IN-VITRO WAVEGUIDE SYSTEM FOR THE DETECTION OF ESCHERICHIA COLI IN DRAIN FLUID AFTER COLORECTAL ANASTOMOTIC LEAKAGE

Masters Thesis

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In-vitro waveguide system for the detection of Escherichia coli in drain fluid after colorectal anastomotic leakage

THESIS

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“The only constant in life is change”

Heraclitus (535 BC – 475 BC)
ABSTRACT

Background Colorectal anastomotic leakage is one of the most feared complications after colorectal surgery and is associated with increased morbidity, prolonged hospital stay and risk of local cancer reoccurrence. One out of ten anastomoses leak, leading to infections. Colorectal anastomotic leakage is currently diagnosed by sniffing and watching drain fluid, which takes six to eleven postoperative days. Clinical research shows the presence of Escherichia coli (E. coli) bacteria in the drain fluid indicates leakage from postoperative day one, while the concentration grows from day four. Therefore measuring E. coli can reduce the time to diagnosis to one day. In this thesis an E. coli measurement system is proposed, which enables early detection and therefore greatly decrease morbidity, mortality, hospital stay and healthcare costs.

Methods An E. coli detection system was designed, simulated and assembled. The system was built around an evanescent waveguide device, which features a reference and sensing path to enable differential measurements. The surface of the sensing path is functionalised to capture E. coli. Light is coupled in and out of the waveguide device by fibres. Evanescent light in the waveguide device is absorbed by captured E. coli. By measuring the light absorption, the concentration of E. coli bacteria is calculated. The proposed system is portable, user-friendly and is tested in clinically relevant simulations. The proposed solution features a microcontroller, a constant current source circuit, an infrared LED, two photodiodes, a transimpedance differential amplifier, a display and computer software. It is powered by USB, communicates over USB and rejects power line noise. Subsystem performance was measured in clinically relevant simulations, i.e. 0.1 dB light absorption measurements. This resembles $10^6$ CFU/ml E. coli on postoperative day five.

Results The subsystems were assembled on experimental PCB and measured. The light source outputs 2.655 mW of light and shows no drift after a warm-up period of five minutes. Electrical measurements show a stable LED driving circuit with no oscillations. Light coupling from the LED to a fibre was not measurable. The photodiode monitoring circuit can measure light absorption in high detail. A drift of $6.92 \times 10^{-5}$ dB per minute was measured. The detection time for individual measurements is 60.8 ms, or 6.08 seconds for an averaged measurement. Electrical measurements show oscillations of 2 kHz in the monitoring circuit, which may limit the functioning. The waveguide device was coupled with 40 dB of light loss. It confines light in the vertical plane, but not in the horizontal plane. Therefore the waveguide device could not be embedded in the measurement system and no biological measurements could be performed.

Conclusions Measurements show a stable infrared light source and precise monitoring circuit were constructed. The proposed measurement system has a detection limit of 9,700 CFU/ml E. coli, thus anastomotic leakage can be detected from postoperative day four. The detection time is 6.08 seconds and allows quick measurements. The designed electronics and waveguide devices are portable, although the complete measurement system is not. The system can calculate the bacteria concentration automatically, adding user-friendliness. In future research the LED can be replaced by an fibre-coupled laser, the differential amplifier stage can be replaced by an instrumentation amplifier and the waveguide device’s functioning can be improved.

Keywords biosensor, evanescent waveguide, colorectal anastomotic leakage, E. coli
This master thesis was written as a part of a graduation project about colorectal anastomotic leakage detection. The graduation project took place in the second and last year of the master study Biomedical Engineering at Delft University of Technology. The assessment was to continue the research project in the Electronic Instrumentation department about colorectal anastomotic leakage detection.

The author assumes the reader has a basic understanding of electrical engineering, human physiology and human anatomy. The aim of this thesis is to inform the reader about the design of an in-vitro waveguide device for the detection of E. coli in drain fluid after anastomotic leakage. Certain basic principles of electrical engineering, human physiology and human anatomy are assumed to be known to the reader and will not be discussed in depth.

Readers with a specific interest in the waveguide device are referred to subchapters 2.7, 3.1 and 5.4. Those with specific interest in the performance of the used measurement equipment are referred to subchapter 5.1. For readers interested in the constant current source to drive a LED, please see subchapters 4.3 and 5.2. Specific information about differential photodiode monitoring can be found in subchapters 4.2 and 5.3.

During this project many people have supported me. First of all I would like to thank Paddy French and Lukasz Pakula for supervising me through the project. Furthermore I would like to thank Xin Yu for cooperating in experiments and our relaxing tea-moments. Piet Trimp, Jeroen Bastemeijer, Zu-Yao Chang and Ger de Graaf, thank you for helping me through technical difficulties. Peter Harmsma, Gregory Pandraud, Agung Purniawan, Tjitte-Jelte Peters and Yulia Meteleva-Fischer have helped me greatly in explaining waveguide theory. Without Marinka Almering and Sandra Vennix the clinical relevance of this research would be unknown to me. Last but not least I would like to thank my parents, sister, girlfriend and friends for the love I receive and providing feedback.

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Delft, May 2014
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1. INTRODUCTION

Anastomotic leakage is one of the most feared complications after colonic surgery [1]. The reported mortality rate can be as high as 33% [2]. Furthermore it is associated with increased morbidity, prolonged hospital stay [3] and risk of local cancer reoccurrence [4]. An anastomosis is applied to prevent intestinal content from leaking into the pelvic and abdominal cavities. One out of ten anastomoses leak. The leaked intestinal content may cause infections, such as peritonitis and sepsis. Currently, anastomotic leakage is diagnosed after six to eleven postoperative days [5], by smelling and watching the drain fluid. Using an Escherichia coli (E. coli) bacteria measuring system, anastomotic leakage can be detected within one day [6]. Although such systems are available, the measurement procedure is expensive, time costly and thus not commonly used. A simpler system for the measurement of E. coli in drain fluid would decrease morbidity, mortality, hospital stay and healthcare costs. Furthermore the system can be used as a surgical quality indicator [3].

The aim of this thesis is to present the design of an in-vitro waveguide device for the detection of E. coli in drain fluid after colorectal anastomotic leakage. To reach the aim, first the medical background, current situation and target group are discussed. Then the specifications are formulated, the research statement follows and relevant research is reviewed. Next, a concept solution is formulated, split into components and the components are discussed in depth. The concept solution is tested with an E. coli dilution simulation. Conclusively, the concept solution is tested if it complies with the specifications. The planning of this project is described in Appendix A. The electrical design, clinically relevant simulations, portability and simple user interface have a high priority in this project. In contrast, the device appearance was of less importance. The waveguide devices and their light coupling mechanism were delivered by another research project and will not be improved in this research. The research statement is:

"Design an in-vitro device for the detection of E. coli in drain fluid after colorectal anastomotic leakage, using a waveguide chip, while prioritizing the electrical design, clinically relevant simulations, portability and simple user interface."

This thesis is structured as followed: First, the accessed literature is reviewed in the background study (chapter 2) and choice of hardware (chapter 3). Next, the design of the concept solution is discussed using a top-down strategy (chapter 4). The concept solution is then realised and tested in experiments (chapter 5). Finally, the discussion reviews the results of this research project (chapter 6) and a general conclusion is presented (chapter 7).
2. BACKGROUND STUDY AND RESEARCH STATEMENT

In this chapter the background study is presented, as it an essential start to the design process. Together with the specifications and research statement, it forms the design brief. In subchapter 2.1 the aim of this research project is stated. In subchapter 2.2 the medical background is discussed and in subchapter 2.3 the current situation of anastomotic leakage detection. The target group is reviewed in subchapter 2.4. From there the specifications follow in subchapter 2.5 and the research statement is formulated in subchapter 2.6. The most significant relevant research is reviewed in subchapter 2.7. This chapter is concluded with a concept solution proposal in subchapter 2.8.

2.1 AIM

The aim of this research project is to develop a device for the detection of anastomotic leakage in patients, by measuring the E. coli concentration in drain fluid. This device should enable fast and simple detection of anastomotic leakage. Once in place, the device should not greatly decrease the patient comfort or influence hospital infrastructure. The advantage of this device is that it replaces expensive and time costly lab measurements.

2.2 MEDICAL BACKGROUND

This subchapter reviews the reasons and procedure of applying a colorectal anastomosis, complications which may arise and indicators of complications. For an in-depth study, please see [7].

The colon, also known as large intestine, is an organ in the digestive system [8]. It is the last part of the human gastrointestinal tract. The colon is located in the abdominal cavity (see Figure 1). Its main functions are to absorb water and electrolytes from the intestinal fluid and to store the remains as faecal matter. Faecal matter is eliminated from the colon via the anus. The colon mainly consists of muscular tissue, which can move the intestinal fluid by peristaltic movements.

![FIGURE 1: THE COLON][9]
In the colon live various bacteria, such as Bacteriodes, Lactobacillus acidophilus and Escherichia coli [10]. These colonic microflora use undigested fibres in the intestinal fluid for their own consumption. In this process, called fermentation, short-chain fatty acids are produced as a waste material. The waste material can be used as energy source for cells in the lining of the colon. Bacteria colonies feature four phases of growth [11][12] (see Figure 2). In the first phase, the lag phase, the bacteria adapt to their new environment. In the second phase, the log phase, the colony grows exponentially because of an abundance of nutrients. Once the nutrients have run out, the third phase starts: the stationary phase. Because of the lack of nutrients, the bacteria die and the colony shrinks. This is the death phase. Although the bacteria in the colon have a symbiotic relationship to the human carrier, they are notorious for causing infections in other parts of the body, e.g. bladder infection.

Colorectal cancer and inflammatory bowel disease are diseases which may require segmental resection of the colon, meaning a part of the intestine needs to be cut out [3]. After removing a part of the intestine, it needs to be reconnected, which is called anastomosis (see Figure 3). Anastomosis is a broad term; it means to reconnect two streams which previously branched out [13] and is used in geology, archaeology, biology and medicine among other professions. In medicine it is used to describe the reconnection of tubular structures, such as the large intestine. Applying a colorectal anastomosis is an intensive surgical procedure.

Two techniques can be used to apply a colorectal anastomosis: hand sewing and stapling, with the latter being the modern alternative [14]. The stapling procedure is faster than hand sewing, however no significant difference in anastomotic leakage rate or mortality was found. After the procedure the wounds on the anastomosis and skin need to heal. A prophylactic drain is applied to release purulence from the abdominal cavity. The purulence stored outside the body is called drain fluid.
Anastomotic leakage is a complication with a defect in the anastomosis, through which non-sterile intestinal content leaks into the abdominal or pelvic cavity [2]. No consensus has yet been reached on the diagnosis of anastomotic leakage. Some diagnose anastomotic leakage as requiring reoperation, while others diagnose anastomotic leakage when signs of leakage have been found [3]. The defect can simply be caused by a loose suture [16]. If the anastomosis is not a water-tight seal, intestinal fluids may leak. Although every effort is made to ensure a good join, there are cases where this is not the case [17]. The defect may also be caused by sutures which are too tight [16]. This may cause blocking of the blood supply, leading to low glucose and oxygen levels, also known as ischemia. Glucose and oxygen are energy sources and are vital to living cells. Their absence may lead to a bad wound healing process. Breakdown of the anastomosis is called dehiscence. Furthermore a tight suture may cause mechanical tension on the anastomosis, leading to tearing of tissue.

Colorectal anastomotic leakage is the most feared complication after colonic surgery [1]. The bacteria in the leaked intestinal content may cause infections [2][10]. The body's immune system will try to clean up the leaked content. However, the bacteria have abundant food available in the abdominal cavity and will reproduce very fast. The first stage of the bacterial infection is peritonitis: inflammation of the peritoneum, the inner lining of the abdominal wall. In the second stage, abscesses form in the abdominal cavity [3]. The third stage is sepsis: infection of the blood. Continual sepsis may lead to metabolic disturbance, multiple-organ failure and ultimately to death. If colorectal anastomotic leakage is diagnosed and the patient’s health turns worse, reoperation is necessary [17].

Both ischemia in the anastomosis and the leaked content can indicate anastomotic leakage. Ischemia can be measured on the anastomotic wound and in the drain fluid by measuring oxygen saturation (sO₂), oxygen pressure (pO₂), carbon dioxide pressure (pCO₂), lactate, free fatty acids, glycerol and temperature levels [2][4][18]. A measurement on the anastomotic wound is called in situ. Such measurements are very difficult and uncomfortable for the patient. The sensor may be dislocated by the intestine, as it constantly moves [19]. Anastomotic leakage due to a loose suture is not detected by ischemia measurements. The leaked content can be measured in the drain fluid, on the wound on the skin and on the anus. If faecal matter is found on the wound on the skin or in the drain fluid, anastomotic leakage is detected [3]. The same goes if purulent discharge is found on the wound on the skin or on the anus. Faecal matter
includes undigested nutrients, waste material, excretory products and bacteria living in the colon. Additionally, the leukocytes or white blood cells trying to clean up the leaked content can be measured as an indicator of anastomotic leakage [2].

In this research project colorectal anastomotic leakage is detected by measuring E. coli bacteria in the drain fluid.

2.3 CURRENT SITUATION

This subchapter reviews the current situation in colorectal anastomotic leakage detection. For an in depth study, please read my literature survey report [7].

Currently, colorectal anastomotic leakage is mainly detected by the patient having a prolonged fever, i.e. an elevated body temperature above 37.5 °C [2]. After surgery it is normal that the patient develops a fever, however it should go down within 24 hours. If the fever does not go down, this may be a sign that anastomotic leakage has occurred. Besides watching the patient’s temperature, the physicians also watch and smell the drain fluid every morning [17]. If the colour or smell changes, this may be a sign that anastomotic leakage has occurred. After six to eight days of prolonged fever or a change of the drain fluid, extra measurements are necessary to determine if anastomotic leakage indeed has occurred. These extra measurements are usually a bacteria culture or a CT scan. Current procedures take a long time, which increases the patient morbidity and mortality. Furthermore the extra measurements require certified personnel and are therefore costly.

A bacteria culture is used to determine which bacteria are present in a sample, and in what concentration. The drain fluid is used for this purpose [20]. If anastomotic leakage has occurred, bacteria from the colon have invaded the abdominal cavity and are measurable in the drain fluid. Specific nutrient culture media are used to grow specific bacteria colonies. Using this technique, one can verify which bacteria are present in the sample. Individual bacteria in a sample reproduce and form colonies (see Figure 4). By counting the number of colonies formed on the growth medium and dividing by the sample volume, the number of colony forming units per millilitre (CFU/ml) can be calculated [12]. This is the concentration of bacteria in the sample. If this concentration is very high, the sample may be diluted to get a countable result. In good practice, cultures of multiple dilutions of a sample are made, so the bacteria concentration can be precisely determined. Making a bacteria culture takes over 24 hours. In case of extreme contamination, the culture may not exactly clarify which bacteria are present. Working with bacteria requires strict safety precautions, to prevent the person performing the measurement from being infected.

![FIGURE 4: BACTERIA CULTURE FROM DRAIN FLUID [21]](image-url)
Anastomotic leakage can be visualized using a CT scan, i.e. a computed tomography scan [3]. In computed tomography, X-ray beams are used to image slices of the body [22][23] (see Figure 5). These slices can be combined to form a three dimensional image of the patient. To enhance the image contrast, contrast medium may be supplied to the patient [2][3]. Contrast medium increases the contrast of the intestinal fluid, so the intestinal content and leaked fluids are clearly visible. This imaging modality is relatively cheap and easy on the patient. Nevertheless X-rays are harmful and the patient should not be exposed to many CT scans. Furthermore small leakages cannot be detected.

Besides making a bacteria culture or a CT scan, the physicians may choose to use polymerase chain reaction [6] [24] or Raman spectroscopy [10] methods to analyze drain fluid, make a MRI scan of the patient or do an endoscopic examination [25] to diagnose anastomotic leakage. These methods are incidentally used, as they are more time-costly and expensive. If the physicians suspect anastomotic leakage, it can be ascertained by reoperation. Reoperation is the most reliable way of diagnosing anastomotic leakage, however this is also the most harmful to the patient [3].

2.4 TARGET GROUP

The target group for this device is hospital personnel. It is used in the micro-organism lab, which is only accessible by certified personnel [17]. The designer may assume the users know about medical terminology and have basic technical understanding [25].

Safety and user-friendliness specifications are to be met. User-friendliness is also known as ergonomics. For an European market introduction, the device should comply with the Medical Devices Directive legislation[26]. This legislation protects the safety of the patient and personnel by setting requirements on safety and user-friendliness aspects. The Medical Devices Directive features several classes of medical devices. A non-invasive monitoring device belongs to Class IIa. In 2007 a revised version of the Medical Devices Directive was adopted [27][28]. It adds ergonomic requirements, namely to design the device for a low risk of use error. Therefore it is obligatory to specify the controls and indicators, as well as to make the visual system understandable (e.g. the display).

To keep the device risks low, one can utilize the ISO standard 14971 on risk management with medical devices during the design process [29]. This includes a risk analysis, risk evaluation, risk
control and production and post-production information. The best practice is to design an inherently safe device.

2.5 SPECIFICATIONS

The specifications result from the background analysis and the product life cycle. All four phases of the product life cycle are considered: production, distribution, consumption and recycling.

1. The system measures accurately and selectively the concentration of bacteria
2. The system has a bacteria detection limit within the clinically relevant region
3. The system has a bacteria detection time within a minute, so an online measurement is possible
4. The system displays the measured concentration of bacteria and an understandable message to the clinician
5. The waveguide chip can be easily removed and replaced
6. The system is portable, i.e. it is low weight and can be carried
7. The system can operate in a clinical environment and does not interfere nearby clinical systems
8. Program code can be read and adapted by colleagues
9. Program code does not contain copyrights
10. The system features a stable stage for the waveguide chip
11. The system features tuneable stages for aligning light transmitters and receivers
12. The system compensates for alignment errors
13. The system works with drain fluid
14. The system has read-out ports for debugging purpose

2.6 RESEARCH STATEMENT

The conclusion that can be drawn from the specifications is the research statement. The research statement reads:

"Design an in-vitro device for the detection of E. coli in drain fluid after colorectal anastomotic leakage, using a waveguide chip, while prioritizing the electrical design, clinically relevant simulations, portability and simple user interface."

2.7 RELEVANT RESEARCH

This subchapter presents the relevant research performed by the Electronic Instrumentation department of Delft University of Technology and the Department of Surgery of Erasmus Medical Center. For an in depth study please see [7].

Research in the Electronic Instrumentation department of Delft University of Technology started by measuring the change of colour of drain fluid after anastomotic leakage [20]. This was a master thesis project by Marcin Pakula in 2005. In the proposed system the colour change of drain fluid is measured over a wide range of wavelengths, namely the visible spectrum (0.4 µm to 1.0 µm) and the infrared spectrum (1.8 µm to 4.8 µm). To generate these light wavelengths, a broadband light source and a monochromator were used (see Figure 6). The monochromator filters out all but the desired wavelength. The light is channelled through a sample of drain fluid. The sample absorbs the light partly, depending on the wavelength and the sample contents. The resulting light power is detected using a photodiode. The results are that no clear distinction in light absorbance of drain fluid in the visible spectrum could be found, especially not for low contamination levels (see Figure 7). In the infrared spectrum two wavelengths were found for
measuring drain fluid contamination, namely 2 µm and 4.3 µm (see Figure 8). From these two
the 4.3 µm wavelength is found to be the best for measuring drain fluid contamination, because
the sensitivity at low contamination is high. The problem however with this system is the
correlation between drain fluid contamination and anastomotic leakage. If anastomotic leakage
has occurred, the drain fluid may be contaminated. However, drain fluid contamination may also
be caused by other factors, e.g. blood loss. Therefore this system could not diagnose anastomotic
leakage.

![Diagram of light measurement system]

**FIGURE 6: CONCEPT FOR MEASURING LIGHT ABSORBANCE IN DRAIN FLUID**

![Graph of light absorbance in the visual spectrum]

**FIGURE 7: DRAIN FLUID LIGHT ABSORBANCE IN THE VISUAL SPECTRUM [20]**

![Graph of light absorbance in the infrared spectrum]

**FIGURE 8: DRAIN FLUID LIGHT ABSORBANCE IN THE INFRARED SPECTRUM[20]**

The drain fluid light absorption analysis was continued by the master thesis project of Sitti
Chaeron. She designed an optical measurement system for the analysis of bacteria in drain fluid
[10]. For this she used a comparable approach as Marcin Pakula, however she replaced the light
source by a near-infrared LED and put the whole system in a portable packaging. The same drain
fluid sample holder was used as in Pakula's research. The LED emits light at 1950 nm
wavelength, on a frequency of 400 Hz and a 20% duty cycle. The resulting current from the photodiode was transamplified by $10^5 \Omega$. The output signal was analyzed using LabView. See Figure 9 for the block diagram of the system. Besides the light absorption, the pH of the sample was measured. The results show no relation between the light absorption and the E. coli concentration was found. Biological measurements were not conducted in laboratory environment and electric circuit functionality was not properly tested.

Research in the Electronic Instrumentation department was continued by the PhD project of Agung Purniawan. The focus was shifted towards waveguide devices [30][31]. A waveguide is a structure which guides waves, in this research optical waves or light. Materials with a high refractive index surrounded by materials with a low refractive index form an optical waveguide. This combination of materials generates total internal reflection in the core material. In this research a rib waveguide was made of TiO$_2$ (titanium dioxide) and deposited by atomic layer deposition (ALD). The surrounding material, the cladding, is simply the air around the waveguide, because it has a smaller refractive index than TiO$_2$. To prevent light from propagating into the Si (silicon) substrate, a SiN (silicon nitride) layer was made between the waveguide and the substrate by low-pressure chemical vapour deposition (LPCVD). This layer also brings mechanical support to the waveguide. A freestanding part of the waveguide was fabricated by removing the substrate using potassium hydroxide (KOH) backside etching. For a cross-section of the complete waveguide see Figure 10. It was verified in a two-dimensional COMSOL Multiphysics RF simulation before realisation. The propagation loss in the waveguide was determined by measuring the light power before and after the waveguide. The propagation loss in the waveguide was found to be 6.7 dB/cm.

This waveguide device was then used to measure E. coli bacteria, by evanescent light absorption [32]. Evanescent light is the part of the light present outside the waveguide [33]. It is generated because the electric and magnetic fields of light cannot be discontinuous at the boundary of the core and the cladding, where total internal reflection takes place. The evanescent wave exhibits
an exponential decay. Objects present on the surface of the waveguide core absorb evanescent light. Therefore *E. coli* bacteria on this waveguide can be measured.

The freestanding structure enlarges the contact area of the waveguide, so it enhances the sensitivity to bacteria. To specifically measure *E. coli* bacteria, while ignoring the other substances which may be present in drain fluid, antibodies are applied to the waveguide. Antibodies are normally generated and used by the body's immune system to identify foreign objects, such as bacteria. Antibodies for *E. coli* can only bind to *E. coli* bacteria. In this research, these specific antibodies are attached to the waveguide by a complex chemical process. First, a thin film of SiO$_2$ (silicon dioxide) is deposited on the TiO$_2$ waveguide by plasma-enhanced chemical vapour deposition (PECVD). Then OH-groups are created by an oxygen plasma. A monolayer of (3-aminopropyl)triethoxysilane (APTES) binds to the OH-groups. To connect the APTES monolayer to the antibodies, Protein A and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) are used. Finally, *E. coli* K99 antibodies (401) are applied.

To test the waveguide sensor, different isopropanyl-alcohol (IPA) dilutions were applied to the waveguide and the light absorption was measured. For measuring the light absorption, the setup depicted in Figure 11 was used. A superluminescent light emitting diode (SLED) transmitting 20 mW of light at 1300 nm wavelength was used as a light source. Optical fibres connect the SLED with the waveguide and the photodiode. The output current of the photodiode was analyzed using LabView. Once functionality was confirmed, the system was tested with *E. coli* dilutions. The results show a logarithmic relation between the concentration of *E. coli* in the sample fluid and the light absorption on the waveguide (see Figure 12). The light absorption by the bacteria is small, the highest absorbance is just 0.30 dB. The total light power reduction from the SLED to the photodiode is around 40 dB. The reported detection limit is 500 CFU/ml. No detection time was reported.
The Department of Surgery of Erasmus Medical Center researches anastomotic leakage indicators from the medical point of view [2][6]. First they have compiled a comprehensive list of anastomotic leakage indicators found in literature. Then, they have used a real-time polymerase chain reaction (RT-PCR) system to measure the daily bacteria concentration in drain fluid of 243 patients after colorectal surgery. Two bacteria were studied: E. coli and Enterococcus faecalis (E. faecalis). Furthermore the emerging of colorectal anastomotic leakage (CAL) was documented. The results show that anastomotic leakage can be diagnosed through E. coli measurements from day one. On day four the bacteria concentration rises sharply, which is easier to measure. Since a rise of bacteria concentration is better measurable than the absolute bacteria concentration, the authors note that E. faecalis is a earlier precursor for anastomotic leakage, as its concentration rises on day two and day three.
**TABLE 1: DAILY BACTERIA CONCENTRATIONS IN DRAIN FLUID AFTER COLORECTAL SURGERY [6]**

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>CAL Y/N</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (CFU/ml)</td>
<td>Y</td>
<td>55</td>
<td>30</td>
<td>55</td>
<td>100,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. faecalis (CFU/ml)</td>
<td>Y</td>
<td>300</td>
<td>7,500</td>
<td>75,000</td>
<td>75,000</td>
<td>100,000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>0</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Other research on the detection of anastomotic leakages focuses on measuring anastomotic ischemia parameters through electrochemical properties [4] [18][19][35][36], or using other methods for measuring E. coli bacteria, e.g. photoluminescence[37]. Using these new techniques, time to diagnosis can be reduced to within eight hours.

From my literature survey various recommendations followed [7]. New research on anastomotic leakage detection should focus on indicators not commonly studied, e.g. the E. faecalis bacteria or leukocytosis. Furthermore when detecting bacteria, one should be able to differentiate between live and dead cells. In optical measurements the choice of light wavelength should be substantiated. An online or continuous detection system is not yet available and could be an unique selling point. Lastly, the achieved detection limit and detection time should be reported, as it is essential for comparing results and was not done by Purniawan.

### 2.8 CONCEPT SOLUTION

The concept solution is a hypothetical answer to the research statement.

The waveguide system for the detection of Escherichia coli in drain fluid after colorectal anastomotic leakage hypothesis is a portable and smart system. The system is portable, so it can be moved by bicycle or public transport to a hospital. The system is smart, so it can perform the measurement and calculate the bacteria concentration by itself. The bacteria concentration is printed on a display. The system is built around the existing waveguide device and features an on-board LED and photodiodes. The detection limit for the E. coli concentration is at maximum $10^5$ CFU/ml. The detection time, from applying a drop of drain fluid to the presentation of measurement results, is within an hour. The detection limit and detection time of the system are determined by clinically relevant simulations.
3. CHOICE OF HARDWARE

In the concept solution the critical hardware components were enumerated. The waveguide chip is delivered by previous research and is investigated in subchapter 3.1. The other mentioned hardware components are commercially available in many forms. Besides the waveguide chip, this chapter reviews and the choice of photodiode (subchapter 3.2), light emitting diode (subchapter 3.3), computing platform (subchapter 3.4) and display (subchapter 3.5).

3.1 WAVEGUIDE DEVICE

The waveguide device was delivered by previous research of the Electronic Instrumentation department of Delft University of Technology. It was designed by Agung Purniawan and tested with E. coli dilutions and patient drain fluid. See the subchapter 2.7 for explanation of the working principles and results. The device is two by two centimetres in size. It features two waveguides, see Figure 13. On the left side of Figure 13 the inputs are located, spaced 0.74 mm apart. Here the light should enter the device. Only one of the inputs needs to be connected, as the waveguides come very close in the next part of the device (the coupler, see Figure 14) and the light is split 50:50 over both waveguides. Then the waveguides split. One waveguide features the freestanding part, where the bacteria should be attached. This is the measurement arm. The other arm, without the freestanding part, is the reference arm. On the right side of Figure 13 the outputs are located, spaced 1.8 mm apart. By measuring the light power from both outputs, a differential measurement can be performed.

![FIGURE 13: TOP VIEW OF THE WAVEGUIDE DEVICE (ILLUSTRATION)](image)

![FIGURE 14: WAVEGUIDES COMING CLOSE TOGETHER](image)
The inputs and outputs of the waveguide device feature tapers (see Figure 15). A taper is where the waveguide narrows, which makes connecting the device to light sources easier. The taper narrows from 125 µm to 3 µm. In previous research, the waveguides were connected to the light source and photodiode by Thorlabs P3-SMF28-FC-2 optical fibres. These single mode patch fibres have a 125 µm cladding diameter, a 8.2 µm core diameter and angled physical contact (APC) connectors [38].

![FIGURE 15: TAPER ON A WAVEGUIDE](image)

The freestanding part of the waveguide is where the actual light absorption by bacteria happens. On Figure 13 it is the square in the measurement waveguide path. On Figure 10 a cross-section is illustrated. A cross-section in the other dimension is illustrated in Figure 16. In this illustration the working principle is depicted: the bacteria partly absorb the evanescent light on the optical waveguide.

![FIGURE 16: FREESTANDING WAVEGUIDE WORKING PRINCIPLE [30]](image)

The devices have been checked for errors. Particular points of interest are: continuous waveguides on the devices, flat and clean edges of the devices and a complete freestanding part. If the waveguide is not continuous, the light loss will be high and therefore the device will be useless. The edges of the devices should be cleanly cut, as the light must couple in and out of the devices without reflections or scattering. The freestanding part of the device is very fragile and can break during fabrication. If it is broken, the light loss will be high and a measurement is impossible.

From previous research 27 devices were investigated for use in this research. Eleven waveguide devices with stains from bacteria measurements were not used in this research, as the status of these devices is unknown. Seven devices with only straight waveguides were not used in this research, since a coupler is needed to perform a differential measurement. A half wafer with waveguide devices was cleaved into eight individual devices. A chip with two devices could not be split and forms one double device. The devices were numbered and checked under a microscope. Five devices with continuous waveguides, an intact membrane and clean edges were found, see Figure 17. These devices will be used in this research. To two devices a piece of
light absorbing foam was attached to block the light that is passing over the waveguide, instead of through.

![Waveguide Devices](image)

**FIGURE 17: WAVEGUIDE DEVICES USED IN THIS RESEARCH**

No functionalisation chemicals were found in the laboratory. Buying new functionalisation chemicals would be a financial risk, since the state of the waveguide devices is unknown. Therefore the available waveguide device were not functionalised and no bacteria measurements were performed.

### 3.2 PHOTODIODE

To measure the light power from the waveguide device, photodiodes, phototransistors, photoresistors and thermopiles can be used. To accurately measure the light power of a 1300 nm wavelength at low light levels, photodiodes are most suitable. Still, photodiodes which can sense light at a wavelength of 1300 nm cannot be found on common component stores, as they are mostly used in professional fibre telecommunication equipment. Most available photodiodes are made of silicon and are made to measure the visible light spectrum. E.g. Farnell [39] and RS Components [40] do not sell photodiodes for measuring 1300 nm light. For measuring the 1300 nm wavelength, germanium (Ge) or indium gallium arsenide (InGaAs) photodiodes can be used. These are sold by specialized optical component companies, such as Thorlabs [41] and Roithner Lasertechnik [42]. For this project photodiodes without a mounting are used to keep the final system small and the design process flexible. Table 2 lists the suitable photodiodes which were found with these companies.
The larger the photodiode’s surface, the easier it is to align the photodiode with a fibre. However, the response time of a large photodiode is longer due to a larger internal capacitance. The response time is no limiting factor in the desired system, as the response time of the functionalisation layer in the waveguide device, which binds the bacteria, is far larger. The Roithner Lasertechnik EPD-1300-5-0.5 has the best specifications for its price, with a workable surface size. However, when trying to order the photodiodes, this type turned out to be discontinued and therefore not deliverable. The second best option is the Roithner Lasertechnik PT511, with comparable specifications, but a smaller surface. Of this type four photodiodes were ordered, two with a flat window (PT511-2) and two with a ball lens (PT511B, see Figure 18). These photodiodes can sense light power up to 10 dBm (10 mW), have a light responsivity of respectively 0.85 A/W and 0.75 A/W and a dark current of 1 nA [43][44].

---

**3.3 LIGHT EMITTING DIODE**

To generate light at a 1300 nm wavelength, previous research has used a SLED. In this research, the aim is to generate the same light in a portable system, so a low power light source must be used. A LED is the best option. The same story goes for the LEDs as for the photodiodes. LEDs which transmit light at 1300 nm wavelength are mostly used in professional fibre
telecommunication equipment and not available through Farnell [39] or RS Components[40]. While most LEDs are made of silicon, suitable LEDs are made of germanium or indium gallium arsenide. These are sold by the same companies which sell the corresponding photodiodes, e.g. Thorlabs [41] and Roithner Lasertechnik [42]. Again, LEDs without a mounting are used for size and design flexibility reasons. Table 3 lists the suitable LEDs found.

<table>
<thead>
<tr>
<th>Company</th>
<th>Name</th>
<th>Material</th>
<th>Wavelength</th>
<th>Current</th>
<th>Power</th>
<th>Angle</th>
<th>Package</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorlabs</td>
<td>LED1300E</td>
<td>InGaAsP</td>
<td>1300 nm</td>
<td>20 mA</td>
<td>2 mW</td>
<td>15°</td>
<td>Epoxy</td>
<td>€15.49</td>
</tr>
<tr>
<td>Roithner</td>
<td>ELD-1300-525</td>
<td>InGaAs/InP</td>
<td>1300 nm</td>
<td>20 mA</td>
<td>2.2 mW</td>
<td>25°</td>
<td>Epoxy</td>
<td>€8.19</td>
</tr>
<tr>
<td>Roithner</td>
<td>LED1300-03</td>
<td>InGaAsP</td>
<td>1300 nm</td>
<td>50 mA</td>
<td>4.5 mW</td>
<td>20°</td>
<td>Epoxy</td>
<td>€12.02</td>
</tr>
<tr>
<td>Roithner</td>
<td>LED1300-35M32</td>
<td>InGaAsP</td>
<td>1300 nm</td>
<td>50 mA</td>
<td>3.0 mW</td>
<td>15°</td>
<td>TO-46</td>
<td>€15.46</td>
</tr>
<tr>
<td>Roithner</td>
<td>LED1300-35K42</td>
<td>InGaAsP</td>
<td>1300 nm</td>
<td>50 mA</td>
<td>2.0 mW</td>
<td>6°</td>
<td>TO-46</td>
<td>€16.61</td>
</tr>
<tr>
<td>Roithner</td>
<td>SMC1300</td>
<td>AlGaAsP</td>
<td>1300 nm</td>
<td>50 mA</td>
<td>0.5 mW</td>
<td>55°</td>
<td>SMD</td>
<td>€13.43</td>
</tr>
</tbody>
</table>

The light from the LED will be channelled into the very small surface of the fibre or waveguide. Therefore the light power must be high at a small radiation angle. For this reason the Roithner Lasertechnik LED1300-35K42 was chosen (see Figure 19) [45].

**FIGURE 19: ROITHNER LASERTECHNIK LED1300-35K42 LEDs**

### 3.4 COMPUTING PLATFORM

Designing a smart system for detecting E. coli bacteria is one of the main goals of this research. A computing platform is essential in such system, as it takes care of control and calculations of the measurement. For a small and portable system multiple computing platforms can be used. Calculation performance is not compared, as all mentioned computing platforms have sufficient performance for the calculations done in this project.

The first option is a Microchip PIC microcontroller (see Figure 20) [46]. These microcontrollers come in various sizes, from very simple ones with only a few digital IO ports to advanced microcontrollers with integrated Ethernet and audio controllers. The advantage of these microcontrollers is the versatility: one can design a custom circuit with total control of the power stabilizers, clock, PCB size et cetera. At the same time, the versatility is the disadvantage.
of this option: designing the PCB, clock circuit et cetera is not relevant to the research topic and only makes the design process more complex. Furthermore many of the available libraries are not easy to include. Once the microcontroller is mounted on a PCB, it is hard to change the circuitry, so this option is not very flexible.

**FIGURE 20: MICROCHIP PIC [46]**

The second option is an Arduino microcontroller platform (see Figure 21) [47]. This open-source platform is based on an Atmel ATmega 328 AVR microcontroller. Actually, it is a complete microcontroller board, with tested and proven circuitry. The device is very portable, easy to connect to a computer, and programming is easy in the Arduino programming language. As the Arduino is pre-assembled, some functionality may be missing, such as onboard operational amplifiers, which makes it less versatile. Adding hardware functionality to the Arduino can be done by designing an extension PCB, called a 'shield', which complies with the pinout and dimensions of the Arduino. Installing or removing a shield is easy and for many libraries are available for commercially available shields, which makes the Arduino very flexible.

**FIGURE 21: ARDUINO UNO SMD [47]**

The third option is using an FPGA as computing platform. If the final solution should be an integrated smart sensor chip, an FPGA implementation comes close to this. An FPGA implementation however has many disadvantages: it is hard to code a smart controller and calculator in VHDL, the programming language of FPGAs, and the product will not be easily adaptable by colleagues. Furthermore an FPGA implementation will need additional analogue circuitry, as no onboard ADC can be programmed.

The last option is an application-specific integrated circuit (ASIC). This can be an integrated smart sensor chip, with the waveguide device, LED, photodiodes and computing platform all in one chip. Commercial medical ASICs are available, however these are made for hearing aids,
pacemakers and common biomedical sensors, such as pressure and temperature\cite{48}\cite{49}. The advantage of this option is its small size and high processing power, which makes it very future proof. However, as it is an highly integrated solution, the design process will be very costly and a prototype will not be flexible at all. Programming an ASIC is harder than programming a microcontroller.

The options for the computing platform are compared in Table 4. A versatile platform can be applied to every situation. A flexible platform can easily be changed after implementation. Easy to program is not only about the programming language, but also about available libraries and the ease of including these. A future proof platform can be made into a chip. The Arduino computing platform is the best option.

<table>
<thead>
<tr>
<th>Computing platform</th>
<th>Versatile</th>
<th>Flexible</th>
<th>Easy to program</th>
<th>Future proof</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microchip PIC</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arduino</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>FPGA</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ASIC</td>
<td>+</td>
<td>--</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

The Arduino runs on 16 MHz and features 14 digital input/output pins, pulse-width modulation (PWM), 6 analogue to digital converters (ADCs), USB programming and USB serial communication\cite{47}. Furthermore it can supply 5 volts and 3.3 volts. The 5 volt is supplied by either an internal voltage regulator or by an attached computer over USB. The 3.3 volts is supplied by an internal voltage regulator. Chip to chip communication is supported over SPI and I\textsuperscript{2}C. As mentioned above, the Arduino will require a custom made shield for analogue signal processing.

### 3.5 DISPLAY

Presenting the bacteria concentration to the user is the end goal of this research. Besides sending the data to a computer, it is interesting to have a live display of measurement results on the system. Usually the Hitachi HD44780 type LCD character displays are used in embedded solutions, as the libraries are widely available and very easy to use. Such an display was available at our laboratory: the Sharp LM16A21 (see Figure 22).

![Figure 22: Sharp LM16A21 Display](image)

The display features 14 pins\cite{50}: two for power supply (5 volts), one for contrast adjustment, three for control signal inputs and eight data lines. When working in 4-bit mode, only four out of eight data lines are used.
4. CIRCUIT DESIGN

With a complete set of requirements and hardware choices made, the device design process was started. This chapter discusses the top level design, the photodiode monitoring circuit, the LED driving circuit, the display circuit and the program codes written for the microcontroller and computer.

4.1 TOP LEVEL DESIGN

The top level design describes the complete device in blocks (see Figure 23). The Arduino computing platform is in the centre of the device. It is programmed and read by a computer. The Arduino controls a LED driver, which drives the infrared LED. For debugging purposes the LED driver features analogue output pins, which can be read using an oscilloscope. The light from the LED is channelled into the waveguide device. The light exiting the waveguide is steered towards the photodiodes. Amplifiers are used to boost the small current to a voltage. These too can be read from analogue output pins using an oscilloscope. The voltage is monitored by the Arduino and sent to a computer. The Arduino calculates the concentration of bacteria and prints the results on a display.

4.2 PHOTODIODE MONITORING CIRCUIT

Monitoring the light power falling on the photodiodes is a delicate process, as the expected light power is very small and the difference in light power caused by the bacteria is even smaller. From the relevant research presented in subchapter 2.7 and in particular Figure 12, the nominal light power received by the photodiode was found to be 3.5 µW (microwatt) and the absorbance of the bacteria at maximum 0.30 dB, which corresponds to an absolute light absorbance by bacteria of 234 nW (nanowatt). Since previous research used a 20 mW SLED as light source and
this research uses a 2 mW LED as light source, the absolute light power is expected to diminish by factor 10: nominally 350 nW with a maximum absorbance of 23 nW by the bacteria.

Photodiodes convert light power to current. The light power to current relation is called the sensitivity or response. The relation is described in formula 1, where \( i(\lambda, t) \) is the generated current, \( S(\lambda) \) is the sensitivity or response of the photodiode and \( P_{\text{light}}(t) \) is the light power falling on the photodiode.

\[
i(\lambda, t) = S(\lambda) * P_{\text{light}}(t)
\]  

(1)

In this research only one wavelength of light is used: 1300 nm. For the Roithner-Lasertechnik PT511-2 photodiode the sensitivity at 1300 nm is 0.85 A/W [43]. For the Roithner-Lasertechnik PT511B photodiode the sensitivity at 1300 nm is 0.75 A/W [44]. In this calculation we will use the worst-case sensitivity: 0.75 A/W. The expected current generated by the photodiodes can now be calculated in formula 2 and 3.

\[
i_{\text{nominal}} = 0.75 \times 350 \times 10^{-9} = 263 \text{ nA}
\]

(2)

\[
i_{\text{max, bacteria}} = 0.75 \times (350 - 23) \times 10^{-9} = 245 \text{ nA}
\]

(3)

The Arduino microcontroller has onboard ADCs for reading out voltages between 0 and 5 volts [47]. Therefore the currents need to be amplified and converted to voltages. However, since the difference between the nominal current and the current with the maximum amount of bacteria is very small, a differential amplifier is necessary. Therefore an photodiode monitoring circuit with two stages is proposed: the first stage is a transimpedance amplifier and the second stage a differential amplifier.

The transimpedance amplifier is the first stage of the photodiode monitoring circuit and amplifies the currents from the photodiodes to voltages. Photodiodes can be read out in two modes: photovoltaic or photoconductive mode [51][52]. In photovoltaic mode the voltage over the photodiode (\( U_{\text{diode}} \)) has a nonlinear relation to the photocurrent, as described in formula 4, with \( k \) being Boltzmann’s constant, \( T \) the absolute temperature in Kelvin, \( q \) the elementary charge, \( I_p \) the photodiode current and \( I_s \) the saturation current of the photodiode.

\[
U_{\text{diode}} = \left( \frac{k \times T}{q} \right) \times \ln \left( 1 + \frac{I_p}{I_s} \right)
\]

(4)

More convenient is photoconductive readout. Using the circuit presented in Figure 24, the output voltage (\( U_{\text{out}} \)) has a linear relation to the photocurrent. This relation is described in formula 5, where again \( I_p \) is the photocurrent and \( R1 \) the resistor value in Ohms.

\[
U_{\text{out}} = I_p \times R1
\]

(5)

Because the photoconductive readout circuit linearly amplifies the photocurrent to a voltage, this method was applied. In Figure 24 a resistor and capacitor are connected to the positive input of the operational amplifier, which seems to be unnecessary. However, the resistor is there to compensate for a bias current through the operational amplifier’s inputs. The capacitor is added to filter the noise generated by the resistor.
The second stage of the photodiode monitoring circuit is the differential amplifier. A standard differential amplifier circuit was adapted by adding offset control (see Figure 25). This gives the user more control while setting up the bacteria measurement system, as small deviations in the setup can cause an imbalance at the input of the differential amplifier. These deviations can then be cancelled out by adjusting the offset of the differential amplifier. In this circuit $R2$ should have a negligible impedance compared to $Rf'$. Then the output voltage is defined as formula 6, with $U_{in}$ being the input voltage, $Rf$ and $Ri$ resistor values in Ohms and $U_{os}$ the voltage over $R2$.

$$U_{out} = -U_{in} \cdot \frac{Rf}{Ri} + U_{os}$$

The photodiode monitoring circuit is the combination of the two stages mentioned above. It is presented in Figure 26. For simulation purposes the photodiodes are depicted as current sources ($I_{sense}$ and $I_{ref}$) with a capacitance ($C_{diodet1}$ and $C_{diodet2}$). The Texas Instruments TLC2274 operational amplifier was chosen for its low noise, low bias current and rail-to-rail output swing [54].
In the first stage, \( R_1 \) is equal to \( R_2, R_3 \) and \( R_4 \). Capacitors \( C_1 \) and \( C_2 \) are added to compensate the internal capacitance of the photodiodes, thus these are equal to \( C_{\text{diode}1} \) and \( C_{\text{diode}2} \). Like mentioned above, \( C_3 \) and \( C_4 \) are there to compensate for the noise generated by respectively \( R_3 \) and \( R_4 \). The (trans)amplification factor of the first stage is \( 680,000 \) Ω.

In the second stage, \( R_5 \) is equal to \( R_7 \) and \( R_6 \) is equal to \( R_8 \). \( R_{\text{pot1}} \) and \( R_{\text{pot2}} \) represent a potentiometer. \( R_{10} \) and \( C_5 \) form a low-pass filter to stabilize the output of the potentiometer. The offset voltage is generated over \( R_9 \), which is negligible to \( R_8 \), as specified above. The differential amplification factor of the second stage is 100 times.

The output voltage of the photodiode monitoring circuit can now be calculated. In formula 2 and 3 the photocurrent of the two photodiodes was calculated, as 263 nA for the reference photodiode and 245 nA for the bacteria sensing photodiode. Formula 5 can be used to calculate the voltages at the output of the first stage, see formula 7 and 8. The difference between the outputs of the first stage is again amplified in the second stage, as described in formula 9. The results may seem small, but the photodiode monitoring circuit has plenty of headroom if the light power is higher than expected, or the bacteria absorbance is higher than expected.

\[
U_{\text{ref}} = I_{\text{ref}} \times R_2 = 263 \times 10^{-9} \times 680,000 = 179 \text{ mV}
\] (7)

\[
U_{\text{sense}} = I_{\text{sense}} \times R_1 = 245 \times 10^{-9} \times 680,000 = 167 \text{ mV}
\] (8)

**FIGURE 26: PHOTODIODE MONITORING CIRCUIT**
The photodiode monitoring circuit was simulated in LTSpice. The above calculated voltages were confirmed.

The bandwidth of the photodiode monitoring circuit is defined by the capacitors and resistors in the signal path of the first stage. If a future implementation of this system would use pulse-width modulation (PWM), the monitoring circuit should have a broad bandwidth. Formula 10 defines the -3 dB bandwidth. It was found to be 23.4 kHz. The bandwidth was also analyzed in a LTSpice simulation, see Figure 27. The simulated bandwidth fits very well with the calculated bandwidth, however for this application the gain should be very stable in order to calculate the proper concentration of bacteria. Therefore the PWM frequency bandwidth should not exceed 1 kHz.

\[
B_{-3dB} = \frac{1}{2\pi * R1 * C1} = \frac{1}{2\pi * 680,000 \times 10^{-12}} = 23.4 \text{ kHz}
\]  

(10)

**FIGURE 27: MONITORING CIRCUIT BANDWIDTH SIMULATION**

### 4.3 LED DRIVING CIRCUIT

The LED driving circuit is a constant current source which can be controlled by a digital pin and was inspired by the LED driving circuit described in previous research [10]. The circuit is presented in Figure 28. Since the Roithner Lasertechnik LED1300-35K42 is specified for a current of 50 mA [45], the constant current source was designed to supply exactly this current. Furthermore the 5 volts power supply and the digital control pin may be noisy, which may cause fluctuations in outputted light power. Since this research is about measuring the light absorbance by bacteria, the light power provided by the LED should be very stable. Therefore the current source has two stabilizing measures.

First, to stabilize the voltage of the digital control pin, a ON Semiconductor LM285Z-2.5G shunt diode is applied [55]. This diode is comparable to a Zener diode, but with an even better voltage stabilizing performance. If the digital control pin is high, 5 volts is supplied and the voltage over the shunt diode will be 2.5 volts. The current is limited by R2 and can be calculated using formula 11. This is well within the specifications of the shunt diode.

\[
U_{out} = -U_{in} \times \frac{R_f}{R_i} + U_{os} = -(0.167 - 0.179) \times \left(\frac{1 \times 10^6}{1 \times 10^4}\right) + 0 = 1.20 \text{ V}
\]  

(9)
Second, the current through the LED is stabilized by a feedback circuit. The same Texas Instruments TLC2274 operational amplifier was used as in the photodiode monitoring circuit, for the same reasons: low noise, low bias current and a rail-to-rail output swing. Because this operational amplifier cannot supply a current of 50 mA, a transistor is added. The STMicroelectronics 2N2219A transistor was used for this purpose, as it can easily provide 50 mA of current [56].

Together, the shunt diode and the feedback circuit make sure the voltage over R1 is 2.5 volts. Resistor R1 then will convert the voltage to a current of 50 mA, which flows through the LED (D1), see formula 12. If \( U_{\text{switch}} \) is off, the current through the LED will be zero.

\[
i_{D1} = i_{R1} = \frac{U_{R1}}{R1} = \frac{U_{\text{shunt}}}{R1} = \frac{2.5}{50} = 50 \text{ mA}
\]  

(12)

![Figure 28: LED Driving Circuit](image)

The LED driving circuit was simulated in LTSpice. The above calculated voltages and currents were confirmed. Furthermore the resistance of R4 was found by trial and error, by having the output voltage of the operational amplifier in the linear region, i.e. 0.15 V to 4.85 V.

**4.4 Display**

The Sharp LM16A21 display presented in subchapter 3.5 is based on the Hitachi HD44780 controller and can easily be attached to the Arduino platform. The Arduino website has a library available, including a tutorial [57]. In the proposed method the display is powered by the Arduino's 5 volts power pin and connected through six digital pins. Furthermore the contrast of the liquid-crystal display (LCD) is set by a potentiometer.
The proposed method has been slightly altered. First of all, after some testing the contrast potentiometer seemed to be superfluous. It suffices to attach the contrast pin to ground. Secondly, the data line pins have been moved from D2-D5 to D7-D10. The result is that the digital pins of the Arduino connected to the display are uninterrupted, D7-D12, and therefore easier to connect on a PCB. The final display circuit is presented in Figure 29.

![Image of display circuit]

**FIGURE 29: DISPLAY CIRCUIT**

### 4.5 MICROCONTROLLER AND COMPUTER

Program code was written for two devices: the Arduino microcontroller and the computer. All codes can be found in Appendix B: codes.

The Arduino microcontroller has four tasks: controlling the LED driving circuit, reading out the voltages of the photodiode monitoring circuit, calculating the concentration of bacteria and displaying results. In the bare Arduino code two functions are defined: `setup()` and `loop()`. The `setup()` function is called when the Arduino is powered up and is used for initialization. The `loop()` function is continuously called after the initialization and thus is being looped.

Controlling the LED driving circuit is simply done by setting a digital output pin low or high. Pin 13 was selected for this purpose. In the `setup()` function pin 13 is defined as an output pin. Subsequently the pin 13 is made high, so the LED turns on.

Reading out the voltages of the photodiode monitoring circuit is done by calling the Arduino’s onboard 10-bit ADCs. Analogue pins A0, A1 and A2 are respectively connected to the photodiode monitoring circuit’s outputs $U_{ref}$, $U_{sense}$ and $U_{out}$. The ADC values are then sent by serial
communication to the computer. Furthermore the light absorbance \((A)\), i.e. the difference between the light power on the photodiodes in decibels, can now be calculated using formula 18, which follows from formula 1, 5, 6 and 13-17. For convenience offset control is set to zero.

\[
\Delta P_{\text{light}} = \frac{P_{\text{light,ref}}}{P_{\text{light,ref}}} - \frac{P_{\text{light,sense}}}{P_{\text{light,ref}}} = 10 \log_{10} \left( \frac{P_{\text{light,ref}}}{P_{\text{light,sense}}} + \frac{\Delta P_{\text{light}}}{P_{\text{light,sense}}} \right)
\]  

\[A = 10 \log_{10} \left( \frac{S \cdot P_{\text{light,ref}} \cdot R1 + \frac{Ri}{Rf} \cdot S \cdot \Delta P_{\text{light}} \cdot R1}{S \cdot P_{\text{light,sense}} \cdot R1} \right) = 10 \log_{10} \left( \frac{U_{\text{sense}} + U_{\text{out}} \cdot \frac{Ri}{Rf}}{U_{\text{sense}}} \right)
\]

If \(\frac{Ri}{Rf} = \frac{1}{100}\) then \(A = 10 \log_{10} \left( 1 + \frac{U_{\text{out}}}{100 \cdot U_{\text{sense}}} \right)

\[U_{\text{ADC}} = 5 \cdot \frac{\text{ADC}_{1023}}{1023}\] with \(\text{ADC} = \{A0, A1, A2\}

\[A = 10 \log_{10} \left( 1 + \frac{U_{\text{out}}}{100 \cdot U_{\text{sense}}} \right) = 10 \log_{10} \left( 1 + \frac{5 \cdot \frac{A2}{1023}}{100 \cdot 5 \cdot \frac{A1}{1023}} \right) = 10 \log_{10} \left( 1 + \frac{A2}{100 \cdot A1} \right)
\]

The detection limit \((A_{\text{min}})\) of the electronics can now be calculated, i.e. the smallest absorbance that can be read out. For this purpose \(U_{\text{sense}}\) is taken from formula 8 and \(A2 = 1\) (one ADC step). The detection limit is presented in formula 19.

\[A_{\text{min}} = 10 \log_{10} \left( 1 + \frac{1}{100 \cdot 1023 \cdot 0.167} \right) = 0.0013 \, \text{dB}
\]

Finally, the Arduino microcontroller presents the concentration of bacteria on a display. The concentration of bacteria is calculated using formula 20, which was deduced from Figure 12.

\[[E. \text{ coli}] = 9200 \cdot e^{44.086 \cdot A} \, \text{CFU/ml}
\]

The display library LiquidCrystalDisplay.h [57] is included and initialized with the correct pins (see subchapter 4.4) at the start of the code. In the `setup()` function the LCD’s number of character columns and rows is set up and the first line is printed. In the `loop()` function the LCD’s cursor is set to the first character of the second row and then the second row is filled with information.

The loop runs four times per second. Therefore every second four measurements are made and presented to the user.
On the computer the serial data sent by the Arduino is received and processed in a Processing script. Processing is a JAVA-based scripting language and IDE. Arduino was designed to interface with Processing. The script listens to the data sent by the Arduino over the serial port. It plots the ADC voltages like an oscilloscope, with the voltage from the sensing photodiode as the red line, the voltage from the reference photodiode as the green line and the differential voltage as the blue line (see Figure 30, note that the red line is hidden under the green line, as the voltages are almost equal). Furthermore it prints the light absorption and E. coli concentration on the screen and saves the measurement data as a comma-separated values (CSV) file ‘measurement.csv’. This file can be used for further analysis in Matlab or Excel.

![Figure 30: Processing GUI on the computer](image)

During early prototyping noise was found to be present at $U_{out}$ with a maximum ADC output of 14, see subchapter 5.3. The noise found at $U_{sense}$ and $U_{ref}$ was found to be negligible. The signal level and the noise level define the signal-to-noise ratio (SNR). The signal level is defined by the power of light falling on the photodiodes, thus by the amount of light generated by the LED and the light losses in the system. The light losses are taken to be 40 dB, see subchapter 2.7. Using these figures, the noise in the calculated absorption level can be simulated for the light power generated by the LED. This was done in a Matlab simulation by adding noise to $U_{out}$ in formula 18 and sweeping the LED light power from 0 to 45 mW. The result for three noise vectors is displayed in Figure 31. To get an accurate absorption measurement, the LED light power should be at least 1 mW.
FIGURE 31: CALCULATED ABSORPTION VERSUS LIGHT POWER
5. EXPERIMENTS

In this chapter the experiments of this research are presented. The experiments consist of the methods and the results. First the measurement equipment is tested. Secondly, the LED and its driving circuit are tested. Next, the photodiodes and the monitoring circuit are tested. Lastly, the waveguides are characterized.

5.1 MEASUREMENT EQUIPMENT

The first experiment is to characterize the measurement equipment which was used in this and previous research. The light losses in various components and the stability of the equipment should be determined before these can be used in further experiments.

For light power measurements, a Thorlabs S122C photodiode power head with germanium detector and a Thorlabs PM100D optical power and energy meter were used. Together, this system can measure light power from 50 nW to 40 mW at wavelengths of 700 to 1800 nm [58]. The PM100D can save measurement data to a SD card [59]. This is used in this experiment to gather measurement data.

First the noise floor of the Thorlabs optical power meter in the optical room is measured, by placing the photodiode in the dark optical room and measuring the received light power during one hour. The result is displayed in Figure 32. The average noise level is 13.7 nW. The highest measured noise value is 26.0 nW.

![Figure 32: Thorlabs Optical Power Meter Dark Measurement](image)

The used light source is an EXALOS EXS1320-2111 SLED mounted on an EXALOS EBD5000 driver board. The current supplied by the driver board to the SLED can be controlled by rotating...
a potentiometer on the driver board [60]. The EXS1320-2111 SLED is capable of generating up to 20 mW of light power, using up to 500 mA of current [61]. The power supply of the driver board is a Delta Elektronika D030-3 lab power supply tuned to 5.00 V, with a maximum output of 3 A. The power supply voltage is monitored by a Hewlett-Packard 34401A multimeter.

The stability of the light source is measured by tuning the driver’s supply current to 300 mA and measuring the light power on the Thorlabs S122C photodiode. Therefore the fibre from the SLED is connected to a Thorlabs PAF-X-2-C FiberPort. The collimated light is pointed on the photodiode. The light power was measured during one hour, see Figure 33. The mean light power generated by the SLED with a driving current of 300 mA is 10.3 mW. The light power drifts from 10.3845 mW to 10.2443 mW.

![Figure 33: EXALOS SLED Stability Measurement](image)

### 5.2 LIGHT EMITTING DIODE AND DRIVING CIRCUIT

The second experiment is to test the LED and its driving circuit. The outputted light power and the stability are measured. Furthermore a fibre is connected to the LED and the light loss is determined. Lastly the electrical characteristics of the LED driving circuit are measured.

The LED driving circuit as shown in subchapter 4.3 was first built on a breadboard (see Figure 34). Once the functionality was confirmed, the circuit was built on an experimental PCB (see Figure 35). This PCB fits on the Arduino as a shield. See Figure 36 for the PCB layout, note that blue components are on the back of the PCB.
FIGURE 34: SYSTEM TEST ON BREADBOARD

FIGURE 35: SYSTEM CONCEPT FOR MEASUREMENTS
The LED was then placed next to the Thorlabs S122C photodiode, the Arduino was powered over USB by a laptop and the power from the LED was measured during one hour. The result is displayed in Figure 37. The outputted light power is 2.651 to 2.668 mW with a mean light power of 2.655 mW. After five minutes of warm-up time, no drift is measured and the standard deviation is 1.50 μW.
After the LED driving circuit’s functionality was confirmed, a fibre was connected to the LED by placing it to the centre of the LED’s lens. The other end of the fibre was connected to the Thorlabs S122C photodiode. The light power from the fibre was measured during one hour. The result is displayed in Figure 38. The mean light power is 18.9 nW. To improve the light coupling a Thorlabs F240APC-C collimator lens was used. Again, the light power was measured during one hour. The result is displayed in Figure 39. The mean light power is 19.6 nW.
To measure the electrical characteristics of the LED driving circuit, it was powered up and the voltage at several circuit nodes was measured using a Yokogawa DL9140 oscilloscope. The system is powered by USB, the attached laptop was powered from its battery to prevent ground loops in the measurement setup. In every measurement the top windows is the transient view and the bottom windows is a fast Fourier transform (FFT) view. In the following figures the voltage of the ground node (Figure 40), the Vdd or 5 V node (Figure 41), the shunt diode node (Figure 42) and the output node of the operational amplifier (Figure 43). The voltage at the ground node is 1.55 mV, at Vdd it is 4.91 V, over the shunt diode there is 2.50 V and the operational amplifier outputs 3.28 V. All signals are DC and show no prominent oscillations.
FIGURE 40: GROUND VOLTAGE AND FFT

FIGURE 41: VDD VOLTAGE AND FFT
Additionally a connector was made to connect the Arduino to the EXALOS SLED. Therefore the SLED can be turned on and off through the same pin as the LED driver.
The third experiment is to test the photodiodes and the monitoring circuit. The noise floor and the stability of the photodiode monitoring circuit are determined in the same fashion as subchapter 5.1. Lastly, the electrical characteristics of the photodiode monitoring circuit are measured.

The photodiode monitoring circuit as shown in subchapter 4.2 was first built on a breadboard (see Figure 34). Once the functionality was confirmed, the circuit was built on an experimental PCB (see Figure 35). This PCB fits on the Arduino as a shield. See Figure 44 for the PCB layout, note that blue components are on the back of the PCB. A ground plane was added to reduce the noise coupling from the microcontroller to the monitoring circuit. It fits between the Arduino PCB and the shield PCB.

First the noise in the system on zero light input is tested by placing the photodiode monitoring circuit in the dark optical room. Since the light absorption cannot be calculated at low light conditions, i.e. when $U_{\text{sense}}$ in formula 17 is zero, the ADC output values are displayed below. Measurements are performed during one hour. In Figure 45 the ADC output value of $U_{\text{sense}}$ is displayed. It is zero for most of the measurement. The same goes for Figure 46, which displays $U_{\text{ref}}$. In Figure 47 the ADC output value of $U_{\text{out}}$ is displayed. Here noise is present; the mean $U_{\text{out}}$ ADC value is 0.96, which corresponds to a voltage of 4.7 mV. The highest noise ADC value measured is 14, which corresponds to a voltage of 68.4 mV.
FIGURE 45: MONITORING CIRCUIT USENSE DARK MEASUREMENT

FIGURE 46: MONITORING CIRCUIT UREF DARK MEASUREMENT

FIGURE 47: MONITORING CIRCUIT UOUT DARK MEASUREMENT
Next, the stability of the photodiode monitoring circuit is measured by directing the light from the SLED to the photodiodes. The fibre from the SLED was connected to the Thorlabs PAF-X-2-C FiberPort and tuned to a divergent light beam. The measurement system was fixated to a Standa 034023 rotating table, so the light power reaching the photodiodes could be precisely tuned to a simulated absorption of 0.10 dB (see Figure 48). During one hour the light power was measured and the absorption was calculated. In Figure 49 the ADC value at $U_{\text{sense}}$ is plotted. The same decrease of light power from the SLED as in Figure 33 can be recognized. In Figure 50 the calculated absorption is plotted. It drifts from about 0.1005 dB to 0.1045 dB. From minute 20 minute to minute 30 minute the standard deviation was $6.21 \times 10^{-4}$ dB.

![Figure 48: Monitoring circuit stability measurement setup](image)

![Figure 49: Monitoring circuit vsense stability measurement](image)
Noise can be reduced by averaging samples. In Matlab this concept was tested on the monitoring circuit absorption stability measurement. See appendix B: codes for the Matlab script. Every hundred samples were averaged. The result is displayed in Figure 51. Although the drift does not change, the standard deviation is reduced to $2.22 \cdot 10^{-4}$ dB in minutes 20 to 30.
The signal to noise ratio (SNR) will be reduced when the inputted light power is reduced, as described in subchapter 4.5. This was tested on the measurement system by using the same setup as in the previous experiment and tuning the SLED current from 170 mA to 0 mA. At 170 mA the first stage of the monitoring circuit was found to be at the edge of saturation, i.e. $U_{\text{ref}} = 5.0$ V. The result is displayed in Figure 52. The calculated absorption is constant for different input light power values, however at low light input noise influences the measurement.
The electrical characteristics are determined with small light power on the photodiodes and using a Yokohama DL9140 oscilloscope. The voltages and their FFT’s are measured at various nodes in the circuit, under the same circumstances as in the previous subchapter. There the ground node and Vdd node measurements can be found. In the following figures the voltage and FFT of the Vsense node (Figure 53), the Vref node (Figure 54) and the Vout node (Figure 55) are presented. Although the DC signals are most prominent, some oscillations are present around 2 kHz.
FIGURE 53: MONITORING CIRCUIT VSENSE VOLTAGE AND FFT

FIGURE 54: MONITORING CIRCUIT VREF VOLTAGE AND FFT
The measurement system returns 16.45 measurements per second. The detection time is therefore 60.8 ms without averaging or 6.08 seconds with averaging.

### 5.4 Waveguide Device

The last experiment is to characterize the waveguide devices. The waveguide devices are tested for light coupling and guiding. This was first done with light of 1300 nm wavelength and then with visible red light.

The test with light of 1300 nm was done using the EXALOS SLED as a light source and the Thorlabs S122C photodiode as a detector. Thorlabs P3-SMF28-FC-2 fibres were used to transfer the light towards the waveguide device. These fibres were cut in two pieces, called pigtailed fibres. A Reggefiber G657A dual fibre cable was used to transfer light from the waveguide device to the photodiode. The endings were stripped using a normal wire stripper and deft using a Fitel S325A precision cleaver. The endings were checked under a microscope for irregularities. Norland 61 optical glue was used to improve light coupling. For curing the glue a Macam Flexicure UVLS202/8/1000 UV light source was used. The fibres were mounted on Thorlabs MBT616/M stages, which feature precision control in three directions (no rotation). A Zeiss Stemi SV11 microscope was placed above the measurement setup, so the positions of the fibres in the horizontal plane could be precisely monitored. Additionally, a Duratool BW-400X USB microscope was placed facing the setup from the side, so the positions of the fibres in the vertical plane could be monitored. For a photo of the setup, see Figure 56.
First the light coupling from fibre to fibre was tested. The fibre positions were tuned so that the fibres face each other with almost no space in between. Without glue, a coupling loss of 10 dB was achieved. Then optical glue was added and the experiment was repeated. This time a coupling loss of 3 dB was achieved.

Second the light coupling from fibre to fibre through two centimetres of air was tested. Because the waveguide devices measure two by two centimetres, this is the normal distance between the fibres. Therefore the fibres were cleaned and pulled two centimetres apart. Using the stages, the positions of the fibres were tuned to optimal coupling. Light coupling through two centimetres of air renders a coupling loss of 47 dB.

Now a waveguide device with a light absorbing foam strip (see Figure 17) was placed between the two fibres and the fibre positions were tuned to optimal coupling. Optimal coupling was reached when the output fibre is aligned with the reference output waveguide and the input fibre is aligned with the output fibre (see Figure 57). The coupling loss is 40 dB in this scenario. After moving the fibres to the sensing output waveguide (with the freestanding bridge), no light coupling was measured.
To confirm these results, a test with visible red light was done at TNO's optical lab. A visible fibre tester was connected to the fibre and functions as a visible light source. A piece of paper was placed at the output side of the waveguide device. An Olympus SP-510UZ camera was used to picture the light on the paper. The input fibre was aligned with the input waveguide. The resulting light on the paper is displayed in Figure 58. The image shows one half ellipse shape on the paper. Using a microscope, the waveguide was inspected from the top. No light leakage from the waveguide device was visible.

Since the waveguide device does not have two functional outputs, the designed measurement system could not be tested with the waveguide devices.
6. DISCUSSION

In this chapter the results from previous chapter are discussed. After the review of the results in subchapter 6.1, recommendations for further research follow in subchapter 6.2.

6.1 REVIEW OF RESULTS

To review the results, first the results presented in the previous chapter are discussed per topic. Then the results are compared to the specifications set in subchapter 2.5.

The measurement equipment results show that the Thorlabs optical power meter and the EXALOS SLED are well suitable for this research. In the Thorlabs optical power meter a noise floor of maximum 26.0 nW was found. This is well within the manufacturer specifications, which state a minimum measurable light power of 50 nW. Drift in the noise floor measurement may be caused by sunlight coming into the optical room through small holes. The drift however is very small and not significant in this research. The EXALOS SLED outputs 10.3 mW of light power when driven at 300 mA. This is lower than the manufacturer specifications, which state 14 mW of light power. Furthermore the SLED shows a negative drift after a cold start. The drift is -0.059 dB during the measurement of one hour, which corresponds to +115,000 CFU/ml and may significantly influence an absolute bacteria measurement as was done in previous research. However, in this research differential measurements are performed, so the SLED is well suitable.

The LED driving circuit itself performs very well, however the coupling to the fibre was not measurable. The LED driving circuit outputs 2.655 mW of light power. That is 33% more than the 2.0 mW of light power specified by the manufacturer. The LED driving circuit shows a sharp decrease in light power of 0.028 dB upon a cold start, however after five minutes no drift was measured. Furthermore the electric measurements show a very stable circuit, with low noise levels and no oscillations. Measurements show that no light is coupled into the fibre, even when using a collimator. One of the reasons this might happen, is because the LED outputs multimode light while the fibre is single mode. Furthermore the LED outputs a divergent beam of light. Although the radiation angle is just 6°, it will limit the collimator coupling to the fibre.

The photodiode monitoring circuit can measure the light absorption in high detail. The theoretical light absorption detection limit was found to be 0.0013 dB in formula 19. In the clinically relevant region of 0 to 0.1 dB absorption, a drift of 6.92·10⁻⁵ dB per minute was measured. This is within the theoretical detection limit of 0.0013 dB, if the measurement duration is shorter than 18 minutes. The cause of drift might be a poor common-mode rejection ratio of the differential stage and the drift of the SLED, causing a change in common-mode signal, or movement in the measurement setup. The standard deviation during 10 minutes of measurement was 6.21·10⁻⁴ dB or 9,500 CFU/ml without averaging. With averaging the standard deviation was 2.22·10⁻⁴ dB or 9,300 CFU/ml. By averaging every hundred samples, the noise in the system is reduced to below the theoretical detection limit. Noise was mainly measured in the differential stage. The detection time of the measurement system is 60.8 ms, or 6.08 seconds for an averaged measurement. Using formula 20, the detection limit of the system can be calculated. The light absorption by bacteria can be monitored from 0 to 0.1 dB with a detection limit of 0.0013 dB or 9,700 CFU/ml E. coli.

The waveguide device improves the light coupling when placed between the fibres. Light losses are reduced from 47 dB without the waveguide device to 40 dB with the waveguide device. This is the same light loss as reported in previous research. The waveguide device only seems to confine the light in the vertical plane. In the horizontal plane light is not confined in the waveguide core.
In subchapter 2.5 specifications were set for the measurement system. All specifications are met, except the ones which should be tested in hospital environment, i.e. 6, 7 and 13. Although the designed electronics and waveguide devices are portable, the complete measurement setup with the SLED and microscope is not portable. The system was not transported to a hospital and not built in hospital environment. Furthermore the system was not tested in drain fluid. All other specifications are met.

6.2 RECOMMENDATIONS

Recommendations for further research follow from the background study, experiences during the research project and the results. This subchapter enumerates all recommendations.

During the background study was found that the E. faecalis bacteria show an earlier rise in concentration after anastomotic leakage than E. coli bacteria. Therefore future research should focus on measuring the E. faecalis concentration in drain fluid. Furthermore the current formula for calculating the E. coli concentration, i.e. formula 20, has a lower limit of 9,200 CFU/ml and can only detect anastomotic leakage from day 4. The presented method for measuring the bacteria concentration cannot differentiate between live and dead bacteria cells, or even cell walls of dead bacteria. The influence of dead bacteria on the concentration measurement should be investigated. Some papers by previous researcher Purniawan show illustrations of flow cells attached to the waveguide device, however this concept has not yet been tested. Attaching a flow cell to the waveguide device enables online measurements on a defined waveguide surface area. Other research on anastomotic leakage detection is based on the measurement of oxygen pressure, pH and more. By combining several sensing modalities anastomotic leakage detection accuracy may be improved. To ease the interface between the proposed measurement system and other hospital equipment, in future research a smart sensor system with a common interface should be investigated, e.g. with Bluetooth or I2C communication. A wireless measurement system allows the patient to be more mobile.

Practical experiences in the optical lab show that future research in our lab should focus on the 1200 nm to 1600 nm infrared light spectrum, as most of the optical components in our lab are designed to operate on these wavelengths. Nonetheless having a visible fibre tester available would be handy for quick visual experiments. The current stages are hand-tuned, while computer-tuned stages are available, which may automate the fibre aligning process. By integrating the LED and photodiodes on the waveguide device, no fibres or alignment process would be needed at all. An alternative is to etch holes in the waveguide devices to fit the LED and photodiodes.

In the results was found that the differential stage introduces noise and drift. Therefore the differential amplifier should be improved, for example by replacing the stage with an instrumentation amplifier. Noise can also be reduced by designing low-pass filters before the ADC inputs and by averaging a number of samples. These options however reduce the system bandwidth and the sample frequency, making PWM impossible. Since the noise is originating from the digital lines, better shielding would reduce the noise, e.g. by designing a PCB with a ground plane and keeping distance between the digital and analogue circuits. Also the LCD should get its 5 V power supply directly from the Arduino, instead of from the photodiode monitoring circuit. The proposed photodiode monitoring circuit can sense a clinically relevant bacteria concentration with a maximum light input of 43 mW. This is much more than the maximum light output of the SLED and LED. While a reduction of light losses in the system, currently 40 dB, was taken into account, no reduction was achieved. Therefore the gain of the photodiode monitoring circuit can be set higher, so a better measurement resolution can be achieved. Furthermore the proposed photodiode monitoring circuit saturates its differential
stage long before the transimpedance stages are saturated. Therefore a better balance in the amplification factors should be made. The offset control circuit works well, but can be automated by applying a digital potentiometer controlled by the Arduino. The light from the used LED cannot be channelled into a fibre, so it should be replaced by a fibre-coupled laser diode. If the SLED is used in experiments, it should be turned on one hour before doing absolute measurements.

About the waveguide devices used in this research many factors are unknown. The results show that the waveguide devices act as optical waveguides in the vertical direction, but not in the horizontal direction. Therefore the waveguide functionality should be further investigated. Only five devices from one wafer were used in experiments, therefore a batch of devices from another wafer should be tested. The coupling efficiency is very low, with a loss of 40 dB. This can be improved by designing waveguides with square end surface, because that matches better with a round fibre. The function of the freestanding bridge, in particular in adding sensitivity, could not be verified, while it makes the devices more fragile. Therefore future experiments should focus on measuring how much the freestanding bridge increases the sensitivity in bacteria measurements. No area has been defined to perform put the bacteria dilutions or drain fluid, which makes it hard to compare different measurements with the same waveguide devices.

Once new waveguide devices are fabricated, they should be checked on functionality. First should be checked if the waveguides split and guide the light to the two outputs. Then the measurement system designed and built in this research can be connected to the waveguide device. The measurement system can then be tested with IPA measurements and compared to previous IPA measurements. If the measurement system can measure the IPA concentration accurately, it should be calibrated with E. coli dilutions and the detection time and detection limit should be determined. By calibrating the system, a more accurate formula can be found to calculate the E. coli concentration from the measured light absorption. The last step is to measure the E. coli concentration in drain fluids using this system, while comparing this to the hospital lab measurement.
In this final chapter conclusions are drawn from this master thesis research project. The research statement reads:

"Design an in-vitro device for the detection of E. coli in drain fluid after colorectal anastomotic leakage, using a waveguide chip, while prioritizing the electrical design, clinically relevant simulations, portability and simple user interface."

A measurement system was designed and assembled. This is a concept solution, not a complete prototype. It features an Arduino Uno microcontroller platform, an infrared LED as a light source with a constant current LED driver, two photodiodes as light sensors with monitoring circuitry, a LCD display and computer software. The Arduino controls the light source through switching the LED driver on or off. Light is channelled into the waveguide device by an optical fibre. The waveguide device can capture E. coli bacteria from patient drain fluid, which absorb light. From the waveguide device light is channelled to the photodiodes. The photodiode monitoring circuit amplifies the signals and is read out by the Arduino. The Arduino calculates the light absorption and the E. coli concentration, which improves user-friendliness. Furthermore the Arduino puts the calculated light absorption and E. coli concentration on LCD display and sends ADC values to the computer. The computer displays the ADC values like an oscilloscope and saves the data to a CSV-file. The electronics and waveguides devices are portable, although the complete measurement setup is not.

Measurements show a very stable light source was constructed, with 2.655 mW of light power and an initial drift of -0.028 dB in the first five minutes after a cold start. After the warm-up period, no drift was measured. This is more stable than the commercial SLED, which shows a drift of -0.059 dB during one hour. Coupling light from the LED to a fibre was not achieved. The photodiode monitoring circuit can sense the light absorption in this system in the clinically relevant region with a detection limit of 0.0013 dB or 9,700 CFU/ml E. coli. This means anastomotic leakage can be detected from day four after surgery, enabling resurgery in an earlier phase of infection and therefore less morbidity, mortality and shorter hospital stay. The detection time between measurements is 60.8 ms, or 6.08 seconds when averaging is enabled. The waveguide devices confine light in the vertical plane, however in the horizontal plane the waveguides do not function. Therefore the measurement system could not be combined with the waveguide device and no biological measurements could be performed.

The specifications were not fully met, because the measurement system was not tested in a hospital. Simulations show the specifications could be met if a functioning waveguide device would be available.

Future research on improving this measurement system might focus on replacing the LED for a fibre-coupled laser and reducing the noise and drift in the measurement system by replacing the differential stage for an instrumentation amplifier. In the broad view, the waveguide device might be improved so that light is confined in both the vertical and horizontal plane, which would allow performing a differential measurement.
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<tr>
<td>A</td>
<td>Ampère</td>
</tr>
<tr>
<td>ADC</td>
<td>Analogue-to-digital converter</td>
</tr>
<tr>
<td>ALD</td>
<td>Atomic layer deposition</td>
</tr>
<tr>
<td>APC</td>
<td>Angled physical contact</td>
</tr>
<tr>
<td>APTES</td>
<td>(3-Aminopropyl)triethoxysilane</td>
</tr>
<tr>
<td>ASIC</td>
<td>Application-specific integrated circuit</td>
</tr>
<tr>
<td>CAL</td>
<td>Colorectal anastomotic leakage</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CSV</td>
<td>Comma-separated values</td>
</tr>
<tr>
<td>dB</td>
<td>Decibels</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>ECs</td>
<td>European Credits</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier transform</td>
</tr>
<tr>
<td>Ge</td>
<td>Germanium</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>InGaAs</td>
<td>Indium gallium arsenide</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropanyl-alcohol</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid-crystal display</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LPCVD</td>
<td>Low-pressure chemical vapour deposition</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed circuit board</td>
</tr>
<tr>
<td>PECVD</td>
<td>Plasma-enhanced chemical vapour deposition</td>
</tr>
<tr>
<td>PWM</td>
<td>Pulse-width modulation</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-time polymerase chain reaction</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>SiN</td>
<td>Silicon nitride</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Silicon dioxide</td>
</tr>
<tr>
<td>SLED</td>
<td>Superluminescent light emitting diode</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>TiO₂</td>
<td>Titanium dioxide</td>
</tr>
<tr>
<td>W</td>
<td>Watt</td>
</tr>
</tbody>
</table>

**Used prefixes:**

- p: Pico (10⁻¹²)
- n: Nano (10⁻⁹)
- µ: Micro (10⁻⁶)
- m: Milli (10⁻³)
- k: Kilo (10³)
- M: Mega (10⁶)
REFERENCES


[61] EXALOS AG, EXALOS releases NEW High Power (20mW) Large Bandwidth (60 nm) 1300nm Superluminescent Light Emitting Diode, Zurich: EXALOS AG, 2005.
APPENDICES

Three appendices are added to this thesis. Appendix A is the planning used during this project. Appendix B contains the written codes. Appendix C is an overview of all circuits and components.

A. PLANNING

In Appendix A the planning for this master thesis project is presented.

The master thesis project for the Biomedical Engineering master at Delft University of Technology is split into four courses. The first course is BMA0206: BME Literature Study of 7 ECs (European Credits), which is completed by writing a literature survey. The second course is BMA0203: Literature Colloquium of 3 ECs, which is completed by presenting and defending the literature survey results. The third course is BMA0302: Introductory Colloquium of 3 ECs, which is completed by presenting and defending the plan and concept of thesis research. The last course is BMA0332: BME MSc Thesis, Colloquium and Defence of 32 ECs. This means the total amount of credits obtained in the thesis research process is 45 ECs. That stands for 32 weeks of full-time work. Taking into account the individual courses, their weight and their deadlines, the following planning was created.

TABLE 5: PLANNING

<table>
<thead>
<tr>
<th>Work</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature survey</td>
<td>September and October 2013</td>
</tr>
<tr>
<td>Literature Colloquium</td>
<td>Wednesday 6 November 2013</td>
</tr>
<tr>
<td>Hardware study</td>
<td>November 2013</td>
</tr>
<tr>
<td>Introductory Colloquium</td>
<td>Wednesday 11 December 2013</td>
</tr>
<tr>
<td>Circuit design and simulation</td>
<td>December 2013 and January 2014</td>
</tr>
<tr>
<td>System realisation and testing</td>
<td>February 2014</td>
</tr>
<tr>
<td>Measurements with E. coli dilutions</td>
<td>March 2014</td>
</tr>
<tr>
<td>Measurements with drain fluid</td>
<td>March 2014</td>
</tr>
<tr>
<td>Writing the thesis</td>
<td>April 2014</td>
</tr>
<tr>
<td>Defence</td>
<td>May 2014</td>
</tr>
</tbody>
</table>
In Appendix B the written code is presented. First the Arduino firmware code, then the Processing GUI code and lastly the Matlab noise simulation code.

Arduino firmware code:

/*
 * Light absorption readout

This firmware is part of the E. coli detection system. It reads out the photodiode monitoring circuit voltages, calculates the light absorption and bacteria concentration, puts the results on the display and sends the data over the serial port to the attached computer.

Stephen van 't Hof, 2014
*/

#include <LiquidCrystal.h>

// initialize the LCD's number of columns and rows
LiquidCrystal lcd(12, 11, 10, 9, 8, 7);

void setup() {
  // initialize the serial communication
  Serial.begin(9600);
  // set up the LCD's number of columns and rows
  lcd.begin(16, 2);
  // power on the LED
  pinMode(13, OUTPUT);
  digitalWrite(13, HIGH);
}

void loop() {
  // read the values of the ADCs
  double Uref = analogRead(A0);
  double Usense = analogRead(A1);
  double Uout = analogRead(A2);

  // calculate the absorption and E. coli concentration
  double absorption = 10*log10((1+Uout)/(100*Usense));
  double ecoli = 0;
  if(absorption > 0.15) {
    ecoli = INFINITY;
  } else {
    ecoli = 9200*exp(44.086*absorption);
  }

  // send the values over serial
  Serial.print("Uref = ");
  Serial.print(Uref);
  Serial.print("\t Usense = ");
  Serial.print(Usense);
  Serial.print("\t Uout = ");
  Serial.print(Uout);
  Serial.print("\t A = ");
  Serial.println(absorption, 4);
// print a message to the LCD
String space = " ";
lcd.setCursor(0,0);
lcd.print("E.coli: ");
lcd.print(ecoli, 0);
lcd.print(" ");
lcd.setCursor(0,1);
lcd.print("A = ");
lcd.print(absorption, 3);
lcd.print(" dB ");

// wait for the ADC to stabilize after the last reading
delay(1);
}
Processing GUI code:

/*
   Data grapher and saver

   This program is part of the E. coli detection system. It reads
   ASCII-encoded strings from the serial port at 9600 baud and graphs
   them. It expects ADC values in the range 0 to 1023 from the
   Arduino. Furthermore it calculates and prints the E. coli
   concentration and saves the data to the text file
   "measurement.csv". The program is terminated upon the pressing of
   a button.

   Stephen van 't Hof, 2014
*/

// import the serial communication library code
import processing.serial.*;

// initialize
Serial myPort; // the serial port
int xPos = 1; // horizontal position of the graph
PrintWriter output; // create text file
PFont f; // create font for texts

void setup () {
   // set the window size
   size(400, 300);

   // list all the available serial ports
   println(Serial.list());
   // open serial port 3 (works for me)
   myPort = new Serial(this, Serial.list()[3], 9600);
   // don't generate a serialEvent() unless you get a newline character
   myPort.bufferUntil('\n');

   // set inital background to white
   background(255);

   // create a new text file
   output = createWriter("measurement.csv");
   output.println("Uref \t Usense \t Uout \t A");

   // create font
   f = createFont("Arial",16,true);
}

void draw () {
   // everything happens in the serialEvent(), so nothing here
}

void serialEvent (Serial myPort) {
   // get the ASCII string
   String inString = myPort.readStringUntil('\n');

   if (inString != null) {
      // strip string to four strings
      int part1 = inString.indexOf("\t");
      int part2 = inString.indexOf("\t", part1 + 1);
      int part3 = inString.indexOf("\t", part2 + 1);
      String Uref = inString.substring(6, part1);
String Usense = inString.substring(part1 + 10, part2);
String Uout = inString.substring(part2 + 8, part3);
String A = inString.substring(part3 + 6);

// strip away empty spaces
Uref = trim(Uref);
Usense = trim(Usense);
Uout = trim(Uout);
A = trim(A);

// write to text file
output.println(Uref + "\t" + Usense + "\t" + Uout + "\t" + A);

// calculate e. coli concentration
float Abyte = float(A);
float Ecoli = 9200*exp(44.086*Abyte);

// convert to an int and map to the screen height minus text box
float UrefByte = float(Uref);
float UsenseByte = float(Usense);
float UoutByte = float(Uout);
UrefByte = map(UrefByte, 0, 1023, 1, height - 51);
UsenseByte = map(UsenseByte, 0, 1023, 1, height - 51);
UoutByte = map(UoutByte, 0, 1023, 1, height - 51);

// draw the lines:
stroke(255,0,0); //red
point(xPos, height - UrefByte);
stroke(0,255,0); //green
point(xPos, height - UsenseByte);
stroke(0,0,255); //blue
point(xPos, height - UoutByte);

// at the edge of the screen, go back to the beginning and whipe screen
if (xPos >= width) {
xPos = 0;
background(255); //white
} else {
    // increment the horizontal position:
xPos++;
}

// clear text area
fill(255); //white
noStroke();
rect(0,0,width,50);

// print absorption and e. coli concentration on screen
String Atext = "Light absorption = " + A + " dB";
String Etext = "E. coli concentration = " + Ecoli + " CFU/ml";
textFont(16); //16
fill(0); //black
text(Atext,10,20);
text(Etext,10,40);
}

void keyPressed() {
    output.flush(); // writes the remaining data to the file
}
output.close(); // finishes the file
exit(); // stops the program
}
Matlab code for the SNR simulation:

```matlab
c%% initialize
N = 1000; % number of samples
S = 0.75; % photodiode sensitivity
light = linspace(0,0.043,N); % create light power vector (x-axis)
noise1 = 3*randn(1,N); % create noise vectors
noise2 = 3*randn(1,N);
noise3 = 3*randn(1,N);

c%% calculate absorption
Aperfect = 0.1*ones(1,N); % perfect absorption is 0.1 dB
iref = light*10^(-4)*S; % calculate photocurrent
isense = light*10^(-4.01)*S;
Uref = iref*680000*(1023/5); % calculate transimpedance voltage ADC value
Usense = isense*680000*(1023/5);
Uout1 = 100*(Uref-Usense)+noise1; % calculate output voltage ADC value
Uout2 = 100*(Uref-Usense)+noise2;
Uout3 = 100*(Uref-Usense)+noise3;
Anoisy1 = 10*log10(1+Uout1./(100*Usense)); % calculate noisy absorption
Anoisy2 = 10*log10(1+Uout2./(100*Usense));
Anoisy3 = 10*log10(1+Uout3./(100*Usense));

c%% plot
plot(1000*light,Aperfect,1000*light,Anoisy1,1000*light,Anoisy2,1000*light,Anoisy3)
xlabel('Light power (mW)')
ylabel('Absorption (dB)')
axis([0,43,0,0.2]);

c%% clean mess
clear all
```

Matlab code for averaging every 100 samples of A:

```matlab
%% Average every x samples of A and plot result
x = 100;

% Create timeline (based on one hour measurement)
N = length(A);
time = linspace(1,60,N/x);

% Average every 100 samples
Aavg = zeros(1,N/x);
for t = 1:N/x
    temp = A(((t-1)*x+1:t*x);
    Aavg(t) = mean(temp);
end

% Create plot
plot(time,Aavg)
xlabel('Time (min)')
ylabel('Absorption (dB)')

% Clear temporary workspace
clear x
clear N
clear t
clear temp
```
C. CIRCUITS AND COMPONENTS

Photodiode monitoring circuit

LED driving circuit
Display circuit

PCB layout (blue components are on the back of the PCB)
List of electronic components

1× Arduino Uno
1× Sharp LM16A21 display
1× Texas Instruments TLC2274CNE4 quad operational amplifier
1× Texas Instruments TLC2272CP dual operational amplifier
1× ON Semiconductor LM285Z-2.5G voltage reference
1× STMicroelectronics 2N2219A transistor
1× CMEC 2.2kΩ linear potentiometer
1× 50Ω resistor
1× 100Ω resistor
1× 470Ω resistor
1× 1kΩ resistor
3× 10kΩ resistor
4× 680kΩ resistor
2× 1MΩ resistor
2× 10pF capacitor
3× 100nF capacitor
1× 4.7µF capacitor
1× Roithner Lasertechnik LED1300-35K42 light emitting diode
2× Roithner Lasertechnik PT511B photodiode