FACULTY OF ENGINEERING AND ARCHITECTURE

Development of an optical/electronic turbidimetric blood culture system for rapid detection of bacterial infection

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PREFACE

This thesis was made in collaboration with several people both at Ghent University and at the Institute of Tropical Medicine. I want to thank prof. dr. ir. Roel Baets and dr. Liselotte Hardy for being excellent promotors and teaching me a lot. I also want to thank my counsellors dr. Mohammadamin Ghomashi and Federico Marchesin for guiding me and helping with the day-to-day work. Next, I want to thank Ellen Corsmit for performing all of the blood cultures used during testing and helping me install the turbidimeters in the lab. Also, many thanks to the rest of the SIMBLE-team for aiding me and answering my questions, Barbara Barbé and Kamalpreet Kaur. I would like to thank Matthias de Schepper as well for assisting me during the assembly and debugging of the PCBs. Lastly, I want to thank my girlfriend and family for the mental support they provided.

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Jens Cornelis, May 2023

REMARK ON THE MASTER'S DISSERTATION AND THE ORAL PRESENTATION

This master's dissertation is part of an exam. Any comments formulated by the assessment committee during the oral presentation of the master's dissertation are not included in this text.

ABSTRACT

Bloodstream Infections (BSIs) cause serious illnesses that can lead to sepsis. Sepsis accounted for almost 20% of all global deaths in 2017 according to the World Health Organisation (WHO) [1]. The SIMBLE-team [2] has recently developed a diagnostic device to aid in the diagnosis of BSIs designed for use in Low- and Middle-Income Countries (LMICs) called the "turbidimeter". It operates based on the change in turbidity of the fluid inside the blood culture bottle. The objective of this master's thesis was to further improve the device, for faster detection of more types of bacterial species. The first way of doing this was by adding more sensors to the device and looking at the effect of the measuring height. Secondly, the impact of the sample volume on the results was tested. Lastly, there are some bacterial families that can as of yet not be reliably detected based on turbidity alone, so it was examined if other signs of growth, like pellicle formation at the liquid-air interface could be detected with this setup.

To do this, new sensor boards were designed, assembled, and tested at Ghent University (UGent). When the prototype was proved functional, in vitro testing with human blood spiked with bacteria was done at the Institute for Tropical Medicine (ITM) to examine the performance of the new measuring setup.

The results showed that the sensing mechanism should be placed near the top of the liquid to allow for the earliest possible detection of bacteria. Additionally, it was concluded that an increase in both blood and broth volume led to a longer sedimentation phase, during which bacterial growth cannot be detected. Finally, pellicle formation on top of the liquid could not be detected with the used measuring setup.

Keywords:
Turbidimeter, blood culture, bloodstream infection, low-resource settings

EXTENDED ABSTRACT (ENGLISH)

DEVELOPMENT OF AN OPTICAL/ELECTRONIC SYSTEM FOR RAPID DETECTION OF BACTERIAL INFECTION

Jens Cornelis
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Abstract - Bloodstream Infections (BSIs) cause serious illnesses that can lead to sepsis. Sepsis accounted for almost 20% of all global deaths in 2017 according to the World Health Organisation (WHO) [1]. The SIMBLE-team [2] has recently developed a diagnostic device to aid in the diagnosis of BSIs designed for use in Low- and Middle- Income Countries (LMICs) called the "turbidimeter". It operates based on the change in turbidity of the fluid inside the blood culture bottle. The objective of this master's thesis was to further improve the device, for faster detection of more types of bacterial species. The first way of doing this was by adding more sensors to the device and looking at the effect of the measuring height. Secondly, the impact of the sample volume on the results was tested. Lastly, there are some bacterial families that can as of yet not be reliably detected based on turbidity alone, so it was examined if other signs of growth, like pellicle formation at the liquid-air interface could be detected with this setup.

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I. INTRODUCTION

To help detect bacterial growth in blood cultures with the turbidimeter, several tests were done to see where performance of the second generation turbidimeter could be improved. The turbidimeter operates based on the turbidity (or cloudiness) of the blood culture inside [3]. To measure this turbidity, an LED shines light from one side of the blood culture bottle (BCB) [3]. One detector is positioned on the opposite side of the BCB to detect the light transmitted through the sample [3]. A second sensor is mounted orthogonally to be able to detect the amount of light that is scattered by the sample [3]. The light intensities that these sensors detect will depend on the turbidity of the sample.

A new turbidimeter generation was developed to examine possible improvements that can be made to the second generation. This new generation has several additions on top of the second generation, that is in use now by the SIMBLE-team [2]. One of the additions was to use multiple sensors at different heights to see if this affected the measuring results. Another additional feature that was added was to try to detect more signs of growth other than turbidity, namely pellicle formation. To implement these features, two custom PCBs were designed, assembled, and tested. The second generation turbidimeter augmented with these new capabilities will be referred to in this work as the third generation turbidimeter.

II. THIRD GENERATION TURBIDIMETER

The main goal of this thesis was to develop and test a third generation turbidimeter. This included designing and assembling new PCBs as well as updating the software for the microcontroller. The third generation turbidimeter serves as a test vehicle to see where future improvements could be made and to test them. One of these improvements is measuring turbidity at different heights. This might be beneficial because light does not get scattered, absorbed, or transmitted the same throughout the bottle. This means that there might be places where a change in turbidity can be detected more easily or faster. This would mean that bacteria can be detected earlier in some locations rather than others.

To test the effect of the measuring height on the turbidimetric measurements, new vertical boards had to be designed. The schematic and PCB layout of these boards are provided in appendices A through C.

The original PCB design of the second generation turbidimeter was a combined PCB for the emitter and detector, and the components placed on it determined its function. For the new design, two separate PCBs were designed, one for the detector and one for the emitter. Each PCB has eight LEDs or sensors placed at the following heights with the bottom of the BCB as a reference: 20mm, 25mm, 30mm, 37.5mm, 45mm, 55mm, 65mm and 75mm. The tested measuring heights are more concentrated towards the bottom of the BCB because it was expected that the most useful information would be concentrated here.

A. *Emitter*

The emitter consists of eight LEDs (Luminus, Sunnyvale, United States of America) that are driven by a TLC59108 LED driver (Texas Instruments, Dallas, United States of America). These LEDs and the LED driver are the same components that were used for the combined emitter/detector board previously. It has eight constant current output channels and communicates with the I²C-protocol. As with the previous emitter/detector board, a PCA951A I²C buffer (NXP, Eindhoven, Netherlands) was placed before the driver to provide hot-swap capability to the board.

B. *Detector*

In previous versions, the detector used a TCS3772 RGBC-sensor (ams-OSRAM, Premstaetten, Austria) but due to availability issues, a different sensor had to be selected. The TCS3400 (ams-OSRAM, Premstaetten, Austria) was chosen as a replacement because it has the same spectral sensitivity, the same pinout, uses I²C and is made by the same manufacturer. Eight of these sensors are used on each detector board. But because all of these sensors have the same I²C address, an I²C multiplexer has to be used. This multiplexer ensures that only one sensor is on the main I²C bus at a time. The multiplexer selected for this purpose is the PCA9547 (NXP, Eindhoven, Netherlands) mainly because it has eight channels and has a configurable I²C-address. This configurable address is a requirement because there are two detector boards used in the system and they need to be individually addressable. A PCA951A I²C buffer is used on the detector board too to give it hot-swap capability, like the emitter.

Finally, a TMP116 (Texas Instruments, Dallas, United States of America) temperature sensor was added to be able to measure the temperature inside the turbidimeter. This feature was however not used in this master's thesis but will be useful for future use.

C. *PCBs*

The PCBs were made so that the width of the detector and emitter boards is the same as in the second generation emitter/detector board. This way, the PCBs would still fit in the already existing 3D-designed turbidimeter case with only minimal modifications. This did however limit the available space for the components severely. For the emitter PCB this wasn't a problem because there aren't a lot of components on this board. The detector however has eight I²C sensors which all have separate SDA and SCL lines coming from the multiplexer. Each of these traces also needed a 1kΩ pullup resistor. These 16 traces and their resistors took up a lot of the available space when put next to each other. Especially the width was a limiting factor. The multiplexer was instead put in the middle of the PCB to avoid this problem. This way, half of the traces could be routed up and half could be routed down so they take up half as much of the width.

The ground plane covers the top and bottom of the PCB. Care was taken to avoid separating parts of this plain with traces. This has the purpose of maintaining a stable and equal ground potential throughout the PCB. When there was space available, bridging vias were placed around the traces that made cuts in the ground plane. This way, a current can go under a trace instead of having to go around it. This avoids electromagnetic interference (EMI) problems and makes sure no voltage potential can build up between both sides of the trace.

III. METHOD

Tests with the third generation turbidimeter were divided into 4 parts. The first test was used to confirm the functionality of the device before the testing on bacteria began. This was necessary because of the change in design, software, and the use of a new RGBC-sensor. The second, third and fourth parts of the testing were conducted at the Institute for Tropical Medicine (ITM) in Antwerp. In all of these tests the turbidimeter was configured such that measurements at different heights were done one by one, from the bottom to the top. Measuring at the 8 heights took 12.8 seconds and a period of 26.4 seconds was left between two measurement cycles.

Since there are only two third generation turbidimeters, the testing happened over five weeks with one or two samples being tested at a time. The data from these tests was logged by a computer connected to the turbidimeters. To examine the data and plot graphs, a python script was used.

As mentioned above, the first test was to confirm the functionality of the device and will not be used

in the results here.

The second part of the tests were focused on determining the effect of measuring height on the blood culture measurements. Eight blood cultures were set up with 30ml of tryptic soy broth to which 0.3mg/ml of SPS was added. 2ml of human blood was used to inoculate the sample. The human blood was kindly donated by several volunteers at ITM (ethics approval was received for sampling of blood). The blood of the volunteers was spiked in 7 out of the 8 bottles with a certain bacterial strain (*Escherichia coli*, *Salmonella* Typhimurium, *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Burkholderia cepacia*), to mimic blood samples of infected patients.

The third part of testing examined the effect of sample volume. Eight more BCBs were filled with 2 different blood-to-broth ratios and were spiked with 2 different bacterial strains (*Escherichia coli* and *Salmonella* Typhimurium). This together with two samples from the previous part gave three different blood-to-broth ratios and two different bacterial strains to compare against each other as well as one blank for each blood-to-broth ratio.

The goal for the fourth and final part of the testing was to see if features other than turbidity could be detected with the third generation turbidimeter. Particularly the formation of pellicle on the surface of the blood/broth mixture was investigated. To measure this, two extra measurement profiles were added. These measured diagonally through the surface by activating the right LED and sensors. The complete measuring profile is listed in appendix E.

This test consisted of two BCBs spiked with *Pseudomonas aeruginosa*. The two cultures consisted of 30ml of broth and 2ml of blood. These volumes were chosen so the liquid-air interface would end up between 37.5mm and 55mm.

IV. RESULTS

The tests conducted at the Institute for Tropical Medicine used BCBs containing a mixture of broth and blood spiked with bacteria. The results will therefore be in the form of bacterial growth curves. These curves traditionally consist of four bacterial growth phases [4]. The results of the tests done here will show the same four phases. The first bacterial phase is the lag phase where no change in the growth curve can be seen because the number of bacteria stays relatively constant [4]. Next is the log phase or growth phase. In this phase, the number of bacteria grows exponentially and signs of growth start showing (in this case an increase in turbidity) [4]. After this comes the stationary phase, this is where the bacterial concentration has reached its maximum [4]. This maximum is caused by the limited availability of nutrients in the BCB and thus capping the population [4]. The last phase is the death or mortality phase [4]. In this phase, the number of bacteria start to decline because nutrients have run out and the medium can't sustain the current bacterial concentration anymore [4].

To make growth curves, the concentration of bacteria inside the medium should be measured over time. However, this is not what is measured here. The turbidimeter measures the turbidity of the medium and thus not the microbial concentration directly. Because of this, the growth curves shown here might not exactly line up with the actual growth curves in time or intensity, but they should show the same phases. There will also be an additional phase before the lag phase (unrelated to bacterial growth), the sedimentation phase. During this phase, some particles within the blood will fall

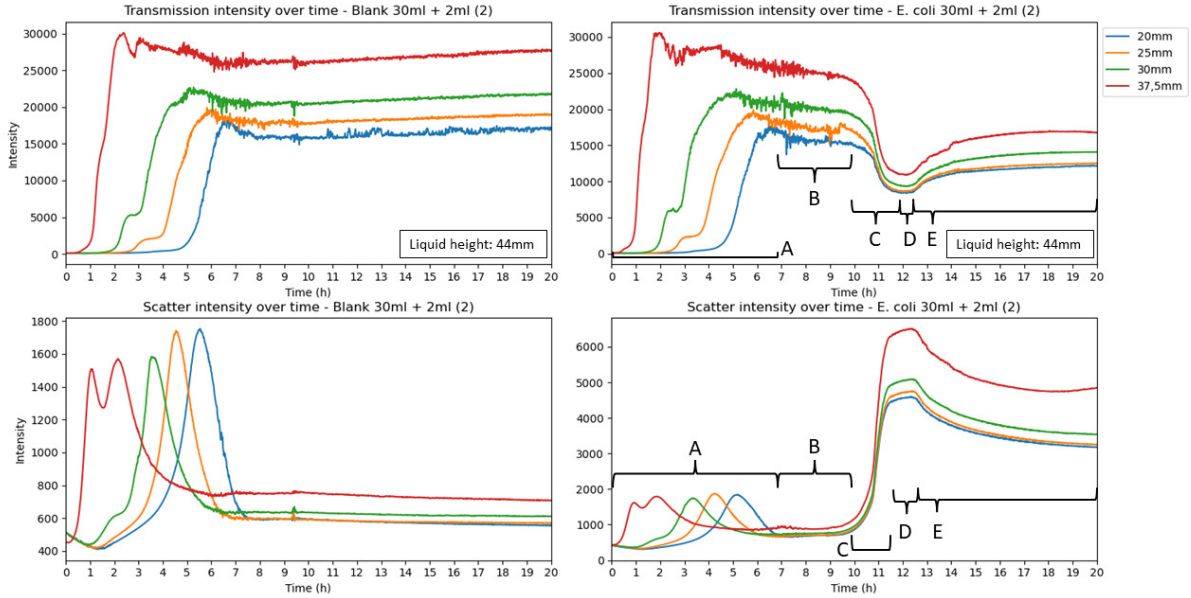

Figure 1: Transmission and scattering measurements at 20mm, 25mm, 30mm and 37.5mm height. The left graphs are of a blank (not spiked) BCB, the right graphs are from a BCB spiked with E. coli. Sedimentation phase (A), lag phase (B), log phase (C), stationary phase (D), mortality phase (E).

to the bottom of the BCB and remain there as sediment.

A. *Effect of Measuring Height*

Figure 1 shows two results from the second part of testing. On the left are the transmission (top) and scattering (bottom) results from the blank test sample. On the right are the transmission and scatter results from the sample spiked with *E. coli*. The graphs only show the bottom four measurement locations because these are the ones that measure through the liquid (when using this sample volume). The four sensor locations above this are above the liquid in the BCB.

In the first couple of hours, the transmission and scatter intensities rise for both samples. This is the result of sedimentation. There are less particles in front of the sensors at the end of this phase so the transmission intensity rises because more light can get through the liquid. The decrease in particles also leads to an increase in scattering which is a bit counterintuitive. The reason for this is that the decrease in absorption has a greater effect on the measurements than the decrease in scattering. When the two effects are combined this leads to an increase in scattering instead of the expected decrease.

The progress of the sedimentation throughout the BCB can be clearly followed by the transmission and scatter peaks on the graphs. In the transmission graphs, the end of the sedimentation phase is indicated by the first sharp increase in transmitted intensity. After this, a small decrease is visible, particularly at a height of 37.5mm. In the scatter graphs, the sedimentation phase shows a sharp increase in intensity at first because the absorption of the sample is going down. This is followed by a sharp decrease in intensity due to the scattering particles in the liquid sinking down to the bottom. Since the sedimentation occurs from top to bottom, the sedimentation phase of a blood culture goes by quicker when the sensors are placed higher in the liquid. If the sedimentation phase lasts longer than the incubation time of the bacteria, it can occlude the growth phase. Because of this, the height at which turbidity is measured should be as high as possible.

There is another interesting phenomenon embedded in the sedimentation phase. The 37.5mm curves show a double peak in both the transmission and scatter graphs. The lower measurement locations also show this in the form of a plateau when they are rising. After this plateau the intensity rises more rapidly than before. A possible explanation for this is that there are multiple particles in the blood which are sinking at different speeds. This would then cause there to be multiple layers in the liquid which have different absorption and scattering parameters and thus result in parts of the curve having a different slope.

For the blank sample, the sedimentation phase is the only thing that can be seen. When this phase is over, transmission slowly and linearly rises while the scattering linearly decreases. This may be because small particles are still falling to the bottom of the BCB, decreasing the turbidity slowly.

In the results of the sample spiked with bacteria, the four microbial growth phases are visible. After the sedimentation, there is a lag phase, log phase, stationary phase, and mortality phase. The most important phase for determining bacterial growth is the log phase. It is this phase that indicates a difference between a spiked sample and a blank sample the quickest. Figure 1 shows this phase clearly as a sharp decrease in transmission intensity and an increase in scatter intensity on the right graphs. The measuring height does not have a significant effect on the time at which the growth phase takes place. This means that relocating the sensors will not improve the time to detection of the turbidimeter when the samples are incubated statically. Some bacterial species grow better when they are shaken during incubation [5, 6]. Different sensor placement might be advantageous in this scenario.

The phenomena discussed so far were observed in all of the samples used for this part of testing. All of the spiked samples showed growth. The labelling of a positive sample happened manually by inspecting the growth curves. An automated way of detecting positive samples with the turbidimeter this way is in development by the SIMBLE-team [2]. The positivity of samples was also confirmed by subculturing a drop of the BCB content on solid agar after the incubation.

The different growth curve phases could be clearly distinguished in all but two samples. The samples spiked with *Burkholderia cepacia* do not show a clear growth phase like the Escherichia coli sample in Figure 1 or any of the other samples. Instead, they show a slower decrease in transmitted intensity and a slower increase in scattered intensity especially in one of the samples. This is actually the start of the growth phase. Sometimes the culturing of *Burkholderia cepacia* takes longer than expected, which was the case for these two samples. Because of this, no conclusions on time to detection can be made for these samples.

B. *Effect of Volume*

All samples in this part were tested according to plan except for one of the blank samples with 40ml broth and 2ml blood. Its subculture after the incubation period showed growth. This means that the sample was contaminated and not usable as a blank. This contaminated sample was not included in the results of any test in this thesis. All of the spiked samples showed growth at the expected incubation time.

To illustrate the effect of different broth and

blood volumes, Figure 2 shows the transmission and scattering results of three samples with different blood-to-broth ratios. Each of these samples was spiked with *E. coli* in this case. Only the results of the sensors below the liquid surface are shown as to not clutter the graph. A consequence of increasing the liquid volume inside the BCB, is that more sensors will be below the surface of the liquid. This is why more curves are shown at higher volumes.

The first observation is that when the sample volume is increased (either blood or broth), the sedimentation phase at a particular height takes longer. This was expected because the sedimentation phase in a sample with more liquid lasts longer. This observation strengthens the previously made statement that the measuring locations should be as high as possible, but still under the liquid surface. In the results of the 40ml broth + 10ml blood sample in Figure 2, it can be seen that the bottom 3 sensors (20mm, 25mm and 30mm) had barely passed the sedimentation phase when the growth phase started. This means that bacteria that started growing before the 11-hour mark would not have been detectable by the bottom most sensor for example.

When the broth volume alone was increased (from 30ml to 40ml), a decrease in transmitted intensity can be seen for the measurement locations at 30mm and 37.5mm. The same is true for the samples spiked with *S.* Typhimurium (not shown in Figure 14). The scattering intensities show the same phenomenon.

For the blank samples, the decrease in transmitted intensity is visible at every location below the liquid (20mm, 25mm, 30mm, 37.5mm). The scattering results here do not show the same pattern. This is likely because the intensities here are low and thus small variations can't be seen because the resolution is too low.

When comparing 10ml of blood with 2ml of blood in combination with 40ml of broth, another significant decrease in transmitted intensity across the whole sample can be observed in Figure 2. This decrease was seen in all of the 10ml blood samples that were tested. The same conclusion can't be drawn from the scattering results. The visible variations are too small between the samples to draw an accurate conclusion, like mentioned above.

Another observation is that the 20mm result (the one closest to the bottom) has a higher intensity than the intensities at 37.5mm and below, at the end of the measuring period. This is again true for all of the tested 10ml blood samples, also for *S.* Typhimurium and the blank samples. A possible explanation for this is that the sedimentation layer at the bottom of the BCB is higher in the 10ml samples and that this has an effect on the measured intensity of the bottom sensors.

The 25mm and 30mm also show this increase in comparison to the 37.5mm results although it is less pronounced. This supports the theory that the cause of this phenomenon is at the bottom of the BCB.

Finally, the highest sensor in the liquid, in both the 40ml broth + 2ml blood sample and 40ml broth + 10 blood does not have the maximum observed intensity, which was always the case in the previous measurements. Measurements from these sensors also fluctuate more frequently and intensely. This is especially true for the transmission sensors. This means that the measuring location should not be right against the liquid-air interface. These sensors were located 3mm and 4mm beneath the surface of the liquid (measured from the centre of the sensor). The sensor width is 2mm. The lowest distance between the liquid surface and the sensor where a stable measurement was still observed, was 6.5mm. It is therefore recommended that a sensor is placed at least 6.5mm below the liquid-air interface.

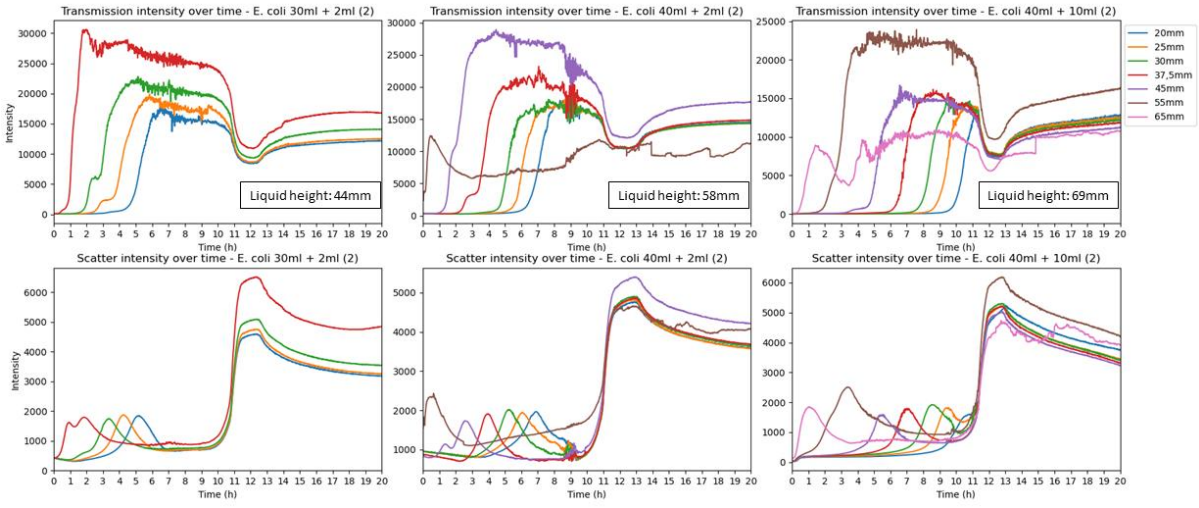

Figure 2: Transmission and scatter intensities at multiple heights of 3 samples spiked with E. coli and with different blood-to-broth ratios. 30ml broth + 2ml blood (left), 40ml broth + 2ml blood (middle), 40ml broth + 10 ml blood (right). Only the results of sensors below the liquid surface are shown.

C. *Diagonal Measurements*

Figure 3 shows the results of the fourth part of testing. The graphs show the transmission and scatter results for at 20mm and 37.5mm as well as the two diagonal measurements D0 (from 55mm to 37.5mm) and D1 (from 37.5mm to 55mm). The 20mm and 37.5mm results act as a reference so comparisons with the previous figures are possible.

D0 and D1 were the main focus of this test. Their curves have a lower intensity than the 20mm or 37.5mm curves. This is caused by several factors. First, the emission and detection efficiency of the LEDs and sensors depend on the angle at which the light is emitted/detected. This means that the intensity of the light will be lower because the light is emitted/detected at an angle. Secondly, the path of the light from the LED to the transmission sensor is not straight anymore and has to penetrate the liquid-air interface to get detected. Because part of the light gets reflected at this interface, the detected intensity is lower. Finally, the light travels along a longer path and thus will be absorbed and reflected more.

The sedimentation phase of the D0 and D1 curves is a little shorter than the sedimentation phase at 37.5mm. This is because the light path of D0 and D1 goes through higher parts of the liquid where the sedimentation phase is done quicker. After the sedimentation phase, the curves largely follow the same trends as the 20mm and 37.5mm curves. This means that this measuring setup was unable to detect the pellicle formation on top of the liquid.

There also is a valley in the D1 transmission curve between 10 hours and 15 hours in the first (left) sample. This could be a sign of the pellicle formation but because the D0 transmission curve does not show the same valley and there is nothing visible in the scattering curves at the same time, this can't be said with certainty.

V. CONCLUSION

The results obtained from testing the third generation turbidimeter show that the design works and that it can detect most of the relevant organisms in a blood culture.

The ideal measurement location is near the top of the blood-broth mixture. This is where the sedimentation phase goes by the quickest which means that organisms can be detected there earlier than in other locations. There is however no decrease in the time to detection at this location. If the sedimentation phase has passed throughout the whole BCB, all measurement locations will have a similar time to detection. However, a measuring distance of 6.5mm below the top of the liquid should be maintained to make sure the signal stays stable.

When blood and/or broth volumes in the BCB were increased, the sedimentation phase took longer to end. This means that growth in the sample was undetectable for longer at a certain measuring height. When blood volume alone was increased from 2ml to 10ml, the transmitted and scattered intensity went down significantly across the whole sample period. This limited the part of the input range that the signal covered. A careful trade-off should therefore be made between maximising blood volume, minimising the non-functional time, and preventing the transmitted and scattered intensities from dropping too much.

Detecting pellicle formation on top of the sample was not possible by measuring diagonally through the liquid-air interface with this setup. While a change in transmitted and scattered intensity was observed, it is likely that this was as a result of the change in turbidity of the liquid inside the BCB.

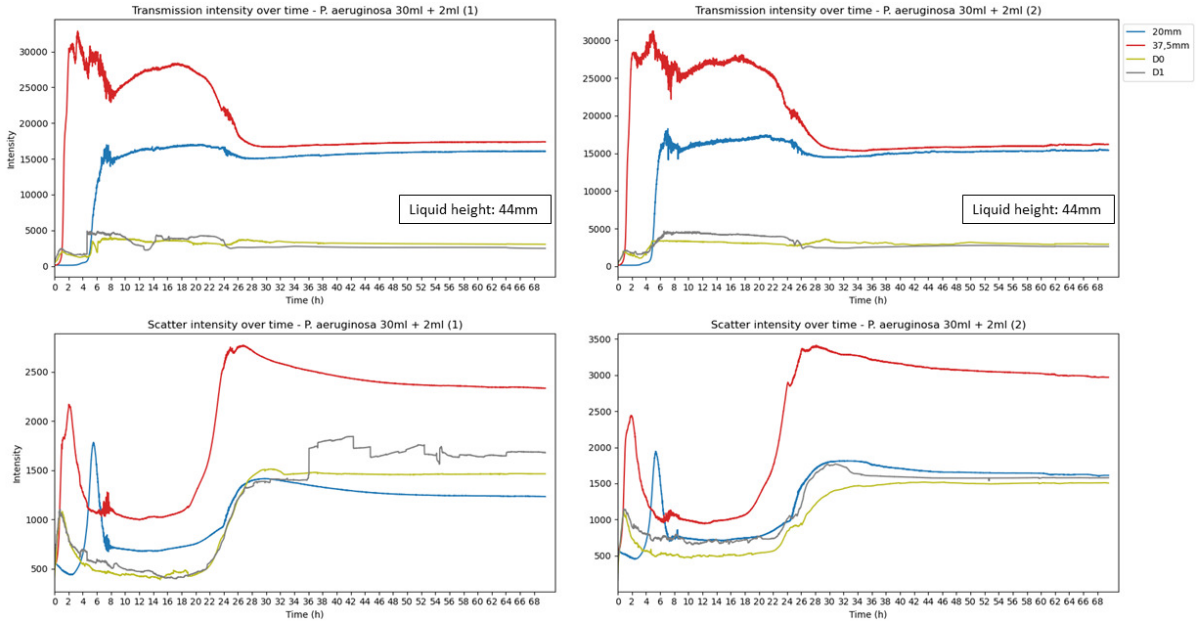

Figure 3: Transmission and scatter results at multiple measuring heights of two samples spiked with Pseudomonas aeruginosa. The samples contained 30ml broth and 2ml blood.

It should be noted that the tests in this master's thesis were performed on a small number of samples. Only six bacterial strains were tested over 22 samples (not equally distributed).

Future studies, including field studies, will be performed with the second generation turbidimeter by the SIMBLE-team [2], while continuing to improve its performance.

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EXTENDED ABSTRACT (DUTCH)

Ontwikkeling van een optisch/elektronisch turbidimetrisch hemocultuursysteem voor snelle detectie van bacteriële infectie

Jens Cornelis
Promotoren: prof. dr. ir. Roel Baets, dr. Liselotte Hardy
Begeleiders: dr. Mohammadamin Ghomashi, Federico Marchesin

Abstract - Bloedbaaninfecties (BSIs) veroorzaken ernstige ziektes die tot sepsis kunnen leiden. Sepsis was verantwoordelijk voor bijna 20% van alle wereldwijde overlijdens in 2017 volgens de Wereldgezondheidsorganisatie (WHO) [1]. Het SIMBLE-team [2] heeft recent een diagnostisch apparaat ontwikkeld om te helpen het detecteren van BSIs. Het apparaat werd de turbidimeter genaamd en is bedoeld voor gebruik in landen met een laag en gemiddeld inkomen (LMICs). Het werkt op het principe van de turbiditeit van de vloeistof in een bloedcultuur. Het doel van deze masterproef was om dit apparaat verder te verbeteren, zodat het meer soorten bacteriën sneller kan detecteren. De eerste manier om dit te doen was door meer sensoren toe te voegen en door te kijken naar het effect van de meethoogte. Ook de impact van het specimenvolume werd getest. Ten slotte zijn er enkele bacteriefamilies die tot op heden nog niet betrouwbaar gedetecteerd kunnen worden op basis van de turbiditeit alleen. Daarom werd er ook gekeken of andere tekenen van groei, zoals vliesvorming aan de vloeistof-lucht barrière kunnen gedetecteerd worden met deze opstelling.

Om dit te verwezenlijken, werden er nieuwe sensorborden ontwikkeld, geassembleerd en getest aan Universiteit Gent (UGent). Wanneer bewezen was dat het prototype werkte, begonnen de in vitro testen aan het Instituut voor Tropische Geneeskunde (ITG) om de performantie van de nieuwe meetopstelling te testen.

De resultaten toonden aan dat de meetapparatuur best zo dicht mogelijk bij de bovenkant van de vloeistof geplaatst wordt om zo snel mogelijk bacteriën te kunnen detecteren. Bovendien werd er geconcludeerd dat een verhoging van het volume van zowel bloed als medium leidde tot een langere sedimentatiefase. Tijdens die fase kan er geen bacteriële groei gedetecteerd worden. Ten slotte kon er geen vliesvorming gedetecteerd worden met deze meetopstelling.

Kernwoorden:
Turbidimeter, bloedcultuur, bloedbaaninfectie, schaarse middelen omgevingen

I. INLEIDING

Om te kunnen helpen met het detecteren van bloedbaaninfecties met de turbidimeter, werd er gekeken waar de prestaties van de tweede generatie turbidimeter konden verbeterd worden. De turbidimeter werkt op basis van de turbiditeit (of troebelheid) van een bloedcultuur [3]. Om dit te kunnen meten, schijnt een led licht aan één kant van de bloedkweekfles [3]. Een lichtsensor aan de andere kant van de fles detecteert dan het doorgelaten licht [3]. Een tweede sensor is loodrecht gemonteerd ten opzicht van de led en eerste sensor om de hoeveelheid verstrooid licht te detecteren [3]. De hoeveelheid licht die door elk van deze sensoren gedetecteerd wordt, hangt af van de turbiditeit van de bloedcultuur.

Een nieuwe generatie turbidimeter werd ontwikkeld om mogelijke verbeteringen aan de tweede generatie te onderzoeken. Deze nieuwe generatie heeft enkele toevoegingen ten opzichte van de tweede generatie, die nu gebruikt wordt door het SIMBLE-team [2]. Eén van deze toevoegingen is de aanwezigheid van meerdere sensoren op verschillende hoogtes om te kunnen evalueren of de hoogte ten opzichte van de bodem van de fles een effect heeft op de meetresultaten. Een tweede toevoeging is een systeem om tekenen van bacteriële groei anders dan turbiditeit te detecteren, voornamelijk vliesvorming. Om deze nieuwe mogelijkheden te voorzien werden er twee op maat gemaakte printplaten ontworpen, geassembleerd en getest. De tweede generatie turbidimeter uitgerust met deze nieuwe eigenschappen zal verder in dit werk de derde generatie turbidimeter genoemd worden.

II. DERDE GENERATIE TURBIDIMETER

Het hoofddoel van deze thesis was om de derde generatie turbidimeter te ontwikkelen en te testen. Dit hield in dat er nieuwe printplaten zowel ontworpen als geassembleerd moesten worden. Bovendien moest ook de software op de microcontroller geüpdatet worden. De derde generatie turbidimeter functioneert als een testapparaat om toekomstige uitbreidingen op uit te proberen. Eén van deze uitbreidingen is het meten

van de turbiditeit op verschillende hoogtes. Dit kan voordelig zijn omdat licht niet gelijk doorgelaten, verstrooid of geabsorbeerd wordt doorheen de bloedkweekfles. Dit betekent dat er plaatsen kunnen zijn waar een verandering in turbiditeit makkelijker of sneller te detecteren kan zijn. Dit zou betekenen dat ook bacteriën sneller zouden kunnen gedetecteerd worden op sommige locaties.

Om het effect van de meethoogte te testen werden er nieuwe verticale dochterborden ontwikkeld. De schema's en lay-out van deze borden zijn te vinden in appendices A tot en met C.

Het originele pcb-ontwerp van de tweede generatie turbidimeter was een gecombineerd emitter/detector bord. De componenten die erop geplaatst werden bepaalden de functie van het bord. Voor het nieuwe ontwerp werden er twee nieuwe pcb's getekend, één voor de detector en één voor de emitter. Elke pcb heeft acht leds of sensoren die op de volgende hoogtes werden geplaatst, met de onderkant van de bloedkweekfles als referentie: 20mm, 25mm, 30mm, 37.5mm, 45mm, 55mm, 65mm en 75mm. De meethoogtes zijn meer geconcentreerd aan de onderkant van de bloedkweekfles omdat er verwacht werd dat de meest nuttige informatie zich daar zou bevinden.

A. *Emitter*

De emitter bestaat uit acht leds (Luminus, Sunnyvale, Verenigde Staten van Amerika) die aangedreven worden door een TLC59108 led *driver* (Texas Instruments, Dallas, Verenigde Staten van Amerika). Deze leds en de led *driver* zijn dezelfde componenten die ook eerder gebruikt werden bij de tweede generatie turbidimeter. De *driver* heeft acht constante-stroom uitgangskanalen en communiceert via het I²C-communicatieprotocol. Net zoals bij het tweede generatie emitter/detector bord werd er ook een PCA951A I²C buffer (NXP, Eindhoven, Nederland) toegevoegd voor de *driver* om de *hot-swap* mogelijkheid toe te voegen aan het ontwerp.

B. *Detector*

In vorige versies werd voor de detector de TCS3772 RGBC-sensor (ams-OSRAM, Premstaetten, Oostenrijk) gebruikt maar door beschikbaarheidsproblemen moest hier een andere sensor gebruikt worden. De TCS3400 (ams-OSRAM, Premstaetten, Oostenrijk) werd gekozen als vervanger omdat deze dezelfde *pinout* heeft, ook gebruik maakt van I²C, en gemaakt wordt door dezelfde fabrikant. Acht van deze sensoren werden gebruikt op elk detector bord maar omdat elk van deze sensoren hetzelfde I²C adres heeft, moest er ook een I²C multiplexer geplaatst worden. Deze multiplexer zorgt ervoor dat er slechts één sensor zich tegelijk op de hoofd-I²C-bus bevindt. De multiplexer geselecteerd voor deze toepassing is de PCA9547 (NXP, Eindhoven, Nederland), hoofdzakelijk omdat deze acht kanalen heeft alsook een configureerbaar I²C adres. Dit configureerbaar adres is een vereiste omdat er zich twee identieke detectorborden tegelijk in de turbidimeter zullen bevinden en ze individueel geadresseerd moeten kunnen worden. Een PCA951A I²C buffer werd ook hier toegevoegd om het bord *hot-swap* mogelijkheden te geven.

Ten slotte werd er ook een TMP116 temperatuursensor (Texas Instruments, Dallas, Verenigde Staten van Amerika) toegevoegd om de omgevingstemperatuur binnenin de turbidimeter te kunnen meten. Deze sensor werd echter niet gebruikt in deze masterproef maar zal handig zijn voor toekomstig gebruik.

C. *Pcb's*

De pcb's werden zo gemaakt dat de breedte van zowel de detector en emitter borden dezelfde is als bij de tweede generatie turbidimeter. Op deze manier kon de reeds bestaande, 3D ontworpen turbidimeterbehuizing gebruikt worden met enkel minimale aanpassingen. Dit zorgde er echter voor dat er aanzienlijk minder ruimte op de pcb beschikbaar was. Voor de emitter-pcb zorgde dit niet voor problemen aangezien er hier niet veel componenten op aanwezig waren. De detector daarentegen heeft acht I²C sensoren die elk aparte SDA- en SCL-lijnen afkomstig van de multiplexer hebben. Elk van deze banen had bovendien een 1kΩ pullup-weerstand nodig. Deze 16 banen en hun weerstanden namen een aanzienlijk deel van de beschikbare ruimte op wanneer ze naast elkaar geplaatst werden. Vooral de breedte was een beperkende factor. Om dit probleem op te lossen werd de multiplexer in het midden van de pcb geplaatst. Op deze manier konden de helft van de banen omhoog geleid worden en de andere helft naar beneden. Hierdoor werd maar de helft van de breedte opgenomen door deze banen.

Het grondvlak bevindt zich zowel aan de bovenkant als aan de onderkant van de pcb. Er werd voor gezorgd dat er geen delen van het grondvlak afgesneden werden door één van de banen. Dit zorgt ervoor dat het grondvlak overal dezelfde, stabiele potentiaal heeft. Wanneer er plaats beschikbaar was, werden er ook *bridging vias* rond de banen geplaatst die voor onderbrekingen van het grondvlak zorgden. Op deze manier kan er stroom vloeien onder een baan in plaats van dat deze er rond moet vloeien. Dit verkleint de kans op EMI-problemen en zorgt ervoor dat er zich geen spanningsverschil kan opbouwen langs de twee kanten van de baan.

III. METHODE

De testen met de derde generatie turbidimeter werden verdeeld in vier delen. Het eerste deel werd gebruikt om de functionaliteit van het apparaat te verifiëren vooraleer de testen met bacteriën begonnen. Dit was noodzakelijk vanwege de

aanpassingen in het ontwerp, de software en door het gebruik van een nieuwe RGBC-sensor. Het tweede, derde en vierde deel van de testen werd uitgevoerd in het Instituut voor Tropische Geneeskunde (ITG) te Antwerpen. In elk van deze testen werd de turbidimeter geconfigureerd zodat de metingen op verschillende hoogtes één voor één gebeurden, van onder naar boven. De metingen op de acht hoogtes duurde 12.8 seconden en een periode van 26.4 seconden werd gelaten tussen twee meetcycli.

Omdat er slechts twee turbidimeters van de derde generatie gemaakt werden, gebeurden deze testen overheen vijf weken met één of twee testen tegelijk. De data van deze testen werden opgenomen door een computer verbonden met de turbidimeters. Om de data te onderzoeken en om grafieken te maken, werd er gebruik gemaakt van een Python-programma.

Het tweede deel van de testen was gefocust op het bepalen van het effect van de meethoogte op de resultaten. Acht bloedculturen werden uitgevoerd met elk 30ml *tryptic soy* medium waaraan 0.3mg/ml van SPS was toegevoegd. 2ml menselijk bloed werd gebruikt in de bloedculturen. Het bloed werd gedoneerd door verschillende vrijwilligers bij het ITG (ethische goedkeuring werd verkregen voor het verzamelen van het bloed). Bepaalde bacteriesoorten werden aan zeven van de acht bloedkweekflessen toegevoegd (*Escherichia coli*, *Salmonella* Typhimurium, *Streptococcus pneumoniae*, *Staphylococcus aureus* of *Burkholderia cepacia*) om bloedafnames van besmette personen te imiteren.

Bij het derde deel van de testen werd het effect van het monstervolume bekeken. Nog acht flesjes werden gevuld met twee verschillende bloed-tot-medium verhoudingen, hieraan werden ook twee verschillende bacteriesoorten toegevoegd (*Escherichia coli* en *Salmonella* Typhimurium). Dit, samen met twee monsters van het vorige deel, gaf drie verschillende bloed-tot-medium verhoudingen en twee bacteriesoorten alsook één blanco (zonder bacteriën) per soort om met elkaar te vergelijken.

Het doel van het vierde en laatste deel van de tests was om te zien of tekenen van groei anders dan turbiditeit konden gedetecteerd worden met de derde generatie turbidimeter. Vooral de vliesvorming op de vloeistof-lucht barrière werd onderzocht. Om dit te kunnen meten werden er twee extra meetprofielen toegevoegd. Deze meten diagonaal door het oppervlak door de juiste led en sensoren te activeren. Het complete meetprofiel wordt gegeven in appendix E. De test bestond uit twee bloedkweekflessen waaraan *Pseudomonas aeruginosa* toegevoegd. De twee culturen bestonden uit 30ml medium en 2ml bloed. Deze volumes werden gekozen zodat de vloeistof-lucht barrière tussen 37.5mm en 55mm belandde.

IV. Resultaten

De testen die werden uitgevoerd in het ITG maakten gebruik van bloedkweekflessen waaraan medium, bloed en bacteriën werden toegevoegd. De resultaten zullen dus in de vorm zijn van bacteriële groeicurves. Deze curves bestaan traditioneel uit vier groeifases [4]. De resultaten van de testen hier zullen ook deze vier fases tonen. De eerste fase is de *lag*-fase, waarin er geen verschil te zien is aangezien het aantal bacteriën relatief constant blijft [4]. De volgende fase is de logfase of groeifase. In deze fase groeit het aantal bacteriën exponentieel en worden er tekenen van groei zichtbaar (in dit geval turbiditeit) [4]. Hierna komt de stationaire fase, waar de concentratie aan bacteriën zijn maximum bereikt [4]. Dit maximum wordt veroorzaakt door de beperkte aanwezigheid van voedingsstoffen in de bloedkweekflessen [4]. De laatste fase is de *mortality*-fase of sterftefase [4]. In deze fase neemt het aantal bacteriën af omdat er niet genoeg voedingsstoffen meer aanwezig zijn en omdat het medium de huidige concentratie aan bacteriën niet kan onderhouden. Om groeicurves te maken, wordt het aantal bacteriën doorheen de tijd geplot. Dit is echter niet wat hier wordt gemeten. De turbidimeter meet de turbiditeit van het monster en dus niet rechtstreeks de concentratie aan bacteriën. Hierdoor kan het zijn dat de groeicurves die hier getoond worden niet overeenkomen met de werkelijke groeicurves van de bacteriën in tijd of in intensiteit maar ze zouden wel dezelfde vier fases moeten vertonen. Er zal ook een vijfde fase aanwezig zijn (die niets te maken heeft met de bacteriële groei), de sedimentatiefase. Tijdens deze fase vallen partikels in het bloed naar de onderkant van de bloedkweekfles en blijven ze daar achter als sediment.

A. *Effect van de meethoogte*

Figuur 1 toont twee resultaten van het tweede deel van de testen. Links zijn de transmissie- (bovenaan) en verstrooiingsresultaten (onderaan) van het blanco monster te zien. Rechts zijn de transmissie- en verstrooiingsresultaten van het monster met *E. coli* te zien. De grafieken tonen enkel de vier onderste meetlocaties omdat enkel deze door de vloeistof meten bij dit volume. De vier sensorlocaties erboven zitten boven de vloeistof in de bloedkweekfles. In de eerste paar uren, is er een stijging te zien in de transmissie- en verstrooiingsintensiteit voor beide monsters. Dit is de sedimentatiefase. Er zijn minder deeltjes voor de sensoren aan het einde van deze fase dus stijgt de transmissie-intensiteit omdat er meer licht door het monster kan. Het lager aantal deeltjes leidt ook tot stijging in verstrooiingsintensiteit, wat niet intuïtief verwacht werd. De reden voor deze stijging is dat de verlaging van de absorptie hier een groter effect heeft dan de verlaging van de verstrooiing. Wanneer de twee effecten gecombineerd worden is

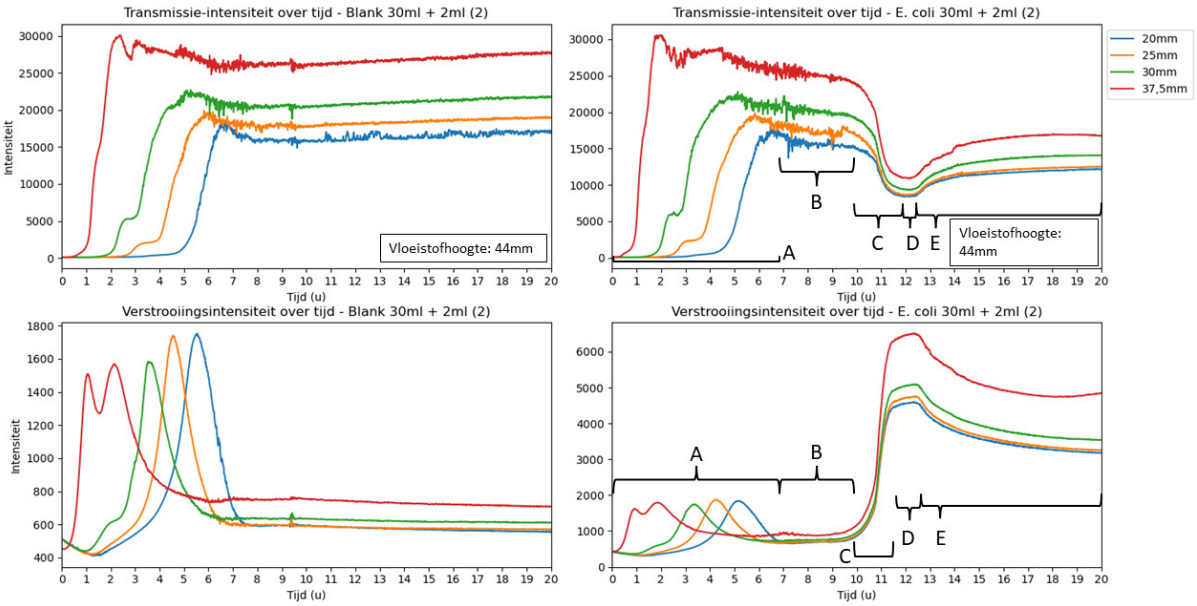
er een stijging in de verstrooiingsmeting zichtbaar in plaats van de verwachte daling. De voortgang van de sedimentatie doorheen de bloedkweekfles kan duidelijk gevolgd worden op de grafieken. Op de transmissiegrafieken is de sedimentatiefase te zien als de eerste sterke stijging van de intensiteit. Hierna is er ook nog een kleine daling zichtbaar, vooral op een hoogte van 37.5mm. Op de verstrooiingsgrafieken, wordt de sedimentatiefase gekarakteriseerd door een stijging van de intensiteit (veroorzaakt door de daling van de absorptie) met daarna terug een daling van de intensiteit (veroorzaakt door de daling van de verstrooiing in het monster). Omdat de sedimentatie in de bloedkweekfles gebeurt van boven naar onder, is het einde van deze fase sneller te zien bij de bovenste sensoren. Als de sedimentatiefase langer zou duren dan de incubatietijd van de bacteriën, kan het de groeifase van deze bacteriën verbergen. Hierdoor zou de turbiditeitsmeting dus best zo hoog mogelijk uitgevoerd worden.

Er zit nog een ander interessant fenomeen verborgen in de sedimentatiefase. Op de 37.5mm grafiek is er een dubbele piek te zien op zowel de transmissie- als de verstrooiingsgrafieken. Bij de lagere sensoren is ditzelfde fenomeen te zien als een plateau tijdens de eerste stijging van de intensiteit, waarna de intensiteit sneller stijgt dan ervoor. Een mogelijke verklaring hiervoor is dat er meerdere soorten partikels in het monster zitten die elk met een andere snelheid naar beneden zinken. Dit zorgt dan voor verschillende lagen in de vloeistof die verschillende optische parameters hebben en dus resulteren in een andere richtingscoëfficiënt op de grafiek.

Voor het blanco monster is de sedimentatiefase de enige fase die er te zien is. Wanneer deze fase voorbij is, stijgt de transmissie geleidelijk en lineair, terwijl de verstrooiing lineair daalt. Dit zou kunnen veroorzaakt worden door kleine deeltjes die nog steeds naar de bodem aan het zinken zijn en dus langzaamaan de turbiditeit verlagen.

In de resultaten van de monsters die bacteriën bevatten, zijn de vier bacteriële groeifases te zien. Na de sedimentatie is er een *lag*-fase, een groeifase, een stationaire fase en een sterftefase. De belangrijkste fase voor het detecteren van bacteriële groei is de groeifase. Deze fase duidt het snelst het verschil aan tussen een blanco monster en een monster dat bacteriën bevat. Figuur 1 toont deze fase als een steile daling van de transmissie-intensiteit en een steile stijging van de verstrooiingsintensiteit aan de rechterkant van de figuur. De meethoogte heeft geen significant effect op de tijd waarop de groeifase plaatsvindt op deze intensiteitsgrafieken. Dit betekent dat het verplaatsen van de sensoren de tijd tot detectie niet zal verbeteren wanneer, zoals hier, de monsters statisch geïncubeerd worden. Sommige bacteriën groeien beter wanneer het monster geschud wordt tijdens de incubatie [5, 6]. Andere sensorlocaties zouden hier dan wel een voordeel kunnen bieden.

De fenomenen die dusver besproken werden, werden geobserveerd in alle monsters die tijdens deze testen gebruikt werden. Alle monsters waar bacteriën aan toegevoegd werden, toonden groei. Dit werd manueel gecontroleerd aan de hand van de groeicurves. Een automatische manier om dit te detecteren wordt momenteel getest door het SIMBLE-team [2]. De positiviteit van de bloedcultuur werd ook gecontroleerd door een subcultuur te maken met een druppel van de vloeistof in de bloedkweekfles op een agarplaat na de incubatie.



Figuur 1: Transmissie- en verstrooiingsintensiteitsmetingen op 20mm, 25mm, 30mm en 37.5mm hoogte. De linker grafieken zijn van een blanco monster, de rechter grafieken zijn van een monster waaraan E. coli werd toegevoegd. Sedimentatiefase (A), lag-fase (B), groeifase (C), stationaire fase (D), sterftefase (E).

De verschillende groeifases konden duidelijk onderscheiden worden bij bijna alle monsters. Bij twee monsters waaraan *Burkholderia cepacia* werd toegevoegd, was er geen duidelijke groeifase te zien. In plaats daarvan, was er een tragere daling in de transmissie-intensiteit te zien alsook een tragere stijging van de verstrooiingsintensiteit aan het einde van de meting. Dit duidde eigenlijk het begin van de groeifase aan. Soms duurt het culturen van *Burkholderia cepacia* langer dan verwacht en dat was ook het geval bij deze twee monsters. Hierdoor kunnen er geen conclusies voor de detectietijd gemaakt worden voor deze twee monsters.

B. Effect van het volume

Alle monsters in dit deel van de testen werden getest volgens plan behalve een van de blanco monsters met 40ml medium en 2ml bloed. De subcultuur hiervan toonde groei na de incubatie. Dit wil zeggen dat het monster gecontamineerd werd en dus niet bruikbaar is als een blanco monster. Dit gecontamineerde monster werd nergens in de resultaten van dit werk gebruikt. Alle andere monsters toonden groei na de verwachtte incubatietijd.

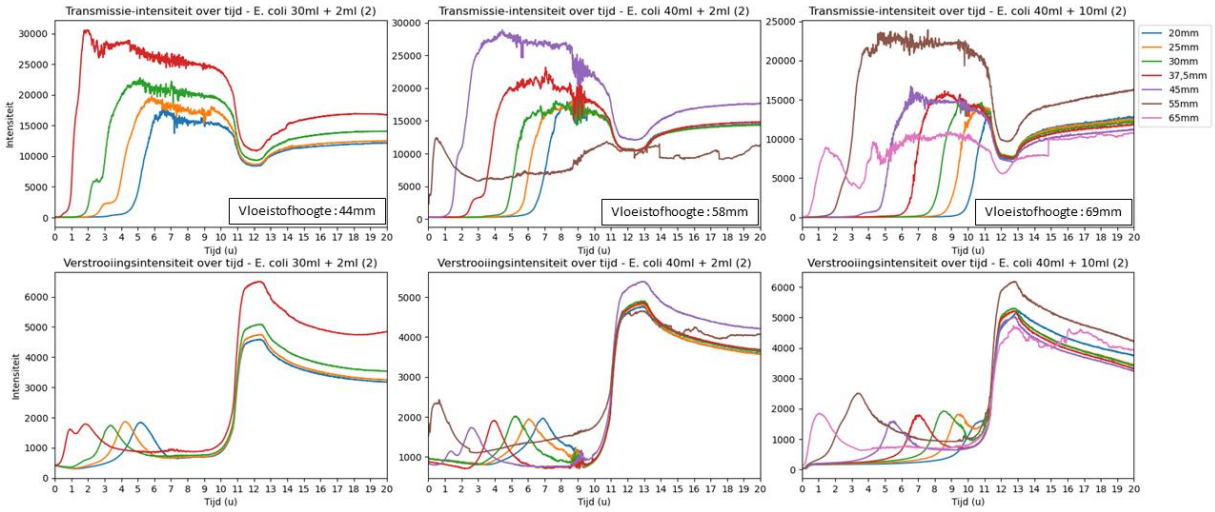
Om het effect van verschillende medium- en bloedvolumes aan te duiden, toont Figuur 2 de transmissie- en verstrooiingsresultaten van drie monsters met elk een andere bloed-tot-medium verhouding. Aan elk van deze monsters werd ook *E. coli* toegevoegd. Enkel de resultaten van de sensoren onder de vloeistof-lucht barrière worden getoond om de grafieken niet te druk te maken. Een gevolg hiervan is dat er meer resultaten te zien zullen zijn voor de monsters die een groter volume hebben.

De eerste observatie is dat wanneer het volume van het monster verhoogd werd (meer bloed of meer medium), de sedimentatiefase langer duurde op een bepaalde hoogte. Dit werd verwacht aangezien de volledige sedimentatie in een monster met een groter volume langer duurt. Dit draagt bij aan het hierboven vermelde argument dat de meetlocaties best zo hoog mogelijk worden geplaatst, maar nog altijd onder het vloeistofoppervlak. In de resultaten van het 40ml medium + 10ml bloed monster op Figuur 2, kan er gezien worden dat de drie onderste sensoren (20mm, 25mm en 30mm) maar net uit de sedimentatiefase kwamen vooraleer de groeifase begon. Dit betekent bijvoorbeeld dat bacteriesoorten die voor 11 uur incubatie zouden starten met de groeifase, niet gedetecteerd zouden kunnen worden door de onderste sensor.

Wanneer alleen het volume van het medium verhoogd werd (van 30ml tot 40ml), is er een daling te zien van de transmissie-intensiteit voor de meetlocaties op 30mm en 37.5mm. Hetzelfde fenomeen is te zien bij de monsters waaraan *S.* Typhimurium werd toegevoegd (niet getoond op de grafiek). Ook de verstrooiingsintensiteiten tonen ditzelfde fenomeen.

Bij de blanco monsters is de daling in transmissie-intensiteit te zien op elke meetlocatie onder het vloeistofoppervlak (20mm, 25mm, 30mm en 37.5mm). De verstrooiingsresultaten tonen hier niet hetzelfde patroon. Dit is waarschijnlijk omdat de intensiteiten hier te laag zijn waardoor kleine variaties niet kunnen gezien worden omdat de resolutie van de sensoren te laag is.

Wanneer de monsters met 10ml bloed en 40ml medium vergeleken worden met de monsters met 2ml bloed en 40ml medium, is er een significante daling van de transmissie-intensiteit te zien overheen de hele meetperiode. Deze afname was te zien bij alle 10ml bloedmonsters die getest werden. Hetzelfde kan niet gezegd worden over de verstrooiingsresultaten. De zichtbare variaties



Figuur 2: Transmissie- en verstrooiingsintensiteitsmetingen op meerdere hoogtes van 3 monsters waaraan E. coli werd toegevoegd en met verschillende bloed-tot-medium verhoudingen. 30ml medium + 2ml bloed (links), 40ml medium + 2ml bloed (midden), 40ml medium + 10 ml bloed (rechts). Enkel de resultaten van de sensoren onder het vloeistofoppervlak worden getoond.

tussen de monsters is te klein om hier een accurate conclusie uit te trekken.

Nog een andere observatie is dat de resultaten op 20mm (degene het dichtste bij de onderkant) een hogere intensiteit hebben op het einde van de meting dan die op 37.5mm en daaronder. Ook dit werd weer gezien in alle geteste 10ml bloedmonsters, ook bij de blanco en *S.* Typhimurium monsters. Een mogelijke verklaring hiervoor is dat de sedimentatielaag aan de onderkant van de bloedkweekfles hoger is bij de 10ml bloedmonsters en dat dit een effect heeft op de metingen van de onderste sensoren.

De 25mm en 30mm curves tonen ook een lichte toename van de transmissie-intensiteit ten opzichte van de 37.5mm metingen, maar in mindere mate. Dit is een extra teken dat de oorzaak van dit fenomeen zich aan de onderkant van de bloedkweekfles voordoet.

Ten slotte is er te zien dat de hoogste sensor onder het vloeistofoppervlak bij de 40ml medium + 2ml bloed en 40ml medium + 10ml bloed monsters, niet de hoogste intensiteit heeft, wat hiervoor steeds het geval was. Metingen van deze sensoren variëren ook vaker en harder. Dit is vooral zo bij de transmissiemetingen. Dit betekent dat de ideale meetlocatie in deze gevallen niet te dicht bij het vloeistofoppervlak is. Deze sensoren zaten 3mm en 4mm onder het vloeistofoppervlak (gemeten vanaf het midden van de sensor). De sensorbreedte is 2mm. De laagste afstand tussen een sensor en het vloeistofoppervlak, waarbij nog steeds een stabiele meting werd gezien, was 6.5mm. Hierdoor wordt het aangeraden om ten minste 6.5mm onder het vloeistofoppervlak te meten.

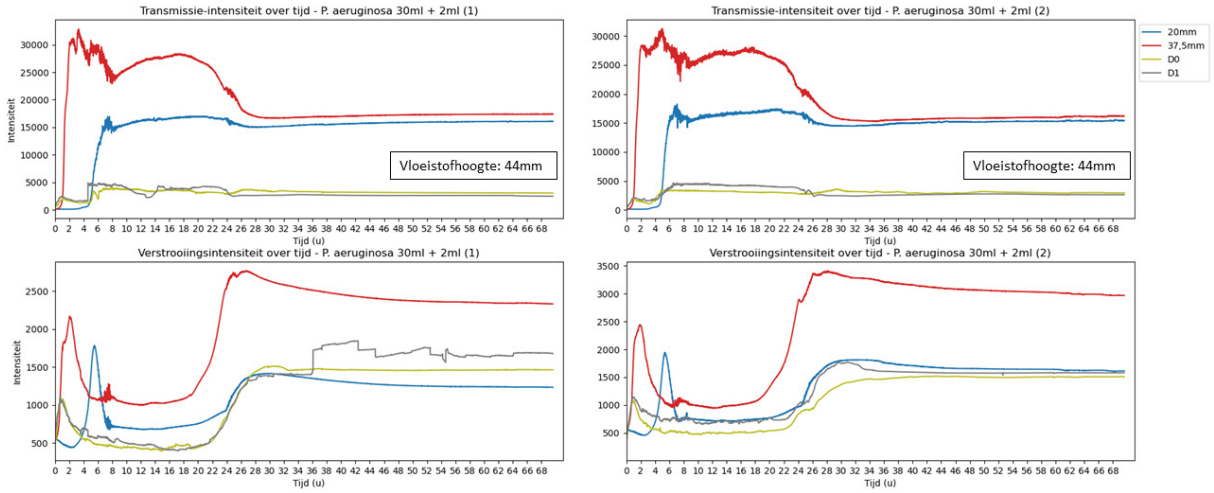
C. Diagonale metingen

Figuur 3 toont de resultaten van het vierde en laatste deel van de testen. De grafieken tonen de meetresultaten op 20mm en 37.5mm hoogte alsook de twee diagonale metingen D0 (van 55mm naar 37.5mm) en D1 (van 37.5mm naar 55mm). De 20mm en 37.5mm resultaten dienen als referentie zodat vergelijkingen met de vorige grafieken kunnen gemaakt worden.

D0 en D1 waren de focus van deze testen. Hun curves hebben een lagere intensiteit dan die van de 20mm en 37.5mm meetlocaties. Dit wordt veroorzaakt door meerdere factoren. Ten eerste hangt de emissie- en detectie-efficiëntie van respectievelijk de leds en sensoren af van de hoek waarin het licht uitgestraald/gedetecteerd wordt. Aangezien het licht hier onder bepaalde hoeken wordt uitgestraald/ontvangen zal de intensiteit dus lager liggen. Ten tweede is het pad dat het licht neemt niet meer recht en gaat het door het oppervlak van de vloeistof. Dit betekent dat een deel van het licht zal gereflecteerd worden en de juiste sensoren niet zal bereiken waardoor de uiteindelijke intensiteit lager zal liggen. Ten laatste is het pad dat het licht aflegt ook langer, waardoor het meer geabsorbeerd en gereflecteerd zal worden.

De sedimentatiefase van D0 en D1 is een beetje korter dan die van de 37.5mm meting. Dit komt doordat D0 en D1 door de hogere delen van de vloeistof gaan waar de partikels in het monster sneller weg zijn. Na de sedimentatiefase volgen de resultaten grotendeels dezelfde trends als de 20mm en 37.5mm resultaten. Dit betekent dat de meetopstelling de vliesvorming op het vloeistofoppervlak niet kon detecteren.

Er is ook een dip te zien in de D1 transmissiegrafiek tussen 10 uur en 15 uur incubatietijd in het linkse monster. Dit zou een teken kunnen zijn van vliesvorming maar aangezien de D0 transmissiecurve niet dezelfde dip toont en er geen tekenen te zien zijn in de verstrooiingsresultaten, kan dit niet met zekerheid gezegd worden.



Figuur 3: Transmissie- en verstrooiingsintensiteitsmetingen op meerdere hoogtes van twee monsters waaraan Pseudomonas aeruginosa werd toegevoegd. De monsters bevatten 30ml medium en 2ml bloed.

V. CONCLUSIE

De resultaten verkregen door het testen van de derde generatie turbidimeter tonen dat het design werkt en dat het de meeste relevante bacteriesoorten kan detecteren.

De ideale meetlocatie is dicht bij het oppervlak van de vloeistof. Daar gaat de sedimentatiefase het snelst voorbij wat betekent dat organismen daar mogelijks sneller kunnen gedetecteerd worden dan op andere plaatsen. Er is echter geen afname in de detectietijd voor organismen op deze locatie. Als de sedimentatiefase doorheen de hele bloedkweekfles gepasseerd is, zullen alle locaties een gelijkaardige detectietijd hebben. Er moet wel op gelet worden dat de meetlocatie zich minstens 6.5mm van het vloeistofoppervlak bevindt om er zeker van te zijn dat er een stabiele meting verkregen wordt.

Wanneer de volumes bloed en/of medium in de bloedkweekfles verhoogd werden, duurde het langer vooraleer de sedimentatiefase op een bepaalde plaats ten einde kwam. Dit betekent dat groei in een cultuur langer ondetecteerbaar was. Wanneer alleen het bloedvolume verhoogd werd van 2ml naar 10ml, gingen de transmissie- en verstrooiingsintensiteit naar beneneden overheen de hele meetperiode. Dit beperkte het deel van het ingangsbereik van de sensor dat het signaal innam. Er moet dus een goede balans gevonden worden tussen bloedvolume, het beperken van de ondetecteerbare tijd en de intensiteit van de transmissie- en verstrooiingssensoren.

Het detecteren van vliesvorming aan het vloeistofoppervlak bleek niet mogelijk met de gebruikte meetopstelling. Hoewel er variaties in intensiteit van de signalen te zien waren, werden deze waarschijnlijk veroorzaakt door de verandering in turbiditeit van de vloeistof en niet door de vliesvorming.

Er moet rekening mee gehouden worden dat de testen uitgevoerd in deze masterproef slechts een bepekt aantal monsters gebruikten. Slechts zes bacteriesoorten werden getest overheen 22 monsters (niet gelijk verdeeld).

In de toekomst zullen er nog studies uitgevoerd worden door het SIMBLE-team [2] met de tweede generatie turbidimeter, terwijl er ook blijft gezocht worden naar mogelijke verbeteringen.

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FIGURES AND TABLES

ABBREVIATIONS

ADC: Analog-to-Digital Convertor
BCB: Blood Culture Bottle
BSI: BloodStream Infection
EMI: ElectroMagnetic Interference
FSM: Finite State Machine
I^2C: Inter-Integrated Circuit
ITM: Institute of Tropical Medicine
LMICs: Low- and Middle- Income Countries
PCB: Printed Circuit Board
SCL: Serial CLock
SDA: Serial DAta
SPS: Sodium Polyanethole Sulfonate
UGent: Ghent University
WHO: World Health Organisation

1 INTRODUCTION

A BSI occurs when bacteria get in the blood of a patient and start overpowering the immune system [5]. Because BSI are such a big problem it is important that it gets diagnosed and treated quickly, this increases the chance of survival of the patient [7]. Diagnosis of BSI relies on performing blood cultures. These cultures are incubated for several days and check for growth either manually or with an automated system. In LMICs diagnostic tools are often limited [8]**.** LMICs rely on manual blood cultures to diagnose BSIs which are less objective and less reliable than their automated counterparts.

To help improve this situation, Barbé, et al. [3] started development and testing (under the SIMBLE-project [2]) of a simple yet effective diagnostic device to detect BSI in blood cultures. This device, the turbidimeter, is specifically designed to operate in LMICs. The aim of this device is to make manual blood cultures more objective and ideally shorten the time to detection. It is currently in its second generation and will be tested in the field in the coming months. The main detection mechanism of the device is based on the turbidity (or cloudiness) of the sample, hence the name turbidimeter.

For this master's thesis, the objective was to give the turbidimeter some new functionalities and test them to see if they increased the diagnostic capabilities of the turbidimeter in a meaningful way. One of the additions was using multiple sensors at different heights to see if this affected the measuring results. Another additional feature was to try to detect more signs of growth other than turbidity, namely pellicle formation. To implement these features, two custom PCBs were designed, assembled, and tested. The second generation turbidimeter augmented with these new capabilities will be referred to in this thesis as the third generation turbidimeter.

This thesis starts by explaining some background information about blood cultures and the associated manual and automated detection systems in section 2. This section also sketches some of the challenges of performing blood cultures in LMICs. In section 3 some details about the first generation turbidimeter are described, like the essential components and operating principals. Only the information relevant to this thesis is explained. For the full evaluation of this first generation the reader is referred to the work of Barbé, et al. [3]. Section 0 focuses on the changes and results of the second generation turbidimeter. Sections 5 through 11 detail the work done for this master's thesis, designing, assembling, and testing the third generation turbidimeter.

2 BACKGROUND

2.1 Blood cultures

Blood cultures are the main way to detect bloodstream infections (BSIs) in patients [5, 9, 10]. They can indicate to a physician whether or not an infection is present [5]. With additional tests after the culture, the bacteria can also be identified and its resistance to antibiotics can be investigated [5]. This is all essential information for a physician to select the appropriate treatment [5].
To perform a blood culture, blood is drawn from a patient and incubated in a bottle containing a growth medium for several days [5]. During this time, it is checked periodically for signs of growth either manually or with the help of an automated system [5]. Different methods of detecting bacterial growth are explained further in section 2.2.

Figure 4: Example of a BCB right after inoculation (left) and after incubation (right).

2.1.1 Broth and Additives

A broth or growth medium is a liquid substance to which the blood is added. It contains the necessary nutrients and provides the right environment to facilitate growth. There exist multiple kinds of broth and some bacteria grow better in a specific broth than other ones. This means that there is no perfect growth medium. The choice of the medium depends on the situation. [5, 6]

The growth medium is not only there to provide nutrients to the bacteria. It is also used to dilute the blood. This is mandatory because there could be substances in the blood that prevent microbial growth. These substances are made as part of the human immune system or can be in the form of antibiotics that might have been taken by the patient. Diluting the blood also lowers the chance of clotting. The recommended dilution is around 5 to 10 times more growth medium than blood. [6, 11]
Another advantage of dilution is that the mixture becomes more transparent, which makes it easier to monitor changes in turbidity. Why this is important is explained in section 2.2.2.

There are several additives that can enhance the performance of the growth medium. These additives can, for example, provide a better environment for a specific type of bacteria or even fungi. Other additives can remove antibiotics from the sample. [5, 6, 12]
“One of the most important additives is sodium polyanethole sulfonate (SPS)” according to Ombelet, et al. [5]. The main purpose of this substance is to prevent the blood from clotting [5, 11]. Besides this, it also has a stabilizing effect on the microbial growth [5]. A disadvantage of SPS however, is that it can make it more difficult to culture some uncommon bacteria [11].

2.1.2 Incubation

When the blood is added to the broth, the sample gets transferred into an incubator. An incubator is a climate-controlled cabinet that regulates the temperature and in some cases the humidity of the air inside. The temperature inside the incubator is normally kept at around 35°C [5], providing the optimal climate for bacteria to start duplicating.
The sample is kept in this environment for several days to give the bacteria time to grow. The recommended incubation period is at most 5 days when using an automated system, and 7 days when using a manual system to detect growth [6, 13]. When growth is detected in a sample, the incubation is stopped, and further laboratory steps are taken to determine which bacterial strain caused the BSI and to which antibiotic it is susceptible. This information is important for treatment. [5]

2.2 Bacterial Detection in Blood Cultures

2.2.1 Commercial Automated Systems

Instead of placing the samples in an incubator and periodically checking them for signs of growth, many laboratories (especially in high-income countries) use automated systems to monitor the samples. These automated blood culture systems are all-in-one systems that incubate, shake, and monitor the samples in them simultaneously [5]. This has several advantages like more accurate and objective results, and less handling of the BCBs [11]. Since the systems monitor the sample continuously, the time to detection is also lower than with manual techniques thanks to embedded detection algorithms [5, 11]. These systems are however very expensive, require specialized maintenance personnel, and aren’t robust enough to operate in the tropical environment in LMICs [5]. These disadvantages mean that automated blood culture systems are less common in LMICs than their manual counterparts [5].

Several of these automated systems are made by different manufacturers. They can operate on multiple of the previously mentioned detection mechanisms. The three most common commercial systems will be explained further. These are the BD BACTEC (BD), BACT/ALERT (bioMérieux) and VersaTREK (Thermo Fisher Scientific) systems [12].

The BACTEC and BACT/ALERT BCBs are based on the same principle, the production of CO_2 by bacteria [6, 11, 12, 14]. Bacteria consume oxygen and produce CO_2. By measuring the amount of CO_2 present in a bottle, it is possible to detect their presence. Both the BACTEC and BACT/ALERT system do this by means of a CO_2 indicator at the bottom of the bottle. In the case of BACT/ALERT, the CO_2 indicator is actually a pH indicator that changes colour. Above this indicator sits a membrane that is only permeable to CO_2. When CO_2 goes through the membrane it reacts with the water present on the indicator side and produces hydrogen atoms. These

hydrogen atoms then cause a change in colour of the pH indicator. An automated system can then monitor the colour of this indicator and report on the presence of bacteria. [6, 12, 14]
The BACTEC BCBs also have a CO_2 indicator on the bottom but instead of a colour indicator, it is a fluorescent indicator. [6, 12]

Figure 5: pH sensors on the BACT/ALERT BCBs. Downloaded from the biomérieux-usa website [15].

The VersaTREK system operates differently than the other two. It tries to detect bacteria based on the concentration of gasses inside the BCB and what gasses are present [6, 11, 12]. The system can monitor the consumption of O_2 and the production of CO_2, H_2 and N_2 [6, 12]. When the concentration of these gases in the BCB change enough, the system will flag a bottle as positive [11, 12].

2.2.2 Manual Systems

Laboratories in LMICs don't always have the means to purchase and maintain the expensive automated blood culture systems [5, 16]. Instead, they use manual systems, but these are less objective than their automated counterparts. These systems are a combination of a static incubator and a BCB. These BCBs can be the same ones as in the commercially available automated systems, or they can be produced locally [17]. The bottles are checked periodically (at least daily) by a laboratory staff member for signs of growth [6]. Since no detection system is used, only visual signs of growth can be used to determine positivity of a sample.

The most important sign of growth is the turbidity of the sample. When a blood culture contains duplicating bacteria, it will get more turbid (or "cloudy") over time. However, a small increase in turbidity can be difficult to see when comparing a sample across several hours. It is also a subjective measurement because the turbidity threshold is determined by the staff member performing the measurement.

There are also some other signs of growth that can be exhibited by a blood culture: formation of gas bubbles or pellicle on the surface of the liquid, hemolysis, or puff balls forming inside the liquid [5, 17]. Five of these visual signs of growth are depicted in Figure 6.

Visual signs of growth (including turbidity) are not universal across all bacteria or fungi and depend on the organism being cultured. It is also possible that there is bacterial growth in a sample, but none of the visual signs listed above are present. Visual inspection might also be impractical due to the addition of resins or

activated charcoal, which occlude the sample on their own [5].

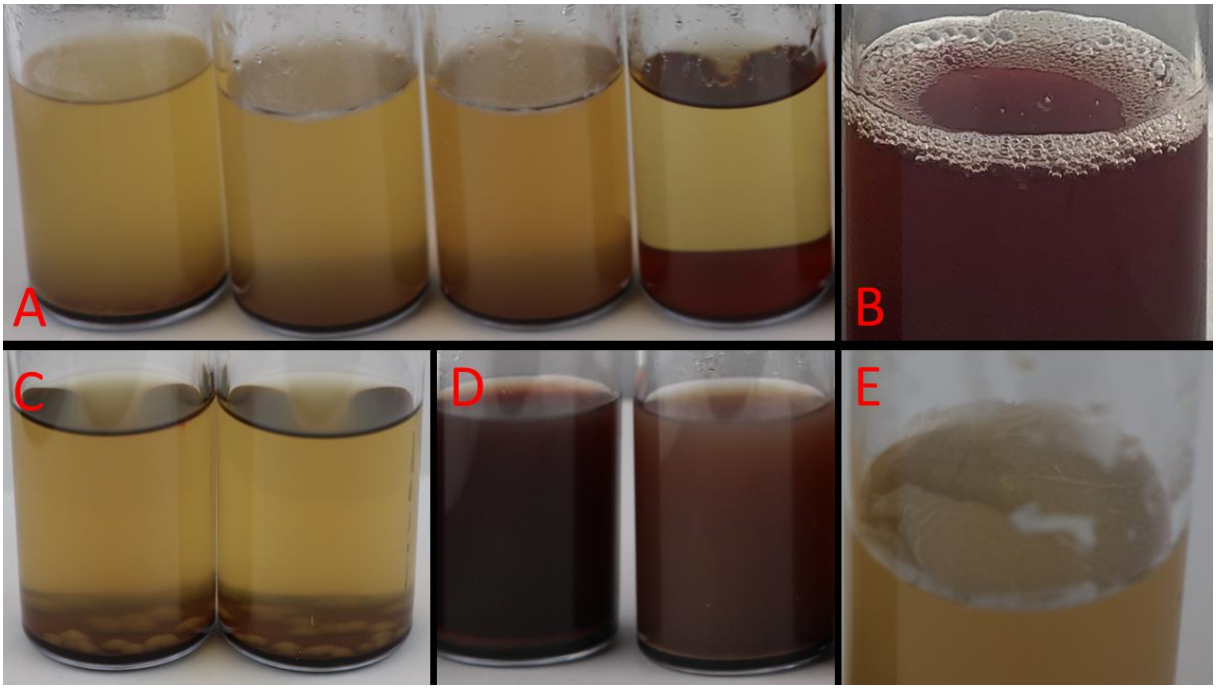

Figure 6: Five visual signs of growth: turbidity (A, 3 BCBs on the left versus a clear "blank" on the right), gas production (B), puff balls (C), hemolysis (D), pellicle formation (E).

2.3 Blood Cultures in LMICs: Challenges

A system designed to operate in a laboratory in LMICs has some specific challenges that need to be overcome. An obvious factor is cost. If a blood culture system has a high cost associated with each test, its likeliness to be used will go down. An optimal target is a cost per test under 5 USD [5, 18].

A second challenge is contamination of the blood sample. Contamination occurs when a bacterial species grows in a blood culture that was not present in the blood withdrawn from a patient's body. This contamination can have various sources, from the flora on the patient's skin to the bacteria present on dust in the environment. Not only is contamination reported to be higher in LMICs, but which bacterial species are considered a contaminant also vary around the world. [5, 18]

Another problem is the less regulated use of antibiotics. In LMICs, antimicrobial agents are often more readily available. Because of this, antibiotics can be present in a blood sample that is meant to be cultured. The presence of these compounds can inhibit microbial growth and thus increase time to detection or even prevent detection. An ideal system would have ways of decreasing the concentration of antibiotics in the blood culture or even completely removing it. [5, 18]

Environmental factors also have to be considered when designing a blood culture system. In many LMICs there is a higher humidity and temperature. These can affect the performance and lifespan of the system. Often

there is also more dust in the air, which can hinder sensing equipment further. The measurement system should therefore be made robust enough, so it can withstand these conditions. An optimal system would be able to operate between 5°C and 40°C and in a 90% relative humidity environment. It should also be able to operate at 3km above sea level. These requirements were composed by Dailey, et al. [18] by interviewing 9 specialists. [5, 18]

A last requirement of such a system is user-friendliness. Laboratory staff in LMICs are not always trained a lot for various reasons, like low staff retention time [5, 18]. This means that a system designed to operate in LMIC laboratories should be intuitive to operate and require a minimal amount of training [5, 18].

The challenges above are only a limited selection. Ombelet, et al. [5] as well as Dailey, et al. [18] have compiled a more extensive list of challenges pertaining to blood cultures in LMICs.

3 FIRST GENERATION TURBIDIMETER

3.1 Operating Principles

Around July of 2021, development of the turbidimeter was started by a team with members from both ITM and UGent. The goal was to remove the subjectivity associated with manual blood cultures. These manual blood cultures are often based on a visual inspection of the turbidity of the sample, like mentioned in section 2.2.2. The turbidimeter operates on the same principle, but it measures the turbidity of the sample objectively by using sensors. This means that the turbidity can be quantitatively plotted over time and can be compared across samples.

The earliest version of the turbidimeter consisted of two measuring systems. The first system is the turbidity measurement. The system determines turbidity of the liquid inside a BCB by looking at how much light got transmitted through it and how much light gets scattered. To measure the turbidity of the liquid, a combination of nine LEDs and nine photodiodes was used. Each LED had a corresponding photodiode that was placed on the opposite side of the BCB. The resulting nine pairs of LEDs and photodiodes were arranged in a ring around the BCB. To accomplish this, the components were placed on a flexible PCB, which had a connector on one side so it could connect to a motherboard. Not all sensors and LEDs were used in a single measurement. They were mainly there to provide flexibility during the prototyping phase, by allowing the angle between the LED and photodiode to be changed easily without hardware changes.

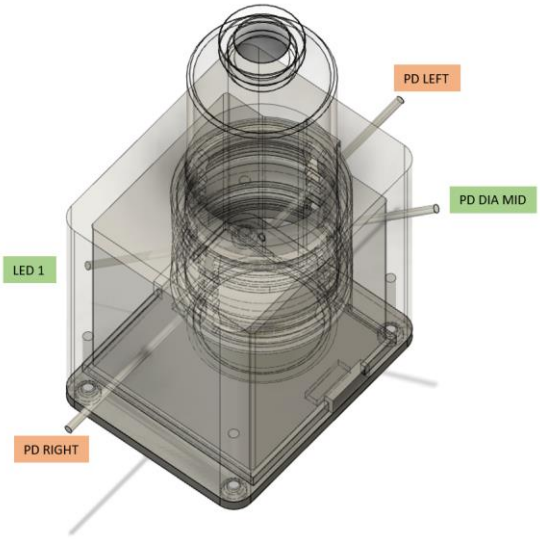

Figure 7: A possible measuring setup with the first generation turbidimeter. An LED shines light on 3 photodiodes that are mounted at different angles relative to the LED.

The second measuring system was based on the colour-changing CO_2 sensor in commercial BACT/ALERT BCBs. To measure its change in colour, an RGB-colour sensor was used in combination with four white LEDs to illuminate the pH indicator.

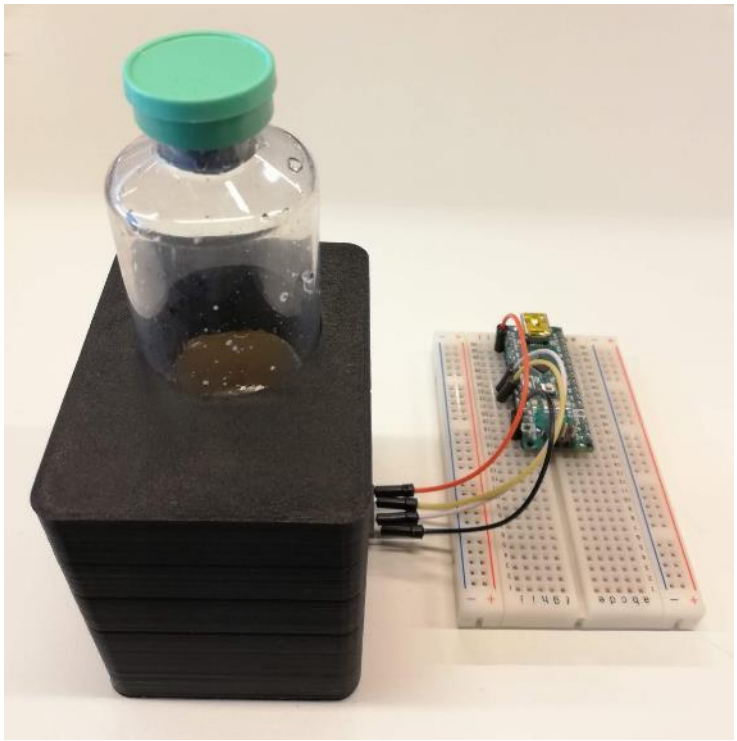

Figure 8: First generation turbidimeter with BCB inside.

3.2 Main Components

3.2.1 Microcontroller

To control all the various components of the prototype, an Arduino Nano development board was used. This board has an ATmega328 microcontroller, 32kB of flash memory, 2kB of SRAM, 16 MHz clock speed, 22 digital I/O pins and 8 analog pins [19]. The digital I/O pins were connected to a motherboard that connected all the various components together. The Arduino Nano was also responsible for reporting the results to a computer. This computer would be connected to the Arduino by USB. The computer could than issue measure commands the Arduino and receive the results back over a serial connection.

The communication between the microcontroller and the components on the motherboard happened via I²C (Inter-Integrated Circuit). This is a simple two-wire protocol that is relatively easy to implement. Many off-the shelf components use this protocol for their communication. The two wires consisting of an SDA (Serial DAta) and SCL (Serial CLock) line, act a bus to which all of the members are connected. One of these devices (in this case the microcontroller) controls al of the communication on the bus. Communication speed can be set from 100kbps up to 3.4Mbps.

3.2.2 LEDs

There were two kinds of discrete SMD LEDs in this prototype. White LEDs to illuminate the pH indicator, and amber LEDs to do the turbidity measurements. The amber LEDs had a peak wavelength of 598nm and were made by Inolux [20]. The white LEDs were 4000K LEDs made by Luminus [21]. The white light enabled the RGBC-sensor to give a more accurate representation of the colour of the pH indicator than if another colour was used.

3.2.3 Photodiodes

The photodiodes on the flexible PCB were discrete silicon photodiodes. To read their signal value, an OpAmp circuit was used to form a transimpedance amplifier. The schematic of this configuration can be seen in Figure 9. The non-inverting input of the amplifier is connected to ground, this means that the photodiode operates in photovoltaic mode. This has the advantage of having zero dark current running through the diode, linear operation and lower noise compared to photoconductive mode. The capacitor C12 is used to ensure stable operation of the amplifier.
The output of this amplifier circuit is connected to a dedicated analog-to-digital convertor (ADC), the MAX11611 by Analog Devices. It has 12 input channels to which the nine amplifier outputs are connected. Three of the inputs are left unconnected. The ADC has a 10 bit resolution and can be read by the microcontroller via I²C. [22]

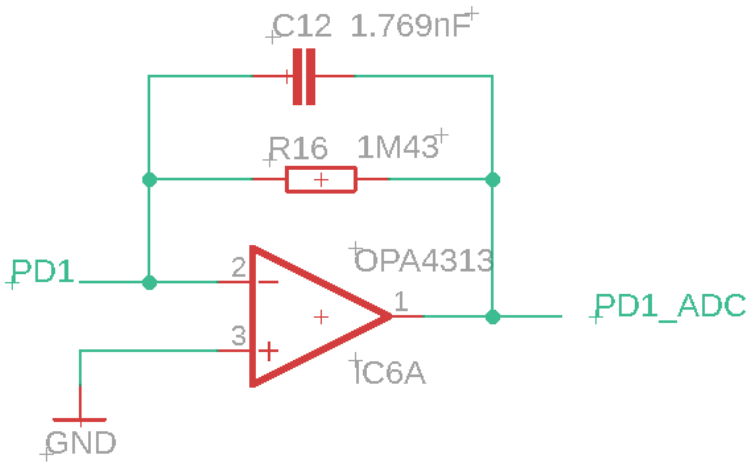

Figure 9: Transimpedance amplifier photodiode in first generation turbidimeter.

3.2.4 RGB-Sensor

The RGB-sensor used for the colour sensing of the pH indicator was a TCS3772 sensor from ams. It had an array of 4x3 pixels to do colour measurements (3 pixels for red, green, blue, and clear) and stored these measurements as 16-bit numbers in an internal register. It could also use 4 more photodiodes to do proximity measurements when combined with an external IR-LED [23]. This was however not implemented in this prototype system. To read the measurement data from the sensor the I^2C protocol was used.

3.3 Results

To evaluate the performance of the first version of the turbidimeter, 60 growth experiments were done with 10 different bacterial strains by Barbé, et al. [3]. In these experiments, the turbidimeter was compared with an automated reference system. The results showed that, in 7/10 species tested, the turbidimeter performed equal to or better than the reference system. These results were mainly due to the colour measurement of the pH indicator in the bottle. When looking at only the turbidimetric detection rates, growth of four out of ten species could be detected. [3]

4 SECOND GENERATION TURBIDIMETER

The first generation turbidimeter had some problems with reliability and robustness so the UGent/ITM team started development of a second generation in 2022. Several changes to the design were made but the device was still based on the same operating principles, namely the turbidity measurement and colour of the pH indicator. In the next section, the changes that are relevant to this thesis are listed.

4.1 Changes

The Arduino Nano microcontroller was replaced with a PAN1780 module which is based on the nRF52840 microcontroller. The PAN1780 is superior to the Arduino Nano in several ways: it has 1MB of flash memory, 256kB of RAM, 64MHz clock speed and has Bluetooth communication build-in [24, 25]. With these specifications more advanced software could be created to improve the turbidimeter.

Some custom PCBs were made by the team to join all parts of the turbidimeter. The first PCB is the main board. It consists of the microcontroller, power system, indicator LEDs and 3 connectors to insert other PCBs. These connectors are used to connect vertical daughterboards that have the necessary LEDs and sensors. This way, multiple variations on the measuring system can be tested without needing to redesign a new motherboard. The connectors expose 3,3V power, ground, and the I²C bus to the daughter boards. The PCBs made to fit in these connectors are a combined emitter/detector board. This board has connection points for all the necessary parts for the emitter and the detector. The function of the board is then determined by which components are placed on it. Figure 10 shows the board in its detector configuration (left) and its emitter configuration (right) and marks the main components.

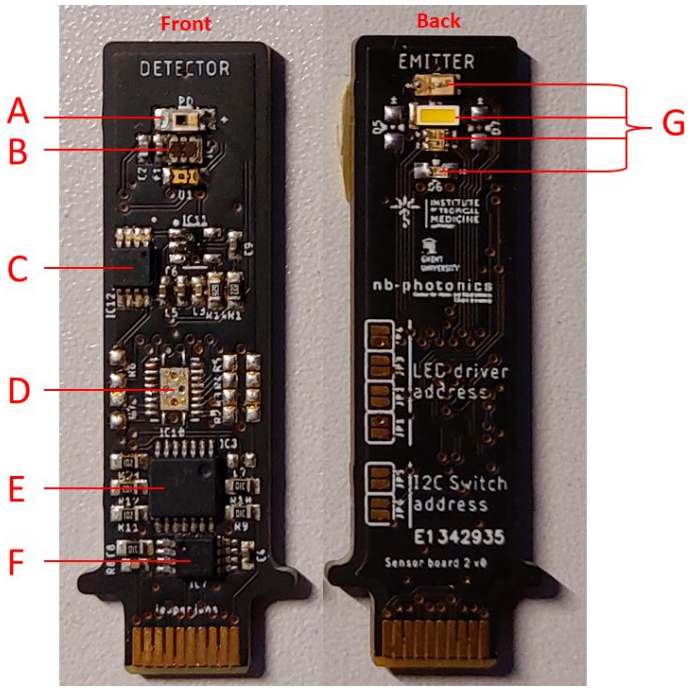

Figure 10: Second generation turbidimeter detector/emitter PCB. Populated as detector (left) and as emitter (right). Photodiode (A), RGBC-sensor (B), ADC (C), LED driver (D), I²C switch (E), I²C buffer (F), LEDs (G).

When the board is used as an emitter the main components are 4 different LEDs, an LED driver, and an I²C buffer. The four LEDs each have a different colour. This was done to try to cover a large part of the visible spectrum. This way, the effect of the light colour on the measurements could also be tested. The work of McBirney, et al. [26] has shown that looking at multiple wavelengths could be advantageous. Another change comparted to the first version, is that the LEDs are no longer placed in a ring around the bottle but are placed close to each other at around 38mm from the bottom of the BCB. Figure 11 shows this light path schematically. The LEDs get driven by a constant current LED driver. For this, the TLC59108 LED driver from Texas Instruments is used. This driver has eight channels that can be individually controlled by an internal register. This register can be written to via I²C communication. It also has a configurable I²C address by means of address pins on the chip. The I²C lines go from this chip via an I²C buffer trough the connector to the motherboard. The I²C buffer, the PCA951A, makes hot swapping of the daughterboard possible. This is necessary because hot swapping these boards without the buffer could lead to disruptions on the I²C lines.

When the board is in the detector configuration, the main components are: an RGBC-sensor, an I²C switch and the previously mentioned I²C buffer. The RGBC-sensor is the same one as in the first version, namely the TCS3772. Because the address of this sensor is not configurable, communication conflicts would arise when they are placed on the same bus. To circumvent this problem an I²C switch is used to connect and disconnect the sensors to and from the main I²C bus.

Figure 11 shows the PCBs of the second generation turbidimeter in their assembled state, including the main board.

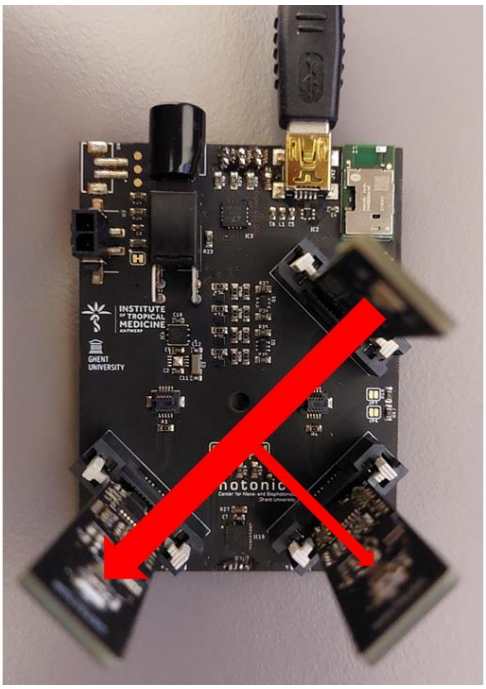

Figure 11: Top view of second generation turbidimeter without case. The red arrows are a schematic representation of the light traveling from the LED to the transmission and scatter sensors.

4.2 Results

4.2.1 Characterising Scattering and Absorption

The work performed in this master's thesis started by testing the second generation turbidimeter to see if the transmission and scattering sensors would behave as expected. The objective was to see what effect absorption and scattering had on the measuring results. For this, blood culture bottles were filled with diluted milk to vary the amount of light that is scattered and absorbed by de fluid. Several runs were then performed to see the impact of each of the concentrations of milk.

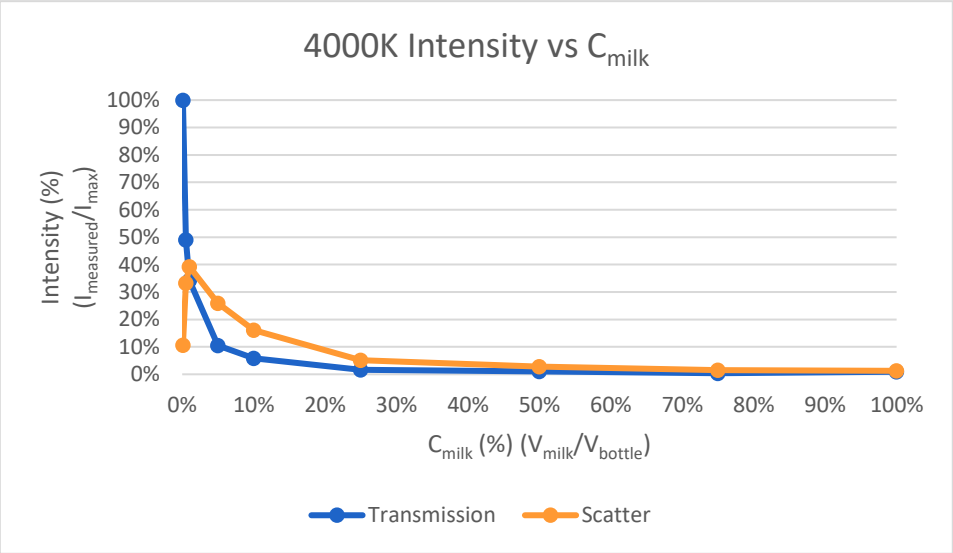

Figure 12: Measured transmission and scatter intensity vs milk concentration in sample.

Figure 12 shows the effect of the milk concentration on the measured transmission and scatter intensity. It can be seen that the transmission immediately goes down in an exponential manner. For example, when going from a milk concentration of 0,1% to 0,5%, the measured transmission intensity drops by 51%. Part of this drop is caused by the absorption of light by the milk, which keeps increasing as the milk concentration increases. But looking at the scattering results shows that part of the light does get scattered by the milk because there is an increase in the intensity between 0% and 1% concentration. When the milk concentration goes above 1% in these tests, the scatter intensity also drops in an exponential way like the transmission measurements. This is most likely caused by the increase of the absorbance of the sample which prevents more light from scattering.

4.2.2 Testing on bacteria

The second generation turbidimeter developed by the SIMBLE project group [2] is still being tested by the team. For the last couple of months many tests have been run with the turbidimeters at ITM. None of these results have been published yet, but this will happen in the future. On top of the laboratory tests, two field studies will be performed with the turbidimeter. These tests on bacteria with the second generation turbidimeter are not part of this master's thesis. The preliminary results however show that the second generation turbidimeter can detect more bacterial species than its first generation.

5 THIRD GENERATION TURBIDIMETER

The main goal of this thesis was to develop and test a third generation turbidimeter. This included designing and assembling new PCBs as well as updating the software for the microcontroller. The third generation turbidimeter serves as a test vehicle to see where future improvements could be made and to test them. One of these improvements is measuring turbidity at different heights. This might be beneficial because light does not get scattered, absorbed, or transmitted the same throughout the bottle. This means that there might be places where a change in turbidity can be detected more easily or faster. This would mean that bacteria can be detected earlier in some locations rather than others.

To test the effect of the measuring height on the turbidimetric measurements, new vertical boards had to be designed. The schematic and PCB layout of these boards are provided in appendix A through C.
The original PCB design of the second generation turbidimeter was a combined PCB for the emitter and detector, and the components placed on it determined its function. For the new design, two separate PCBs were designed, one for the detector and one for the emitter. Each PCB has eight LEDs or sensors placed at the following heights with the bottom of the BCB as a reference: 20mm, 25mm, 30mm, 37.5mm, 45mm, 55mm, 65mm and 75mm. The tested measuring heights are more concentrated towards the bottom of the BCB because it was expected that the most useful information would be concentrated here.

5.1 Emitter

The emitter consists of eight LEDs that are driven by a TLC59108 LED driver from Texas Instruments. These LEDs and the LED driver are the same components that were used for the combined emitter/detector board previously. It has eight constant current output channels and communicates with the I^2C-protocol. As with the previous emitter/detector board, a PCA951A I^2C buffer was placed before the driver to provide hot-swap capability to the board.

5.2 Detector

In previous versions, the detector used a TCS3772 RGBC-sensor made by ams but due to availability issues, a different sensor had to be selected. The TCS3400 was chosen as a replacement because it has the same spectral sensitivity, the same pinout, uses I^2C and is made by the same manufacturer. Eight of these sensors are used on each detector board. But because all of these sensors have the same I^2C address, an I^2C multiplexer has to be used. This multiplexer ensures that only one sensor is on the main I^2C bus at a time. The multiplexer selected for this purpose is the PCA9547 by NXP mainly because it has eight channels and has a configurable I^2C-address. This configurable address is a requirement because there are two detector boards used in the system and they need to be individually addressable. A PCA951A I^2C buffer is used on the detector board too to give it hot-swap capability, like the emitter.
Finally, a TMP116 temperature sensor was added to be able to measure the temperature inside the turbidimeter. This feature was however not used in this master's thesis but will be useful for future use.

5.3 PCBs

The PCBs were made so that the width of the detector and emitter boards is the same as in the second generation emitter/detector board. This way, the PCBs would still fit in the already existing 3D-designed turbidimeter case with only minimal modifications. This did however limit the available space for the components severely. For the emitter PCB this wasn't a problem because there aren't a lot of components on this board. The detector however has eight I²C sensors which all have separate SDA and SCL lines coming from the multiplexer. Each of these traces also needed a 1kΩ pullup resistor. These 16 traces and their resistors took up a lot of the available space when put next to each other. Especially the width was a limiting factor. The multiplexer was instead put in the middle of the PCB to avoid this problem. This way, half of the traces could be routed up and half could be routed down so they take up half as much of the width.

The ground plane covers the top and bottom of the PCB. Care was taken to avoid separating parts of this plain with traces. This has the purpose of maintaining a stable and equal ground potential throughout the PCB. When there was space available, bridging vias were placed around the traces made cuts in the ground plane. This way, a current can go under a trace instead of having to go around it. This avoids EMI problems and makes sure no voltage potential can build up between both sides of the trace.

6 ASSEMBLY

Testing was done with two turbidimeters, this meant that two emitter boards and four detector boards had to be assembled. The two required main boards were already available since they are the same as in the second generation turbidimeters. The work for this thesis also included assembling these six boards. Only the manufacturing of the blank PCBs for the emitter and detector boards was outsourced to Eurocircuits. The further assembly was done in-house at iGent, located in the Tech Lane Ghent Science Park. When the boards were received from Eurocircuits, solder paste was first applied to the PCBs by means of a stencil. The components were then placed on the PCBs with the help of a manual pick-and-place machine. Following that, the PCBs were placed inside a reflow oven to let the solder paste melt. The six finished boards are shown in Figure 13.

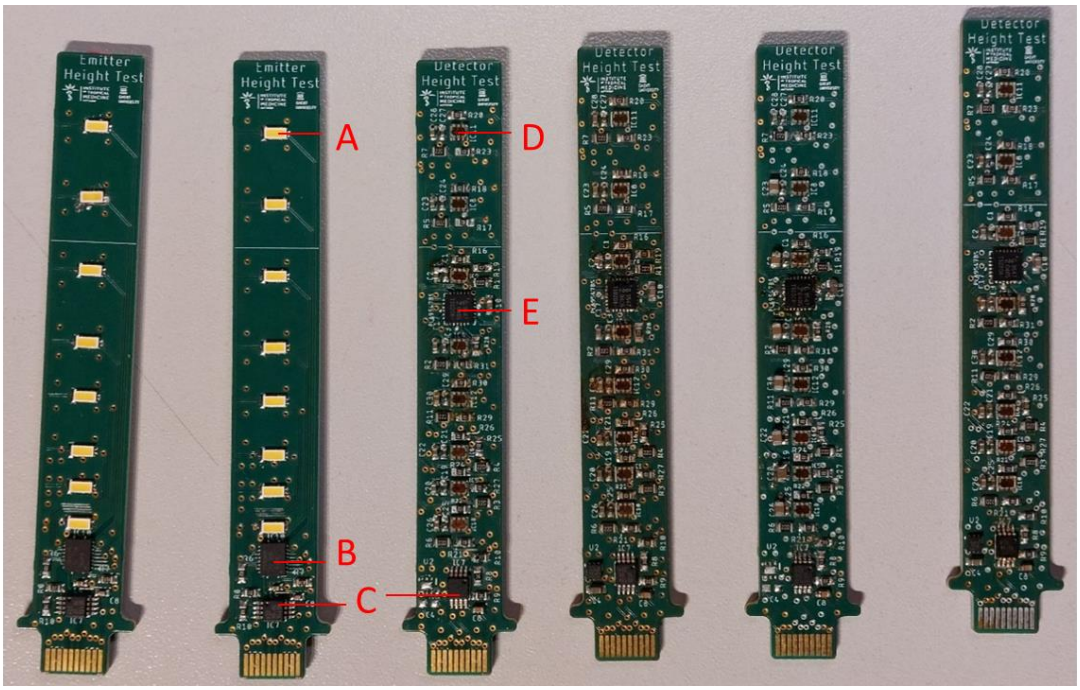

Figure 13: Assembled PCBs. Two emitters (left) and four detectors (right). LED (A), LED driver (B), I²C buffer (C), RGBC-sensor (D), I²C multiplexer (E).

Some defects were found when testing the boards. Most of these were due to components that weren't properly connected to their pads. The bad connections happened in the same place across several PCBs and only on the detector boards. The reason is a design flaw in the custom PCBs. Vias were placed in the same place as some of the connection pads to save space on the PCBs, as shown in Figure 14. This caused the solder to flow inside these vias during the reflow process instead of making a connection with the component. The components that suffered the most from this problem were the RGBC-sensors and the multiplexer because their pads are small and thus contain little solder paste. Vias were also placed on the connection pads of some resistors and capacitors, but they experienced no connection problems on these four boards. The problem for the unconnected sensors and multiplexers was solved by using more solder in these places to saturate the vias which allowed the rest of the solder to connect with the components. In the future however, a redesign of the PCB would be a more practical solution.

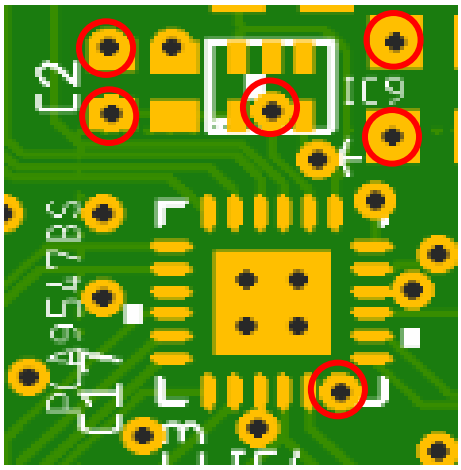

Figure 14: Zoomed in view of 3rd generation detector PCB around multiplexer. The red circles show problematic vias.

7 SOFTWARE

The software for the nRF52840 microcontroller was also updated as part of this master's thesis so that it would be compatible with the new daughterboards. Care was taken to make sure it would also still work with the second generation emitter/detector board. The software built for the second generation turbidimeter by the SIMBLE-team [2], is based on the principle of a finite state machine (FSM). An FSM has a predefined number of states it can be in. This makes development easier and improves reliability because the program flow is easy to follow, and each state has a very specific function. The states that this software can be in, are shown in Figure 15 as well as the possible transitions between the states. The FSM was not made as part of this thesis, but the states were modified so that it would be compatible with the new measuring setup. There is no difference in the number of states between the second and third generation of the turbidimeter. The only difference is the amount of emitter profiles and what sensors and switches get activated, because they have different hardware.

The red states in Figure 15 are states that are disabled. These states make the bottom sensor (for the pH indicator) and the photodiodes work. These were not necessary in the third generation of the turbidimeter because the hardware isn't present in this version.

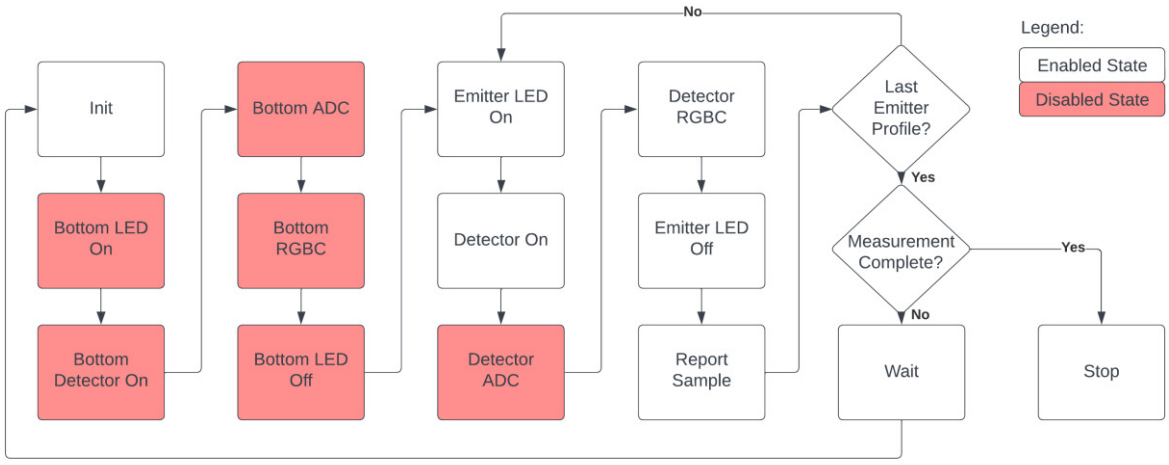

Figure 15: Flowchart of program flow turbidimeter.

The init state prepares the turbidimeter for a measurement cycle. When in this state, the microcontroller clears all registers that hold measurement results and determines which emitter profiles are active.
Emitter profiles are a collection of settings that define a measurement. These settings include which LEDs have to activate, which sensors have to measure, the name of the profile and whether or not the profile is active.
The next five states are meant for the bottom colour sensor that isn't present in the third generation, but they work the same way as the other sensors.
In the seventh state, "Emitter LED On", the LEDs corresponding to the first emitter profile get activated. After that, the program waits 100ms to give the LEDs time to ramp up and get to their maximum brightness.
Next, the detectors get configured and activated. Detectors in this case refers to the RGBC-sensors that are defined in the emitter profile. As previously mentioned, the configuration of these sensors happens over I^2C by setting some registers to the right values. In the case of the TCS3400 sensor, used in the third generation turbidimeter, this would first be register 0x81. This register sets the integration time of the sensor, which

should be set to its maximum to detect the maximum amount of light and to minimize the effect of noise. 100ms later, the value 0x03 is written to register 0x80, which enables the sensor. The wait time of 100ms is necessary to give the TCS3400 time to change the integration time setting. When all of the sensors are enabled, the program waits 700ms to give the sensors time to finish their measurement. Then it transitions to the next state. Only one sensor can be enabled in each emitter profile to keep the number of output samples fixed.
When in the ”Detector ADC” state, the program reads a register in the OpAmp amplifier that is paired with a photodiode. These two components are however only present on the second generation of the turbidimeter, so this state is disabled for the third generation that is used for this master’s thesis.
In state ten, the result registers are read on each of the previously enabled RGBC-sensors. For the TCS3400 these are registers 0x94 to 0x9B. There are eight results registers per sensor because there are four kinds of measurement (red, green, blue and clear) and each result is placed in two 8-bit registers. These results are copied from each sensor to the RAM memory of the microcontroller.
The next state, “Emitter LED Off” has the sole function of turning off all LEDs on the vertical emitter board. When this is done, the program waits 100ms again to make sure the LEDs have turned off completely.
The next state reports the results obtained from the sensors back to the user via the USB serial interface. For the third generation turbidimeter, the results are reported in the format shown in Table 1. All of the values are sent as a single string with commas between each value.

Table 1: Data structure of the information sent over the USB-interface.

General			Bottom Board					Transmission				Scattering				General
Board id	Sample number	Profile name	Red	Green	Blue	Clear	Photo-diode	Red	Green	Blue	Clear	Red	Green	Blue	Clear	Sensor id

These results are read by a logging program on the connected computer so the results can be analysed later. When the sample is reported, the program checks if there are any more enabled emitter profiles left. If so, it goes back to “Emitter LED On” state and continuous with the settings described in that profile. If there are no more emitter profiles left, it compares its current runtime with the runtime configured by the user. If the runtime exceeds the configured runtime, the FSM stops. If not, then the FSM goes back to the init-state after waiting a certain amount of time that gets configured by the user.

8 METHOD

Tests with the third generation turbidimeter were divided into 4 parts. The first test was used to confirm the functionality of the device before the testing on bacteria began. This was necessary because of the change in design, software, and the use of a new RGBC-sensor. The second, third and fourth parts of the testing were conducted at the Institute for Tropical Medicine (ITM) in Antwerp. In all of these tests the turbidimeter was configured such that measurements at different heights were done one by one, from the bottom to the top. Measuring at the 8 heights took 12.8 seconds and a period of 26.4 seconds was left between two measurement cycles. A diagram of the measuring setup is shown in Figure 16.

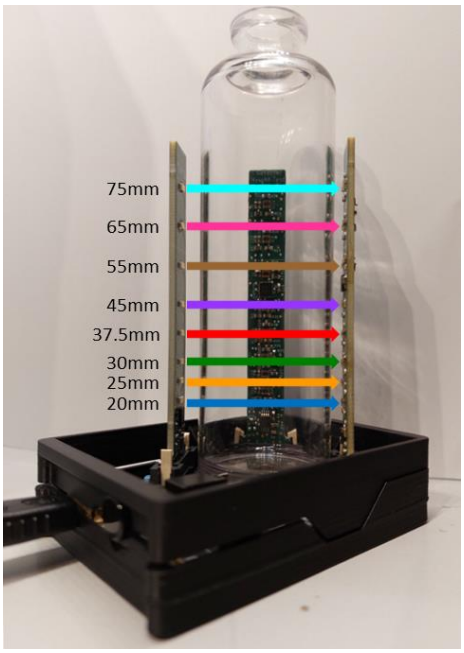

Figure 16: Diagram of the first measuring setup.
The colours of the arrows correspond to the colours of the graphs in the results.

Since there are only two third generation turbidimeters, the testing happened over five weeks with one or two samples being tested at a time. The data from these tests was logged by a computer connected to the turbidimeters. To examine the data and plot graphs, a python script was used.

As mentioned above, the first test was to confirm the functionality of the device. To do this, part of the tests with milk mentioned in section 4.2.1 were redone. Three different bottles were tested filled with a mixture of tap water and semi-skimmed milk. One bottle had no milk, one was filled with 1% milk (by volume), and one was filled with 2% milk. Each BCB was measured for 1 hour and its data was averaged to get one value for each sensor in the turbidimeter. Results could then be compared with the previous turbidimeter generation.

The second part of the tests were focused on determining the effect of measuring height on the blood culture measurements. Eight blood cultures were set up with 30ml of tryptic soy broth to which 0.3mg/ml of SPS was added. 2ml of human blood was used to inoculate the sample. The human blood was kindly donated by several volunteers at ITM (ethics approval was received for sampling of blood). The blood of the volunteers spiked in 7 out of the 8 bottles with a certain bacterial strain (*Escherichia coli*, *Salmonella* Typhimurium, *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Burkholderia cepacia*), to mimic blood samples from infected patients. An overview of the tested samples is given in Table 2.

Table 2: Part 2 of testing N° of samples, bacterial strain blood/broth volume and runtime.

Number of Samples	Bacterial Strain	Broth Volume (ml)	Blood Volume (ml)	Runtime (h)
1	None (blank)	30	2	20
1	*Escherichia coli*	30	2	20
1	*Salmonella* Typhimurium	30	2	20
1	*Streptococcus pneumoniae*	30	2	36
2	*Staphylococcus aureus*	30	2	44
2	*Burkholderia cepacia*	30	2	44

The third part of testing examined the effect of sample volume. Eight more BCBs were filled with 2 different blood-to-broth ratios and were spiked with 2 different bacterial strains. This together with two samples from the previous part gave three different blood-to-broth ratios and two different bacterial strains to compare against each other one blank for each blood-to-broth ratio. The blood and broth volumes as well as the bacterial strains are listed in Table 3.

Table 3: Part 3 of testing, N° of samples, bacterial strain, broth/blood volume and runtime.

Number of Samples	Bacterial Strain	Broth Volume (ml)	Blood Volume (ml)	Runtime (h)
1	None (blank)	30	2	20
2	None (blank)	40	2	24
2	None (blank)	40	10	72
1	*Escherichia coli*	30	2	20
2	*Escherichia coli*	40	2	20
2	*Escherichia coli*	40	10	20
1	*Salmonella* Typhimurium	30	2	20
2	*Salmonella* Typhimurium	40	2	20
2	*Salmonella* Typhimurium	40	10	20

The goal for the fourth and final part of the testing was to see if features other than turbidity could be detected with the third generation turbidimeter. Particularly the formation of pellicle on the surface of the blood/broth mixture was investigated. To measure this, two extra measurement profiles were added. These measured diagonally through the surface by activating the right LED and sensors. For one of these extra measurements, LED 3 was activated, and the light measurement was done by RGBC-sensors 5. For the other one, LED 5 was activated and sensors 3 did the measuring. The complete emitter profile is listed in appendix E.
This test consisted of two BCBs spiked with *Pseudomonas aeruginosa*. The two cultures consisted of 30ml of broth and 2ml of blood. These volumes were chosen so the liquid-air interface would end up between 37.5mm and 55mm.

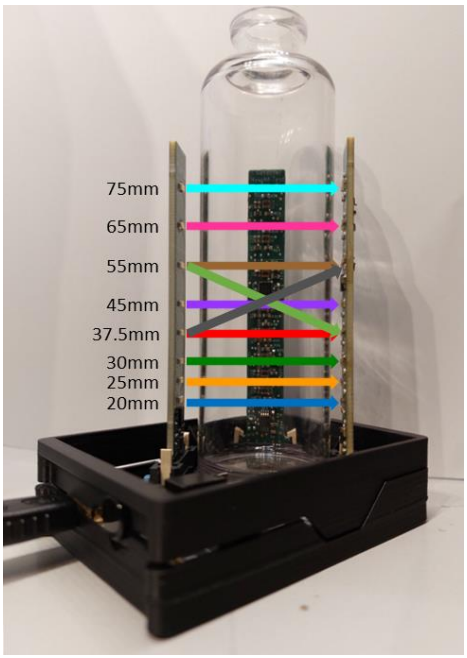

Figure 17: Diagram of the second measuring setup. The green arrows show the two extra measurements. The colours of the arrows correspond to the colours of the graphs in the results.

Table 4: Part 4 of testing, N° of samples, bacterial strain, broth/blood volume and runtime.

Number of Samples	Bacterial Strain	Broth Volume (ml)	Blood Volume (ml)	Runtime (h)
2	*Pseudomonas aeruginosa*	30	2	70

9 RESULTS

9.1 Tests with Phantoms

The results of the tests with a varying milk concentration can be seen in Figure 18. The same trends can be seen as the ones mentioned in section 4.2.1, where the same test was performed on the second generation turbidimeter. The transmission intensity goes down with an increasing milk concentration because of the increase in scattering and absorption of the milk. The increase in scattering can be seen on the bottom part of the graph. Between 0% and 1% there is an increase in scattering intensity. At 2% milk concentration, the scatter intensity begins to drop because the absorption of the milk starts to be the dominating effect. This peak at 1% milk concentration on the scatter graph was also present in the tests with the second generation turbidimeter. From these results it can be concluded that the third generation turbidimeter works as expected. It should be noted however, that because of the change in sensor, no direct comparisons can be made between the third and second generation turbidimeter in terms of absolute intensity. Only relative changes in measurements can be made.

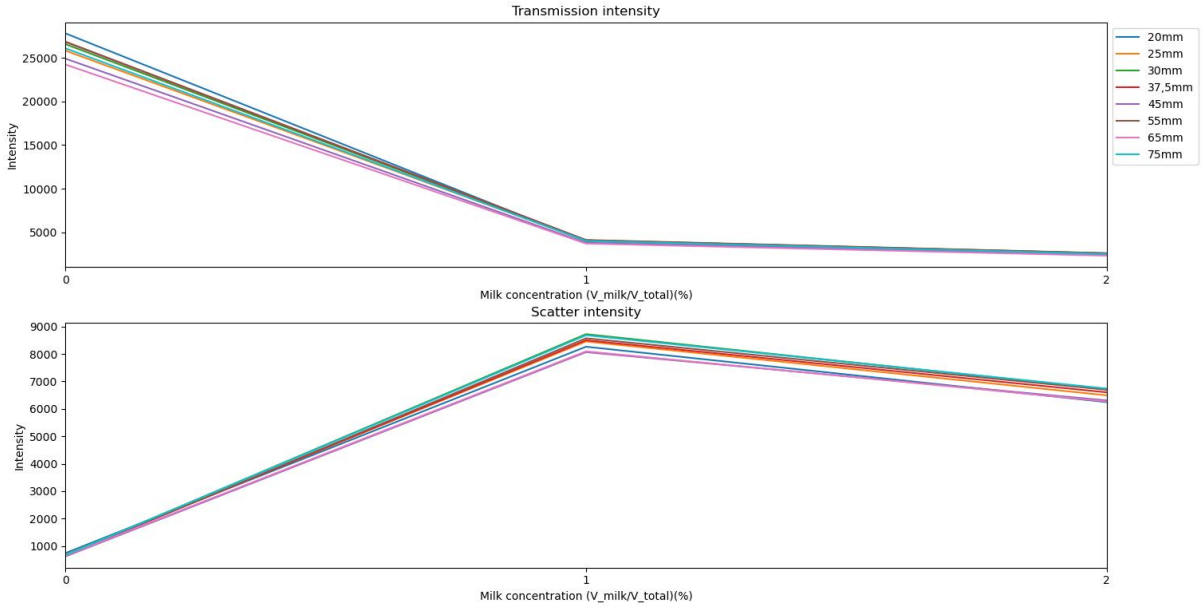

Figure 18: Transmission and scatter intensity vs. milk concentration.

9.2 Tests at ITM

The tests conducted at the Institute for Tropical Medicine used BCBs containing a mixture of broth and blood spiked with bacteria. The results will therefore be in the form of bacterial growth curves. These curves traditionally consist of four bacterial growth phases [4]. The results of the tests done here will show the same four phases. The first bacterial phase is the lag phase where no change in the growth curve can be seen because the number of bacteria stays relatively constant [4]. Next is the log phase or growth phase. In this phase, the number of bacteria grows exponentially and signs of growth start showing (in this case an increase in turbidity) [4]. After this comes the stationary phase, this is where the bacterial concentration has reached its maximum [4]. This maximum is caused by the limited availability of nutrients in the BCB and thus capping the population [4]. The last phase is the death or mortality phase [4]. In this phase, the number of bacteria start to decline because nutrients have run out and the medium can't sustain the current bacterial concentration anymore [4].

To make growth curves, the concentration of bacteria inside the medium should be measured over time. However, this is not what is measured here. The turbidimeter measures the turbidity of the medium and thus not the microbial concentration directly. Because of this, the growth curves shown here might not exactly line up with the actual growth curves in time or intensity, but they should show the same phases. There will also be an additional phase before the lag phase (unrelated to bacterial growth), the sedimentation phase. During this phase, some particles within the blood will fall to the bottom of the BCB and remain there as sediment.

9.2.1 Effect of Measuring Height

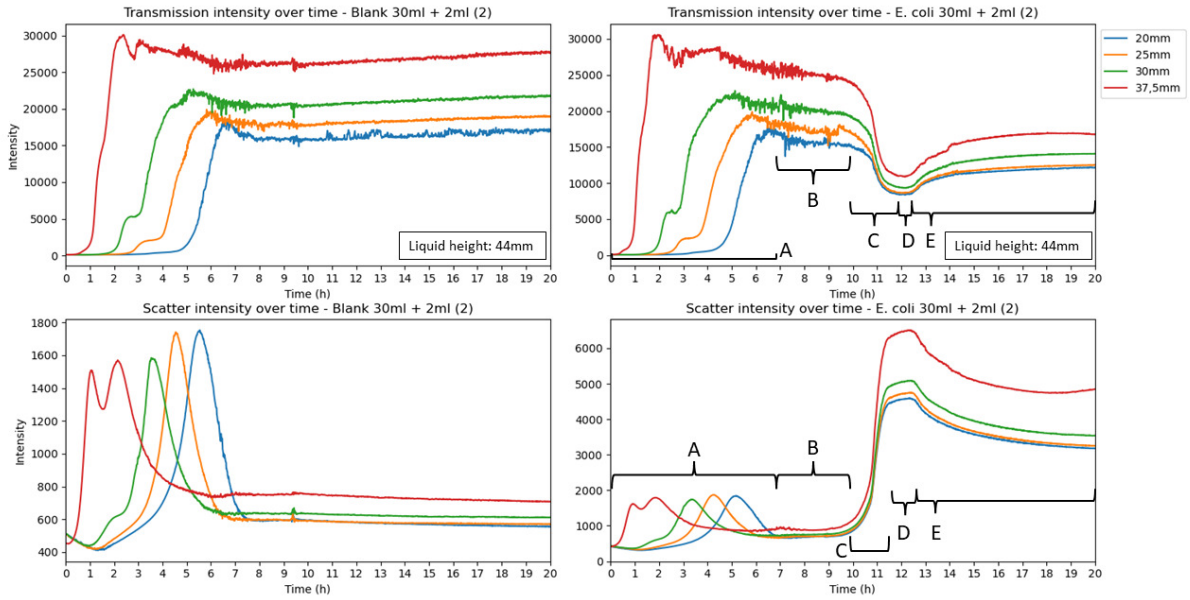

Figure 19: Transmission and scatter measurements at 20mm, 25mm, 30mm and 37.5mm height. The left graphs are of a blank (not spiked) BCB, the right graphs are from a BCB spiked with E. coli. Sedimentation phase (A), lag phase (B), log phase (C), stationary phase (D), mortality phase (E).

Figure 19 shows two results from the second part of testing. On the left are the transmission (top) and scatter (bottom) results from the blank test sample. On the right are the transmission and scatter results from the sample spiked with *E. coli*. The graphs only show the bottom four measurement locations because these are the ones that measure through the liquid (when using this sample volume). The four sensor locations above this are above the liquid in the BCB.
In the first couple of hours, the transmission and scatter intensities rise for both samples. This is the result of sedimentation. There are less particles in front of the sensors at the end of this phase so the transmission intensity rises because more light can get through the liquid. The decrease in particles also leads to an increase in scattering which is a bit counterintuitive. The reason for this is that the decrease in absorption has a greater effect on the measurements than the decrease in scattering. When the two effects are combined this leads to an increase in scattering instead of the expected decrease.

The progress of the sedimentation throughout the BCB can be clearly followed by the transmission and scatter peaks on the graphs. In the transmission graphs, the end of the sedimentation phase is indicated by the first sharp increase in transmitted intensity. After this, a small decrease is visible, particularly at a height of 37.5mm. In the scatter graphs, the sedimentation phase shows a sharp increase in intensity at first because the absorption of the sample is going down. This is followed by a sharp decrease in intensity due to the scattering particles in the liquid sinking down to the bottom. Since the sedimentation occurs from top to bottom, the sedimentation phase of a blood culture goes by quicker when the sensors are placed higher in the liquid. If the sedimentation phase lasts longer than the incubation time of the bacteria, it can occlude the growth phase. Because of this, the height at which turbidity is measured should be as high as possible.

There is another interesting phenomenon embedded in the sedimentation phase. The 37.5mm curves show a double peak in both the transmission and scatter graphs. The lower measurement locations also show this in

the form of a plateau when they are rising. After this plateau the intensity rises more rapidly than before. A possible explanation for this is that there are multiple particles in the blood which are sinking at different speeds. This would then cause there to be multiple layers in the liquid which have different absorption and scattering parameters and thus result in parts of the curve having a different slope.

For the blank sample, the sedimentation phase is the only thing that can be seen. When this phase is over, transmission slowly and linearly rises while the scattering linearly decreases. This may be because small particles are still falling to the bottom of the BCB, decreasing the turbidity slowly.

In the results of the sample spiked with bacteria, the four microbial growth phases are visible. After the sedimentation, there is a lag phase, log phase, stationary phase, and mortality phase. The most important phase for determining bacterial growth is the log phase. It is this phase that indicates a difference between a spiked sample and a blank sample the quickest. Figure 19 shows this phase clearly as a sharp decrease in transmission intensity and an increase in scatter intensity on the right graphs. The measuring height does not have a significant effect on the time at which the growth phase takes place. This means that relocating the sensors will not improve the time to detection of the turbidimeter when the samples are incubated statically. Some bacterial species grow better when they are shaken during incubation [5, 6]. Different sensor placement might be advantageous in this scenario.

The phenomena discussed so far were observed in all of the samples listed in Table 2. All of the spiked samples showed growth. The labelling of a positive sample happened manually by inspecting the growth curves. An automated way of detecting positive samples with the turbidimeter this way is in development by the SIMBLE team. The positivity of samples was also confirmed by subculturing a drop of the BCB content on solid agar after the incubation.

The different growth curve phases could be clearly distinguished in all but two samples. The samples spiked with *Burkholderia cepacia* do not show a clear growth phase like the *Escherichia coli* sample in Figure 19 or any of the other samples. Instead, they show a slower decrease in transmitted intensity and a slower increase in scattered intensity especially in one of the samples, as can be seen in Figure 20. This is actually the start of the growth phase. Sometimes the culturing of *Burkholderia cepacia* takes longer than expected, which was the case for these two samples. Because of this, no conclusions on time to detection can be made for these samples.

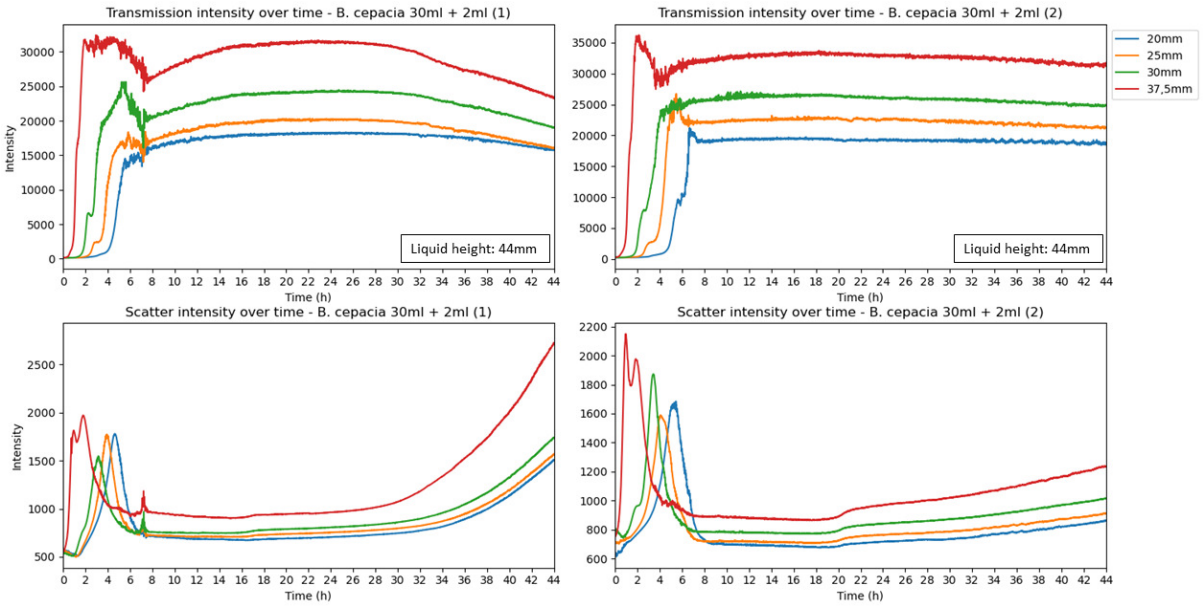

Figure 20: Transmission and scatter intensity at heights below the liquid surface of two samples spiked with Burkholderia cepacia.

Figure 21 shows the results from the measurement locations not shown in Figure 19. These are the measurements at 45mm, 55mm, 65mm and 75mm relative to the bottom of the BCB. The growth curve at 45mm (purple line) follows the same trends as the curves at lower heights albeit at a lower intensity. This is because this sensor is right on the edge of the liquid-air interface. It might have been partly above the liquid surface and partly below. Since the used sensors average multiple pixels spread across the surface, this results in the lower intensities seen in Figure 21.

The LEDs and sensors above 45mm are above the liquid in the BCB. Their intensities behave rather chaotically and seem to jump at random times. A possible explanation for this is that the light from these LEDs is reflecting on the surface of the medium and reflecting back to the sensor. If there are bubbles or particles disturbing this surface, the light will reflect differently and may result in the observed intensity jumps. Another cause could be condensation on the inside of the BCB. This condensation would have an effect on path the light takes between the LED and sensors and might influence the detected intensity.

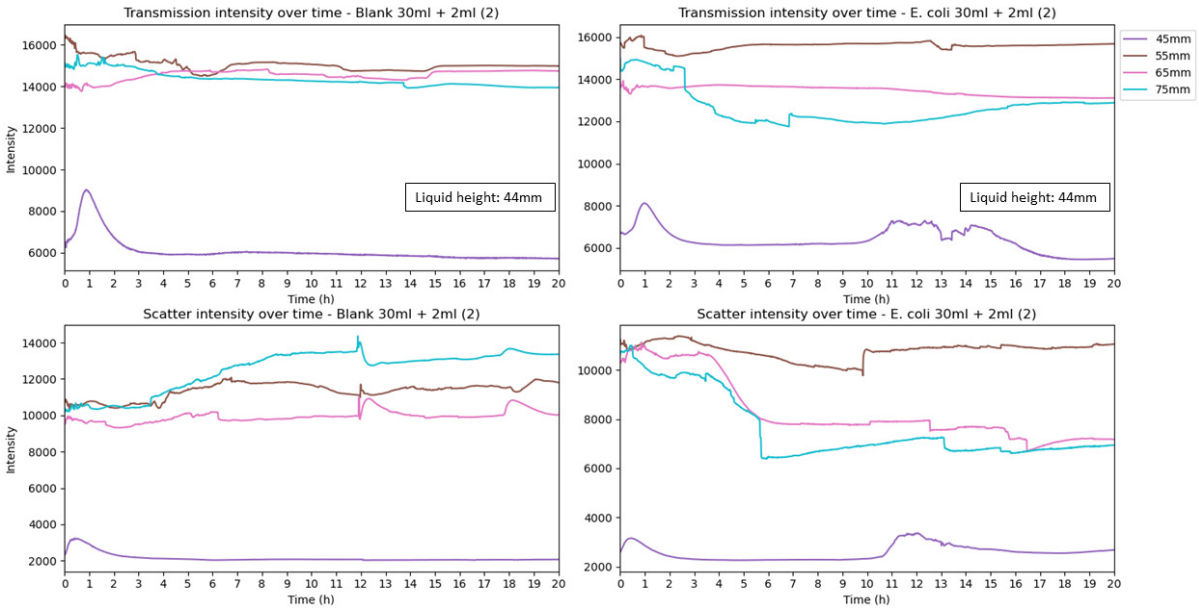

Figure 21: Transmission and scatter measurements of sensors above the liquid (45mm, 55mm, 65mm, 75mm). The left graphs are of a blank (not spiked) BCB, the right graphs are from a BCB spiked with E. Coli.

9.2.2 Effect of Volume

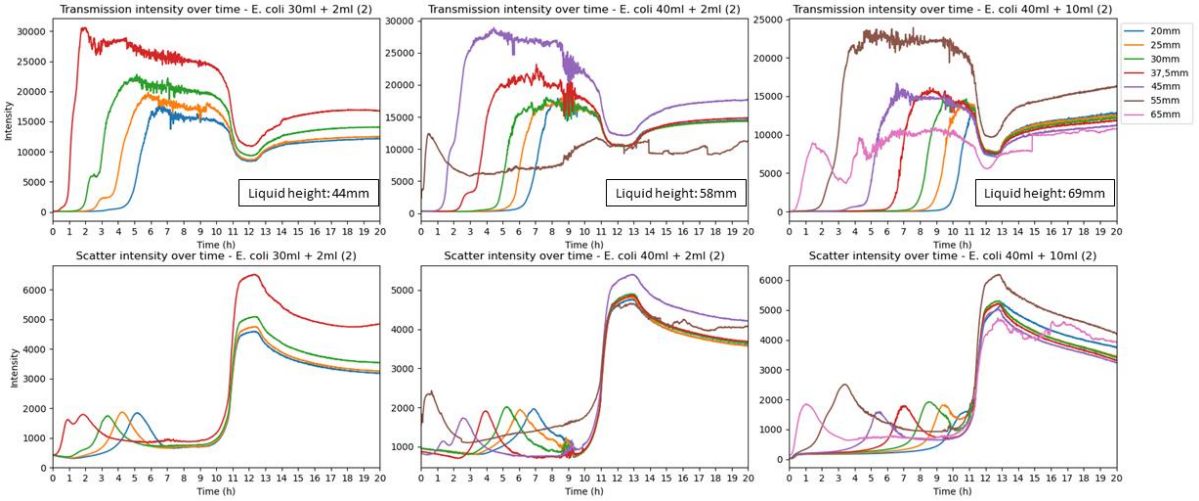

Figure 22: Transmission and scatter intensities at multiple heights of 3 samples spiked with E. coli and with different blood-to-broth ratios. 30ml broth + 2ml blood (left), 40ml broth + 2ml blood (middle), 40ml broth + 10 ml blood (right). Only the results of sensors below the liquid surface are shown.

All samples mentioned in Table 3 were tested according to plan except for one of the blank samples with 40ml broth and 2ml blood. Its subculture after the incubation period showed growth. This means that the sample was contaminated and not usable as a blank. This contaminated sample was not included in the results of any test in this thesis. All of the spiked samples showed growth at the expected incubation time.

To illustrate the effect of different broth and blood volumes, Figure 22 shows the transmission and scattering results of three samples with different blood-to-broth ratios. Each of these samples was spiked with *E. coli* in this case. Only the results of the sensors below the liquid surface are shown as to not clutter the graph. A consequence of increasing the liquid volume inside the BCB, is that more sensors will be below the surface of

the liquid. This is why more curves are shown at higher volumes.

The first observation is that when the sample volume is increased (either blood or broth), the sedimentation phase at a particular height takes longer. This was expected because the sedimentation phase in a sample with more liquid lasts longer. This observation strengthens the previously made statement that the measuring locations should be as high as possible, but still under the liquid surface. In the results of the 40ml broth + 10ml blood sample in Figure 22, it can be seen that the bottom 3 sensors (20mm, 25mm and 30mm) had barely passed the sedimentation phase when the growth phase started. This means that bacteria that started growing before the 11-hour mark would not have been detectable by the bottom most sensor for example.

When the broth volume alone was increased (from 30ml to 40ml), a decrease in transmitted intensity can be seen for the measurement locations at 30mm and 37.5mm. The same is true for the samples spiked with *S.* Typhimurium (not shown in Figure 22). The scattering intensities show the same phenomenon.
For the blank samples, the decrease in transmitted intensity is visible at every location below the liquid (20mm, 25mm, 30mm, 37.5mm). The scattering results here do not show the same pattern. This is likely because the intensities here are low and thus small variations can't be seen because the resolution is too low.

When comparing 10ml of blood with 2ml of blood in combination with 40ml of broth, another significant decrease in transmitted intensity across the whole sample can be observed in Figure 22. This decrease was seen in all of the 10ml blood samples that were tested. The same conclusion can't be drawn from the scattering results. The visible variations are too small between the samples to draw an accurate conclusion, like mentioned above.

Another observation is that the 20mm result (the one closest to the bottom) has a higher intensity than the intensities at 37.5mm and below, at the end of the measuring period. This is again true for all of the tested 10ml blood samples, also for *S.* Typhimurium and the blank samples. A possible explanation for this is that the sedimentation layer at the bottom of the BCB is higher in the 10ml samples and that this has an effect on the measured intensity of the bottom sensors.
The 25mm and 30mm also show this increase in comparison to the 37.5mm results although it is less pronounced. This supports the theory that the cause of this phenomenon is at the bottom of the BCB.

Finally, the highest sensor in the liquid, in both the 40ml broth + 2ml blood sample and 40ml broth + 10 blood does not have the maximum observed intensity, which was always the case in the previous measurements. Measurements from these sensors also fluctuate more frequently and intensely. This is especially true for the transmission sensors. This means that the measuring location should not be right against the liquid-air interface. These sensors were located 3mm and 4mm beneath the surface of the liquid (measured from the centre of the sensor). The sensor width is 2mm. The lowest distance between the liquid surface and the sensor where a stable measurement was still observed, was 6.5mm. It is therefore recommended that a sensor is placed at least 6.5mm below the liquid-air interface.

9.2.3 Diagonal Measurements

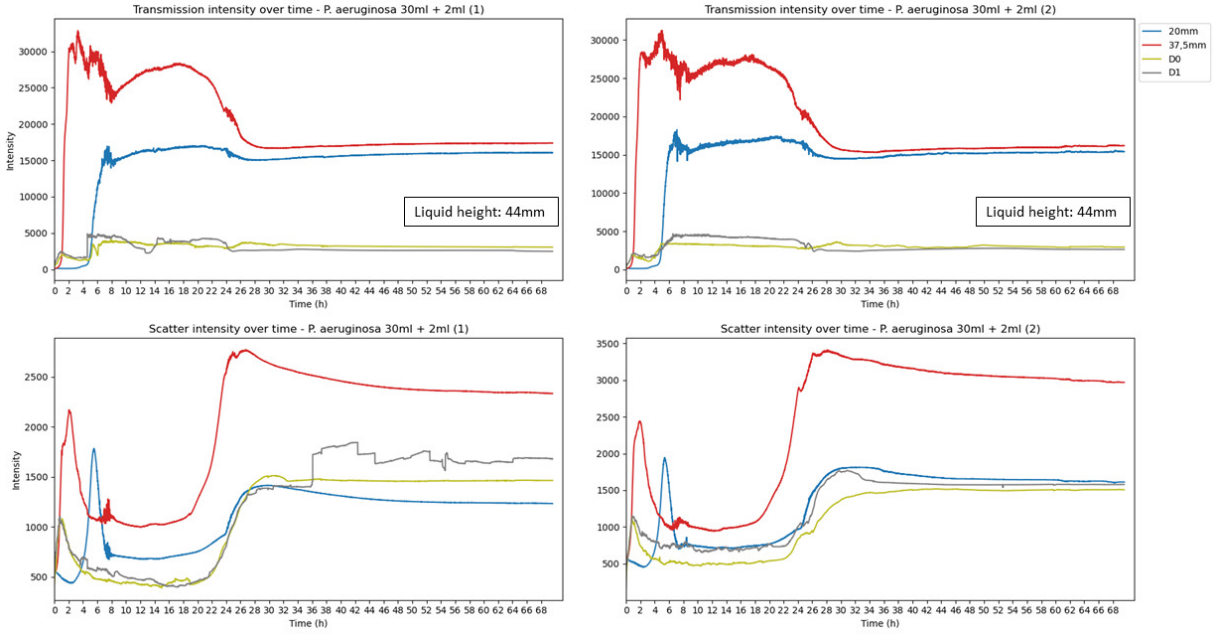

Figure 23: Transmission and scatter results at multiple measuring heights of two samples spiked with Pseudomonas aeruginosa. The samples contained 30ml broth and 2ml blood.

Figure 23 shows the results of the fourth part of testing. The graphs show the transmission and scatter results for at 20mm and 37.5mm as well as the two diagonal measurements D0 (from 55mm to 37.5mm) and D1 (from 37.5mm to 55mm). The 20mm and 37.5mm results act as a reference so comparisons with the previous figures are possible.

D0 and D1 were the main focus of this test. Their curves have a lower intensity than the 20mm or 37.5mm curves. This is caused by several factors. First, the emission and detection efficiency of the LEDs and sensors depend on the angle at which the light is emitted/detected. This means that the intensity of the light will be lower because the light is emitted/detected at an angle. Secondly, the path of the light from the LED to the transmission sensor is not straight anymore and has to penetrate the liquid-air interface to get detected. Because part of the light gets reflected at this interface, the detected intensity is lower. Finally, the light travels along a longer path and thus will be absorbed and reflected more. A diagram of the approximate diagonal light path is shown in Figure 24. Only the light that reaches the sensor directly is shown. To calculate the angles, a refractive index of 1 was used for the air and a refractive index of 1.33 was used for the liquid.

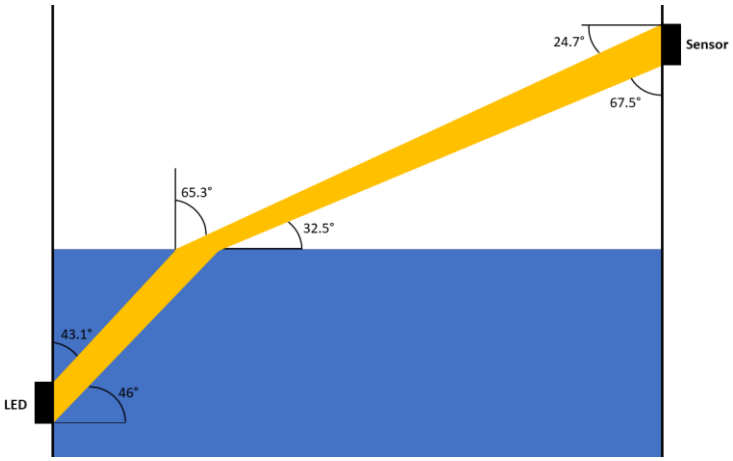

Figure 24: Diagram of approximate diagonal light path. Only the light that reaches the sensor directly is shown.

The sedimentation phase of the D0 and D1 curves is a little shorter than the sedimentation phase at 37.5mm. This is because the light path of D0 and D1 goes through higher parts of the liquid where the sedimentation phase is done quicker. After the sedimentation phase, the curves largely follow the same trends as the 20mm and 37.5mm curves. This means that this measuring setup was unable to detect the pellicle formation on top of the liquid. Figure 25 shows that there was pellicle formation on the tested samples.

There also is a valley in the D1 transmission curve between 10 hours and 15 hours in the first (left) sample. This could be a sign of the pellicle formation but because the D0 transmission curve does not show the same valley and there is nothing visible in the scattering curves at the same time, this can't be said with certainty.

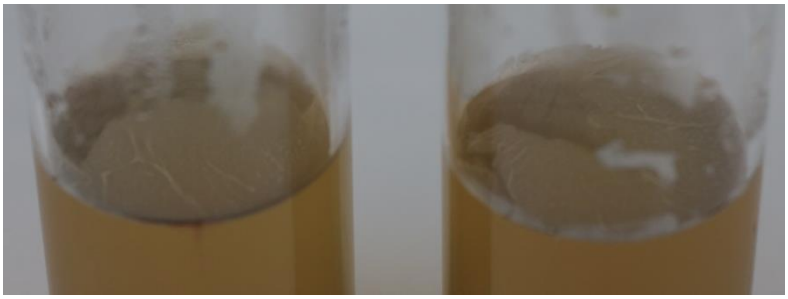

Figure 25: Pellicle formation on top of liquid after incubation with Pseudomonas aeruginosa.

10 SUSTAINABILITY

Sustainability is an essential part of any project and sustainability can come in many forms. The United Nations has created a list of 17 global sustainable development goals (SDGs) to keep track of all the things we as a society should strive to. The list of al the SDGs is shown in Figure 26. Each goal also has several "targets" that define a specific problem that needs to be solved. On top of this, there are "indicators" for each "target" that provide the factors that can show the progress for this "target". [27]

Figure 26: The 17 SDGs made by the UN. Downloaded from the UN website [27].

The work done in this thesis focusses mainly on two of these goals. The first one is goal 3 "Ensure healthy lives and promote well-being for all at all ages" [27]. Since the turbidimeter is a diagnostic device to detect bloodstream infections, its objective is to contribute to a better physical health. This is done by trying to detect these infections as fast as possible to be able to start a treatment sooner and decrease the mortality rate due to BSIs. An indirect consequence of a more correct diagnosis is that a more targeted antimicrobial treatment can be started. This helps to reduce antimicrobial resistance which is one of the major targets of this goal [27].

The general goal of this thesis was to see if the turbidimeter could be further improved by looking at multiple measurement locations. A second goal was to see if more signs of growth than only turbidity could be detected. This would give the turbidimeter the ability to detect more infections than it is currently able to which also increases its effectiveness in aiding in achieving goal 3.

The second goal to which this thesis tries to contribute, is goal 17 “Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development” [27]. The turbidimeter is designed to be operated in LMICs. It is made to provide better diagnostic capabilities for detecting BSI worldwide. The SIMBLE-project [2], under which it was developed has members from Belgium but also partners with people from Benin and Burkina Faso, promoting global partnership.

11 CONCLUSION

The results obtained from testing the third generation turbidimeter show that the design works and that it can detect most of the relevant organisms in a blood culture. The new detection sensors used in this generation did not seem to have a meaningful impact on the results, so they serve as a good replacement for the no longer available sensor in the second generation turbidimeter.

The ideal measurement location is near the top of the blood-broth mixture. This is where the sedimentation phase goes by the quickest which means that organisms can be detected there earlier than in other locations. There is however no decrease in the time to detection at this location. If the sedimentation phase has passed throughout the whole BCB, all measurement locations will have a similar time to detection. However, a measuring distance of 6.5mm below the top of the liquid should be maintained to make sure the signal stays stable.

When blood and/or broth volumes in the BCB were increased, the sedimentation phase took longer to end. This means that growth in the sample was undetectable for longer at a certain measuring height. When blood volume alone was increased from 2ml to 10ml, the transmitted and scattered intensity went down significantly across the whole sample period. This limited the part of the input range of the sensor that the signal covered. A careful trade-off should therefore be made between maximising blood volume, minimising the non-functional time, and preventing the transmitted and scattered intensities from dropping too much.

Detecting pellicle formation on top of the sample was not possible by measuring diagonally through the liquid-air interface with this setup. While a change in transmitted and scattered intensity was observed, it is likely that this was as a result of the change in turbidity of the liquid inside the BCB.

It should be noted that the tests in this master’s thesis were performed on a small number of samples. Only six bacterial strains were tested over 22 samples (not equally distributed).

Future studies, including field studies, will be performed with the second generation turbidimeter by the SIMBLE-team [2], while new ways of improving its performance will be continually investigated.

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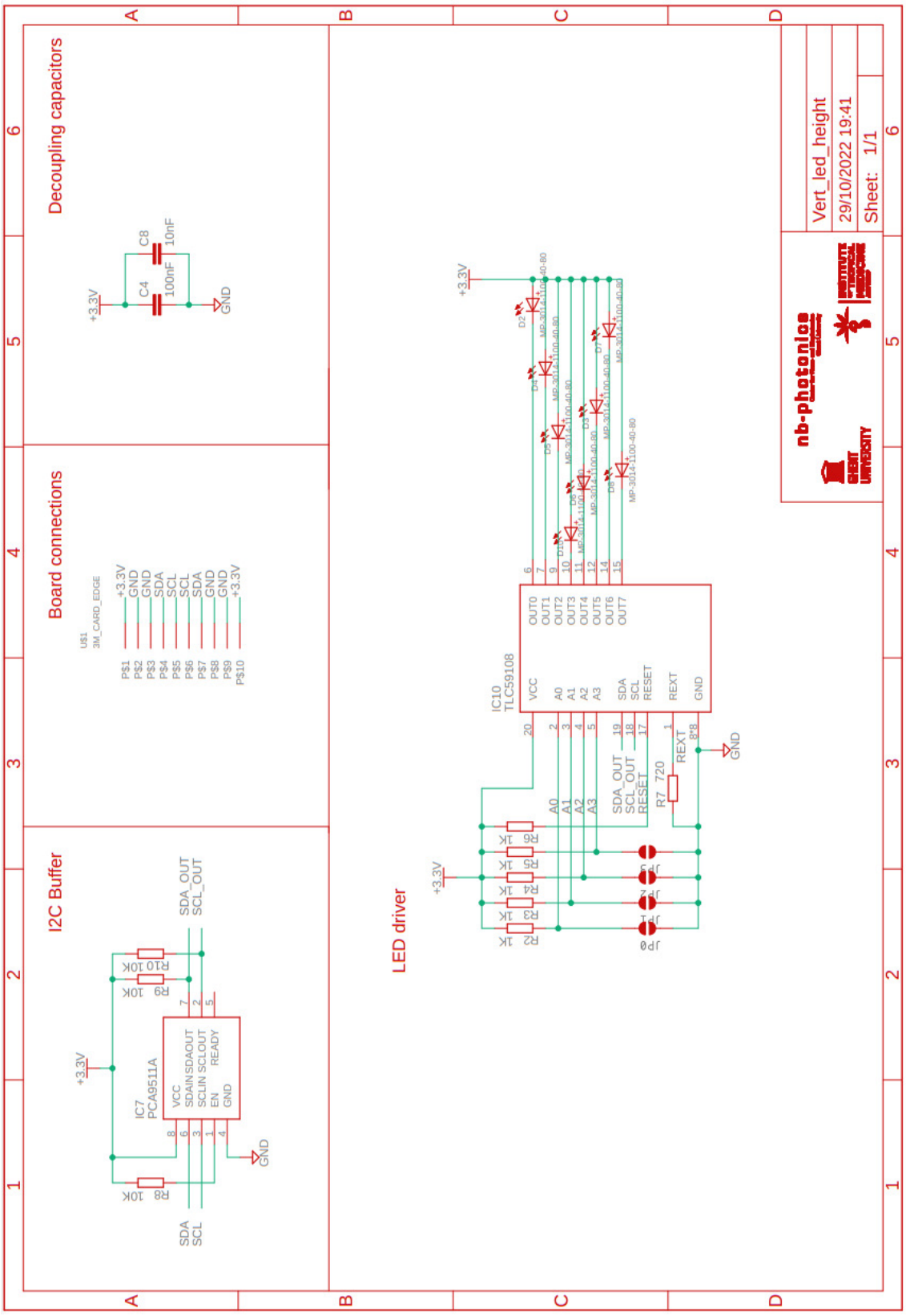
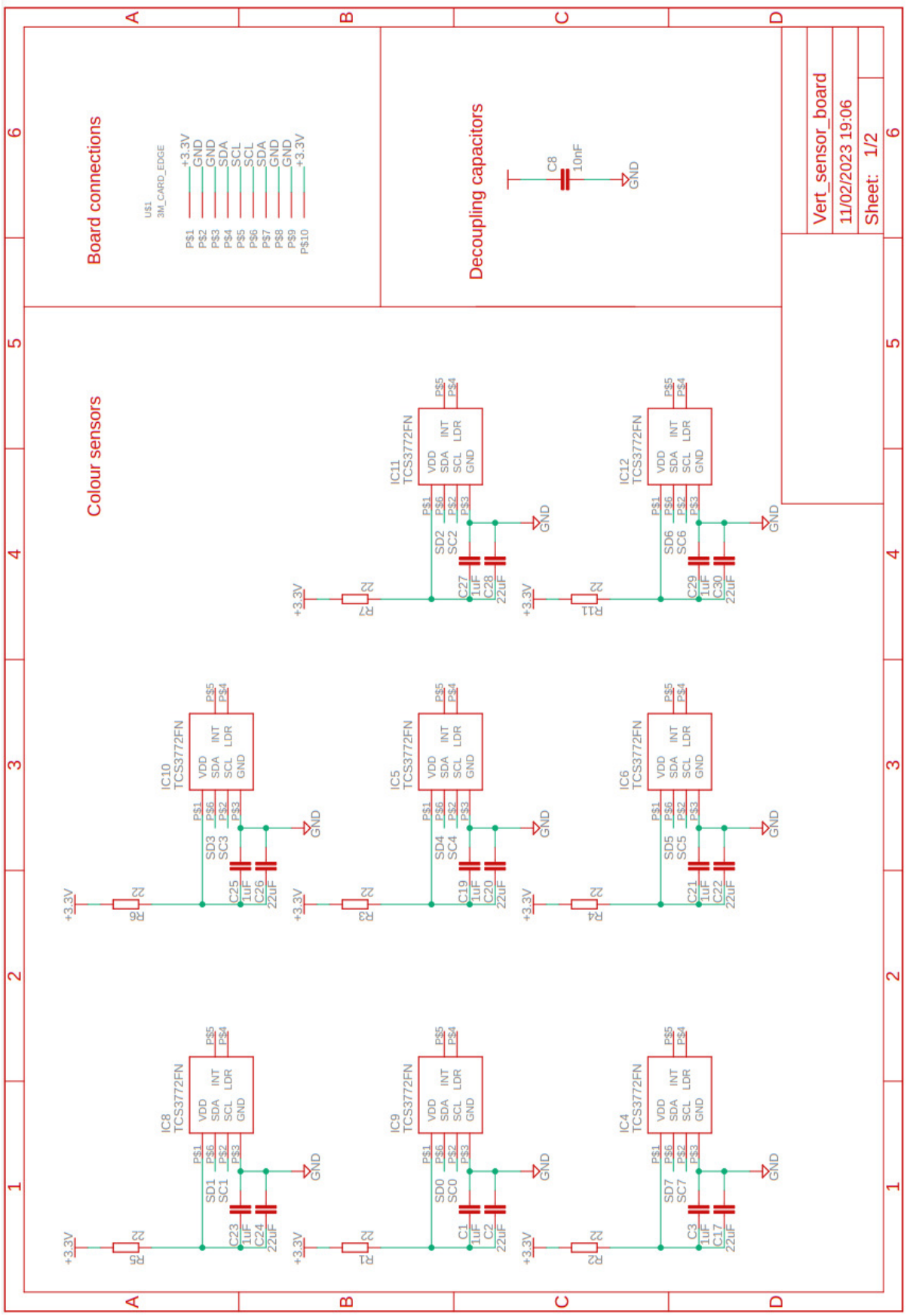
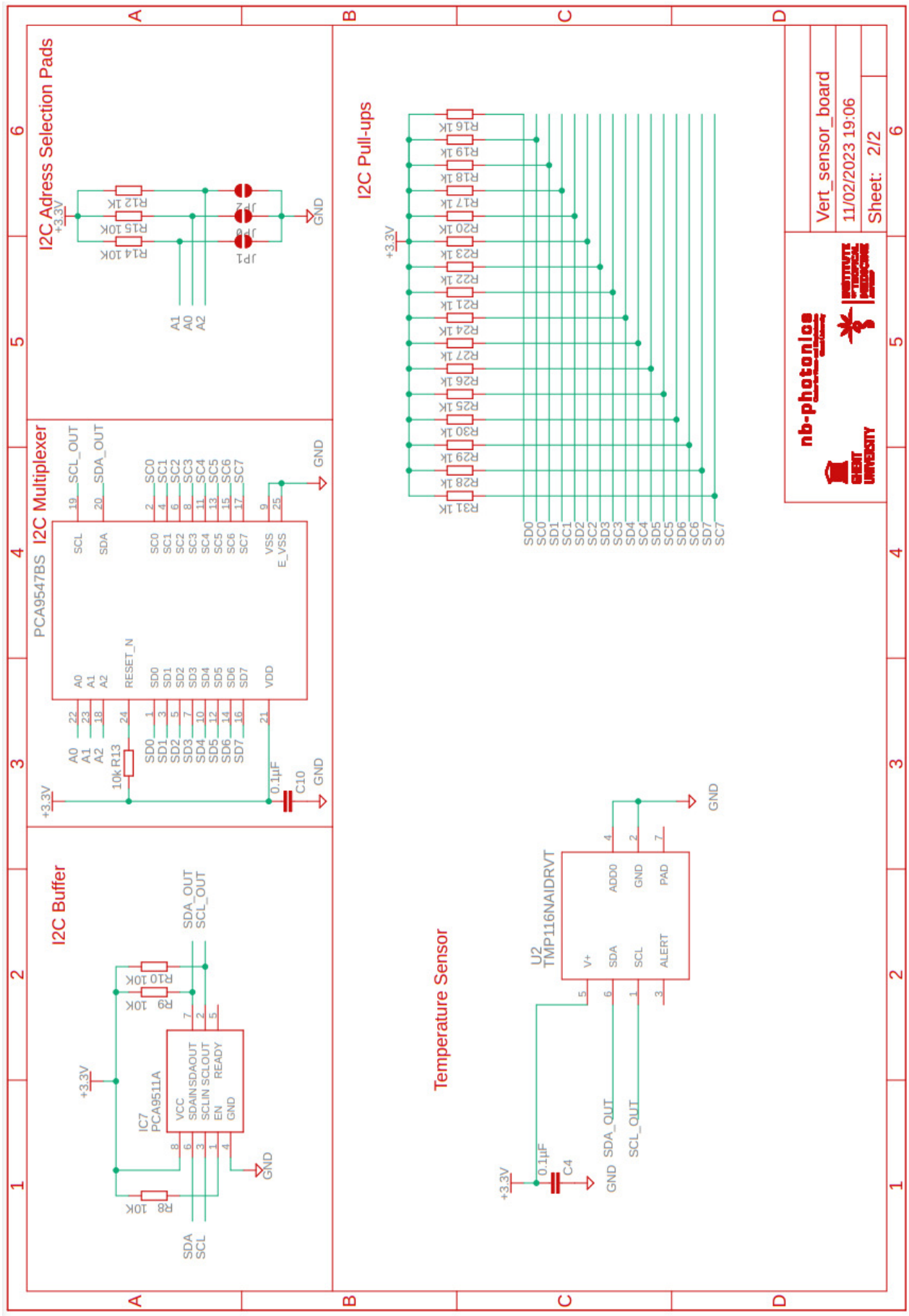
APPENDIX

A. Schematic Emitter PCB



B. Schematic Detector PCB




I2C Adress Selection Pads
I2C Pull-ups
I2C Multiplexer
PCA9547BS
I2C Buffer
Temperature Sensor
U2
TMP116NAIDRVT
IC7
PCA9511A
Vert_sensor_board
11/02/2023 19:06
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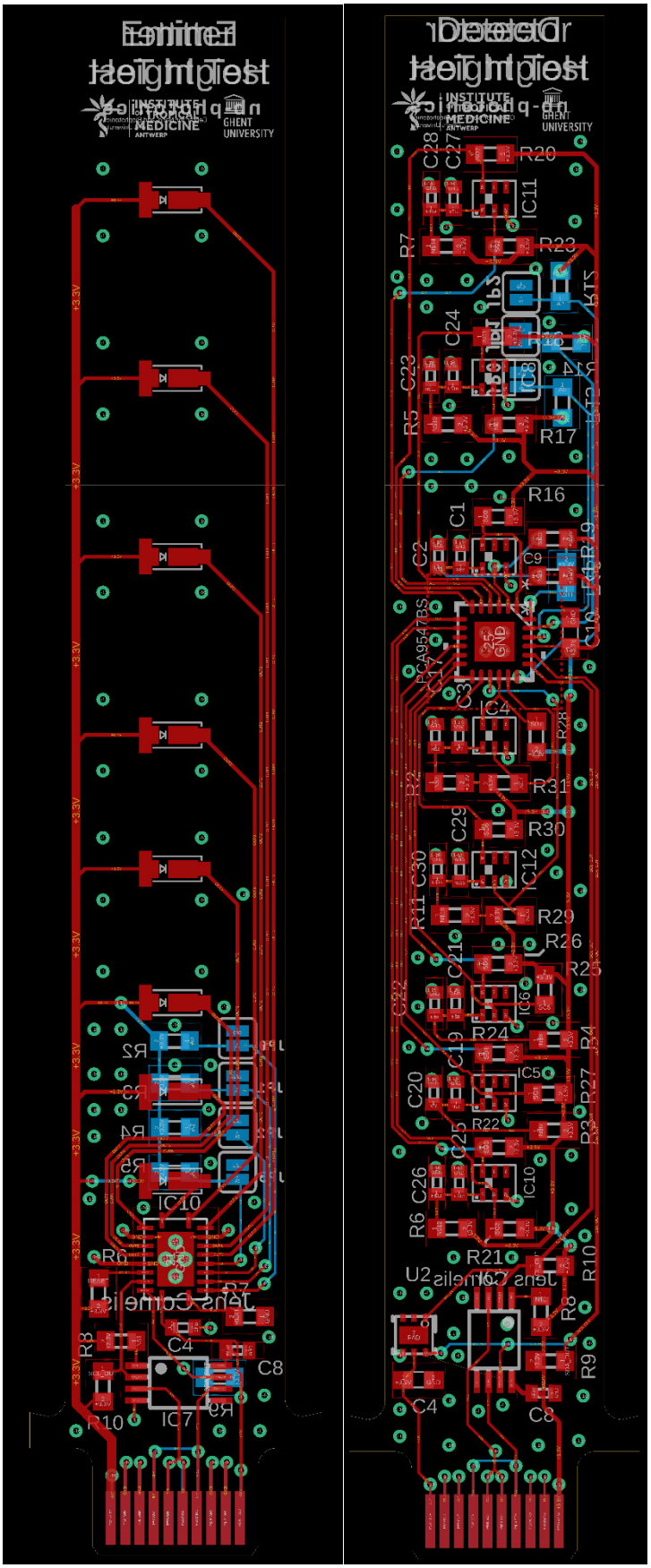
nb-photonics
GHENT UNIVERSITY

C. Layout Emitter and Detector PCB



D. Emitter Profile 1

Name	LEDs	Sensors
LED0	0	0
LED1	1	1
LED2	2	2
LED3	3	3
LED4	4	4
LED5	5	5
LED6	6	6
LED7	7	7

The LEDs and sensors are numbered from the bottom (0) to the top (7).

E. Emitter Profile 2

Name	LEDs	Sensors
LED0	0	0
LED1	1	1
LED2	2	2
LED3	3	3
LED4	4	4
LED5	5	5
LED6	6	6
LED7	7	7
DIAG0	5	3
DIAG1	3	5

The LEDs and sensors are numbered from the bottom (0) to the top (7).