An Optical Measurement System for the Analysis of Bacteria in Drain Fluid

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Abstract

When a patient needs to undergo a colon surgery and an anastomosis is constructed to connect the two ends of the colon, an anastomotic leakage may occur. The need for an ex vivo miniaturized system that is able to improve current diagnostic methods for detecting this leakage is important. The mortality rate of patients with anastomotic leakage is high due to the inability to detect bacteria growth at the intervention site. After a colon anastomosis, an early post-operative monitoring system to detect bacteria growth is an essential tool to prevent infection of the patient.

This thesis presents a sensor system based on infrared absorption to detect bacteria growth in two different types of liquid. The first type is a liquid media culture where bacteria are able to metabolize and the second type is peritoneal drain fluid originating from the intervention site in the abdomen of a patient.

The sensor system is built up of two main components: a near-infrared LED and a photodiode to detect the transmitted near-infrared light. A near-infrared LED emitting at a spectral range around 1950 nm is driven by a pulsed current of 30 mA. This pulse is generated with a 20% duty cycle and frequency of 400 Hz. The photodiode is sensitive in the mid-infrared with a spectral range from 800 nm to 2400 nm. A sample holder, which consists of two disc glasses with thickness of 0.4 cm and a diameter of 2 cm, are made of CaF2. The sample holder is placed between the LED and photodiode with a distance of approximately the total thickness of the two discs. The photocurrent that is generated by the photodiode is converted to a voltage with an amplification of $10^5 \Omega$ by a transimpedance amplifier. This output voltage is processed in LabView (National Instruments) and projected on a PC monitor.

The results that are presented in this thesis lead to a better understanding of the absorption of infrared by the samples that are used in the experiment. The liquid samples that were used in the experiment complicates the explanation of the infrared spectrum. There are several effects that cause a molecule to absorb infrared light. The chemical and the bonding structures between atoms play a significant role. In this way, the technique that is used for the analysis of bacteria detection in biological fluids points out some difficulties leading to a discussion about whether this method is applicable for the detection of bacteria present in biological fluids.
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The digestive system performs the complex processes involved in converting food into energy for the activities required to keep the human body functioning. During these processes waste is created as a by-product and it has to be eliminated from the body. The digestive tract is made almost entirely of muscular tissue. These muscles coordinate the movement of food from the moment it enters the mouth, through the stomach and the intestines until it reaches the rectum. Here the solid waste is stored until it is excreted via the anus. After passing through the small intestine, water and electrolytes are absorbed from the food in the large intestine, also called the colon. Many bacteria like *Bacteriodes sp.*, *Lactoboscillus acidophilus*, *Klebsiella sp.* and *Escherichia coli* (E.coli) are present in the colon to help with the digestion process. These bacteria are essential for digesting food, however, due to malfunction on the colon, bacteria could enter the abdominal cavity causing severe infections. Examples of diseases caused by the best known pathogen, E.coli, in human beings are traveler’s diarrhea and urinary tract infections.

Some diseases that could lead to a surgical resection of the colon (colectomy) are colon cancer, trauma, bowel infarction and polyposis. After resection, an anastomosis (suturing) is often the surgical intervention (Figure 1.1) to restore the continuity of the colon.

![Figure 1.1: Anastomotic intervention.](image)

The construction of this anastomosis introduces the risk of dehiscence (insufficiency/breakdown of the suture), which leads to anastomotic leakage. This is a complication that occurs when the content of the intestine leaks into the abdominal cavity, due
to a defect in the anastomosis [1]. Infections caused by non-sterile substances originating from the colon result in peritonitis, which is the inflammation of the peritoneum (the tissue layer of cells lining the inner wall of the abdomen), followed by abscesses inside the abdominal cavity. This infection can further lead to sepsis - otherwise known as blood poisoning-, multiple-organ failure and even death. In the Netherlands, the incidence of anastomotic leakage is 7% with a mortality rate up to 33% [1]. The total number of patients receiving an antomosis between 2000 and 2004 is 10,000, which means that each year approximately 700 patients will suffer from anastomotic leakage and that each year at least 200 patients will die because of it [1]. Furthermore, the number of patients with primary anastomosis is increasing per year (see Figure 1.2).

![Figure 1.2: Numbers of colorectal procedures performed per year in the Netherlands [1].](image)

For the purpose of detecting complications due to the insufficiency of an anastomosis, a prophylactic drain may be placed at the surgical site (Figure 1.3). By performing a microbiological analysis on this drain fluid, the quantity and type of bacteria due to the spill of intestinal content could be determined. The problem of current diagnostic methods is the required time to diagnose anastomotic leakage. The time that is needed to diagnose anastomotic leakage causes the patient to be ill already before a treatment could take place. In addition to this, multiple tests cause an increase medical cost. Therefore, a great challenge is to detect anastomotic leakage in an early postoperative phase.

Currently, there are three existing experimental methods that can be used to perform a microbiological analysis on drain fluid [1]. The first method is bacteria culture, which determines the type of bacteria present in the drain fluid. A sample from the drain fluid is placed on a glass plate on which known nutrients for a specific bacteria type are provided. The identification of bacteria types is highly complex and laboratories have different identification systems. Most of the time, identification is based on the colonial and microscopic morphology, growth on selective media, oxygen tolerance and biochemical characteristics. Unfortunately, this method can only specify the type of bacteria and not
1.1. MOTIVATION OF THE PROJECT

Figure 1.3: The collection of drain fluid.

The trend of bacterial growth. Furthermore, this method is time consuming, taking at least 24 hours to obtain the results.

The second method is Raman Spectroscopy, which is based on inelastic light scattering of a molecule. Raman scattering occurs together with the change in vibrational, rotational or electronic energy of a molecule. The difference in energy between the incident photon and the Raman scattered photon is equal to the vibrational energy of the molecule. A plot of intensity of scattered light versus energy difference is known as the Raman spectrum. Analyzing the spectrum of these photons identifies the molecules. The disadvantage of this method is the high purchase costs of the instrumentation.

The third method is Polymerase Chain Reaction (PCR); this method is an enzymatic in vitro amplification of a piece of 'target' DNA [2]. This amplification of DNA to make million of copies, allows for identification using visual techniques based on size and charge. Due to the high sensitivity of PCR, it is also sensitive to contamination errors, which can lead to unreliable results. Another disadvantage is the high cost of a PCR-machine, which makes this method not suitable for large-scale or routine experiments.

An alternative method is required to enhance the microbiological analysis without having the disadvantages mentioned above. The objective of this study is to fabricate a low cost measurement system, which can be used for monitoring the growth of bacteria in drain fluid clinically.

1.1 Motivation of the project

A number of studies are currently in progress to enhance the diagnostic method to detect anastomotic leakage. The diagnostic methods currently used in clinical practice include observation of clinical symptoms and imaging. Nevertheless, the results of these methods are not absolutely reliable in detecting an infection in the abdominal cavity of the patient. On the other hand, the mortality rate for the intervention of an anastomotic leakage
worldwide is 39% [1], which points out the severity of the problem. Early diagnosis of anastomotic leakage is desired to prevent complications for the patient. The goal of this work is to help clinicians detect anastomotic leakage in the early postoperative period. In this respect, an optical monitoring system was fabricated by means of which clinicians should be warned on time so that infection of the patient can be prevented.

The optical measurement system that is meant for the detection of bacteria in drain fluid proposed in this report consists of a single-wavelength near-infrared LED and a photodiode which is sensitive to this wavelength.

The bacteria analysis is done by analyzing drain fluid. The prophylactic drain in Figure 1.3 is placed at the surgery site to withdraw this drain fluid, which passively flows through the drain from the abdominal cavity.

In a previous study [3], a method to detect bacteria based on the absorption of near-infrared radiation in drain fluid has been investigated. Using a spectroscopic setup to measure absorption, a set of discrete wavelengths was found with strong contrast between contaminated and clear samples. Based on these findings we developed the optical measurement system that should detect the trend of bacteria growth in time. The size and weight of the optical sensor should be small, bedside applicable (so that the drain analysis can be performed directly) and have low fabrication costs.

In this study, bacteria in basal culture media in liquid form are supplied from the Erasmus Medical Centre (EMC) in Rotterdam. The fluid samples to be examined are exposed to an infrared light source. When the photons interact with the molecules in the sample, some photons will be reflected, and others absorbed or transmitted. The degree of absorption of photons by the sample gives information about the chemical and physical properties of the fluid. The challenge is to use this principle to detect the presence of microorganisms in contaminated samples. The fluids that are examined in this study are liquid nutrient medium for the growth of bacteria, with different percentages of bacteria contamination and peritoneal drain fluid.

1.2 Structure of the thesis

The thesis is organized as follows. The medical background regarding the analysis of drain fluid is described in chapter 2. The different diseases that may occur in the colon are described with the main focus on the explanation of anastomotic intervention. The analysis of the composition of drain fluid is also described in this chapter. The design of the optical measurement system (OMS) is presented in chapter 3. This chapter also describes the concept and basic principle of the sensor system. Further in the chapter, the constructive steps of the design are presented from the mechanical and electrical point of view. The measurement results obtained from experimental testing of the bacteria detection in contaminated liquid medium and the analysis of peritoneal drain fluid are presented in chapter 4. Finally, chapter 5 gives the general discussions of the interpretation of the results and the future work recommendations.
In this chapter, a general description of the anatomy and the function of the colon are given. Due to the development of colonic cancer, a patient might have to undergo a colectomy, which involves the removal of a section of the colon. Clinicians may construct an anastomosis to restore the continuity of the colon. Risks that could be encountered in this procedure are described in this chapter. In order to monitor the post-operative progression, a new diagnostic method is desired. The chapter also covers a description of the composition of the samples that are used for the experiment. The chapter ends by introducing the different bacteria types, which are present in the sample used for the in-vitro testing of the measurement setup. Also the composition of the sample that is used for the experiment is described.

2.1 Medical overview: the large intestine

The large intestine consists of the colon and the rectum (see Figure 2.1) and is the end of the digestive tract. When the chyme (the semi-fluid mass of the digested food expelled by the small intestine) passes through the large intestine, the digestion process is complete. Furthermore, when chyme enters the large intestine, water and ions (Na+, K+, Cl-) are extracted [4]. With the help of microorganisms the liquid is converted into feces. These feces are a semi-solid mixture stored in the large intestine until they are eliminated through the anus during defecation.

![Figure 2.1: The anatomy of the large intestine.](image-url)
The amount of chyme that enters the large intestine varies between 100mL to 500mL per day, one third being excreted as feces and the rest being absorbed back into the body to prevent dehydration. During the process of the extraction of water, salts and vitamins, intestinal bacteria, such as *E. coli* and *Bacillus sp.*, play an important role in synthesizing these vitamins. Furthermore, they make up almost the half the weight of the feces.

Bacteria are very useful for the digestion process and as long as they remain in the colon there will be no complications. However, if the content of the colon enters the abdominal cavity, severe inflammation due to the invasion of bacteria on healthy tissues will take place.

### 2.2 Anastomosis of the colon

A colon anastomosis is the connection of two ends of the colon with the purpose of restoring colonic continuity. One of the diseases that leads to anastomosis of the colon is colorectal cancer. Colorectal cancer develops from the growth of malignant tissue that occurs on the inside lining of the large intestine, which the incidence increases as individuals get older.

Inflammation diseases that can lead to an anastomosis of the colon are Crohn disease and ulcerative colitis, which is a chronic inflammation of the colon caused by bacteria infection and frequently referred to as inflammatory bowel disease (IBD). Another colon disease is diverticulitis, which is an inflammation of small out-pouches along the wall of the colon due to weakening of the walls of the colon and elevated pressure due to constipation.

When surgery is conducted for the treatment of cancer, the tumor, a margin of the surrounding healthy bowel and adjacent lymph nodes are removed. Subsequently, the two healthy sections of the bowel are reconnected.

Figure 2.2 shows an anastomosis after a sigmoid (the lower portion of the large bowel) resection. A segment of the sigmoid colon is removed and the descending colon is reconnected to the rectum.

![Figure 2.2: A sigmoid colectomy, where the whole part of the sigmoid is removed.](image)

There are two possible surgery techniques to place an anastomosis: stapling and suturing. A large number of studies are currently being conducted to attenuate risk factors
that lead to anastomotic insufficiency. The main focus is to optimize the restoration of the intestine wall of the colon ends after a resection took place.

2.3 The cause of anastomotic leakage

As a result of an unsuccessful restoration of the colon anastomosis, a defect in the intestinal wall can develop. In some cases, due to ischemia (inadequate blood supply) the intestinal content leaks in the abdominal cavity. When anastomotic leakage occurs, inflammation of the peritoneum (the tissue layer of cells lining the inner wall of the abdomen and pelvis) also called peritonitis will develop at the leakage site. As a result, pus will accumulate locally (abscess) and after a while it spreads into the abdominal cavity. This pus, which contains bacteria will penetrate through organ tissues and eventually enters the blood stream. This condition will lead to blood stream infection also called sepsis. Sepsis can be a life threatening disease that needs urgent and comprehensive care.

In colorectal surgery, anastomotic insufficiency is the major cause of the high mortality rate. Several risk factors for anastomotic leakage after resection of the colon are malnutrition, long course radiotherapy and chemotherapy, preoperative steroid use, placement of the anastomosis at a lower part of the colon, intra-operative blood loss and intra-operative adverse events [5],[6]. Uncertainty about the actual cause of anastomotic leakage is still present, despite the identification of several potential risk factors.

2.4 Diagnosis of anastomotic leakage

Effective methods are needed to confirm the diagnosis of anastomotic leakage. When a surgical intervention takes place in the colon, several substances will be present at the surgical site. These substances can be divided into two categories: the clinical chemical parameters and the microorganisms for bacteria analysis. These substances will be used as diagnostic parameters for the detection of anastomotic leakage.

2.4.1 The clinical chemical parameters

The diagnostic parameters of the clinical chemical substances after a surgical intervention are described below [1]. Immune parameters:

1. Interleukins, a group of proteins that enhance the communication between cells. The level of interleukin will significantly increase due to inflammation response after injury. If there are no complications, this level will decrease.

2. Pancreatic enzyme, the enzyme in the pancreas that is used for the digestion of food. It is also present in the feces and may be present in the abdominal cavity when leakage occurs.

3. Tissue repair parameters: Matrix metalloproteinase’s (MMPs), a group of enzymes that can break down proteins. These enzymes need zinc or calcium atoms for tissue repair and regeneration.
4. Ischemia parameters: When blood supply becomes inadequate, organs will develop oxygen shortage. The blood flow decelerates which will cause a decrease of the pH and a lowering the the glucose level. The damaged healthy tissue degenerates and expels phospholipids. The amount of fatty acids and glycerol, which result from the splitting up of phospholipids, can be used as a diagnostic parameter.

5. Inflammatory parameters: CRP (C-reactive protein), a plasma protein that rises in the blood with the inflammation from surgery, infection and advanced cancer.

2.4.2 Microorganisms for bacteria analysis

During a colectomy, a spill of intestinal content is inevitable, when this occurs bacteria will be present in the abdominal cavity. For this reason, some bacteria that reside in the colon are examined in this study. The bacteria analysis used in this study are the following:

1. *Escherichia coli* (*E*.coli) (gram negative)
   This bacterium is commonly found in the lower intestine of warm-blooded beings. Some *E*.coli strains posses flagella that are used to swim and are motile. Figure 2.3.a shows a picture with 10.000x magnification of an *E*.coli cluster. *E*.coli has a bacillary shape and has an average size of 1.1 – 1.5µm wide by 2.0 – 6.0µm long. Most *E*.coli strains are harmless; these strains are part of the normal flora of the gut and can be beneficial for the host by producing vitamin K2 or by preventing the establishment of pathogenic bacteria in the intestine. *E*.coli uses mixed-acids fermentation in anaerobic conditions, producing lactate (C3H6O3), succinate (C4H6O4), ethanol (C2H5OH), acetate (CH3COO-) and carbon dioxide (CO2). *E*.coli falls under facultative anaerobes, thus able to grow in the presence or absence of oxygen.

2. *Klebsiella sp.* (gram negative)
   These bacteria species are ubiquitous in nature. Like *E*.coli, *Klebsiella sp.* has also a bacillary shape with a size of 0.5µm wide and 2µm long. They are non-motile because they do not have flagella, and have a polysaccharide-based capsule. Klebsiellas are rod-shaped, aerobic bacteria and some produce an extra cellular toxic complex. They are composed of 63% capsular polysaccharide, 30% lipopolysaccharide and 7% protein. As can be seen in Figure 2.3.b, the bacteria form a planarity colony, as opposed to *E*.coli, forming a complex piled up cluster (Figure 2.3.a).

3. *Staphylococcus warneri* (gram positive)
   These bacteria species do not have flagella either, thus are non-motile. They have a round or ovoid shape also called cocci (see Figure 2.3.c) and live in pairs or chains of varying length. The size of this bacterium varies with a diameter of around 0.2 – 0.8µm. They grow in clusters, pairs and occasionally in short chains. They mainly grow by aerobic respiration or fermentation that produces lactic acid (C3H6O3).

Several engineering methods to detect anastomotic leakage are currently under development. One of the proposed techniques is the use of an oxygen tension (pO2) sensor.
to monitor the oxygenation of tissues. The outcome of this sensor system will reveal the sufficiency of the tissue during and after anastomosis is constructed on the colon [7]. Another method introduces the use of infrared spectroscopy to detect anastomotic leakage. This study covers the absorption of infrared light by bacteria due to the leakage [3]. Based on this, the discussion and development of a miniature bacteria detection system will be the main topic in this report.

2.5 Peritoneal drain fluid analysis

In paragraph 2.4.1 the chemical substances that may be present during or after a surgical intervention were described. In this paragraph, the description of the two types of fluids used for bacteria analysis are presented.

Peritoneal drain fluid is the fluid that originates from the peritoneum which is the smooth membrane lining the cavity of the abdomen. When a patient has undergone colonic anastomosis, this fluid is eliminated from the anastomotic site through a prophylactic drainage. Firstly, this method is meant to improve the post-operative healing process. Secondly, the drain fluid is an appropriate sample to monitor a selection of the diagnostic parameters presented in the previous paragraph.

In the following subparagraphs, the composition of drain fluid and of the samples that are used for the initial tests is described.

2.5.1 Composition of the drain fluid

The drain fluid consists of peritoneal fluid, wound fluid and blood.

1. The peritoneum is composed of a layer of mesothelium (see Figure 2.4.a), a membrane that forms the lining of several body cavities, which is supported by a thin layer of connective tissue. This connective tissue is derived from the germ layers: ectoderm, endoderm and the mesoderm.
2. The wound fluid is generated by the angiogenesis process where certain protein catabolism occurs by hydrolysis to develop new blood vessels.

3. Blood is a fluid that circulates through the blood vessels of human beings, transporting oxygen, nutrients and hormones to tissue and cells, carrying away waste products to be excreted. Also, blood is important to maintain a uniform body temperature. Blood consists of cells that flow in plasma and is composed of red blood cells (also called RBC or erythrocytes), white blood cells (including leukocytes and lymphocytes) and platelets (also called thrombocytes), see Figure 2.4.c. Blood consists of cells that flow in plasma. The dissolved substances in plasma are electrolytes, nutrients and vitamins, hormones, clotting factors and proteins such as albumin and immunoglobulin.

![Image of blood cell structure](image)

Figure 2.4: a) The mesothelium cell structure; b) L is white blood cell, E is red blood cell and P is platelet; c) Enlarged blood composition.

### 2.5.2 Samples provided by Erasmus Medical Centre (EMC)

The Erasmus Medical Centre (EMC), Rotterdam, provided the samples that were used for the experiments. These are preliminary samples that are used to perform an analysis on bacteria detection in liquid nutrient media (culture media). The basic element of these culture media is peptone. Peptone is a derived protein or various soluble compounds produced by partial hydrolysis of natural proteins; it is also used as a nutrient in bacteria culture media. Examples of the derived proteins are polypeptides and amino acids from the enzymatic digestion of meat.

In addition to peptone, Brain-Heart Infusion (BHI) solids are used in the culture media. This BHI is composed of a dehydrated infusion of porcine brain and hearts (meat extract). It provides nitrogen, amino acids and vitamins in bacterial culture media. BHI solids are processed from large volumes of raw material, retaining nutritive and growth stimulating properties of fresh tissues. This composition is an appropriate media to allow bacteria to grow in a normal in vivo condition.

As described in paragraph 2.4.2, the bacteria that are grown in culture media provided by EMC are the most common organisms associated with intra-abdominal abscesses: *E. coli* together with *Klebsiella species*. Also, *E. coli* is the most common causative agent of peritonitis, as mentioned in paragraph 2.3. This is the reason for which these
bacteria are the main study in this thesis. The first attempt is the interpretation of bacteria behavior based on the measurement results obtained from the optical measurement setup (OMS). A detailed description of the OMS will be given in the next chapter.

2.5.3 Samples provided by Reinier de Graaf Groep in Delft (RGGD)

The peritoneal drain fluid that is used in this experiment is provided by a health institution in Delft called Reinier de Graaf Groep that also participates in the multicohorent research, the APPEAL-study. The peritoneal drain fluid is freshly tapped from the patient each day over the course of 5 days. The practical procedures of the experiment will be described in the following chapters.

Conclusion

The search for a diagnostic parameter that would lead to the detection of anastomotic insufficiency is crucial for the prevention of complication that may develop by a patient who has undergone an anastomosis. There are two diagnostic parameter categories; chemical and microbiological. This study in thesis analyzes the infrared absorption by different types of bacteria that are commonly present in the colon. The focus is to interpret the obtained results to study the growth and property changes of the bacteria in a span of several days.
The optical measurement system (OMS) introduced in this chapter is based on infrared spectroscopy. Infrared spectroscopy is the most important and most common analytical technique used for the study of a variety of samples. Samples in all physical forms (liquid, solution, paste, powder, film, fiber or gas) can be examined. In this thesis, the infrared light absorption by a liquid sample containing bacteria is investigated as a function of the contamination concentration and measurement time.

In this chapter, the basic principle of infrared spectroscopy is presented and its application to analyze bacteria in fluids is described. Furthermore, a design proposal for an OMS is introduced with design criteria that are needed to perform the experiments for this study. The design of the OMS will be characterised from a mechanical and electrical point of view.

3.1 Near-infrared spectroscopy

Spectroscopy is the study of the interaction between radiation and matter. Near-infrared (near-IR) spectroscopy uses the spectrum from about 750 nm to 2000 nm wavelength (see Figure 3.1 for the division of near-IR in the EM spectrum). The basic principle of this technique is related to the vibration of the atoms of a molecule. The absorbed fraction of the incident radiation at a particular energy corresponds to the vibration frequency of a sample molecule. The vibration of molecules forms the fundament for the interpretation of infrared spectra.

![Figure 3.1: Electromagnetic radiation spectrum.](image-url)
CHAPTER 3. OPTICAL MEASUREMENT SYSTEM (OMS) FOR BACTERIA ANALYSIS IN BIOLOGICAL FLUIDS

To explain the varied interactions between radiation and many forms of matter, we explore Maxwell’s classical and Einstein’s quantum theories. According to Maxwell, radiation is composed of two mutually perpendicular electric and magnetic waves (Figure 3.2).

![Figure 3.2: Representation of the electromagnetic wave.](image)

The velocity of a wave traveling a fixed distance is equal to the product of the wavelength ($\lambda$) and the frequency ($\nu$). The wavelength can also be defined as the distance between two adjacent peaks (see Figure 3.2) and the frequency relates the number of cycles per second [8].

$$c = \lambda \nu. \quad (3.1)$$

where $c$ is the velocity of light ($c = 2.99725 \times 10^8 m/s$) in vacuum.

To determine the number of waves in a length of one centimeter, the wavenumber is defined as:

$$k = \frac{1}{\lambda} = \frac{\nu}{c}. \quad (3.2)$$

The advantage of this unit is that it is directly proportional to the true frequency and the quantum energy. In the near-infrared wavelength region, the wavenumber lies between the regions $13.000 - 4.000 cm^{-1}$ [8].

According to Einstein, Planck and Bohr, electromagnetic radiation should be regarded as a flow of particles carrying defined amount of energy. Energy $E$, is given by Bohr’s equation [8]:

$$E = h\nu. \quad (3.3)$$

where $h$ is Planck’s constant ($h = 6.626 \times 10^{-34} Js$) and is equal to the classical wave frequency. With this concept, spectroscopy can be further elaborated.

The process of molecular change, e.g. vibration and rotation associated with infrared spectroscopy, can be represented in terms of quantized discrete energy levels $E_0$, $E_1$, $E_2$, etc.
3.2. NEAR-INFRARED ABSORPTION SPECTROSCOPY OF BIOLOGICAL FLUIDS

etc. (Figure 3.3) [8]. Whenever a molecule interacts with radiation, a quantum of energy (or photon) is either emitted or absorbed. This occurs as long as the quantum energy is exactly fitting the energy gap $E_1 - E_0$ or $E_2 - E_1$, etc. The quantum energy is related to the frequency as follows:

$$\Delta E = h\nu. \quad (3.4)$$

The frequency of the emitted or absorbed photons corresponds to the vibration and rotation of the atom, is given by:

$$v = \frac{(E_2 - E_1)}{h}. \quad (3.5)$$

As can be seen in Figure 3.3, the uptake of quantum energy is associated with absorption meaning that a molecule returns to its original state (deactivation). Whereas, loosing quantum energy is associated with emission (excitation) [8].

![Figure 3.3: Illustration of quantized discrete energy levels.](image)

3.2 Near-infrared absorption spectroscopy of biological fluids

Infrared absorption spectroscopy utilizes the physical properties of molecules for analysis. In order for a molecule to interact with electromagnetic radiation, i.e., absorb it, it has to interact with either the fluctuating electrical field or the fluctuating magnetic field at right angles to it. Each molecule has a specific frequency at which it vibrates or rotates due to interaction with electromagnetic radiation at that frequency, which corresponds to the discrete energy levels. The specific requirement for a molecule to absorb infrared radiation is that the electric dipole moment of the molecule must change during vibration.
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Infrared (IR) Vibration of Biological Molecules

It is known from literature that the application of IR vibration on biological materials has some limitations regarding the information obtained from the results [9]. Among other factors, the large size and the asymmetrical structure of biological molecules complicate the analysis. Also, biological molecules are frequently investigated in a solution state, aqueous or non-aqueous, causing the exclusion of partial vibrational information due to absorption or scattering interference by the solvent. In aqueous solution, it is desirable to remove the relatively large effects of hydrogen bonding between water and its solution such that the bonding between solute molecules themselves can be studied. Thus, a solvent is needed which lacks both donor and acceptor sites for hydrogen bond formation. A hydrogen bond is a special type of dipole-dipole force that exists between an electronegative atom and a hydrogen atom bonded to another electronegative atom such as nitrogen, oxygen or carbon. Hydrogen bonding is a very important effect in infrared spectroscopy. This bonding influences the bond stiffness and so alters the frequency of vibration. Hydrogen attractions can occur between molecules (intermolecular) or within different parts of a single molecule (intra-molecular) [8].

A bond between two atoms has an average length and not a specific length, because the bond behaves as if it were a vibrating spring connecting two atoms. Furthermore, a bond vibrates with both stretching and bending motions. Figure 3.4 gives the vibration complexities of molecules containing three or more atoms.

Figure 3.4: Stretching and bending vibrations of bonds in organic molecules. This picture is taken from [10].

As mentioned before, if the frequency is matched between the incident radiation and
the vibration frequency of the molecule, it will cause the molecule to absorb energy. This allows the bonds to stretch and bend more, thus, increasing the amplitude of the vibration. Each type of vibration of a bond in a molecule happens due to a specific frequency.

The intensity of absorption bands depends on the following factors [10]:

1. The greater the change in dipole moment associated with the vibration, the higher the intensity of the absorption.

2. The larger the number of bonds responsible for the absorption increases the intensity of the absorption.

3. The strength of the bond and the masses of the bonded atoms. The stronger the bond between the atoms, the higher the energy frequency that is needed to vibrate the molecule. The heavier the mass of the atoms, the lower the required frequency.

4. Symmetrical molecules lead to a weak infrared absorption. This is due to the fact that no changes occur in the dipole moment when the bond stretches.

To investigate and analyze these effects in the samples used in this research, we need to study the chemical properties of the molecules contained in the samples. The two main samples that are used in this study are bacteria culture media (in liquid form) to grow bacteria and peritoneal drain fluid that originates from the abdominal site of a patient after an anastomotic surgery on the intestine.

3.2.1 Analysis of the bacteria culture media (in liquid form) for the growth of \(E.\text{coli}\), \textit{Klebsiella sp.} and \textit{Staphylococcus warneri}

As mentioned in the previous chapter, the main chemical compound that forms the liquid nutrient media for the growth of bacteria is peptone. Polypeptides are the main chemical compound of peptone, as hydrolysis is used to decompose proteins in watersoluble structures. Hydrolysis is the decomposition of a chemical compound by reaction with water. This chemical reaction consists of water and another reactant exchanging functional groups to form two products: one containing the \(H^+\) and the other the \(OH^-\) group. This means that peptone contains a hydrogen bond donor. This will give a broad absorption spectrum in the infrared absorption band. Due to the single wavelength spectrum (\(\approx 5260 \text{ cm}^{-1}\)) that is used in this sensor system, this effect may increase the complexity to interpret the absorption behavior of the sample. Variations in temperature, concentrations of electrolytes, pH and also strong interaction with biopolymers such as protein will produce significant changes in the absorption spectrum. It will lead to broadening of the absorption bands due to large variations in the molecular force field and structures resulting from intermolecular interactions [9].

Another factor that should be considered is the effect of a small amount of \(E.\text{coli}\), \textit{Klebsiella sp.} or \textit{Staphylococcus} being inoculated into the medium. Metabolism of the bacteria will take place due to the consumption of the available nutrition in the media and favourable atmospheric conditions. The temperature of the environment which permits the growth of the bacteria is between 20 – 50°Celsius and pH of the media needs to be
between 5 – 9 with an optimal growth at pH neutral (pH = 7). The most important metabolic system that occurs is the synthesizing of protein into amino acids. Due to N-H stretching, amino acids have characteristic infrared absorption bands around 3000 cm\(^{-1}\) [10]. The waste products from this process are e.g. lactate, succinate, ethanol, acetate and carbon dioxide.

### 3.2.2 Analysis of the peritoneal drain fluid

The peritoneal drain fluid has a more complex molecular structure. The relevant physiology of the drain fluid has been described in Chapter 2.5.1. A significant characteristic of peritoneal drain fluid is the large molecule structure that applies to the four conventional biopolymers: nucleotides, proteins, carbohydrates and lipids.

In a previous study, [3], it is stated that infrared absorption is able to distinguish between drain fluid samples with varying percentages of contaminant. There were two kinds of samples used: clean drain fluid (assumed 0% contamination) and highly contaminated drain fluid (assumed 100% contamination) with significant color change. The samples were mixed such that test tubes contained 10 cm\(^3\) of solution with each successive sample containing 1 cm\(^3\) more contaminant and 1 cm\(^3\) less drain fluid respectively.

The result of this study is a clear visible trend of decreasing transmission in infrared due to the increase of contaminant volume in the test samples. This trend has an optimal gradient change in two wavelengths: \(\approx 1900\) nm and \(\approx 4400\) nm.

However, the exact composition of the samples that have been tested remains undefined. As it is mentioned before, drain fluid has a very complex molecule structure with different infrared-active molecule groups. It is unclear whether the change of absorption is due to the increase of contaminant or the reduction of clear drain fluid. Thus, a thorough examination of the method for the analysis of drain fluid should be performed.

### 3.3 Design of the optical measurement setup (OMS)

To perform the experiments in an accurate and convenient way, the design of the measurement setup demands a well-defined design which will be described in chapter 3.3.1. The main components of the OMS are the Light Emitting Diode (LED), the photodiode and a holder to keep the liquid sample. With this design the aim is to minimize all external perturbations (mechanical and electrical) that can influence the measurement results. The description of all the conditions will be given from an electrical and mechanical point of view.

#### 3.3.1 Electrical part

The main electrical elements and software used to develop the optical measurement system are: LED drive, photodiode readout and LabView readout software.

- **Light source** (Figure 3.5). The LED has a spectral region around 1950 nm (wavenumber 5263 cm\(^{-1}\)). The model type is LED19-PR, where a parabolic reflector is used to align the beam spectrum. Heterostructures are grown on a GaSb substrate to enhance the output power. The emitting area is 300 × 300 \(\mu m\).
3.3. DESIGN OF THE OPTICAL MEASUREMENT SETUP (OMS)

Figure 3.5: The LED19-PR.

- **Light detector** (Figure 3.6). The photodiode has a detection area in the spectral range from 800 to 2400 nm. Heterostructures with the InGaAsSb active layer and the AlGaAsSb "window" are grown on GaSb substrates. The photodiode has a sensitive circular area with a diameter of 2 mm.

Figure 3.6: The PD24-20.

- **Data acquisition** (Figure 3.7). The National Instruments (NI) USB-6259 is the data acquisition device that is used to generate the signals. The device has the capability to handle up to 32 analog inputs with a resolution of 16 bits and a maximum sample rate of 1.25 MS/s. The obtained data is sent to a PC and processed using LabView (Version 8.0).

Figure 3.7: The NI USB-6259 and LabView Software.
The block diagram that represents the optical measurement setup is shown in Figure 3.8. The output of the LED driver electronics is a modulated analog voltage, which controls the current through the LED. The light that passes through the sample holder is detected by the photodiode. The photodiode generates a photocurrent, which corresponds to the intensity of the incoming light. A transimpedance amplifier converts the photocurrent into an analog output voltage. By processing the ratio of the two output voltages - the voltage where the LED drive current goes through a load resistor and the output voltage of the transimpedance amplifier - gives the amount of transmitted light through the sample.

![Figure 3.8: The electrical block diagram.](image)

### 3.3.1.1 LED driver

The light source in this OMS is a LED that emits narrow-spectrum light when the p-n junction is forward-biased. In order to maintain the output level of the LED, a well-defined current is needed to drive the LED. Due to the high light intensity of the LED, the current has to be pulsed at a low duty cycle to minimize the thermal effect that would degrade the intensity of the LED. Furthermore, a modulated signal is also needed to exclude DC and low frequency noise. The current is controlled by the circuit drive given in Figure 3.9.

The circuit driver shows three main parts: power supply, 555-timer, voltage follower and bipolar transistor. The values of the components in Figure 3.9 are given in Table 3.1.
Figure 3.9: The LED driver circuit.

<table>
<thead>
<tr>
<th>Passive components</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 = C2</td>
<td>12nF</td>
</tr>
<tr>
<td>R1</td>
<td>220kΩ</td>
</tr>
<tr>
<td>R2</td>
<td>56kΩ</td>
</tr>
<tr>
<td>R3 = R4</td>
<td>2kΩ</td>
</tr>
<tr>
<td>R_B</td>
<td>470Ω</td>
</tr>
<tr>
<td>R_E</td>
<td>56Ω</td>
</tr>
</tbody>
</table>

Table 3.1: The values of the passive components for the 555-timer.

**Power supply**

Two 5V regulators (positive and negative) from two 9V batteries supply the power of the optical system. The ripple rejection (dV_in/dV_out) of the LM7805C (positive) and LM7905C (negative) is 80 dB and 70 dB, respectively. The variation in the voltage source would be attenuated significantly by the ripple rejection value.

**555-timer**

The 555-timer generates voltage pulses based on the charge and discharge of the timing capacitor, C1, which operates in astable mode. An astable circuit produces a square wave, a digital waveform that oscillates between a low (0V) level and high level (+Vcc). The capacitor C1 is charged by current flowing through R1 and R2 and sets the output to high (V+). The threshold and trigger inputs monitor the capacitor voltage and when it reaches 2/3Vcc (threshold voltage) the output becomes low and the discharge pin is connected to ground. C1 is then discharged through R1 into the discharge pin. When 1/3Vcc (trigger voltage) is reached, the output becomes high again and the discharge pin is disconnected, allowing the capacitor to charge again.

By determining the right values for the passive components (R1, R2 and C1), the
CHAPTER 3. OPTICAL MEASUREMENT SYSTEM (OMS) FOR BACTERIA ANALYSIS IN BIOLOGICAL FLUIDS

Figure 3.10: The voltage regulators supplying the stabilized power of the OMS

The modulation frequency of the square wave is set to be around 400 Hz. This frequency has been chosen due to noise considerations in the detection part (see equation 3.20 and 3.21). The modulation frequency can be calculated with the formula:

\[ f = \frac{1.4}{(R2 + R1) \times C1} \approx 400\,\text{Hz}. \]  

(3.6)

The time period of the square wave:

\[ T = 0.7 \times (R2 + R1) \times C1 \approx 2.5\,\text{ms}. \]  

(3.7)

The time period can be split in two parts, \( T_H \) (high level) and \( T_L \) (low level):

\[ T = T_H + T_L, T_H = 0.7 \times (R2 + R1) \times C1, T_L = 0.7 \times R1 \times C1. \]  

(3.8)

A duty cycle of 20% was chosen to drive the LED. This duty cycle limits the degradation of the light intensity due to the thermal effects. A much lower duty cycle would increase the number of harmonics of the signal. This would require high frequency opamps in order to reproduce the original wave without filtering high frequency components, which would increase costs as well as complexity. To achieve 20% duty cycle, a diode was added in parallel with R1. This bypasses R1 during the charging part of the cycle so that \( T_H = 0.7 \times R2 \times C1 \). The duty cycle is thus calculated as follows:

\[ \frac{T_H}{T_H + T_L} = \frac{R2}{R1 + R2} = \frac{56k}{220k + 56k} = 0.2028 \approx 20\% \]  

(3.9)

The output of the 555-timer comes from a high-current totem-pole stage, a circuit that has two stacked transistors of the same type, that provides sourcing and sinking current. The internal transistors at the output stage of the timer chip provide source-type loads causing the high-state output voltage to be 1.7\,\text{V} less than the supply voltage (Vcc). The voltage 1.7\,\text{V} is needed for the upper totem-pole which consists of a Darlington pair transistor to switch on. For the low-state loads, the internal output stage transistor that
is connected to the ground has a low saturation voltage (for the NE555C, \( V_{OL} = 0.2V \)).
Thus, the 555-timer output would switch between 0.2V (low-state) – 3.3V (high-state).

**Voltage follower**

To maintain the stability of the voltage transfer from the 555-timer signal source to the load resistor \( R_E \), a voltage follower is placed between the source signal and \( R_E \). An opamp is used (in a non-inverting configuration) to provide very high input resistance for the control of the input voltage. It outputs a low impedance voltage that is identical to the input \( (V_{out}/V_{in} \approx 1) \). This implementation avoids the loading problem of the 555-timer output and therefore stabilizes the transfer from the source to the load. Some relevant specifications of the OP07CN are given in table 3.2:

<table>
<thead>
<tr>
<th>Electrical Parameter of the OP07CN</th>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large signal voltage gain</td>
<td>( A_o )</td>
<td>100V/mV</td>
</tr>
<tr>
<td>Open loop output resistance</td>
<td>( R_o )</td>
<td>60Ω</td>
</tr>
<tr>
<td>Common mode input resistance</td>
<td>( R_{ic} )</td>
<td>33MΩ</td>
</tr>
<tr>
<td>Common-mode Rejection Ratio</td>
<td>CMR</td>
<td>100dB</td>
</tr>
<tr>
<td>Supply current</td>
<td>( I_s )</td>
<td>2mA</td>
</tr>
<tr>
<td>Equivalent input voltage noise</td>
<td>( e_n )</td>
<td>10nV/√Hz</td>
</tr>
<tr>
<td>Equivalent input current noise</td>
<td>( i_n )</td>
<td>0.2pA/√Hz</td>
</tr>
<tr>
<td>Gain Bandwidth Product</td>
<td>GBP</td>
<td>0.5MHz</td>
</tr>
</tbody>
</table>

Table 3.2: The parameter values of the OP70CN.

<table>
<thead>
<tr>
<th>Electrical Parameters of the NPN 2N3904</th>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base resistance</td>
<td>( r_{bb} )</td>
<td>30 50Ω</td>
</tr>
<tr>
<td>Base-Emitter resistance</td>
<td>( r_{be} )</td>
<td>3kΩ</td>
</tr>
<tr>
<td>Emitter resistance</td>
<td>( r_e )</td>
<td>100Ω</td>
</tr>
</tbody>
</table>

Table 3.3: Some parameter values of the NPN 2N3904.

**Bipolar Transistor**

The load resistor \( (R_E) \) is connected to an NPN power transistor because the load resistor requires more current than can be provided by the output of the opamp. The voltage across the load resistor determines the current according to Ohm’s Law and is also the transistor’s collector current. The base current, which is supplied from the output current of the opamp, is much less than the collector current because of the high current gain (beta) of the transistor.

Figure 3.11 includes the small signal parameters of the NPN bipolar transistor that is used to provide the required current gain. The values of the parameters are given in table 3.3.

The schematic representation of the voltage follower and the small signal parameters of the Bipolar Transistor is depicted in 3.11.
Using the known parameters in equation 3.9, the unity gain transfer can be calculated,

$$V_o = I_L R_E = \frac{R_E}{R_E + R_{o} + r_{bb} + r_{be}} \times \frac{R_{ic}}{R_{ic} + R_{in}} \times V_s.$$  \hspace{1cm} (3.10)

The data acquisition hardware (DAQ-NI USB-6259), monitors the LED current by measuring the voltage $V_{RE}$ over the emitter resistor $R_E$, which has the following equation:

$$V_{RE} = i_{LED} \times R_E.$$  \hspace{1cm} (3.11)

The load resistor has a value of 60 Ω, which leads to a collector current of $\approx 30 \, mA$. When this current goes through the LED it corresponds to an optical power of $0.2 \, mW$.

The relation between the optical power generated by the LED and the current flowing through it is \[11\]:

$$P_{\text{source}} = \left( \frac{i_{LED}}{e} \right) \eta_i h \nu,$$  \hspace{1cm} (3.12)

where $i_{LED}$ is the current through the LED, $e = 1.6022 \times 10^{-19} \, C$ is the elementary charge, and $\nu$ is the frequency of the light wave. $\eta_i$ is the internal efficiency, which is also the electrical-to-optical conversion efficiency and has the following relation:

$$P_{\text{source}} = \eta_i P_{\text{elec}}$$  \hspace{1cm} (3.13)

and

$$P_{\text{elec}} = i_{LED} \times V_F.$$  \hspace{1cm} (3.14)

where $\frac{h \nu}{e} = \frac{E_g}{e} \approx V_F$, $V_F$ is the forward voltage and $E_g$ is the bandgap energy of the diode material.

Not all the electrical energy ($P_{\text{elec}}$) is converted into optical energy. The non-radiative mechanism causes the conversion of electrical energy into heat due to the vibration of
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the lattice atoms [11]. This temperature effect has been managed by decreasing the duty cycle to 20%, as mentioned before.

Concluding remarks regarding the LED-driver

The current through the LED is regulated by a 555-timer that produces a pulsed voltage signal. This signal is transferred to the load resistor by a voltage follower and optimized by a NPN switch transistor that determines the current through the LED. This current is set to be 30 mA. According to the datasheet, the optical power that is illuminated by the LED at 30 mA is 0.2 mW. There are several factors to be considered in this drive circuit in order to maintain the radiation output of the LED. The LED is driven in a succession of pulses at high repetition rate to appear continuous. The frequency is chosen to be 400 Hz for noise considerations and a duty cycle of 20% is used to reduce heating effects.

3.3.1.2 Photodiode readout

The part of the optical energy that has succeeded in reaching the photodiode is detected and subsequently converted into a current. The responsivity ($\mathcal{R}$) of the PD24-20 is 1 A/W at a wavelength of 1900 nm. The relation between the generated photocurrent and the responsivity of the detector is given by the following formula [12]:

$$\mathcal{R} = \frac{I_{ph}}{P_{\text{incident}}}[A/W].$$

(3.15)

Where $I_{ph}$ is the generated photocurrent and $P_{\text{incident}}$ is the optical power that reached the photodiode after passing through the sample. $P_{\text{incident}}$ depends on the absorption coefficient ($\alpha$) of the propagation medium, the thickness of the sample (L) between the transmitted light source to the detector and the emitting optical power by the source ($P_{\text{source}}$). This relation is also called as the Beer-Lambert law and is given in the following formula:

$$P_{\text{incident}} = P_{\text{source}}e^{-\alpha L}.$$  

(3.16)

And from equation 3.13 the formula above can be written as,

$$P_{\text{incident}} = \eta_i P_{\text{elec}}e^{-\alpha L} = \eta_i i_{\text{LED}} \times V_F.$$  

(3.17)

The generated photocurrent is converted into a voltage by the transimpedance amplifier with a gain of $10^5$ (with 10% error), see Figure 3.12. The reason for this value is to amplify the input current into a significant voltage level for better accuracy readings. Table 3.4 and 3.5 give a summary of the specification of the source and load respectively.

The transfer function of the readout circuit is depicted in Figure 3.12. 

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1Refer to page 57 for Errata on this section
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<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. effective signal [DC]</td>
<td>50\mu A</td>
</tr>
<tr>
<td>Signal bandwidth</td>
<td>400Hz</td>
</tr>
<tr>
<td>Source impedance</td>
<td>303\Omega</td>
</tr>
<tr>
<td>Grounding</td>
<td>One sided</td>
</tr>
</tbody>
</table>

Table 3.4: The specification of the signal source (the input quantity is a current source).

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. effective signal</td>
<td>5V</td>
</tr>
<tr>
<td>Load impedance</td>
<td>10k\Omega</td>
</tr>
<tr>
<td>Grounding</td>
<td>One sided</td>
</tr>
</tbody>
</table>

Table 3.5: The specification of the load (the output quantity is voltage driven).

Figure 3.12: The photodiode readout circuit.
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The derivation of the transfer function of the readout-circuit is given in Equation (3.18).

\[
V_{pd} = -V^- \cdot A(s) \\
V^- = -\frac{V_{pd}}{A(s)} \\
0 = I_{feedback} - I_{ph} + I_i \\
0 = \frac{(V^- - V_{pd})}{Z_f} - I_{ph} - \frac{V_{pd}}{A(s)} \\
V_{pd} = \frac{-1}{(\frac{1}{A(s)Z_p}) + \left(\frac{1}{Z_f}\right)(1 + \frac{1}{A(s)})} \\
\]

**Substituting:**

\[
A(s) = \frac{A_{OL}}{s\tau_1 + 1}, \tau_1 \approx \frac{1}{2\pi 20} \text{sec} \approx 8\text{msec} \\
\]

**Becomes:**

\[
\frac{V_{pd}}{I_{ph}} = \frac{-1}{\frac{A_{OL}}{Z_f} + \frac{1}{Z_f}(1 + \frac{A_{OL}}{\tau_1})} = -(Z_f + Z_p)(s\tau_1 + 1) + Z_pA_{OL} \\
V_{pd} = -Z_f \cdot \frac{1}{\frac{(s\tau_1 + 1)(Z_f + Z_p)}{Z_pA_{OL}} + 1} = -Z_f \cdot \frac{1}{Z_f + Z_p} s\tau_1 + 1 + \frac{1}{A_{OL}} \\
\frac{V_{pd}}{I_{ph}} = -\frac{R_f}{1 + sR_fC_f} \cdot \frac{1}{\frac{R_f(1 + sR_p(C_p + C_f))}{R_p(1 + sR_fC_f)} \cdot \frac{s\tau_1 + 1}{A_{OL}} + 1} \\
V_{pd} = -\frac{R_f}{1 + sR_p(C_p + C_f)}(s\tau_1 + 1) + \frac{R_pA_{ol}}{R_f} + sC_fR_pA_{ol} \\
\frac{V_{pd}}{I_{ph}} = \frac{R_pA_{ol}}{s^2R_p\tau_1(C_p + C_f) + s(\tau_1 + R_p(C_p + C_f) + C_fR_pA_{ol}) + \frac{R_pA_{ol}}{R_f} + 1} \\
(3.18)
\]

From the derivation in 3.18, we have the following important gain considerations of the transfer function:

\[
Feedback \ gain : \frac{1}{\beta} = \frac{Z_f + Z_p}{Z_p} \\
(3.19)
\]

**Open loop gain :** \(A(s) = \frac{A_{OL}}{s\tau_1 + 1}\)

This response delivers the ideal output signal, \(V_{pd} = I_{ph}Z_f\), as long as the loop gain, \(A_{OL}\beta\), remains large. However, due to non-ideal conditions of the components,
the declining of $\beta$ and $A_{OL}$ at high frequency is an inconvenient fact. Nevertheless, the analysis of the frequency behaviour of the circuit continues. We will derive the feedback equation to find the significant factors, which determine the bandwidth of the circuit.

$$\beta = \frac{Z_p}{Z_p + Z_f}$$

$$= \frac{R_p}{1 + sR_pC_p} \cdot \frac{R_f}{1 + sR_fC_f} + \frac{R_p}{1 + sR_pC_p}$$

$$= \frac{1}{(1 + sR_fC_f) + (1 + sR_fC_f)}$$

Because $\frac{R_f}{R_p} >> 1$

$$= \frac{1 + sR_fC_f}{R_f\left(\frac{1}{R_p} + sC_f\right) + (1 + sR_fC_f)}$$

$$= \frac{1 + sR_fC_f}{(1 + sR_fC_f) + sR_f(C_f + C_p)}$$

The $\frac{1}{\beta}$ becomes:

$$\frac{1}{\beta} = \frac{R_f}{R_p} \cdot \left[1 + sR_p(C_p + C_f)\right]$$

$$\left[1 + sR_fC_f\right]$$

$$= \left[1 + sR_fC_f\right]$$

$$\left(3.20\right)$$

In the DC mode, the current will flow through the feedback resistor and generate the output voltage. This output voltage is given by the following equation:

$$V_{out} = -I_{ph} \times R_f,$$

where $R_f$ is the feedback resistor and the negative sign indicates the direction of the photocurrent.

To avoid instability caused by the high input capacitance of the photodiode and the opamp, a feedback capacitor ($C_f$) is placed parallel to $R_f$. The value of this $C_f$ is given as follows [13]:

$$C_f = \sqrt{\frac{C_p}{2\pi R_f f_c}}, C_f \approx 50 pF$$

$$\left(3.22\right)$$

With help from the mathematical software tool MatLab, we calculated the total transfer function. The values of the components of the PD-readout circuit is given in table 3.6.

$$Transfer \ function: \frac{V_{pd}}{I_{ph}} = \frac{3.03 \cdot 10^7}{1.224 \cdot 10^2 s^2 + 0.009517 s + 304}$$

$$\left(3.23\right)$$

With a dominant pole at: $p_1 \approx 30 kHz$
3.3. DESIGN OF THE OPTICAL MEASUREMENT SETUP (OMS)

### Parameters of the PD24-20 Photodiode

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Typical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum responsivity at 90%</td>
<td>µm</td>
<td>1.3 – 2.2</td>
</tr>
<tr>
<td>Responsivity, $R$</td>
<td>A/W</td>
<td>1</td>
</tr>
<tr>
<td>Dark current, $i_{dark}$, at reverse bias 0.2V</td>
<td>µA</td>
<td>100</td>
</tr>
<tr>
<td>Capacitance, $C_{sh}$</td>
<td>pF</td>
<td>4120</td>
</tr>
<tr>
<td>Impedance, $R_{sh}$</td>
<td>kΩ</td>
<td>0.3</td>
</tr>
<tr>
<td>Feedback resistor, $R_f$</td>
<td>kΩ</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3.6: Specifications of Photodiode, type PD24-20.

### Parameters of the CA3140 BiMOS-OpAmp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Typical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open loop gain, $A_O$</td>
<td>kV/V</td>
<td>100</td>
</tr>
<tr>
<td>Input Capacitance, $C_{in-OpAmp}$</td>
<td>pF</td>
<td>4</td>
</tr>
<tr>
<td>Equivalent input noise voltage, $e_N$</td>
<td>nV/√Hz</td>
<td>100 (at 100 Hz bandwidth)</td>
</tr>
<tr>
<td>Input bias current, $I_{bias}$</td>
<td>pA</td>
<td>10</td>
</tr>
<tr>
<td>Gain-Bandwidth Product, $f_c$</td>
<td>MHz</td>
<td>4.5</td>
</tr>
<tr>
<td>Input offset voltage, $V_{i-Offset}$</td>
<td>mV</td>
<td>5 (15 Max.)</td>
</tr>
<tr>
<td>Input offset current, $I_{i-Offset}$</td>
<td>pA</td>
<td>0.5 (50 Max.)</td>
</tr>
</tbody>
</table>

Table 3.7: Specifications of BiMOS-OpAmp, type CA3140.

$R_p$ and $C_p$ are the shunt resistance and capacitance of the photodiode and its value is given in table 3.6 and 3.7.

The passive components are also chosen based on the tuning of the LED light frequency modulation. The contribution of $R_p$ and $C_p$ dominates the bandwidth specification. It gives a pole frequency $f_{zf}$ and sets the bandwidth [13]:

$$f_i = \sqrt{f_{zf} f_c} \approx 30 kHz. \quad (3.24)$$

$f_C$ is the crossover frequency at 50 dB gain that is set by the feedback.

For the optimal use of the detector, the influence of the bandwidth with respect to the noise contribution should be minimal. There are two noise components regarding the photodiode: shot noise of the dark current and thermal noise of the shunt resistance. From table 3.3, the dark current of the PD24-20 is relatively high and should be carefully examined in the noise contribution of the overall readout circuit. This noise current can disturb the detection of the photocurrent and limit the dynamic range the output. The calculations of the noise contributions are given below.

The first noise component results from the statistical uncertainty in photon arrival rate and has the following form:

$$I_{dark} = \sqrt{2ei_{dark}BW_n}. \quad (3.25)$$

Where, $I_{dark}$ is the RMS noise current, $e$ is the electronic charge, $i_{dark}$ is the photo generated signal current and $BW_n$ is the electrical bandwidth over which noise is measured. The second noise source is also known as the Johnson noise and has the form:
\[ I_j = \sqrt{\frac{4kTBW_n}{R_{sh}}} \]  

(3.26)

Where, \( I_j \) is the RMS noise current resulting from Johnson noise, \( k \) is the Boltzman’s constant \((k = 1.38 \times 10^{-23} \text{ J K}^{-1})\), \( T \) is the absolute temperature of the photodiode and \( R_{sh} \) is the shunt resistance of the photodiode. The noise calculations are given below.

**Input OpAmp CA3140**

The close loop bandwidth = \( \frac{\text{GainBW Product}}{\text{NoiseGain}} = \frac{4.5 \text{ MHz}}{300} = 15 \text{ kHz} \). The noise bandwidth, \( BW_n = 15 \text{ kHz} \times 1.57 \approx 23 \text{ kHz} \).

- **Broadband voltage noise:**
  From the datasheet, the dominant noise is the 1/f noise. Thus the broadband voltage noise is negligible.

- **1/f voltage noise:**
  \[
  e_{i_f} = \sqrt{\int_{f_L}^{f_H} e_{a_{i_f}}^2 \times \frac{f_f}{f} df} \\
  = \sqrt{e_{a_{i_f}}^2 \times f_I \ln \left( \frac{f_H}{f_L} \right)} \\
  = 100 \frac{nV}{\sqrt{Hz}} \sqrt{100 \text{ Hz} \times \ln \left( \frac{15 \text{ kHz}}{1 \text{ Hz}} \right)} \\
  = 3.1 \mu V_{rms}
  \]

- **Total voltage noise referred to input OpAmp:**
  \[
  e_{n_v} = 3.1 \mu V_{rms}
  \]

- **Amplifier’s input noise current:**
  \( I_{bias} = 10 pA \)

  \[
  i_{n-input} = \sqrt{2qI_{bias} \cdot BW_n} \\
  = 1.8 fA
  \]

- **Total voltage noise referred to input current noise:**
  \[
  e_{n_i-output} = i_{n-input} \times R_f \\
  = 0.18 nV_{RMS}
  \]

(3.27)
Photodiode

- **Shot noise:**
  \[ I_s = \sqrt{2eI_D}B \approx 0 \]  \hspace{1cm} (3.31)

  \( I_D = 0 \text{A} \) (dark current is zero because the reverse bias voltage is zero)

- **Johnson noise (thermal noise):**
  \[ I_j = \left( \frac{4kTBW_n}{R_{eq}} \right)^{\frac{1}{2}} = 1.13nA \]  \hspace{1cm} (3.32)

- **Total current noise:**
  \[ I_{ni\text{-input}} = 1.13nA \]

- **Equivalent voltage noise referred to output transimpedance amplifier:**
  \[ e_{n\text{-vo}} = 1.13 \times 10^{-9} \cdot 10^2 = 113\mu V_{RMS} \]

- **Total voltage noise referred to input voltage noise:**
  \[ e_{n\text{-vi}} = \frac{e_{n\text{-vo}}}{300} = 0.377\mu V_{RMS} \]

- **Total noise RMS voltage referred to input:**
  \[ e_{n\text{-on}} = \sqrt{e_{n\text{-v}}^2 + e_{n\text{-i}}^2 + e_{n\text{-vi}}^2} \approx 3\mu V_{RMS} \]  \hspace{1cm} (3.33)

- **Total noise RMS voltage referred to output:**
  \[ e_{n\text{-out}} = e_{n\text{-in}} \cdot \text{NoiseGain} \]
  \[ = 3\mu V_{RMS} \cdot 300 \]
  \[ = 0.9m V_{RMS} \]  \hspace{1cm} (3.34)

**Concluding remarks regarding the PD-readout electronics**

The photodiode PD24-20 detects radiation in the mid-Infrared with spectral range between 800 to 2400 nm. The optical responsivity of the detector is around 1 A/W, thus the electrical output (\( I_{\text{ph}} \)) is proportional to the optical input (\( P_{\text{incident}} \)). The generated photocurrent is amplified and converted into a voltage with a gain of \( 10^5 \). This value has been chosen to match the dynamic range of the input signal to the range of the DAQ card. The detectability of a signal depends on how large the signal is compared to the noise. The noise contributions of the circuit have been specified in two primary types of noise: shot noise and Johnson noise. The calculation has made an estimation of the equivalent output noise to be \( 0.9m V_{RMS} \).
Signal-Noise Ratio

An important measure to determine the quality of the signal of interest with respect to background noise that is produced by the electrical components is the signal-to-noise ratio (SNR). The SNR [dB] is simply defined as,

\[
\text{SNR} = 20 \log_{10} \left( \frac{I_{\text{ph}}}{I_{\text{n_PD}}} \right) = 20 \log_{10} \left( \frac{50 \times 10^{-6}}{1.13 \times 10^{-9}} \right) \approx 90 \text{ dB}.
\]

From this calculation we can conclude that the detection range is sufficiently accurate for the desired circuit performance.

3.3.1.3 DAQ with LabView software

The hardware that is used to read the analog signals is the DAQ-NI USB-6259. Finally, the obtained data is sent to a PC where a software program called LabView is used to signal process the data and project it through a user interface screen. The DAQ card has three analog input (AI) ground-reference configurations:

1. DIFF, measures the difference in voltage between two AI signals.
2. RSE, measures the voltage of an AI signal relative to AI GND.
3. NRSE measures the voltage of an AI signal relative to one of the AI SENSE or AI SENSE 2 inputs.

The OMS is a 'plug-in instrument with non-isolated outputs' thus, the DIFF mode is used to perform the AI measurement (see Figure 3.13).

RMS calculations are being used to process the data of the analog signals. RMS is a mathematical function, like average, that reduces a complex function to a single value. The definition of RMS is revealed by the name, Root of the Mean of the Square of the function. First the waveform (or function) to be calculated is squared, then this squared waveform is averaged and finally the square root of the average is taken. The importance of RMS voltage and current lies in the fact that they can be directly used to calculate the average power. In addition to this, the offset factors originating from the electrical components are canceled out due to filtering.

The RMS has the following form:

\[
V_{\text{RMS}} = \sqrt{\frac{1}{T} \int_{0}^{T} V(t)^2 dt},
\]

where T is the time span and V is the signal form.
3.3. DESIGN OF THE OPTICAL MEASUREMENT SETUP (OMS)

The block diagram of the signal processing done by LabView is given in Figure 3.14. The process runs in real-time.

![Block Diagram](image)

**Signal Timing and Data Processing**

The LabView software sets the hardware to acquire the analog inputs at a sample rate of 100 kHz. The output is calculated by the signal processing program with a period of 1 minute, during which 60 values are generated from the acquired data. The 60 output values are averaged and displayed on a chart diagram. In the experiments, the recorded time was chosen to be 3 minutes, thus approximately 180 data points are displayed and plotted in the measurement results.

3.3.2 Mechanical Assembly

The mechanical holder of the OMS was designed by Ing. P. Trimp, (Electronic Instrumentation Laboratory) as shown in Figure 3.15.

The parts of the mechanical box consist of:
Figure 3.15: Design of the mechanical OMS

1. The LED, which includes the parabolic reflector.

2. The Photodiode has a fabrication packaging which included a transparent glass cover.

3. The sample holder, which consists of two pairs of CaF2 glasses and a space ring, made of PVC.

4. The bottom plate where the print plate, connectors and batteries are situated.

5. The middle plate, which gives a firm grip on top of the bottom plate and holds the Photodiode centered through the fitted hole in the middle of the surface of the plate.

6. The top plate that can be removed easily and in addition holds the LED holder.

7. Print plate that is used to solder the electrical components.

8. The LED holder that is connected through a coax cable through the side of the bottom plate to the electronics.

Because the goal of this study is to develop a measurement system that should be used clinically, several criteria need to be fulfilled:
3.3. DESIGN OF THE OPTICAL MEASUREMENT SETUP (OMS)

- Reduced size and portability: It has to be small and portable because the clinical tests have to be conducted in the hospital near the patient. A nurse or medical assistant should perform this test in a period of hours or days. The drain fluids originate directly from patients. Thus, the OMS needs to be a stand-alone electronic device.

- Low cost: The cost should be low to increase the usability of this OMS in comparison to the conventional electronic devices of the present days, such as the Raman spectroscopy machine and PCR instrument.

- Low perturbations: The measurement reliability of the OMS is highly dependent on the stability of the sensor response, so external perturbations need to be isolated and minimized.

- User friendly: the use of the OMS needs to be efficient and easy to use.

The measurement setup is shown in Figure 3.16(a) and the content is displayed in Figure 3.16(b). The case is made of polyvinyl chloride (PVC), which is a low cost and light weighted material. The batteries are located underneath the print plate.

![The experimental optical measurement system.](image1)
![Contents of PVC case.](image2)

(a) The experimental optical measurement system.
(b) Contents of PVC case.

Figure 3.16: The optical measurement system casing.

The sample holder

The sample holder of the OMS consists of two glasses made of $CaF_2$. These glasses were chosen based on the low absorption coefficient of this material in the infrared spectrum. Some relevant optical properties of $CaF_2$ are presented in table 3.8. The $CaF_2$ glasses are also known as the beta cells.

The total optical path is $2 \times 4 = 8 + 0.65 = 8.65 \text{ mm} = 0.865 \text{ cm}$. The total optical path contains the thickness of each beta cell plus the width of the spacer.
Optical properties | value |
---|---|
Transmission range | $0.12 - 7.5$
Absorption coefficient $\lambda = 1.06 \mu m$ | $2 \times 10^{-5} cm^{-1}$
Absorption coefficient $\lambda = 2.70 \mu m$ | $1 \times 10^{-3} cm^{-1}$
Refractive index $\lambda = 2.00 \mu m$ | $1.42386$
Reflection | 5% (2 surfaces)
Thickness of one beta cell | $4mm$

Table 3.8: Optical properties of CaF2.

**Volume liquid between the beta cells**

The volume of the liquid is determined by the cylindrical space between the beta cells. This space is created by the two round surfaces of the two sides of the beta cells and the width of the ring spacer. The diameter of the beta cells is $25 \ mm$, the surface is $\pi(D/2)^2 = 3.14 \times 2.5 = 7.875 \ cm$. The thickness of the spacer is $0.06 \ cm$. Thus, the volume of the liquid between the two beta cells is $0.4725 \ cm^3 = 0.4725 \ ml$. 

This chapter presents the results of the bacteria and drain fluid analysis based on the OMS. To investigate and understand the relation between the theory and the practical results, a series of measurements have been performed. Measurements on biological fluids containing different percentages of bacteria contaminations are performed. The results are presented in this chapter for each type of bacteria (E.coli, Klebsiella sp., and Staphylococcus warneri). The last part shows the measurement results and the discussions relating to the measurements on drain fluid from patients who have undergone a colonic anastomosis surgery.

4.1 Experiments for the analysis of two different fluids

The aim of this study concerns the ability to find a parameter that could be used to enhance the diagnostic method to give an early warning to avoid fatal complication after a colonic anastomosis surgery. The first attempt is to detect bacteria growth in liquid media culture. From the presented results we can gain a better understanding about the application of this method relating to the improvement of current diagnostics.

In the second experiment, we investigate drain fluid from patients who have undergone colonic anastomosis surgery. In this research, the outcome of the measurements based on infrared absorption will be compared with the outcome of the other three methods that are used in the APPEAL-study [1].

The transmission of the liquids is defined as the percentage transmitted light in comparison to the transmission of the reference value. The reference value is the detected transmitted light when the sample holder is empty. This reference value is taken every time before the transmission of the liquids is to be taken.

4.1.1 Bacteria analysis in liquid media culture

The samples to be investigated are supplied by Erasmus Medical Centre in Rotterdam. The different types of bacteria are injected into basal liquid media culture with different percentages of contamination. The experiment is performed with the following bacteria: E. coli, Klebsiella sp., and Staphylococcus warneri.

The goal of this first experiment is to find the correlation between different percentages of bacteria contamination and infrared absorption. This experiment provides the basis for a discussion about infrared absorption by liquid samples containing specific chemical compounds.
A. Necessary equipment

To ensure the accuracy of the measurements, the sterility of the instruments and surroundings should always be maintained during the measurement. The following tools are needed for the measurement:

1. Surgical mask to keep away bad smell.
2. Sterile gloves that are individually packed.
3. Sterile pipettes to inject the liquid from the contaminated media into the beta cells which are also individually packed.
4. Ethanol (70% Alcohol) to sterilize the beta cells before and after use.
5. Sterile gauzes to dry the beta cells.

A sterile environment for the experiment also needs to be maintained during and after the measurements. The sterile objects can only be used once and have to be subsequently disposed of. The sterilization of the equipment is important to ensure that no extra contamination will occur in the samples. This is to prevent random influences on the growth of the contamination.

B. Measurement samples

The samples that were used in this experiment are given in Table 4.1. The experiment was performed on 15, 16, 17 and 18 of November 2007. The total number of samples that were examined in the measurement was 10.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Contamination percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty beta cells</td>
<td>Calibration</td>
</tr>
<tr>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>E.coli-10%</td>
<td>10%</td>
</tr>
<tr>
<td>E.coli-25%</td>
<td>25%</td>
</tr>
<tr>
<td>E.coli-35%</td>
<td>35%</td>
</tr>
<tr>
<td>Klebsiella sp.-10%</td>
<td>10%</td>
</tr>
<tr>
<td>Klebsiella sp.-25%</td>
<td>25%</td>
</tr>
<tr>
<td>Klebsiella sp.-50%</td>
<td>50%</td>
</tr>
<tr>
<td>Staphilococcus-10%</td>
<td>10%</td>
</tr>
<tr>
<td>Staphilococcus-25%</td>
<td>25%</td>
</tr>
<tr>
<td>Staphilococcus-50%</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Total samples</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

Table 4.1: The samples used in experiment with bacteria media culture

The samples are produced by diluting the 100% contaminated liquid with the basal liquids shown in Figure 4.1. The percentage contamination is set by the ratio of the volumes between the 100% contaminated liquid and the 0% contaminated liquid. All of the basal liquids have a volume of 9 cc.
4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

Figure 4.1: The original contaminated liquids with (from left to right): *E.coli*, *Klebsiella* sp. and *Staphylococcus warneri*

The main reason to use these samples as preliminary tests is to find the threshold of bacteria contamination. The characteristic of the contaminated sample is determined, which gives a strong indication that bacteria is present in the liquid.

C. Conditions

In order to maintain the consistency of the measurement the conditions should be determined beforehand. The measurement condition is defined as the protocols to be maintained so that the results can be directly compared to each other. We assume the accuracy of the conditions to be sufficiently accurate. However in practice, some deviations may occur due to improper handling.

- **Temperature**
  The temperature is measured with a temperature sensor, the Pt 3240. The ambient temperature is measured and it also applies to the temperature of the measurement system and the samples. From the fabrication specifications of the LED19-PR it is expected that the band drifts by 1 \( \text{nm/K} \). Additionally, the responsivity of the PD24-20 changes 1.8 \( \text{nm/K} \). The change of the temperature is relevant to indicate the stability of the OMS. Therefore it is important to consider the effects of these characteristics on the eventual results.

- **pH**
  The pH measurement is noted for examining the acidity of the liquid. The uncontaminated media culture liquid is pH neutral (pH = 7).

- **Longitudinal study**
  A longitudinal study is a study that is conducted in a lengthy period of time in order to investigate any long-term effects. In this way, the bacteria analysis
is conducted over several days. With this method, it is possible to examine the dynamic change over time.

**D. Measurement results of the experiment with bacteria media culture**

1) *Escherichia coli*: 0%, 10%, 25%, 35%

The first measurement series was performed with samples contaminated with the bacterium *E. coli*. The 0% contamination is the sample that has not been oculated with any of the three bacteria. This sample gives a basic reference which states that each day the transmission is stable. In contrast to the contaminated samples, we see an increase in the transmission each day.

![Graphs of measurement results](image)

**Figure 4.2**: Various contaminated samples with *E. coli* measured in 3 days

In Figure 4.2(a) we can observe that the change in transmission after 3 days is around 0.5%. This can be caused by the decreased level of sterileness of the liquid resulting from multiple instances of exposure to the surrounding air. The reason for this low transmission level, may be explained by the theory proposed in chapter 3.2.1. The liquid contains a high level of hydrogen bonding, which causes an intense absorption of infrared. In the other three pictures in Figures (4.2(b), 4.2(c) and 4.2(d)), we can see that the increase in transmission is increasing with almost the same slope each day. In relation to the theory in the previous Chapter (3.2.1), a dynamic change in the liquid due to the presence of bacteria causes a change in the composition of the liquid. This causes a change in the absorption spectrum.
4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

A picture is taken to show the turbidity of the samples (see Figure 4.3. It is clear from the pictures that the uncontaminated sample is the most transparent in comparison to the contaminated ones.

![Figure 4.3: A photo of the samples with *E.coli* contamination. From left to right: 10%, 25%, 35% and 0%](image)

For more analytical details, we take into account the pH of the liquid and the room temperature where the measurement took place (Table 4.2).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>≥ 7</td>
<td>22.7</td>
<td>≥ 7</td>
</tr>
<tr>
<td>10%</td>
<td>6</td>
<td>22.6</td>
<td>6</td>
</tr>
<tr>
<td>25%</td>
<td>6</td>
<td>21.6</td>
<td>6</td>
</tr>
<tr>
<td>35%</td>
<td>≥ 5</td>
<td>21.8</td>
<td>≥ 5</td>
</tr>
</tbody>
</table>

Table 4.2: The pH of the samples with *E.coli* contamination and the temperature room of each measurement day.
2) Klebsiella: 0%, 10%, 25%, 50%

The second measurement series are performed with samples contaminated with the bacterium *Klebsiella sp.* From the figures in 4.4, we see that the transmission trend is likely to increase as we saw with *E.coli* contamination.

![Graphs showing transmission results for different contamination levels of Klebsiella](image)

(a) 0% Bacteria contamination
(b) 10% contamination
(c) 25% contamination
(d) 50% contamination

Figure 4.4: Various contaminated samples with *Klebsiella sp.* measured in 3 days

As mentioned in Chapter 2.4.2, *Klebsiella sp.* are non-motile bacterium and are slightly smaller in size compared to *E.coli*. The transmission of the samples saturates slightly after the second day.

In Figure 4.5 we can see the turbidity differences of the samples that are contaminated with the bacteria *Klebsiella sp.* and the uncontaminated samples.

The pH of the samples on each day and the room temperature are given in Table 4.3
4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

Figure 4.5: A photo of the samples with *Klebsiella sp.* contamination

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>pH</td>
<td>pH</td>
</tr>
<tr>
<td>0%</td>
<td>≥ 7</td>
<td>≥ 7</td>
<td>7</td>
</tr>
<tr>
<td>10%</td>
<td>6</td>
<td>≥ 6</td>
<td>6</td>
</tr>
<tr>
<td>25%</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>50%</td>
<td>6</td>
<td>22.3</td>
<td>≥ 6</td>
</tr>
</tbody>
</table>

Table 4.3: The pH of the samples with *Klebsiella sp.* contamination and the temperature room of each measurement day

3) *Staphylococcus warneri*: 0%, 10%, 25%, 50%

The last measurement series on bacteria was performed with *Staphylococcus warneri* contamination. We can observe that apart from the 50% bacteria contamination the transmission of the samples are increasing with increasing contamination.

The pictures of the samples are given in Figure 4.7. It is worth mentioning that the turbidity differences of the four samples are very low. Unfortunately, this is not apparent from the transmission trend.
CHAPTER 4. MEASUREMENTS AND RESULTS

(a) 0% Bacteria contamination
(b) 10% contamination
(c) 25% contamination
(d) 50% contamination

Figure 4.6: Various contaminated samples with *Staphylococcus warneri* measured in 3 days

![Image of contaminated samples]

Figure 4.7: A photo of the samples with *Staphylococcus* contamination

The pH of samples are given in Table 4.4. In contrary to the previous samples, the pH of these samples are consistently neutral. The room temperature is also given in the table.
### 4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>≥ 7</td>
<td>21.7</td>
<td>≥ 7</td>
<td>20</td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td>10%</td>
<td>7</td>
<td>21.9</td>
<td>7</td>
<td>20</td>
<td>7</td>
<td>18.8</td>
</tr>
<tr>
<td>25%</td>
<td>7</td>
<td>22</td>
<td>7</td>
<td>20</td>
<td>7</td>
<td>18.8</td>
</tr>
<tr>
<td>50%</td>
<td>7</td>
<td>22</td>
<td>7</td>
<td>19.9</td>
<td>7</td>
<td>18.7</td>
</tr>
</tbody>
</table>

Table 4.4: The pH of the samples with *Staphylococcus warneri* contamination and the temperature room of each measurement day

**Discussions regarding the measurement results of bacteria in media culture**

Except for Staphylococcus-50% contamination (see Figure 4.6(d)), the transmission of the samples increases rapidly each day. It remains unclear what the cause is of the increase in transmission. However, there are several factors to be considered regarding the transmission change:

- More waste product (metabolite) due to the growth of bacteria
- The change in the molecular structure of the liquid due to the consumption of nutrients by the bacteria
- Multiplication of bacteria

We can conclude from this experimental results, that the pH and the temperature do not have a direct influence on the measurements.

#### 4.1.2 Drain fluid analysis

In this experiment the sample used in the experiment is freshly tapped drain fluid from patients who have recovered from a colonic anastomosis surgery. The goal of this experiment is to monitor the clinical relevance of the OMS that has been developed in this study.

Particular interest go to the correlation between the outcome of this experiment compared to the outcome of the results achieved by PCR, RS and bacteria culture.

**A. Necessary equipment**

To ensure the accuracy of the measurements, the sterile and cleanliness of the instrument and surrounding should always be maintained during the measurement. The following tools are needed for the experiment:

1. Sterile gloves that are individually packed.
2. Sterile pipettes to inject the liquid from the contaminated media into the beta cells and are also individually packed.
3. Ethanol (70% Alcohol) to sterilize the beta cells before use.
4. Sterile gauzes to dry the beta cells.
B. Measurement samples

The drain fluid samples are provided by the nurses of the department of surgery under the supervision of drs. Freek Daams at the medical center Reinier de Graaf Gasthuis in Delft. This medical center participates in the multicenter cohort study, the APPEAL-study [1].

According to the logistics of this study, after the admission of the patient to the hospital, he/she is asked to participate in the APPEAL-study. If he/she is willing to participate, the patient is asked to sign the informed consent form.

After constructing the anastomosis and before closing the abdomen, a drain will be left behind at the anastomatic site. The drain fluid will be collected in two sterile jars, with one being delivered to the laboratory of microbiology and the other being utilize in this research study.

For reasons of anonymity and privacy, each included patient will be given a number, "patient inclusion number" (PIN), starting with "1" in each center. The materials used to collect the drain fluid are labeled with PIN, in combination with a letter (A-E), corresponding with the postoperative day on which the fluid is collected. Example: 1A (= patient nr. 1, postoperative day 1)

C. Measurement results experiment with drain fluid

With the same method as in experiment with bacteria media culture, we performed a series of measurements with drain fluid. The first measurement result is presented below.

![Drainfluid transmission of Patient nr. 18 in 5 days](image.png)

Figure 4.8: The transmission of the drain fluids of Patient nr. 18

The sample photos of Patient nr. 18, the pH and the temperature of the measurements are given in table 4.5.

Some additional details regarding the condition of the patient:

- On the first day, the patient got a fever
- On the second day, the patient is doing better, the fever has reduced
4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

<table>
<thead>
<tr>
<th>Sample 18A</th>
<th>Sample 18B</th>
<th>Sample 18C</th>
<th>Sample 18D</th>
<th>Sample 18E</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = 9</td>
<td>pH = 10</td>
<td>pH $\geq$ 8</td>
<td>pH = 8</td>
<td>pH = 8</td>
</tr>
<tr>
<td>$T = 22.9^\circ C$</td>
<td>$T = 23.3^\circ C$</td>
<td>$T = 22.6^\circ C$</td>
<td>$T = 23.5^\circ C$</td>
<td>$T = 23.7^\circ C$</td>
</tr>
</tbody>
</table>

Table 4.5: pH, room temperature details and pictures of the drain fluids of Patient nr. 18

- On the fourth day, the patient has severe complains in the stomach and the abdomen is swollen. A CT scan is taken to check for anastomotic leakage.

- On the fifth day, the CT scan result is negative, no anastomotic leakage has been detected.

Interestingly enough, from the graph Figure 4.8 and the pictures in figure 4.5 we can see that the transmission follows the color of the samples. The darker the color the lower the transmission. In addition to this, the pH of the samples follows the physical color of the liquid.

In the case of the second patient, the transmission is shown in Figure 4.9. The transmission level is around the same as in the previous patient. However, the levels of transmissions have not changed significantly. By observing the pictures in table 4.6, the colors have not changed drastically, and we can observe only a slight change in the last sample.

![Drainfluid transmission of Patient nr. 19 in 5 days](image)

Figure 4.9: The transmission of the drain fluids of Patient nr. 19
The sample photos of Patient nr. 19, the pH and the temperature of the measurements are given in table 4.6.

<table>
<thead>
<tr>
<th>Sample 19A</th>
<th>Sample 19B</th>
<th>Sample 19C</th>
<th>Sample 19D</th>
<th>Sample 19E</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = ≥ 8</td>
<td>pH = NA</td>
<td>pH = 8</td>
<td>pH = 8</td>
<td>pH = 8</td>
</tr>
<tr>
<td>T = 25.2°C</td>
<td>T = NA</td>
<td>T = 25.4°C</td>
<td>T = 24.4°C</td>
<td>T = 24.7°C</td>
</tr>
</tbody>
</table>

Table 4.6: pH, room temperature details and pictures of the drain fluids of Patient nr. 19

For the samples from Patient nr. 20 the transmission goes up subsequently. The liquid were also getting thinner and lighter (in color) each day.

![Graph showing drainfluid transmission of Patient nr. 20 in 5 days](image)

Figure 4.10: The transmission of the drain fluids of Patient nr. 20

The sample photos of Patient nr. 20, the pH and the temperature of the measurements are given in table 4.7.
4.1. EXPERIMENTS FOR THE ANALYSIS OF TWO DIFFERENT FLUIDS

<table>
<thead>
<tr>
<th>Sample 20A</th>
<th>Sample 20B</th>
<th>Sample 20C</th>
<th>Sample 20D</th>
<th>Sample 20E</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = 8</td>
<td>pH = 8</td>
<td>pH = ≤ 8</td>
<td>pH = NA</td>
<td>pH = NA</td>
</tr>
<tr>
<td>T = 25.3°C</td>
<td>T = 24.7°C</td>
<td>T = 24.8°C</td>
<td>T = 23.9°C</td>
<td>T = 23.6°C</td>
</tr>
</tbody>
</table>

Table 4.7: pH, room temperature details and pictures of the drain fluids of Patient nr. 20

As a comparison with Patient nr. 18 we observe the measurement results of Patient nr. 23. We see the same transmission trend as with Patient nr. 18, the darkest liquid has the lowest transmission percentage. After the third day, the liquid became lighter and the transmission increases.

![Figure 4.11: The transmission of the drain fluids of Patient nr. 23](image)

The sample photos of Patient nr. 23, the pH and the temperature of the measurements are given in table 4.8.

In contrary to Patient nr. 18, the low pH does not account for Patient nr. 23. The only factor that lowers the transmission is the dark-red color of the liquid

**Discussions regarding the measurement results of drain fluid**

There are several factors that should be considered:

- The part of the colon where the anastomosis has taken place will determine the properties of the drain fluid
The medical condition of the patient before and after the surgery will also determine the condition of the drain fluid.

The composition of drain fluid is very complex due to the fact that the liquid is directly originated from the abdominal cavity of the patient.

The multiple data points in the transmission graph show the consistent performance of the measurement. The deviation is very small, which states that the measurement has been performed accurate.

### 4.1.3 Bacteria analysis in liquid media culture with ATR application

Two methods for the analysis of samples with different percentages of *E.coli* contaminations have been conducted. The first method is using an ATR-crystal instrument that is based on infrared spectroscopy. The second method is the OMS that has been developed in this thesis. The goal is to compare the results such that we can acquire more understanding in order to draw possible conclusions.

The samples that were used: bacteria media culture in liquid form (Peptone and BHI) And the contamination percentages: 0%, 1%, 5%, 10%, 100%.

Type of machine: Nicolet 8700 FT-IR from Thermo scientific. Crystal in use: Diamond ($n = 2 \cdot 4$).

1. **ATR-crystal instrument**

   The ATR uses a very thin sampling path length and depth of penetration of the IR beam into the sample. The IR beam is directed into a crystal with relative higher refractive index. The IR beam reflects from the internal surface of the crystal and creates an evanescent wave, which projects orthogonally into the sample that has direct contact with the ATR crystal [15]. The absorption of the samples are calibrated with the background reference absorption of de-mineralized water. From the Figure 4.12, we observe the absorption band around wavenumber 3500 $cm^{-1}$, which shows the N-H amide groups of protein chains [8]. In the wavelength that...
Figure 4.12: The absorbance of E.coli contaminated samples using the ATR

we are interested in (wavenumber \( \approx 5000 \text{ cm}^{-1} \)), we see that the molecular binding of the samples includes in the base band of the absorption spectrum.

2. Optical Measurement System

Figure 4.13: The transmission of E.coli contaminated samples using the OMS

On the first day of occultation of the bacteria in media culture liquid, we detect no transmission change in the different percentages of contamination.
Concluding remarks regarding the measurement results of bacteria media culture using ATR-crystal

With help of the ATR-crystal machine, we are able to see water composition in the bacteria media culture liquid. However, around wavenumber $3500 \text{ cm}^{-1}$ we see an excitation by the molecular binding of the sample that leads to a characteristic of protein. Unfortunately, the identification of the different percentages of contaminated samples were not successfully distinguished. Even the clear liquid (0% percentage contamination) differs very modestly from the samples with a higher percentage of contamination.
The need of ex vivo test avoiding central laboratories by using small-sized instrumentation close to the patient for the analysis of drain fluid is clear. In this thesis a sensor system is presented for an attempt to detect bacteria in two different types of liquid: bacteria media culture and peritoneal drain fluid. The optical measurement system has been fabricated with success. However, difficulties occur in the analysis due to less selective information in near-IR band due to intensive vibration stem from molecular parts with hydrogen atoms involved, e.g. O-H, C-H and N-H.

5.1 Discussion

Remarks on the experiment on bacteria media culture:

1. Hydrogen bonding of the solution of the media culture has an impact in the absorption characteristics of near-IR. The main problem is the presence of strong hydrogen bonding which leads to a large absorption band in the near-IR.

2. The device is not sensitive enough due to the inability to distinguish the different contamination levels.

3. Microbiological analysis is significant for this research.

Remarks on the experiment on peritoneal drain fluid:

1. The transmission chart follows the trend of the color pattern of the fluid.

2. There is a relation between transmission and the pH (acidic or alkalinity) of the fluid. The higher the pH the lower the transmission.

Conclusions from the experiment using the ATR-crystal machine: No clear distinction can be made between the samples between with different percentage contamination levels.

5.2 Future work

The need for a refinement in the measurement method should be carefully considered. The problems with IR absorption by liquid form can be solved by applying biochemical techniques. However, the proposition to improve the measurement method is presented below:

1. A beam splitter can be used to monitor the split light from the LED source. One light goes through the empty sample holder and is to be used as the reference signal. The second light goes through the filled sample holder that will determine the transmission percentage of the sample.
2. A calibration on the infrared spectrum of the known molecular structure of the media culture or drain fluid can be implemented. By subtracting the absorption characteristics of the known basal solution, we will know the transmission that is caused by the sample.

3. To perform the experiment for the analysis of liquid samples, it is recommended to use a multi-grating infrared spectrum device. Thus, different wavelengths can be tuned such that the absorption by the material can be distinguished.
Bibliography


Errata on the thesis “An Optical Measurement System for the Analysis of Bacteria in Drain Fluid” for Chapter 3

2 February 2009

The LED-driver and PD-readout circuit were tested based on the frequency behavior with the following scheme:

The components
- Signal Generator, Model 412
- Tektronix oscilloscope, Model TDS 3034B (300 MHz and 2.5 GS/s)
- Wave functions: sinus, square
- Amplitude of the signal: 200 mV
- $U_{\text{off}} = 110$ mV

The response of the LED-driver circuit:
- The roll-off frequency is between 200 – 300 kHz

The response of the PD-readout circuit:
- The roll-off frequency is around 10 kHz
Corrections

- Page 26, Fig 3.12: The photodiode readout circuit.

Values of the components:
- \( R_p = 303 \, \Omega \)
- \( C_p = 4.2 \, \text{nF} \)
- \( R_f = 10^5 \, \Omega \)
- \( C_f = 50 \, \text{pF} \)

The OpAmp CA3140
- \( A_{OL} = 100 \, \text{kV/V} \)
- \( \tau_1 = 1/2\pi f = 0.008 \, \text{sec} \)
- \( f_T = 4.5 \, \text{MHz} \)
- \( V_{off} = 2 \, \text{mV} \)
- \( I_{off} = 0.1 \, \text{pA} \)

Offset calculation to determine the output error voltage:
- \( U_{IO} (1 + R_f/R_p) = 5 \times 10^{-3} \times 331 = 1.65 \, \text{V} \)
- \( I_{bias} \cdot R_f = 0.1 \times 10^{-12} \times 100 \times 10^3 = 1 \times 10^{-8} \, \text{V} \)
- \( \Delta V_{IO}/\Delta T = 6 \times 10^{-6} \times 10 = 6 \times 10^{-5} \, \text{V} \Rightarrow 6 \times 10^{-5}(1 + R_f/R_p) = 0.02 \, \text{V} \) (for \( \Delta T = 10 \))

Total offset contribution voltage on the output = 1.6\, \text{V}
• The transfer function from current $I_{ph}$ to voltage $V_{pd}$:

$$I_{int} : \frac{V_{pd}}{I_{ph}} = Z_f = -\frac{1}{\beta}$$

Not ideal: $I_{ph} + I_p - I_{feedback} = 0$

$$I_p = \frac{V}{Z_p}, \quad V = -\frac{V_{pd}}{A(s)}, \quad I_{feedback} = \frac{V_{pd} - V}{Z_f}$$

$$I_{ph} + \frac{V}{Z_p} - \frac{V_{pd} - V}{Z_f} = 0$$

$$I_{ph} - \frac{V_{pd}}{Z_p A(s)} - \frac{V_{pd}}{Z_f A(s)} - \frac{V_{pd}}{A(s) Z_f} = 0$$

$$I_{ph} = V_{pd}(\frac{1}{Z_f} + \frac{1}{Z_p A(s)} + \frac{1}{Z_f A(s)})$$

$$\frac{V_{pd}}{I_{ph}} = \frac{1}{\frac{1}{Z_f} + \frac{1}{Z_p A(s)} + \frac{1}{Z_f A(s)}}$$

$$\frac{V_{pd}}{I_{ph}} = \frac{1}{\frac{1}{Z_f} + \frac{1}{Z_p} + \frac{1}{Z_f} + \frac{1}{A(s)}}$$

$$\frac{V_{pd}}{I_{ph}} = \frac{1}{Z_f}

\begin{align*}
V_{pd} = & \frac{R_f A_{ol}}{I_{ph}^2 \left( s^2 R_f (C_p + C_f) \tau_1 + s (R_f C_f A_{ol} + R_f (C_p + C_f)^2 + \frac{R_f}{R_p} \tau_1) + \frac{R_f}{R_p} + A_{ol} \right)} \\
& + \left[ \frac{s R_f (C_p + C_f) + \frac{R_f}{R_p} + 1}{s R_f C_f + 1} \right] A_{ol} \\
& + 1 + \frac{s R_f (C_p + C_f) + \frac{R_f}{R_p} + 1}{s R_f C_f + 1} \right] A_{ol}
\end{align*}$$

Substituting the values,

$$\frac{V_{pd}}{I_{ph}} = \frac{1 \times 10^{10}}{3.4 \times 10^{-6} s^2 + 2.141 s + 9.96 \times 10^4}$$

Calculating the poles of the transfer function:

$$\begin{align*}
P_{1,2} &= \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \\
&= \frac{-2.141 \pm \sqrt{2.141^2 - 4 \cdot 3.4 \times 10^{-6} \cdot 9.96 \times 10^4}}{2 \cdot 3.4 \times 10^{-6}} \\
&= \frac{-2.141 \pm 1.797}{6.8 \times 10^{-6}} \\
P_1 &= -50.58 \times 10^3 \\
P_2 &= -580 \times 10^3
\end{align*}$$

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The inverse Laplace transformation calculation:

\[
\frac{V_{pd}}{I_{ph}} = H(s) = \frac{1 \times 10^{10}}{(s + 51.3 \times 10^3)(s + 580.17 \times 10^3)}
\]

\[
h(t) = \text{Inverse}[H(s)] = \frac{e^{st} \cdot 1 \times 10^{10}}{(s + 51.3 \times 10^3)}|_{s=-580.17\times10^3} + \frac{e^{st} \cdot 1 \times 10^{10}}{(s + 580.17 \times 10^3)}|_{s=-51.3\times10^3}
\]

\[
h(t) = \frac{e^{-580.17\times10^3 t} \cdot 1 \times 10^{10}}{-528.8 \times 10^3} + \frac{e^{-51.3\times10^3 t} \cdot 1 \times 10^{10}}{528.87 \times 10^3}
\]

\[
h(t) = [-18.91 \times 10^3 \cdot e^{-580.17\times10^3 t} + 18.908 \times 10^3 \cdot e^{-51.3\times10^3 t}]\epsilon(t)
\]

The \( h(t) \) is the impulse response of the transfer system in the time-domain. The Laplace transform of \( \delta(t) \) is 1. Thus, when we multiply \( H(s) \) with 1 we will get the impulse response of the transfer function in the time domain. A multiplication in the Laplace domain is a convolution in the time domain. \( y(t) = h(t) \ast \delta(t) = h(t) \).

The impulse response of the system with respect to time is shown below:
This figure is approximating a delta function, which has, ideally, an infinitely large amplitude in an infinitesmaly small amount of time. The total area of the function is approaching 1 ([1] B. Girod, R. Rabenstein and A. Stenger, “Signals and Systems”, John Wiley&Sons, Ltd., Sussex, 2001. 158-159). The drawing shows that the system is stable and the impulse response is created at the output of the system.

The bode plot of the transfer function \( H(s) = 1 \times 10^{10}/3.4 \times 10^{-6} s^2 + 2.141 s + 9.96 \times 10^4 \):

We can see that there is a -3db bandwidth of around 8 kHz \((5.1 \times 10^4/2\pi \approx 8 \text{ kHz})\). This frequency comes near the roll-off frequency that has resulted from the experiment which was conducted and is described at the first page of this document.
The bode plot of the ideal transfer: $H(s) = \frac{R_f}{1 + sR_fC_f}$

We can see that there is a -3dB bandwidth of around 30 kHz ($2 \times 10^{5}/2\pi \approx 30\text{kHz}$).
The transfer function of the LED-driver from voltage $V_s$ to current $I_c$

To calculate the transfer function of the LED-driver circuit, we take approximations of the ideal form of the transistor $\Rightarrow R_o, R_b, r_{bb}$ are not significant because $r_{be} = \infty$ (ideal voltage source), $r_{ce} = \infty$ and $r_e = 0$ (ideal current source).
Values of the components:
\( R_f = 60 \, \Omega \)

The OpAmp OP07CN
\( A(s) = \frac{A_{ol2}}{1+s\tau_2} \)
\( A_{ol2} = 400000 \)
\( \tau_2 = 0.127 \, \text{sec} \)
\( g_m = I_c / V_T \approx 1 \)
\( V_{off} = 60 \, \mu\text{V} \)
\( I_{off} = 0.8 \, \text{nA} \)
\( f_T = 0.5 \, \text{MHz} \)

The bipolar transistor 2N3904
\( f_T = 270 \, \text{MHz} \)

The transfer function is derived bellow:

\[
I_c = g_m V \\
= g_m A(s) v_i \\
v_i = \frac{I_c}{g_m A(s)} \\
V_s = v_i + V_R f = v_i + g_m A(s)v_i R_f \\
V_s = (1 + g_m A(s) R_f) v_i
\]

Substitution of \( I_c \):
\[
V_s = (1 + g_m A(s) R_f) \frac{I_c}{g_m A(s)} = \frac{I_c}{1 + g_m A(s) R_f}
\]
\[
\frac{I_c}{V_s} = \frac{g_m A(s)}{(1 + g_m A(s) R_f)} \\
\frac{I_c}{V_s} = \frac{g_m A_{ol2}}{(1 + s\tau_2)(1 + g_m A_{ol2}) R_f} \\
\frac{I_c}{V_s} = \frac{g_m A_{ol2}}{R_f \tau_2} \cdot \frac{1}{s + \left( \frac{1 + g_m A_{ol2}}{\tau_2} \right)}
\]

Ideal when \( A_{ol2} >> \approx \frac{I_c}{V_s} = \frac{1}{R_f} \)
The transfer function of the LED-circuit from voltage $V_s$ to current $I_c$:

*Substituting the values,*

$$\frac{I_c}{V_s} = \frac{52.5 \times 10^3}{s + 3.15 \times 10^6}$$

The inverse Laplace transformation calculation:

$$\frac{I_c}{V_s} = H(s) = \frac{52.5 \times 10^3}{s + 3.15 \times 10^6}$$

$$h(t) = \text{Inverse}[H(s)] = 52.5 \times 10^3 \cdot e^{(-3.15 \times 10^6)t} \epsilon(t)$$

The $h(t)$ is the impulse response of the transfer system in the time-domain.

The drawing of this impulse response is given below:
The bode plot of the transfer function $H(s) = \frac{52.5 \times 10^3}{s} + 3.15 \times 10^6$:

We can observe a -3dB bandwidth of around 400 kHz ($2.7 \times 10^6/2\pi \approx 400\text{kHz}$). This frequency is different from the bandwidth that has resulted from the experiment described in the first page. This can be explained by the model that has been used in the calculation. There are several unknown values of the components of the transistor, thus we took the ideal model. This may explain the difference between the results. Nevertheless, the bandwidth shows a large enough value that ensures us that the signal will not be limited due to the bandwidth of the circuit.
Simulation with SIMetrix for the LED Driver circuit:
The transfer function of the overall transfer system:
- Transfer system of the LED-driver circuit \( V_s \to I_c \)
- Transfer system of the optical filter \( \to 1 \) (assumption)
- Transfer system of the PD-Readout circuit \( I_{ph} \to V_{pd} \)

Thus, the overall transfer function is

\[
\frac{V_{pd}}{V_s} = \frac{g_m A_{ol} \tau_2}{R_f \tau_2} \cdot \frac{1}{s + \left( \frac{1 + g_m A_{ol} \tau_2}{\tau_2} \right)} \cdot \frac{R_{f_{PD}} A_{ol}}{s^2 R_{f_{PD}} (C_p + C_f) \tau_1 + s (R_{f_{PD}} C_f A_{ol} + R_{f_{PD}} (C_p + C_f) + \frac{R_{f_{PD}}}{R_p} \tau_1) + \frac{R_{f_{PD}}}{R_p} + A_{ol}}
\]

Ideally,

\[
\frac{V_{pd}}{V_s} = \frac{1}{R_{f_{LED}}} \cdot 1 \cdot R_{f_{PD}}
\]