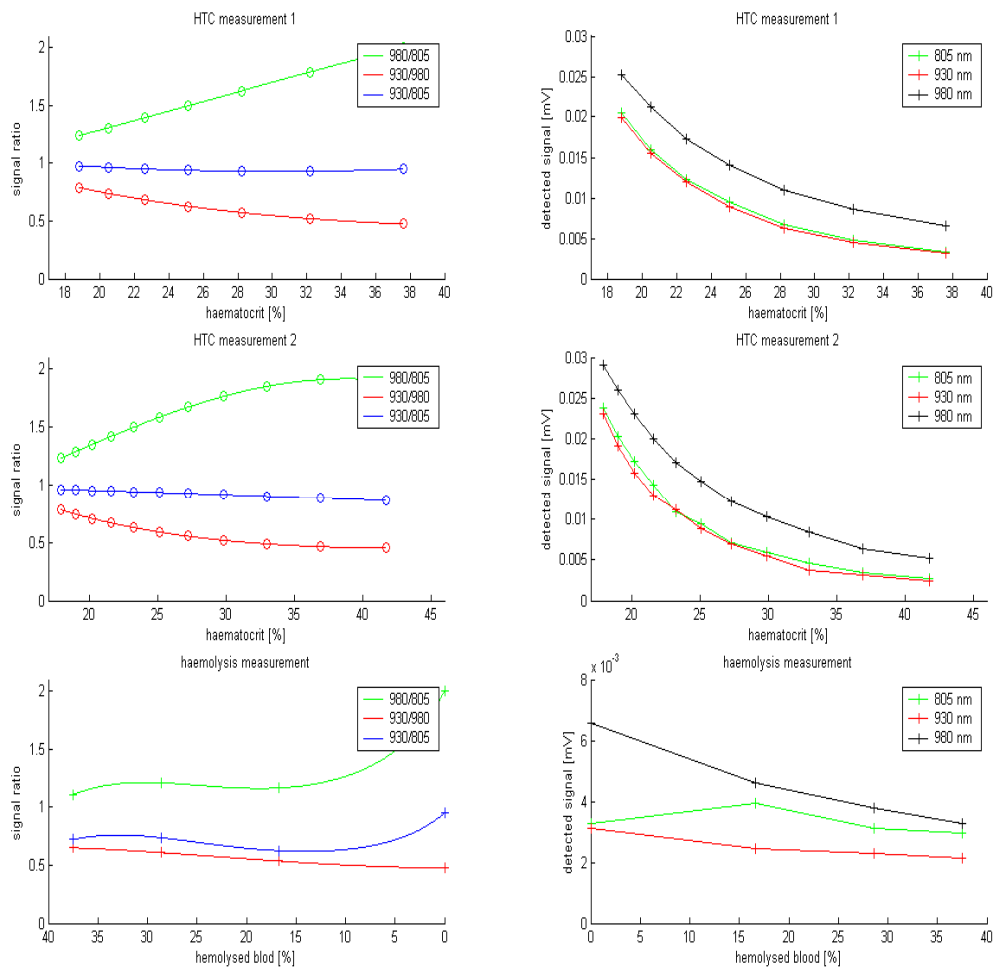


Figure 5.9: The results from the BVS experiments. The most characteristic sign of haemolysis can be seen in the lower right picture where the green 805 nm signal increases while the other two decrease.

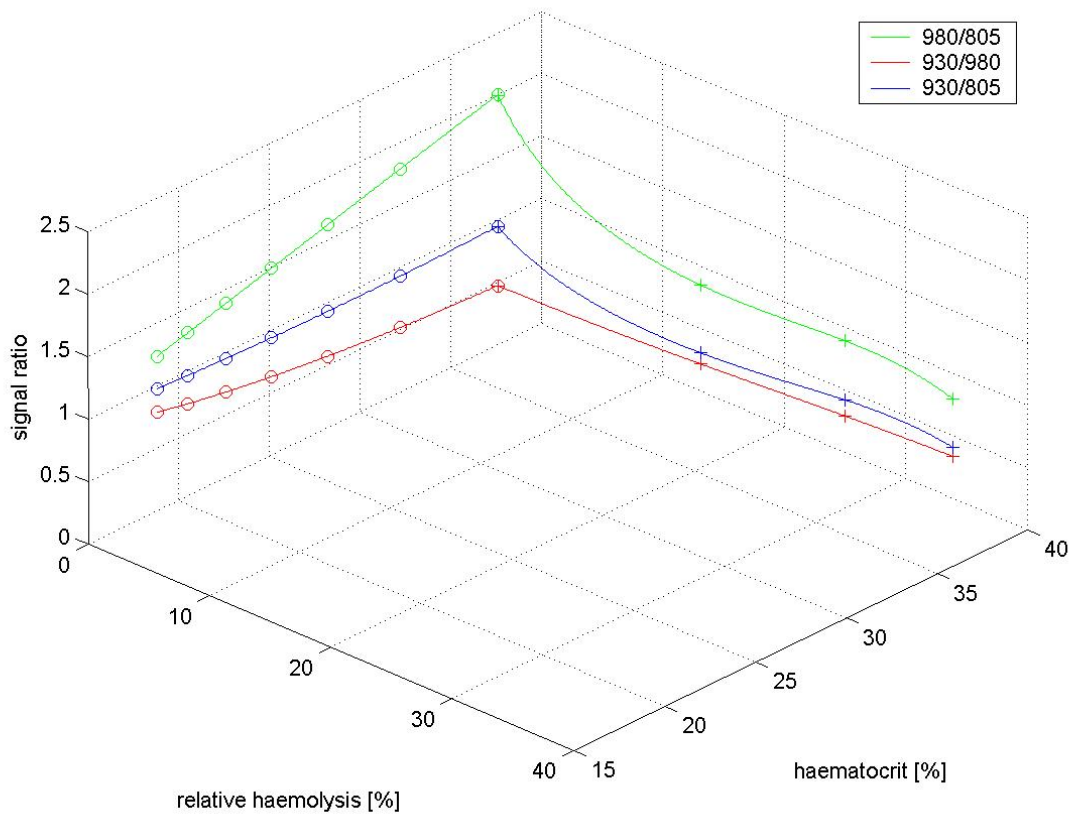


Figure 5.10: The signal ratio dependency on both haematocrit changes and haemolysis.

## 5.5. The Monte Carlo Simulation Results

Figure 5.9 shows the light irradiance in a cross section through the middle of the cuvette made with an absorptive holder and a laser light source with 150 x 150 beams. The laser was polarized 90° counterclockwise. The irradiance distribution shows that the light seems to be concentrated in the middle. The total power detected in the middle of the cuvette was 5.343%.



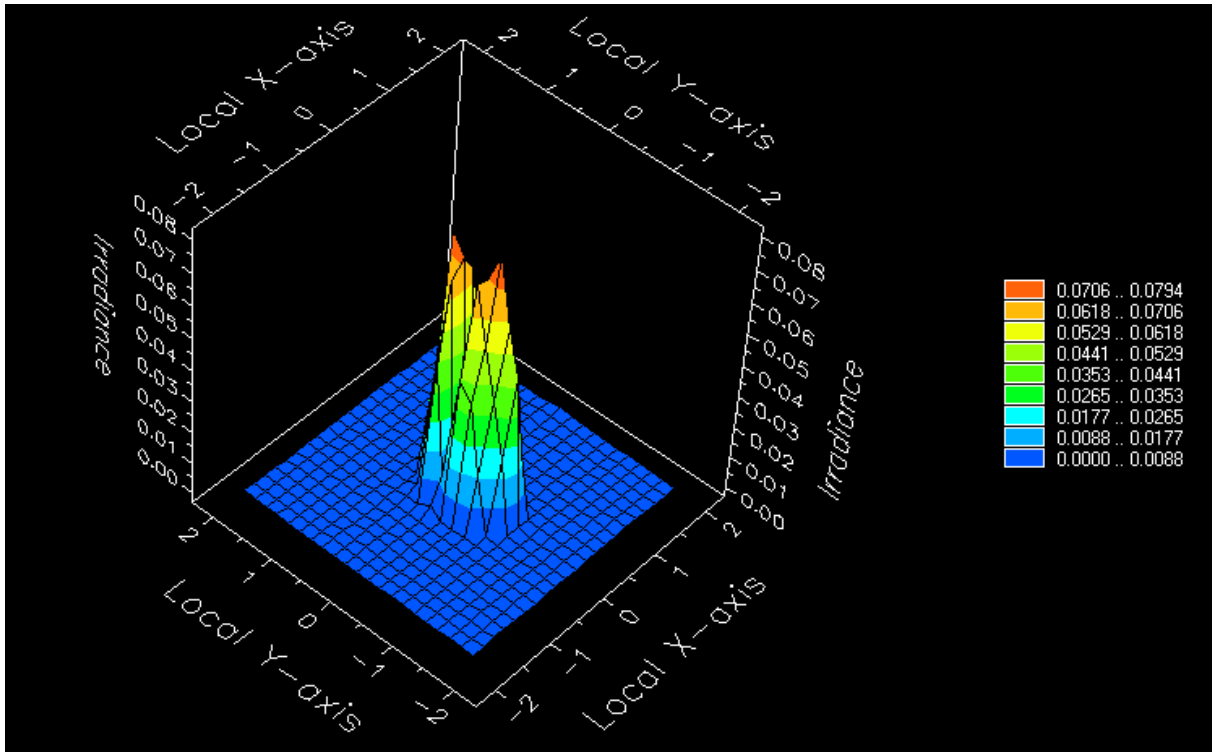


Figure 5.11: The irradiance distribution of laser light in a cross section along the middle of the cuvette. The total integrated power fraction is  $5.3 \times 10^{-2}$ .

Figure 5.10 shows the irradiance distribution across the PD detection surface. The surface is much smaller, but the intensity is still very low. The first half of the propagation the light lost approx. 95% of its intensity, and the other half it was reduced to  $10^{-6}$  of its original power.

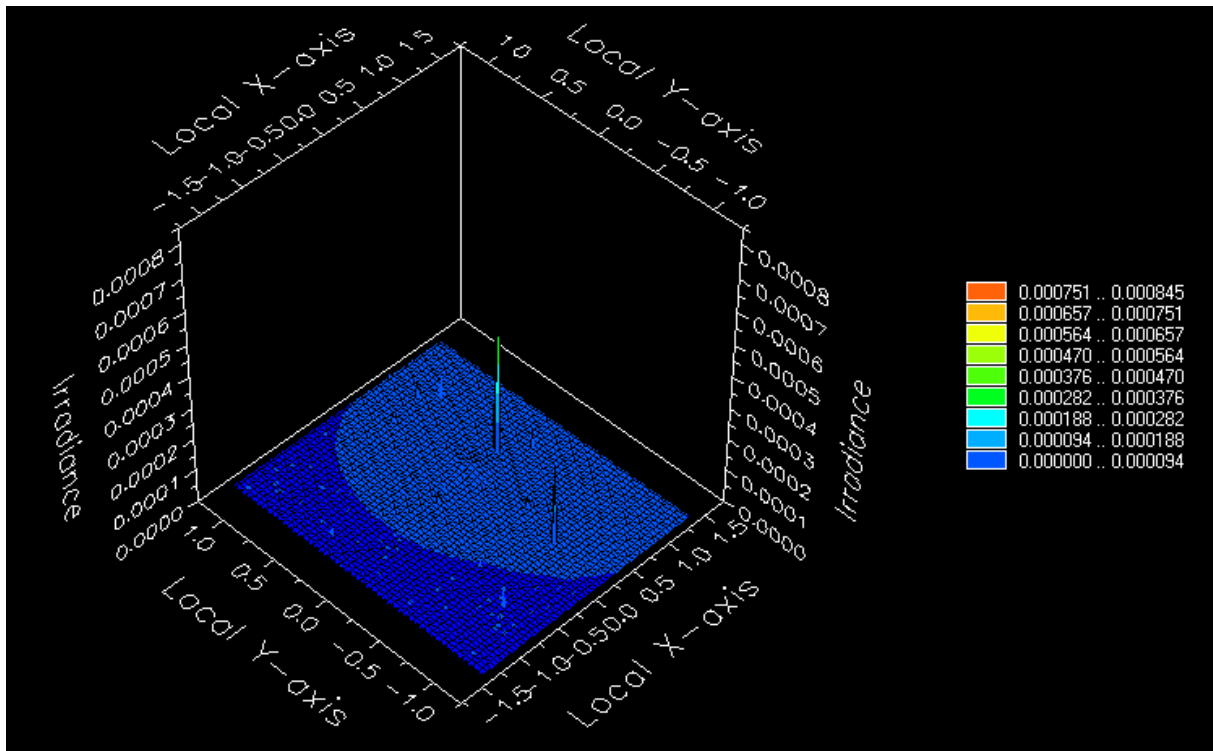
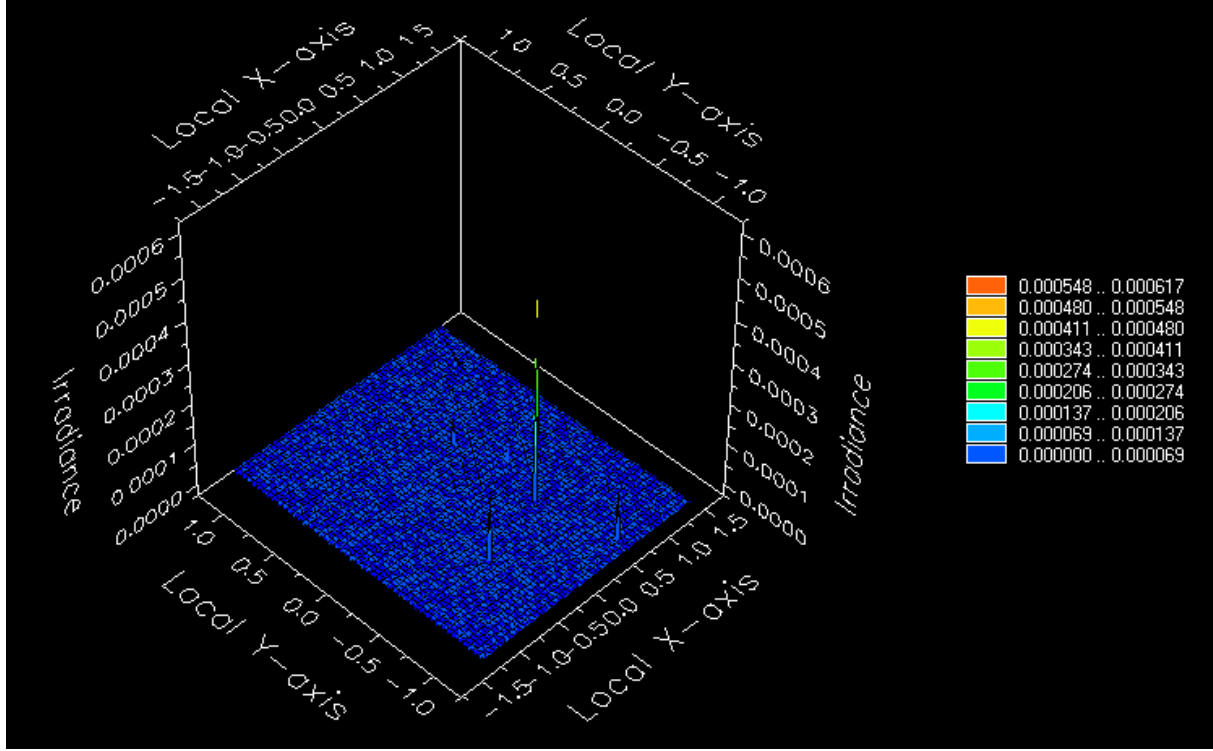


Figure 5.12: The intensity distribution across the PD is very small compared to the middle of the cuvette. The total detected transmittance was  $0.9 \times 10^{-6}$ .

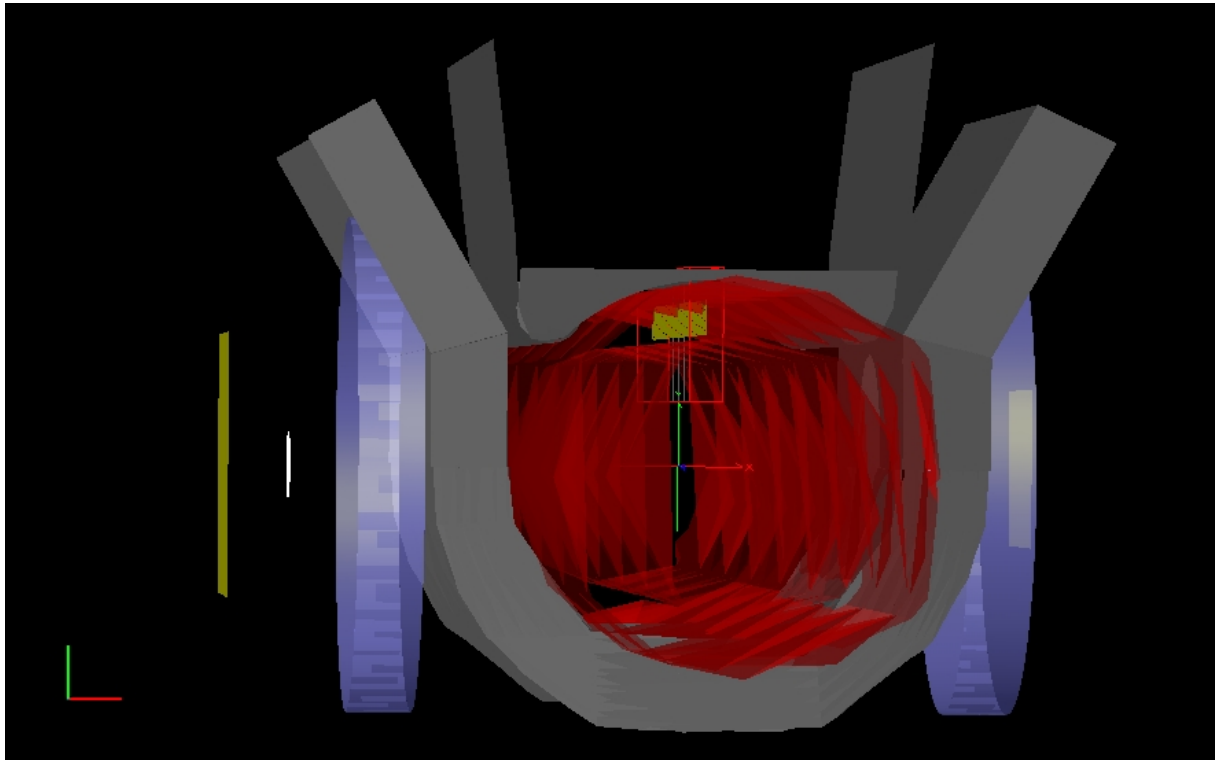
Figure 5.11 shows the PD light irradiance distribution of the reflective holder with the same light source properties as in Figure 5.10.

The transmittance is approximately twice as high as in the absorptive holder, which is consistent with the measurements.



*Figure 5.13:* The irradiance distribution over the PD with a reflective holder. The transmittance was  $1.743 \times 10^{-6}$ .

A detection surface was also placed inside the cuvette above the center of the propagating beam as seen in Figure 5.12. The surface was 5 mm long and 0.5 mm high, so it was just big enough to fit inside the cuvette. The irradiance distribution can be seen in Figure 5.13 and the total fraction of absorbed power reached 0.001248.



*Figure 5.14:* The detection surface inside the cuvette.

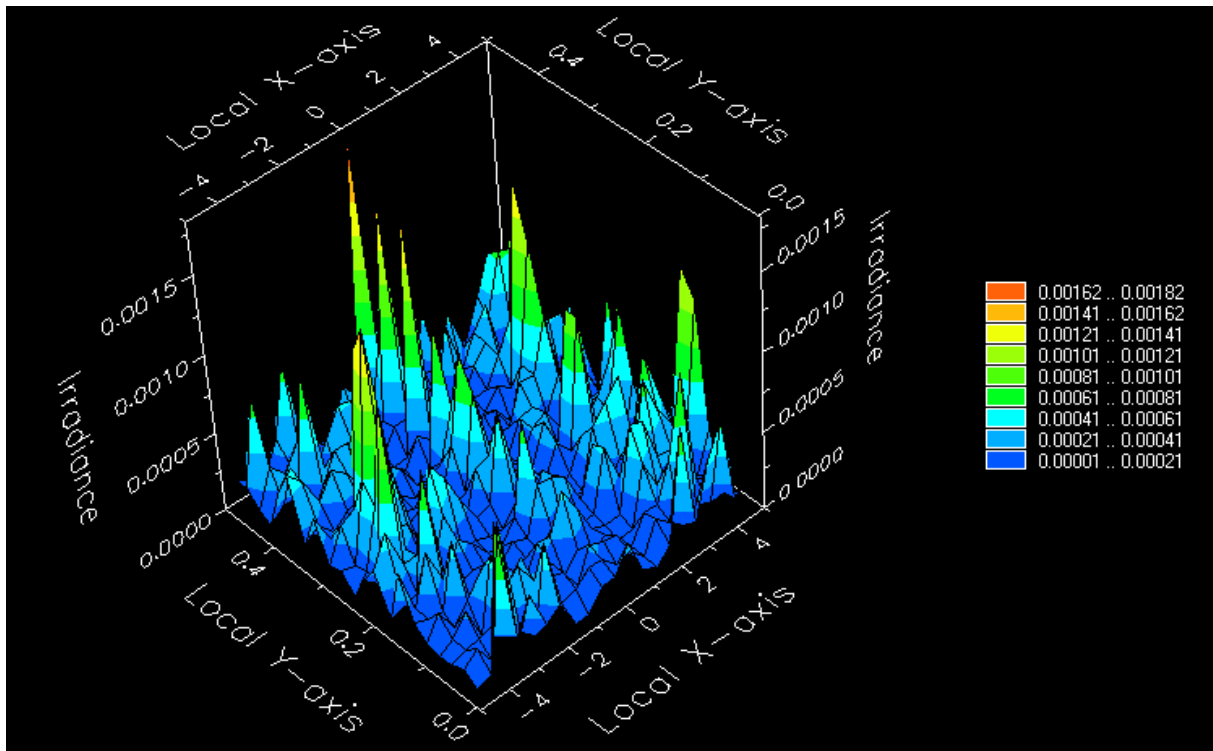


Figure 5.15: The irradiance of the detection surface inside the cuvette. The total absorbed power fraction was  $12 \times 10^{-2}$ , which is quite a lot considering that the power fraction inside the cuvette at the same depth was  $5.3 \times 10^{-2}$ .

## 6. VALIDATION MEASUREMENTS

The results presented in section 5.3 show that haemolysis is possible to detect at high levels. According to Gambro R&D the maximum tolerance for haemolysis in blood was 2% during a haemodialysis session. It was also revealed that complete haemolysis can be obtained by freezing the blood. This information was obtained after the regular experiments had been performed so a new set of validation measurements were done to see how well haemolysis can be detected by using multiple wavelengths. This time absolute measurements were possible since the level of haemolysis was known.

### 6.1. Experimental Setup and Method

Cary 50, a spectrometer from Varian, was used together with a 0.2 mm cuvette to measure the transmission. The spectrometer was set to scan between 780 and 1050 nm with 0.5 nm intervals. Haemolysed blood samples were simulated by mixing 10 ml of regular blood with 100, 200, 400, 600, 800 and 1000  $\mu$ l of completely haemolysed blood.

The haemolysed blood was injected into the cuvette by a syringe. Since the cuvette was so thin it was nearly impossible to wash it between measurements, so for each new measurement the old blood was simply flushed out and new blood was injected. This may have caused the different blood samples in the cuvette to mix to some degree.

### 6.2. Validation Measurements Results and Conclusions

The resulting data was filtered by averaging over the 20 closest samples (10 nm) and it can be seen in Figure 6.1.

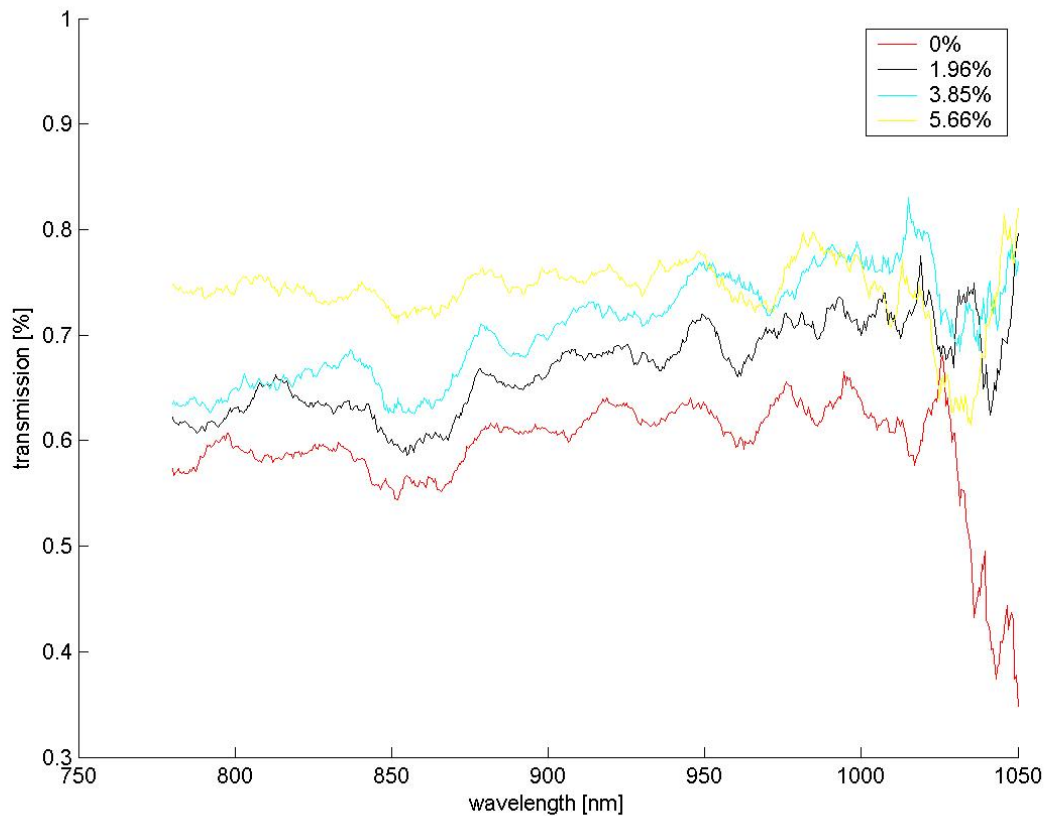


Figure 6.1: The transmission data from the absolute transmission measurements in the 0.2 mm cuvette. The degree of haemolysis can be seen in the box in the upper right corner. The spectra are clearly distinguishable and follow a pattern.

The filtered data shows that it is possible to see a difference in signal at 2 % haemolysed blood which proves that the method is usable for haemolysis detection.

A measurement of 100% haemolysed blood was also done to see how much it differs from whole blood. The results are presented in Figure 6.2 which shows that more than 80 % of the light is transmitted at all wavelengths. The thin cuvette looked like a piece of red-colored glass when observed by the naked eye. Haematocrit measurements by centrifuge showed no segregation of cells and plasma at all; the test tube was completely pink in color.

The signals tend to diverge when the wavelength increases past 1000 nm. This is caused by lower detection efficiency in the NIR spectrum.

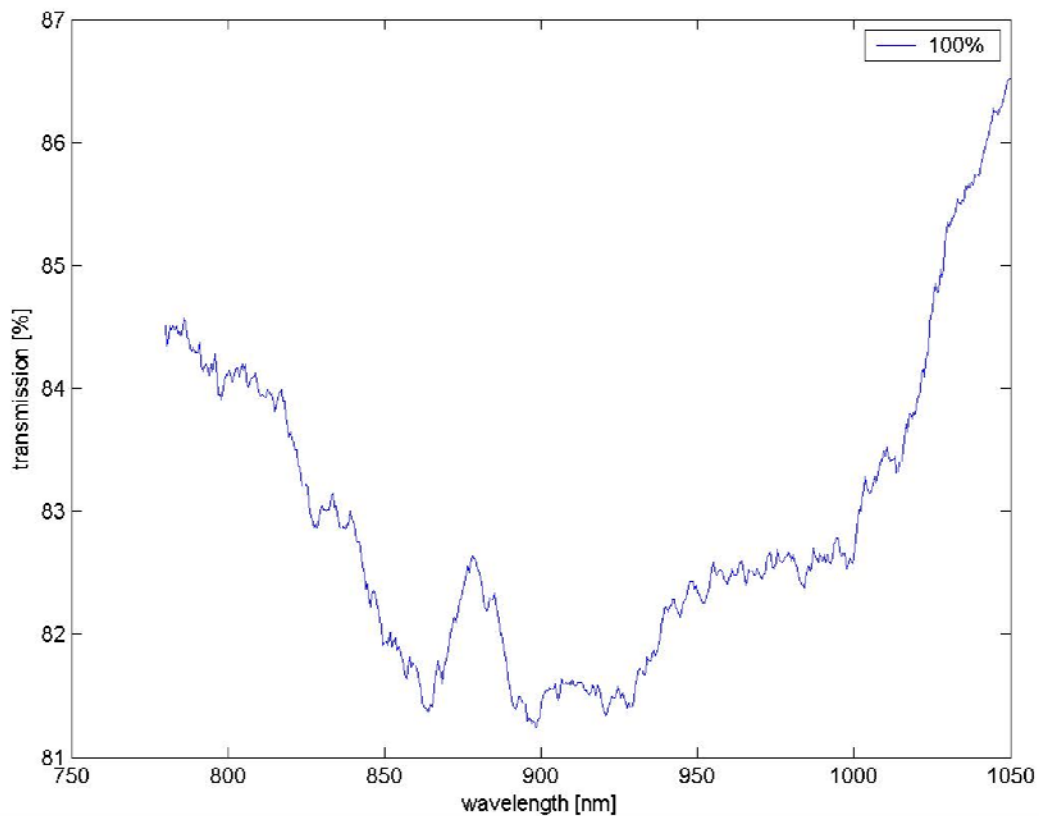


Figure 6.2: The unfiltered transmission spectrum obtained from 100 % haemolysed blood in a 0.2 mm cuvette. More than 80 % of the light is transmitted for all wavelengths.

### 6.3. Validation Measurements Discussion

The validation measurements show that the method can be used to detect low levels of haemolysis in blood. The modified BVS sensor constructed in this thesis had a very different geometry so a direct comparison to relative signal strength at the chosen wavelengths would be meaningless. Prior to the 0.2 mm cuvette measurements a 1 mm cuvette was tested, and the spectrum obtained from haemolysed blood was quite different than the one in Figure 6.2.

The method of freezing and thawing the blood resulted in 100 % haemolysed blood. The blood haemolysed by the mixer had different color and consistence, as well as a completely different haematocrit, so it is safe to assume that the mixer induced haemolysis was far from complete.

Another possibility is that the mixing caused air bubbles to form in the blood which would explain the change of optical properties in the blood. However, during a failed attempt to create haemolysis by bent lines a lot of visible bubbles formed in the blood, but the optical properties didn't change noticeably.





## 7. SUMMARY AND IMPROVEMENTS

### 7.1. Summary of the work presented

The goal of the work presented in this thesis was to create a sensor with the following criteria:

1. To be able to detect if there is any degree of haemolysis while measuring the haematocrit continuously using the BVS cuvette and holder together with multiple wavelengths.
2. To see if it is possible to measure the haematocrit with an absorbing holder instead of a reflective one if a laser module is used.
3. To study how the change of light source from LED to laser and the change of holder affects the measuring and detection quality of the BVS. This could be done both by constructing a theoretical model and by doing measurements.

The first point was achieved by using a single wavelength, 805 nm, to measure the haematocrit. The wavelength is isobestic, which means it isn't affected by variations of blood oxygenation as opposed to the two other wavelengths that were used: 930 and 980 nm. The haemolysis was considered detectable since the signals ratios behave in a specific way when the blood is haemolysed.

The transmission of multiple wavelengths was obtained by switching laser module after each measurement.

The second point proved quite easy to achieve due to the strong light of a laser. Even though the signal was reduced to half strength in comparison to the reflective holder, the total signal strength was greater because of the laser.

The simulations seem to work quite well, since measurements say that at least half of the light is affected by the holder. The irradiance distribution always looks like it contains a lot of transients, which is an indication that too few rays were used. A computation of the distribution took about an hour with 150 x 150 rays on a 1.86 GHz computer. A higher resolution could be obtained by letting the program work with more rays, but since the model wasn't used for quantitative measurements the obtained results were considered sufficient.

The model suggested that the light traveling inside the cuvette is a surprisingly big part of the total emitted light. The detected light (in the PD) is a hundred times weaker than the light detected over the small 5 x 0.5 mm slab. A big portion of the signal may be light that scattered around the blood and hit the detector without ever being inside the blood.

### 7.2. Improvements

This work shows that the method is possible, but it is probable that much better results could be obtained if the BVS could measure two light sources simultaneously instead of sequentially. Perhaps measurements of the absolute degree of haemolysis could be achieved.

Maximizing the laser signal by hand was very tedious and probably a big source of errors so if any further work is done on this the author strongly recommends simultaneous detection of wavelengths.

The secondary wavelengths dependence on the grade of oxygenation of the blood could perhaps be circumvented by using multiple isobestic wavelengths, but they are all located in the visible spectrum and thus they have high attenuation coefficients. The signal strength of the laser modules could make it possible to measure transmission of visible light through a sample as thick as 4 mm.

A better theoretical model of the BVS could also be of use since the sensor is in need of improvements. The effects of the absorbing holder are quite stronger than the model suggested.

The switch of light source had both good and bad effects. Even though the signal strength was much improved the dependence of the polarization of the light is yet another unwanted parameter that can affect the transmission very much due to the anisotropic nature of the plastic cuvette.

The validation measurements showed that it is possible to tell the difference between 0 % and 2 % haemolysis in blood by monitoring the transmittance of multiple wavelengths. Hopefully the next generation of haemodialysis machines will have a blood volume & haemolysis sensor to increase the comfort of the patient.

## Acknowledgements

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Furulund  
July 28, 2008

Christian Ibron

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