Automated Colilert detection for tap water

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Title: Automated colilert detection for tap water

Abstract:
The purity of the tap water, is often something people take for granted. Amphi-Bac is a company focused on testing the water quality in Denmark. Their idea is to have an automatic alert system, if the water is contaminated. Slow alerting can cause sickness if not stopped in time. A solution to this is to have a system monitoring the samples incubation. This system must detect the sample and analyze periodically for growth. This project created a prototype handling the vision aspect of this concept. It was created to localize and analyze all the wells within the Quanti-Tray/2000. The result compared to a sample comparator, for detection threshold. The created prototype showed potential, but was limited but the illumination setup. Thus not being able to deliver consistent well localization. The system however capable of analyzing a complete incubation phase.
Preface

A list explaining the different reference methods, used in the report, can be seen below.

- **Figures:**
  Figures are referenced by numbers. This is shown as *figure ??*, where the first number refers to the chapter number and the second is the figure number.

- **Tables:**
  Tables are referenced by numbers. This is shown as *table ??*, where the first number is the chapter number and the second number is the table number.

- **Equations:**

- **Source references:**
  Source references are referenced by numbers. This is shown as [1], where this is referenced to the numbers in the bibliography.

Throughout the report the term Quanti-tray, Quanti-tray/2000 and quanti-tray is used. From the problem statement and beyond, they all refer to Quanti-tray/2000. The uploaded file beside the report, is the code developed for this prototype.

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Part I

Introduction
In the modern western world, many things are often taken for granted. There is plenty of electricity, food and water. However, to ensure that the water is for example suitable for drinking purposes, rules have been established for the limitations of pollution in the water. This ensures that the tap water in, for example, Denmark stays suitable for drinking and the population can drink it with good conscience. One quality test performed is the test for coliform bacteria.

The family of coliform bacteria, is an organism that can be found in the environment around us, however it is also present in the feces of all warm-blooded animals - including humans. It is not the coliform bacteria itself, that causes the problems. The presence of coliform bacteria indicates, that the water supply can be reached from the environment around it. This is used as an indication of the possibility for pathogens in the water. Pathogens are disease causing organisms, which existence needs to be avoided in the drinking water. Testing for each of the different pathogens, require multiple tests and would be rather time consuming and expensive. When a detection of total coliform bacteria occurs, a test for one of the two other coliforms, fecal coliform or E. coli, is also performed. These two are sub-categories in the coliform bacteria, fecal coliform from the total coliform and E. coli is a sub-form of the fecal coliform bacteria, see figure 1.

![Diagram](image.png)

**Figure 1:** Coliform and its sub-categories, fecal Coliform and E. Coli.

The fecal coliform bacteria comes from the intestines of warm-blooded animals and from humans. If the existence of fecal bacteria is found in the water supply, it is contaminated with feces and thereby has a higher risk of pathogens. With E. coli being a sub-form of the fecal coliform, this is a bacteria form which can have some strains that cause illness. Not all E. Coli are harmful, but E. Coli have become the standard measurement for contamination, since this proves the presence of feces in the water. The most media coverage happens surrounding E. coli detections. [2]

A commonly used method for detection the presence of coliform, is the IDEXX created Colilert test [3]. A Colilert test both indicates the total coliform and E. coli bacteria in the water sample. Before the samples can be examined, an incubation time of 18-24 hours is needed. The inspection of the samples is done manually after incubation. The test is not only used to sustain the quality of drinking water, but also to indicate if its safe to swim in lakes and rivers.

The company Amphi-bac [4] regularly uses the Colilert test to the quality of the water in Denmark, this ranges lakes to tap water. They came to the university with the question: ”Is it possible to build a setup that automatically can analyze and process the results from a water sample, while alerting the user of detections?” This is to get a faster alert for the...
presence of coliform, thereby being able to close off the water and not contamination the people? This also helps with the cleaning of the water lines and saving money on rinsing the water, as many liters of water affected.
Chapter 1

Analysis

1.1 Idexx colilert analysis

This section will be an introduction to IDEXX’s colilert analysis method, covering both some of the chemical reactions and introducing the quanti-tray.

IDEXX is a company with headquarters in Maine, USA. They have departments all over the world, in over 175 different countries with over 7000 employees. They thrive to be the leading company within their area of expertise. IDEXX have three focus areas, these include the Companion Animal Group, Water and Livestock, Poultry and Diary (LPD).

The test for coliform and e-coli bacteria which IDEXX have produced is a product, that can be used on all kinds of water sources, ranges from tap-, waste and source-water. The procedure of the test is very straightforward and therefore ensures there is little to none training involved. There is also very little hands on time in the test, as everything happens in the incubation stage.

1.2 Analysis procedure

1 The procedure for analyzing the samples, can be split into two groups. One group for positive/negative and one for quantization. The test do not differ that much, only in the preparation step. Here the difference is in the container for the sample. The procedure as described by IDEXX for the Colilert test, goes as the following:

1. Add contents of one pack of Colilert to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

This description is for the quantization methods. The difference in the two methods is as mentioned, in the preparation. If only a positive/negative result is wanted, then step three can be skipped. Placing the vessel directly inside the incubator, after dissolving the...
Colilert in the water sample. The steps for quantization can be seen on figure 1.1.

![Figure 1.1: A demonstration of how the Colilert analysis procedure is.](image)

Not pictured is the incubation chamber. The incubation chambers purpose is to keep the sample at a steady 35±0.5°C, under the entire incubation phase. This is because the bacterias grows its best under these temperatures.

### 1.3 Colilert-18/24

#### 1.3.1 Reaction

The Colilert Test separates it from other coliform and e-coli tests, by minimizing the amount of false-positives and false-negatives. The test uses a Defined Substance Technology (DST) reagent system to simultaneously react with both total coliform and E. Coli. The two reactions are possible by using two nutrient-indicators that cause metalization, which makes Coliform and E. Coli possible to detect using simple equipment.

**Coliform**

To create a reaction for the Coliform bacteria to become detectable, the reagent contains o-nitrophenyl-β-d-galactopyranoside (ONPG). Under the incubation stage, where the coliform bacteria grow, it uses β-galactosidase to metabolize with reagent ONPG. The metalization results in the change from colorless to yellow. An illustration can be seen on figure 1.2.
CHAPTER 1. ANALYSIS

Figure 1.2: Coliform bacteria

E. Coli

For E. Coli to be observable a different reagent is used. The one used in coliform only shows coliform bacteria and does not have a reaction with E. Coli. The reagent 4-methylumbelliferyl-beta-d-glucuronide (MUG) is used. Compared to the coliform, E. Coli uses $\beta$-glucuronidase under incubation to grow. MUG attaches to $\beta$-glucuronidase and the bi-product is fluorescence, this can therefor be observed under a Ultra Violet light source. An illustration can be seen on figure 1.3.

The total coliform bacteria is the only bacteria that hydrolyze and turn yellow with the ONPG reagent, ensuring a minimum of false positives and false negatives. The same samples will also be the ones that show if there is E. Coli in the water. These are also fluorescence when the MUG reagent hydrolyze with the E. Coli. However $\beta$-glucuronidase also has a reaction with genus of Escherichia and Salmonella. However the coliform test, is used on water samples, thus only yielding positive results on E. Coli. The DST also contains a numerous amount of suppressive enzymes to ensure suppression of the nontargets. This is necessary since the solution is a nutrient-rich environment and also provides growth for total Coliform and E. Coli, but also the none target bacterias. This is done to further ensure a minimum of false positives, however it must not interfere with the coliform resulting in false negatives. This mixture makes the IDEXX Colilert the most used classification test for Coliform and E. Coli.\footnote{\textit{FiXme} Note: KILDE}
1.3.2 Bacteria quantification

There are several methods for quantification when using this classification method. It can be used for total classification. This is done in the scenario that only concerns whether or not the water sample is contaminated. It does not give any idea regarding the amount of either Coliform or E. Coli present in the sample. The way to do this, is to mix the reagent with the water sample and incubate for the listed 18-24 hours. On **figure 1.4** the absence/presence of the E. Coli is show due to the fluorescence of one sample.

![Figure 1.4](image)  
*Figure 1.4: A test only illustrating the absence or present of E. Coli, where to E. Coli is found in the sample on the right, shown by the fluorescence of the sample.*

In other scenarios a more precise evaluation on the quantity of bacteria within the sample is needed. This is used to give a numeric estimation on how polluted the water source is. There are different levels of pollutions allowed depending on the use of the water. Drinking water, tap water, must be totally free of Coliform and E. Coli contamination, where lakes can have traces of Coli and still be acceptable for swimming. To give some precise estimation on the samples, IDEXX have developed their quanti-tray.

**Quanti-Tray**

The quantification method developed by IDEXX is divided into two separate accuracies. Both apply the same procedure as with the test for absence or not, as described above. The difference is the container unit, that is used when incubating the samples. The first method is the Quanti-Tray shown in **figure 1.5a**. This tray has 51 wells sharing the 100 mL water sample. The second method is the Quanti-Tray/2000 shown in **figure 1.5b**. The difference in these two trays, is the number of wells and the size of them. Quanti-Tray/2000 has two different sizes of wells, divided in the total number of 97 wells.
From the total number of positive wells, wells with Coliform bacteria present, it is possible to give an estimation of the quantity of bacterias in the sample. For calculating the most probable number (MPN) of Coliform in the sample, a computer program provided by IDEXX is used [5]. These MPN numbers, can also be found on a lookup table, provided by IDEXX, the Quanti-Tray can be seen in appendix A and the Quanti-Tray/2000 in appendix B. In the case of the ‘normal’ Quanti-Tray, this gives a MPN from the total number of positive wells and a 95% confidence. Taking an example with a sample containing seven positive wells, this yields in a MPN of 7.5 per 100 mL with a 95% confidence of 3.7 - 15.5. The limitation of the Quanti-tray is that it does not provide MPN over 200. For this purpose, the Quanti-Tray/2000 have been designed. The two different sizes of wells in the tray provides both a more precise and possible higher reading of MPN. Taking the same example with 7 total well, in this case 4 big and 3 small. This yields in a MPN of 7.2 per 100 mL, also with a 95% confidence. Both numbers are found using the lookup tables.

Colilert comparator

When analyzing the samples after incubation, there can often be a fine line between positive and non positive wells. As explained previous, if the inspector is in doubt, it is advised to let the sample incubate for another 4 hours. However the situation whether sample is positive or not, is still an issue. To have a guideline for the readings, IDEXX created a reference comparator with the yellow color of positive Coliform and the fluorescence of positive E. Coli. On figure 1.6 an image of the comparator can be seen. The comparator gives the references when comparing samples with clear water. Not all water samples are clear, some have a coloration due to chemicals or the presence of dirt in the water.
Figure 1.6: Colilert color comparator.
1.4 Conceptual idea

This section will describe the total system idea presented by Amphi-Bac. In addition to this description, the focus will be turned to the machine vision part of the project.

Amphi-Bac have found a niche with the focus on the Danish water control system. This ranges both from the quality on the Danish tap water supply to the purity and quality of the swimming water. They have come with the idea, that even though the procedure for applying the Colilert test is very simple and does not require much effort, some improvements can be made. Since the test requires substantial incubation time, the test has a lot of planning involved. This planning is that all tests should be started at the same time, and from Friday to Sunday not many tests are performed since it requires available staff to analyze the result after the incubation.

Their idea is to create a system which can automatically detect changes in the samples, and thereby send alerts to the observator if positive samples are present. On figure 1.7 an illustration of the total system idea can be seen. In combination with the setup for automatically detection, a system for rotation between the numerous samples is developed as the system needs to be able to handle several samples at a time. The system is designed to handle samples in quanti-trays. This will both provide a positive/negative results and the quantity of MPN in the sample, as different samples may have particular limitation on the MPN of allowed bacteria per sample.

![Figure 1.7: Conceptual model.](image)

The system can be seen as three different parts, a mechanical part, a machine vision part and an alert/interface part.

The mechanical part has the focus of creating a carousel for the quanti-tray, making it possible to have multiple samples at a time. With the addition of multiple samples, the need for an automatic unit control system. The machine vision will be focused on automatically detection of the quanti-trays and recognizing the status of the individual
wells. This includes camera and light setup together with software for automatically assessing each well.

The last part of the system, is the alert part. This has to be able to give the user alerts if samples are positive, but also the ability for the user to go online and check the current status of the samples. Provides as much information about the observations as possible. This enables the users to track the progress of the analysis.

The focus of this project will be the creation of the machine vision part of the system. This includes both the selection and setup for the camera and light, but also the software for analyzing the quanti-trays. This will cover the overall recognition of the tray, but also the per well analysis. Further an analysis of progress of the bacteria development can be used to give an estimation on whether the sample changes to positive within the incubation time. This progress will be regards to the total Coliform, and not the E. Coli. Since the E. Coli is only present when Coliform is detected, the concept will be build to observe Coliform only. Thereby not using UV lights, however if proven successful and a short adoption time for E. Coli is expected.
1.5 System description

An introduction to the components are necessary for creating such a system will be done. This including the setup of a complete machine vision setup and analyzing the quanti-trays to set accuracy requirements for the creation of the system.

The machine vision setup consists of different parts that all needs to work in harmony, to yield the best results. Comparisons can be draw to how a human sees objects. An illustration of this can be see on figure 1.8a. The comparisons can be draw to how the object is perceived of the person regarding the light in the surrounding area. If the light is not proper, it is harder to focus and see what the object looks like. This is the same in machine vision, if the lighting is not proper, then it requires more post-processing to get the right result, if even possible. The eye also needs to be able to focus, as the eye adapts to the light and if necessary, glasses can be used to correct the sight and get everything in focus.

![Diagram of human eye and light source](image1)

(a) An illustration of a human eye, seeing the grass using the sun as a light source.

![Diagram of machine vision setup](image2)

(b) An simple illustration of how a machine vision setup can be used to see the grass

Figure 1.8: The human view of an object vs. the machine vision view.

The same principles is seen when building a machine vision setup. This can be seen in figure 1.8b. For everything to work, each part needs to be specified for the task at hand. The lighting and camera part are crucial, because if these does not work properly, the system will not be able to detect the object of interest. Not even through post-processing. The better the preparation of the light and camera, the less work is needed for the software. When designing a machine vision setup these things must be addressed, further a number of subcategories within camera and illumination plays a role. Some of the important part can be seen on the list below.\(^3\)

- **Camera**
  - Camera type
  - Resolution
  - Lens
  - Field of view

\(^3\)FiXme Note: ref
1.5. SYSTEM DESCRIPTION

- Illumination
  - Light Sources
  - Back/frontlight
- Interfaces
- Software design
- Processing time

Processing time is not a factor since the focus of this project is to create the vision part of the total system. This system does not require a high frequency of the analysis, since the total time of an incubation is 24 hours. An update between each 5 - 10 minutes is expected, yield a total of 300 - 150 samples over the 24 hour test duration.

1.5.1 **Quanti-tray**

Another factor to take into account when creating the system is the object at hand and what information the system needs to gather from it, the elements of interest. The object at hand is the quanti-tray, in particular the quanti-tray/2000. This tray is the most commonly used tray for the Colilert analysis at Amphi-bac, and yield a better resolution for any amount of bacterias detected.

![Figure 1.9: Quanti-tray/2000](image)

The elements of interest are the wells on the quanti-tray, which contain the water samples. There are a total of 97 well, distributed on three different sizes. One big well, which can be seen on **figure 1.10a**, this is located on the top part of the tray and is used to contain the potential overflow of water in the sample. Then there are 48 wells located in the middle of the quanti-tray/2000, an example of the well can be seen on **figure 1.10b** These wells each contain approximately 2 mL of sample water. Finally there are also 48 smaller wells located in the bottom part of the quanti-tray/2000. These provides the possibility to generate the greater span of bacteria concentration. Each well contains 0.2 mL of sample water. One well can be seen on **figure 1.10c**.
1.5.2 Well analysis

Looking further at each of the wells, the size and shape are used to determine the necessary accuracy of the system and knowledge of possible obstacles.

Starting off with the largest of the wells, it can be seen that this well is not a square as the other wells. It can be seen on figure 1.11, providing a view from both the side and the top. The width of the well is 80 mm and the height is 35 mm. A possible obstacle with this well is that the depth range from 2 mm to 10 mm. Since this well is also used to catch the excess water of the sample, there will most likely always be a lot of air in the well. This will make the information gathering harder, since the middle of the well might not be read accurately, because of the air pocket and the sides have a slope. This gives problems regarding the illumination, making it harder to avoid highlights.

The two other sizes of wells both have a square shape. The biggest of these have a size of 15 mm in both width and height. The depth on these is 10 mm. The well can be seen on figure 1.12. A possible obstacle with these is the border area. This area is curved to the edges, making highlights a possibility. The simplest solution is to focus only on the region within the curved edges. This minimizes the region of interest to a size of 9 mm
in both directions.

**Figure 1.12:** An illustration of the medium size well of the quanti-tray/2000, showing the width, height and depth.

The smallest of the wells have the smallest region of interest size. The well can be seen on **figure 1.13**. The size is significantly smaller than the other wells, also indicated by the volume. The width and height is 6 mm. Were the depth is 3 mm. The edges are also rounded, as the larger wells, shrinking the region of information even further. The size of this is 3 mm in both direction.

**Figure 1.13:** An illustration of the smallest well of the quanti-tray/2000, showing its width, height and depth.

With such a small area with information in the small wells, it is necessary to have an accurate system, both when detection the whole tray but also when finding each well. Using a higher resolution on the camera side, it is possible to have more pixels within the area of interest, proving more information for the analysis.

### 1.5.3 Quanti-tray

4 Since the wells are located on the quanti-tray/2000, the first part of the machine vision system is to locate this. Therefore a short overview of this, covering the problems and possible hurdles.

The quanti-tray consists of 97 wells, as stated before. These wells are distributed over the total area of the quanti-tray. The distribution is 49 big wells, including the large in the top, and 48 smaller wells. The total area of the quanti-tray is, 270mm height and 150mm width. An image of the tray can be found on **figure 1.14**.

---

4 Fixme Note: Ny overskrift?
5 Fixme Note: Må last det!!!!
As described in 1.5.2, each of the wells have their own shape and most of them have rounded corners, which can yield problems for illumination. Further problems for the illumination can be found on the “background” of the quanti-tray. The whole back-page is a silvery color/aluminum, this is an ideal surface for reflection and can thus also result in highlights in images. On figure 1.15 the Quanti-Tray/2000 can be seen illuminated with a light source from different angles. This provides evidence for the high amounts of possible highlights.

1.5.4 Water sample

At Amphi-bac the Colilert analysis is used to determine the water quality in both drinking water, but also in lakes and water stream around the danish countrysides. This gives as very different range of clarity in the water samples. However since this project’s focuses
area is particular in the purity of the Danish drinking water, it is assumed that the clarity of the water samples used for testing is more or less clear.
Chapter 2

Problem Specification

This chapter will be a summary on chapter 1 findings. These will be the foundations of the rest of the project. Furthermore a set of requirements and the test specification is defined.

2.1 Problem statement

The analysis in chapter 1 have introduced the solution for Colilert detection and Amphi-bac idea to create an automatic solution.

In chapter 1: Introduction it was determined that the responds time for bacteria detection, has a great influence on how many will be affected when a potential contamination occurs. It is not the E. Coli or total coliform bacteria that causes the diseases, but numerous of pathogens which is elusive and expensive to test for. The colilert test created by IDEXX gives indication for total coliform and E. Coli, providing an indication for contamination of the water and to what degree. A faster responds time will provide the possibility for better contamination control. Amphi-bacs idea with a automatic control system, includes the ability to handle multiple samples and alerting the observers. This also gives flexibility in time and day of incubation, since it limits the need for further user interference. The system in this work will focus on the detection and analysis of the samples. This is the first part of the total system, and a important step to have under control before moving forward with the creation of the rest. This leads to the following problem statement:

Is it possible to create a vision based system to automatically detect quanti-tray/2000 and analyze the water samples within?

2.2 Requirements

2.3 System requirements

The focus of the project will be the detection of the quanti-tray and analyzing the Colilert test. Since both the detection and analysis need to be reliable for the system to be automatic, the requirements will mostly be focused on accuracy. The accuracy of the software handling the quanti-tray detection and ensuring proper detection of positive samples.
2.3.1 Detection and analysis requirements

In this subsection the purpose is to establish requirements for the detection of the quanti-tray/2000 and the analysis of the water samples within the well. These requirements will create the basis for the quality test of the system. They will be made based on the formerly mentioned details of the wells in chapter 1.5.2: Well analysis. The working area of the smallest well is only 3mm x 3mm. The system needs to be able to find all the wells with high accuracy, therefore location and detection of the quanti-tray/2000 is relevant to ensure this accuracy.

1. Location of the quanti-tray should be > 95%.
2. Accuracy of 95 % well detection, within a 3 mm x 3 mm square.
3. 98 % detection on samples that exceeds comparator color.

2.4 Model/System diagram
Part II

Setup
Chapter 3

Conceptual model

This part of the project will be to derive information and selection of the camera and illumination. A set of physical boundaries will be set, and a solution will be created. The setup concept can be seen on figure 3.1.

![Diagram of setup including lighting, camera and object](image-url)

**Figure 3.1:** An illustration of the setup, including the lighting, camera and object.

The test setup is limited in space, since the entire solution must be able to fit inside the incubation chamber. These ranges in size depending on the capacity of each model. This creates some problems, as the idea of the solution, is to make an universal kit that can be deployed in the champers. This project will focus on the incubation chamber used at Amphi-Bac for incubations of the Colilert test samples. This chamber is from the company Binder, model BD 53. The internal size of this chamber is:

- Width: 400mm
- Height: 400mm
- Depth: 330mm

This is still a tight working area for the entire conceptual idea, but should be sufficient for illumination and the camera. Taking a look inside the incubation chamber, it can be seen that the inside is completely covered in aluminum. An image can be seen on figure 3.2. When building the illumination setup, it will be hard to control the reflection from the surroundings inside the chamber.

---

1Fixme Note: Kilde: http://www.binder-incubator.us/us/binder-incubators/bd-53/
To overcome the problem regarding the surroundings inside the chamber, the setup will be deployed in its own package. This package will contain the camera and illumination. The isolation properties of the solution, is not of great concern due to the system being inside of the chamber at all time. This package could in practice fill the entire chamber, since the focus of the project is only the creation of the computer vision setup. However to make the further creation of the remaining parts, this package will be as small as possible. The area must of course be able to fit the quanti-tray/2000, with the dimensions 270mmx150mm, providing some extra area gives the laboratory technician a small amount of free room when placing the samples. This makes the detection more crucial, but the system easier to use. A surface area approximately the size of an A4 page is used, 300mmx210mm. The depth on the other hand, will be determined when chosen the camera, as this is dependent on field of view, in this camera.

Figure 3.2: A inside look of the Binder BD53 incubation chamber.
Chapter 4

Prototype

This chapter will be the construction of the prototype - including the lighting and camera setup.

The psychical model in this project is a prototype, which purpose is to validate whether or not Amphi-Bac’s concept idea, for an automatic analysis for E. Coli, is possible to do with computer vision. In this early state of this concept, some factors are not known and thereby not accounted for. One particular factor that will be excluded from further thought, is the selection of the camera for this prototype. This is done to have a low cost, and a simple solution satisfying for this state.

To both keep low cost, yet still have the flexibility to expand with features, the prototype will be made using the Raspberry Pi platform, as provided by Amphi-Bac. The Raspberry Pi features original modules for GSM, camera and a lot more. Raspberry Pi has gone through many generations, each providing more processing power. The current generation, 3. gen., packs a capable quad core processor at 1.2GHz and 1 GB RAM. With this added processing power, the Raspberry Pi 3 should be able to both handle the lighting setup, computer vision analysis and future features such as, alert system and control of the rotation system. The Raspberry Pi features 40 General Purpose Input/Output pins(GPIO), these makes it possible to do Serial, PWM and a lot more. The layout can be seen on figure 4.1. The GPIO pins can also be used to operate the lights in the prototype.


**Figure 4.1:** The GPIO layout for Raspberry Pi. Source: https://www.raspberrypi.org/documentation/usage/gpio/images/gpio-numbers-pi2.png

4.1 Camera

As the Raspberry Pi platform i provided for this project, the Raspberry Pi Camera Module provides great compatibility. This camera module have the following specifications:

1. Size: 25 x 24 x 9 mm
2. Still resolution: 8 Megapixels
3. Sensor: Sony IMX219
4. Sensor image area: 3280 x 2464 pixels
5. Horizontal field of view: 62.2 degrees
6. Vertical field of view: 48.8 degrees
7. Focal length: 3.04 mm
8. Focal ratio: 2.0

This camera will be used as the optics in this project, and the physical model will be created around its properties. From \(^1\) it is stated that the prototype has physical limitations, since it is placed within the incubation chamber. These limitation is mostly set in the area of the package, since the depth is determined from the camera’s specifications. The camera should be able to see the entire ground floor, but the closer to the test object, the greater the number of pixels in the interest area.

Provided with the information about the cameras specification and the size of the Quanti-tray/2000, in \(^2\). It is possible to find the minimum distance the camera needs to be from the Quanti-tray/2000 to ensure complete capture. This is done using equations for a right-angled triangle. An illustration of this can be seen on figure 4.2.

\[
\begin{align*}
\text{31.1°} \\
14.9 \text{ cm} \\
? \\
\end{align*}
\]

**Figure 4.2:** Show the right-angled triangle, for camera distance calculation.

Thus making the calculations for the distance in question:

\[
b = \frac{a}{\tan A} \quad (4.1)
\]

\[
a = 14.9 \text{ cm} \quad (4.2)
\]

\[
A = 31.1° \quad (4.3)
\]

\[
b = 24.53 \text{ cm} \quad (4.4)
\]

This \(b\) distance, is the minimum distance from the camera to the quanti-tray/2000 and the depth of the prototype. Some headroom is desired here to ensure flexibility for the

\(^1\)\text{Note: Referer til der hvor der bliver bestemt størrelse på kassen.}

\(^2\)\text{Note: ref til quanti-tray/2000 størrelse.}
user, upon loading the system. The depth of the package is chosen to be 360mm. This yielding in the total size of:

- Width: 210mm
- Length: 310mm
- Depth: 360mm

These dimensions are well within the boundaries of the incubation camber. With the camera and platform determent, the illumination method must be found.
Chapter 5

Illumination

The lighting in a machine vision setup, is the a key element in the pursuit for gathering the correct information. In Machine Vision it is often said that "better to light than write(software)" [6]. The general aim for such a statement is to create a lighting setup that gives maximum separation between the elements of interest and their surrounding. For this project, this means maximizing the separation between the Quanti-tray/2000 and the background. But also making sure to be able to detect the color change from neutral to positive, as seen on figure 5.1.

![Figure 5.1: The color shift when the sample becomes positive.](image)

Another concern for the illumination setup, is to minimize the reflection from the quanti-tray/2000 itself. To get an understanding on what reflection is, a short introduction to how light affects the object at hand will give insight to the reflection. There is a split of the light ray as it enters the object. The split separates the light into threes different components:

- Reflected light \( R \)
- Transmission light \( T \)
- Absorbed light \( A \)

To find the properties of the test object many variables must be known. This is not a reasonable task, since this includes information about for example the wavelength and incident angle. However also the test object components must be known, since these affect every part of the reflection. It is however known with the law of energy conversion, that the sum of all light types must be equal to the incoming light [7]. An illustration of the different light can be seen on figure 5.2.
CHAPTER 5. ILLUMINATION

Figure 5.2: Illustration of the lights behavior on an object. Showing both the absorption, transmission and reflection.

5.1 Reflection

In \(^1\) it is shown that the Quanti-tray/2000 can cause a lot of reflection. This section will cover why these reflections occur.

When covering the reflection of a test object it is often its macro structure, that determines diffuse and direct reflection. The micro structure is also a concern. The micro structure determines how plain the surface of the object is. If the surface has rough micro surface, the diffuse reflection will scatter in all direction with different strength, creating a hard to control reflection.

Handling reflection on transparent materials is yet another problem. This creates the phenomenon with double reflection, as a reflection both occurs on the front and the backside. On figure 5.2 the different lights on an object can be seen. The transmission lights, is what creates backside reflection, as not all of the light is transmitted through the back, some are reflected.

The overall shape of the test object is also a concern when dealing with reflection, as different shapes changes the angle of the reflections. In \(^2\) a lot of concern were raised towards the size of the available information area. This is due to the curvature on the edges of the well, creating possible reflection points. In figure 5.3 the impact of rounded corners can be seen.

Figure 5.3: Reflection difference upon differently shaped objects.

\(^1\) Fixme Note: ref til analyse med reflektion
\(^2\) Fixme Note: ref til analyse med wells
5.1. REFLECTION

Furthermore the material of the object at hand also have an influence on how the light gets reflected, absorbed or transmitted.

5.1.1 Shadow free lighting

For this scenario it is ideal that the light source does not introduce any shadows in the image, since these will make it harder for the software to localize the quanti-tray/2000 or even the individual wells. These are the essential elements, and the interest point for further analyze. The name for this lighting setup can be multiple thing, cloudy day illumination, dome lighting, super diffuse illumination or simple shadow free lighting. The principle of this type of lighting setup is to cover the entire object in the illumination setup. This can be done by having a cylinder light or creating a dome of lights over the object, hence the names. An illustration of a shadow free illumination setup can be seen on figure 5.4.

![Shadow free lighting illustration](image)

**Figure 5.4:** The principle behind shadow free lighting.

This lighting form has the characteristics, that there is a minimum of reflections or scattering appearing with the usage of such a solution. This creates a more homogeneous image of the object, due to the high number of diffuse reflections. The illumination creates a smooth surface on the object, while removing the shadows. This can be seen on figure 5.5b, which shows the smooth object compared to a conventional diffuse illumination in figure 5.5a. However one of the downsides to this illumination technique, is the need for having a very short distance to the object. The short distance is needed for the illumination to have the intended properties.

![Normal diffuse lighting on object](image) ![Shadow free lighting on object](image)

**Figure 5.5:** Images comparing normal diffuse lighting and shadow free lighting on an object.

---

Note: ref
5.2 Light Color

The knowledge is that as the water sample become positive, the samples color will turn yellow. There is the possibility to use a light source that will embrace this change in color, so that it can be more pronounced. Using different colored light sources will yield in colored images. Therefor it is necessary to choose the right color for a giving task. Monochromatic lights, are lights that only allow the desired wavelength, color, to emit.

To get the most effective color pronunciation a light source with the same color is required, for example green light for green objects. As it is possible to enhance the correct color, it is also possible to use its complementary color. This will make the color become more dark. Some of the color and it complementary color can be seen on figure 5.6. The colors is possible to mix, as the light is additive. This gives the opportunity to make any color using the red, green and blue.

Figure 5.6: The complementary color wheel, with the complementary color on the opposite side of the wheel.

Fixme Note: lav dit eget

Combining a monochromatic light source with a monochrome camera, it is possible to have high contrast between the color in focus and the irrelevant colors.
Chapter 6

Lighting setup

The lighting setup will be formed around the formerly conducted analysis in chapter 5, this provided knowledge regarding the reflection problem. Further more a couple of solutions on how to create a lighting setup that can deal with the reflection problem.

For the solution a based on the shadow free lighting setup will be created. The shadow free lighting solution is not optimal, since the distance from the lights to the test object is to big and the camera is able to have such a short distance to the object.

The light source used is multiple LED diodes. These do not generate much heat or uses a lot of power, they can therefore be powered directly from the Raspberry Pi. The chosen LED diode also provides a light emission angle of $120°$ [8]. Given the illumination angle from each of the LEDs, using multiple of these will provide a diffuse illumination of the test object. Some inspiration have been drawn from the shadow free lighting, especially the part of how having light covering object. On figure 6.1 an illustration of the lighting setup can be seen.

![Figure 6.1: Drawing of the illumination setup for the prototype.](image)

For the construction of the illumination setup, an arc had to be made, as show in figure 6.1. This arced spans the total length of the prototype, show in figure 6.2. On this arc, multiple arrays of LED’s is placed to imitate the shadow free lighting principle. By using multiple arrays for each side of the arc, it is possible to activate them separately, thus giving higher control over the illumination. As seen on figure 6.2, a layer of thin transparent paper were placed. This layer is placed to help overcome, possible highlights and reflection of the individual LED.
Figure 6.2: The prototypes illumination setup.
Part III

Design
Chapter 7

System Flowchart

The next chapter will cover the knowledge, creation and testing of the system. Firstly a system overview is provided. This helps the gathering of information, as the overall flow and functionality of the system is found. To do this a flowchart for the system is created. The flowchart includes no technical specification or information, as the procedures is not found at this stage of the project.

![Flowchart for the prototype.](image)

Figure 7.1: Flowchart for the prototype.

The process starts with the gathering of the image. This task also includes the control of the lighting and handling the properties of the camera, to ensure similar images every time. After the image has been gathered, the next task is to localize the quanti-tray within the image. With the tray localized, the next step is to localize the individual wells on the quanti-tray. With the wells in place, the final step is to extract the information from these.

Under each of the steps, there can be multiple sub-steps needed. These will be uncovered in the chapter regarding the subject at hand.

\[^1\text{Fixme Note: thomas noter??}\]
Chapter 8

Software

This chapter will gather information regarding the creation of the software for this project. Uncovering the needed steps to complete the system flowchart and providing suggestions for the possible solution. By looking at multiple methods for the solution, a proper choice can be made. At the end of each step, a detailed look towards the chosen method will be made, giving an overview of how a solution can be created using the method. This detailed look will transfer to the next chapter, where the performance of each method will be analyzed.

8.1 Image capturing

Capturing the ideal image, can break or make the rest of the system’s performance. This image is foundation for the rest of the system. Therefore it is important to ensure proper and consistent image capturing for the project. This is therefore the first step in the system.

As this first step needs to be able to take consistent images of the quanti-tray, both the illumination and the cameras variables must be controlled. The image capturing step can therefore be branched out to multiple sub steps. A flow of these steps, can be seen on figure 8.1. It starts with control of the illumination, then adjusting the camera settings and lastly taking the image.

Figure 8.1: Steps for capturing an image of the Quanti-tray.
8.1.1 Illumination

In chapter 6 the construction of the lighting solution is covered, yielding in multiple arrays of LED’s. By dividing the lighting system into four groups, it is possible to exclude separate parts of the lighting. Each group is connected to a separate GPIO on the Raspberry Pi, figure 8.2. The control of lighting will be performed using the GPIO pins found on the Raspberry Pi. These pins can be turned on/off with software, thus not illumination the samples during the entire incubation period. The layout of the GPIO pins have been shown in figure 4.1.

![Separate GPIO for each group of LED’s.](image)

By the change of the resistance, it is possible to reduce or increase the current to each of the lighting groups. This provides the ability to change the illumination, if it proves problematic under testing.

8.1.2 Camera adjustment

The Raspberry Pi camera module is the camera chosen for the project, both for the compatibility and for the low cost at this stage in the prototype phase. Besides from these perks, it also comes with many easy to control features [9]. These features can be controlled automatically by the camera or set manually before taking the images. Capturing the consistent images requires manually selecting how the camera handles the different variables. The possible variables are:

- Shutter speed
- Exposure mode
- White balance
- Frame rate
- Resolution
One of the important variable to set, is the resolution. The resolution play a role in how much information can be gathered from the well. The resolution is therefore set the highest possible:

- Resolution = 3280 x 2464

The camera can also automatically adapt to the illumination present, this can be achieved by letting the camera be turned on for some time, before capturing its image. However to ensure consistent images, from this automatic adjustment, a set of images was taken. These can be seen on figure 8.3, and show a consistency in the adjustment of the camera.

![ Automatically adjusted images.](image)

Lastly the image is saved, for further analysis and to have the possibility to manually inspect the images if desired.

### 8.2 Tray localization

The next step in the flow of the system, after capturing a photo, is to find the Quanti-Tray/2000 within the acquired image. This step is crucial to get correct, as miss localization of the Quanti-Tray can lead to errors in the data extraction later in the system. Therefore the localization must be able to meet the requirements as stated in 1. Before deciding the method for localization of the quanti-tray, the procedure of it must be formed. The quanti-tray localization process has different stages, the stages can be seen in figure 8.4.

1Fixme Note: section KRAV!?!
8.2. TRAY LOCALIZATION

First of all it is necessary to isolate the quanti-tray, this makes it possible to find the edges, which then makes it possible to find the corners of the tray. Afterwards a perspective correction needs to be done, as similar images are wanted for further analysis.

8.2.1 Isolation of quanti-tray

Isolation of the quanti-tray is an important step to get correct, as this determines the outer border. If this detection is off, then the corners will be off and the perspective will be distorted. Yielding the final well analysis impractical.

Isolation of an object is normally done in computer vision using threshold. This is especially good for finding objects that differ in color or brightness. In this case, the object of interest is the quanti-tray, the trays have a silvery color, which makes it possible to distinguish from the black background in the image. This can be verified by looking at the color histogram of an image with the quanti-tray, seen on figure 8.5.

To get a understanding of how the backgrounds histogram would be represented, a slice...
Figure 8.6: Slice of the quanti-tray image, covering both the black background and the sidewalls and the corresponding color histogram.

Figure 8.7: The principle of template matching. [10]

of the previous image have been used. This slice have both the black background and some of the brown sidewall. Its histogram can be seen on figure 8.6. Here it can be seen that below 70 in the red and green colors, can be associated with the background as well as 45 in the blue color. These therefore can be set as thresholds for isolation of the quanti-tray. There is little to non change in color from image to image, making it possible to have a static threshold for isolation of the quanti-tray/2000.

Alternatively different template matching methods are also an option, since the shape of the test object is known. The general method for template matching, is to slide the template over the input image using convolution. This compares the template with each part of the image. From this a gray scaled output is created, from where the area with highest value is the place where the template has the best match. Template matching have many functionalities with finding letters, numbers or specific shapes and also faces in image processing. An example of template matching can be seen on figure 8.7. Also a convolutional neural network(CNN) could be used for detecting the Quanti-tray/2000, the task at hand seems to simple for such an advanced method. There is not enough change from image to image.
8.2. TRAY LOCALIZATION

8.2.2 Corner detection

The next part of the tray localization procedure, is to find the corners of the quanti-tray. By finding the corners of the quanti-tray, it is possible to do a transformation to get a consistent capture of the quanti-trays. This consistency is key, as the well mapping on the quanti-tray, will be done using a world model. The corner detection can be seen as two different steps. One step is to find lines for all the sides on the quanti-tray, and the removal of the tap on the left side of it, see figure 8.8 for reference. The next step is to use these lines to get the corner coordinates, from the point of intersection.

Edge detection

The detection of the outer borders of the quanti-tray, can be done with edge detection followed by a line estimation method. As the edge detection method provides coordinates of potential edges, the line estimation uses these coordinates to approximate a line for the entire edge. An edge can be seen as a change in value. This change in value is often between the two neighboring pixel. The comparison between these two, provides a gradient. If this gradient is greater than a threshold, it is classified as an edge. Edge detectors based upon this method is the Canny and Sobel edge detector. [11]

Focusing on the task at hand, since the quanti-tray have already been localized using thresholding, the difference between it and the background is huge. There it is not deemed necessary to analyze for any slopes as a change in value, will lead to an edge on the quanti-tray. A simpler technique is therefore proposed.

First multiple searching lines are used to find the boundary of the quanti-tray blob. As only the blob is of non zero value in the image, the search lines look for the first coordinate with a value. By using multiple of these, from all sides of the images, the outer lines will be illustrated as a number of coordinates along each side. An illustration of this can be found on figure 8.9, this shows the quanti-tray, next the searching lines and their intersection point.
Figure 8.9: Illustration of the used line search method.

**Line estimation**

To get a proper side detection, different theories have worked on line estimations all have different advantages and require different inputs.

Usually a least square estimator fits a model to all of the data in the dataset. This do not take noise and outliers into account. The estimated model is therefore a fit to all data, inliers, outliers and noise. This will have an negative effect on the final corner detection and an impact in the analysis of the well, as the mapping to world coordinates will not be aligned. As this use case can be very sensitive to outliers, as the tap on the top of the quanti-tray needs to be ignored.

RANSAC on the other hand tries to estimate a fit on only the inliers. It uses a non-deterministic algorithm approach to produce a result which fits with a certain probability. This probability depends on the number of iterations. In the python implementation of RANSAC each iteration have four step:

1. Select a minimum amount of random samples from the original data and check whether the set of data i valid.
2. Fit a model to the random subset and check whether the model is valid.
3. Calculate the residual of each data point to the estimated model. Classify all data as inlier or outlier based on the residual, compared to a residual threshold.
4. If the fitted model has the maximum number of inliers, it is saved as the best model. If the amount is equal, the best scoring model is chosen.

**Corner estimation**

Line-line intersection is a method for finding the intersection point between two lines. This principal is useful in this use-case, as each of the sides will be represented as a line estimation. The corner of the quanti-tray can therefore be seen as the intersection of these estimated lines from the sides. Thus the corner being a line-line intersection.

In line-line intersection there is different methods for calculating the intersection, each method uses a different input from the other. Either two point on each line can be used, one requires to be given the line equations and one uses the homogeneous coordinates.

With both point of intersection and the lines from the line estimation provided. These can be used for different methods. But as the tap of the quanti-tray could yield a prob-

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Note: KILDE!!?!!!???
lem, using the points found in edge detection doesn’t seem reliable. This is because the sometimes they will find tap of the wrong side of the quanti-tray, especially critical with the outer searching lines.

Another problem was discovered, this had to due with the lines provided from the line estimation, figure 8.10. The tool used did not provide the equations for the lines it estimates, but instead the coordinates. One of the line to line uses two points from two lines, to calculate the point of intersection. Taking two points from two provided lines, first and last point, it is possible to calculate the intersection of these lines. The equations used for this can be seen in 8.1 and 8.2. [12]

$$P_x = \begin{bmatrix} x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \\ x_3 & y_3 & 1 \\ x_4 & y_4 & 1 \end{bmatrix} P_y = \begin{bmatrix} x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \\ x_3 & y_3 & 1 \\ x_4 & y_4 & 1 \end{bmatrix}$$ (8.1)

This can then be rewritten as:

$$(P_x, P_y) = \left( \frac{(x_1 y_2 - y_1 x_2)(x_3 - x_4) - (x_1 - x_2)(x_3 y_4 - y_3 x_4)}{(x_1 - x_2)(y_3 - y_4) - (y_1 - y_2)(x_3 - x_4)}, \frac{(x_1 y_2 - y_1 x_2)(y_3 - y_4) - (y_1 - y_2)(x_3 y_4 - y_3 x_4)}{(x_1 - x_2)(y_3 - y_4) - (y_1 - y_2)(x_3 - x_4)} \right),$$ (8.2)
8.2.3 Perspective alignment

The image at this point shows the rest of the working area beyond the borders of the quanti-tray. To move further with the mapping from image coordinates to world coordinates, the quanti-tray needs to be isolated from the rest of the image. As the position of the quanti-tray is random, because the observer have wiggle room inside the cameras FOV to position the sample. This provides a less strict and a more easy to learn work flow, with this prototype. This freedom of position can prove troublesome, as the angle from the camera to each of the well changes all the time, this is also true for the rotation of the quanti-tray. The task at hand is to generate an image only containing the quanti-tray, which is perfectly centered in the image, see figure 8.11.

![Figure 8.11](image)

Methods for such a problem are transformations. Transformations, in particular transformation matrix, is a transformation from one plane to another plane. This transformation can both be rotational, scaling, shearing and reflection. Looking at the pictures at hand, it can be seen that a simpler transformation than the normal 3D perspective transformation could be sufficient. This is due to the fact that the Quanti-Tray/2000 itself may have a slight bend to it, making it hard to do a perfect transformation. However due to the main problem concern being the rotation and scale of the Quanti-Tray. Affine transformations are used to performed these. This is two different matrices used to perform these transformations.

8.3 Locate wells

Provided at this stage of the system, is the outer border of the quanti-tray. This indicate the ROI in the captured image. However the whole tray is not needed for further analysis, as the interest points are located on each of the quanti-trays wells. This is because the water sample is captured within each of these wells, and by limiting the working area to only include these, the further data processing will be smaller than analyzing the whole area within the borders.

This project will only work around the quanti-tray/2000. These quanti-trays are produced by IDEXX and it can be assumed that they are identically in shape and size. This is because they all most fit inside the presser, which distributes the water sample to each of the wells on the tray. This is shown in 3. Given that all quanti-tray/2000 can be seen

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\(^{3}\text{Fixme Note: REF til starten}\)
as identical, the method for locating the wells will therefor be a mapping from the world coordinates to the image.

For a mapping to be done properly, a correct measurement of the tray is needed. If these measurements are off, then the mapping to locate the wells, will not be precise. There are a lot of elements on the quanti-tray, which must be measured.

### 8.3.1 Mapping

With the measurements in place, a conversion to the image must be performed. With the image transformation in section 8.2.3, the image of the quanti-tray is seen from directly above and with its borders as the edges of the image. Provided that the border are correctly found, then the span of the image can be seen as the span of the quanti-tray. A conversion from centimeter to pixel can therefore be performed.

Looking at the creating mapping diagram on figure 8.12, it can be seen that the start of the big well is 1 cm from the top, and it ends 3.5 cm from the top. Providing a center of 2.25 cm from the top. This is furthermore marked as the center of the width of the Quanti-Tray/2000, at 7.15 cm.

The first medium well is positioned 1.9 cm from the side and 5.65 cm from the top. Spacing over 8 row with 6 wells each. The space between the wells on the same row, is found by the total distance from end to end center: 10.4 cm, and dividing this with the amount of wells minus 1: $\frac{10.4 \text{ cm}}{6-1} = 2.08 \text{ cm}$. The same is the the spacing between the rows: $\frac{14.55 \text{ cm}}{8-1} = 2.08 \text{ cm}$. Lastly is the coordinates for the small wells. The first one is positioned 1.95 cm from the side and 5.85 cm from the bottom, 27.9 cm - 5.85 cm = 22.25 cm from the the top. Here there are 10 wells on each row, except the last one, and a total of 5 rows. The math is the same, yielding in a distance on the row to: 1.16 cm. And between the rows: $\frac{5.85 \text{ cm} - 1.25 \text{ cm}}{5-1} = 1.15 \text{ cm}$

To ensure maximum resolution on the small wells, a scale of 10 pixel / 1 mm is used. Making the Quanti-Tray/2000 2790 pixels long.
Figure 8.12: Illustration of the well placement, on a Quanti-Tray/2000.
8.4 Find well color

Knowing the position of the well and therefore the area of interest for analyzing, it is necessary to figure out how big of a working area it is possible to have. There is many factors which have influence in this area, how accurate was the tray localized, where the sample starts being positive, as well as how big of an area is used for measurement. Another factor to take into account, is that the reference sample yield positive at a sudden value, this value must be comparable with the test samples, to get correct positive results.

The first task is therefore to determine this threshold for positivity. This will be done using the comparator made by IDEXX, mentioned in \(^4\). The comparator is used as a guide line for determining whether or not the sample is contaminated. By using this knowledge, the color intensity of the comparator will be the threshold for alerts. The comparator is in a liquid form, and will poured and pressed into a quanti-tray, as the procedure describes in \(^5\). It is then placed within the prototype and images are taken of it. One of the images can be seen on figure 8.13.

![Figure 8.13: Image of the comparator with the prototype.](image)

It can be seen that the color is not identical in all the wells. This could be because of the angle to the camera, the lighting is off and the curvature of the well. Therefore to ensure a proper threshold, a mean of multiple pixels within each well will be found. This mean will be the a mean of means, then the amount of pixels wont be that great. Later a short analysis for the optimal amount of pixel will be conducted. For the purpose of the mean, the image is processed in the software written, and the pixels used for mean calculation is the pixels within a square of 3x3 around the found center. An illustration of this can be seen on figure 8.14. This is then done on all the wells, and a mean is calculated.

\(^4\)Note: ref til comparator afsnit
\(^5\)Note: ref til start omkring hvordan man tager en prøve
For the result to be more reliable, since there is such a change in color from well to well. The tray is also rotated and moved around, for two additional times. This provides a more consistent mean for the threshold. This yields in a mean result of: 156.66. However looking at the histogram, figure 8.15, it can be seen that there is a large spread. The standard deviation of the result is: 23.72.

This huge deviation is caused by the color change that occur throughout the image. A local threshold for alerts will therefore be necessary using the current prototype setup.

8.4.1 Searching radius

The purpose of the analysis, is to get a proper value from the well, to ensure correct alert if a positive sample occurs. However to ensure that the reading of the well is correct and consistent, a mean value is found. This mean value will be found using the same method as in section 8.4, but this time the area for searching will be found, by looking at the change in mean, contra size of area. Since there is a limitation on the area and information, inside the small wells, this searching area must be optimal in regards to gathering the most information of the well.

6Fixme Note: Kom ind på at det kun er den blå kanal der kigges på.
The sample used in the test, is a known positive test. Using a positive sample the color represented will be of positive state. On figure 8.16 the variance of the different searching sizes have been plotted. This shows a minimum of variance using around 100 pixels for mean calculation. With a steady increase afterwards.

![Variance vs. n. pixels](image)

**Figure 8.16:** The variance of the mean, for the number of searching pixels.

**Area within well**

However the maximum possible area also depends on the accuracy of the mapping. If the mapping is not in the center, then the distance to the walls of well is smaller. This is more crucial when processing the data from the small wells, as their area is not that huge to start with. Using a searching area of 100 pixels for color calculation, the required world size is: 1 mm x 1 mm. This is a huge area, compared to the top area of the small wells, 3 mm x 3 mm.
Part IV

Module Tests
8.5 Locating Quanti-Tray/2000

Starting the program off, with the localization of the quanti-tray. This part is a critical component, as an error in this localization will ripple throughout the rest of the system. This is also a set requirement for the prototype, subsection 2.3.1, which states that 95% of the quanti-trays must be localized.

8.5.1 Testing procedure

The proposed system for localization should be able to meet this requirement. To ensure this, the system needs to be thoroughly tested. The test will show the performance of localization system.

For the testing a series of images with the quanti-tray in different positions have been taken. This will simulate how a user of the system will place the quanti-tray differently within the prototype, from time to time. This test both demonstrates the robustness and accuracy of the proposed localization procedure. In figure 8.17 multiple test images is showing how the position of the quanti-tray changes from image to image. On figure 8.17c it can be seen that some of the quanti-tray is not within the working area of the prototype, this could prove to be a problem for the localization system, as the quanti-tray is not completely shown.

To verify whether or not the system can localize the quanti-tray, there will be two way quantification of the test results. One will be done automatically and the next step will be manually. The manually will be to ensure that the fault is caused by the localization and not another part of the system. The automatically result yields from multiple steps of the program. After the image transformation in subsection 8.2.3, if the Quanti-Tray/2000 is not properly found, it will cause black pixel in the image. Counting these will indicate how well the Quanti-Tray/2000 was found.

8.5.2 Test

The test is conducted as stated in the procedure above. The quanti-tray is randomly moved, from image to image, thereby determining the robustness pf the system. A total
of 20 test images is used to give a wide enough variation for the position and skew of the object.

### 8.5.3 Results

The images can be seen in

<table>
<thead>
<tr>
<th>Image nr.</th>
<th>Positive</th>
<th>Negative</th>
<th>Error type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>×</td>
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<tr>
<td>3</td>
<td>×</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>(×)</td>
<td></td>
<td>Out of frame</td>
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<tr>
<td>6</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>×</td>
<td></td>
<td>Out of frame</td>
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<tr>
<td>9</td>
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On **Table 8.1**: Test result of the localization system. *(Sometimes)*

The variation is caused by image number 5. This image was not consistent in the result, sometimes it was found correctly other times not. Even with the possible detection of number 5, the result is far from the 95 % requirement.

- 65-70%

However investigation the error type, shows that all the occurring error yields from the quanti-tray being placed beyond the boundaries of the camera frame or even the frame of the prototype. Examples of this can be seen on **figure 8.18**.
Looking past this kind of error, the system achieves an accuracy of:

- 100 %

This result is however not that reliable, as the sample size is now down to only 13. But it proves that for this set of images, if the Quanti-Tray/2000 is within the boundaries of the prototype, the detection is accurate.
8.6 Locating wells

The test to locate the quanti-tray show imitate results, when looking past the problems with not being able to properly locate the tray, if the tray is not positioned within the working area of the prototype. This proves sufficient enough for further analysis with the prototype. The prototype test, is also conducted as element test before a complete prototype test. Testing each subsystem on their own provides information about their individual performance, showing if they can meet the requirements for the prototype.

The entire procedure for the location of the wells is documented in section 8.3. A short summary is that after the tray has been located in the previous subsystem, a map of the quanti-tray is used to map the quanti-tray to real world coordinates.

This mapping must meet the accuracy requirement set in subsection 2.3.1. The accuracy must be within a square of 3 mm x 3 mm. From the mapping to real world coordinates, this area translates to 30 x 30 pixels.

8.6.1 Testing procedure

The proposed system for locating the wells should be able to meet this requirement. To ensure this, the systems needs to be thoroughly tested. The test will show the performance of the system.

For the testing a series of Quanti-tray/2000 images is used. Here only the Quanti-Tray/2000 is in the scene. The test procedures as the following:

2. Plot 30 x 30 blob on each coordinate.
3. Measure the distance from the blob to the center.

8.6.2 Test

The images selected for this test, is the images of which the localization method was able to locate the Quanti-Tray/2000. This is important, as the first step in this system requires a proper localization. The sample size for this test is therefore down to 13 images.

8.6.3 Results

The images can be seen in appendix C. These results deemed harder to give concrete numbers open.

One thing to none by looking at the images of the mapping result, is that all of the coordinates are within their represented well. However since the angle and perspective
is so difference from well to well, on the outer line, it is hard to determine whether the
found position is on the top part of the well or on a curve. This might show difficulty
upon reading the color value. Therefore this requirement for localization the wells, is not
completed.
8.7 Analyzing of color

Analyzing the colors within the wells, is the task that can completely fail the system. If this system is not able to track the change in color, then it will not be able to alert any authorities of a potential crisis. Therefore the proposed method will be tested, to demonstrate how it performances.

8.7.1 Testing procedure

For testing the color analysis process, a different approach is used. Since the mapping from image to real world, did not meet the requirements some of the mapping will not be used. Instead this process will focus on the color change of one well. Thereby somewhat isolation this test from the previous processes.

The test will therefore also use the optimal searching area of 10 x 10 pixels, see subsection 8.4.1. The procedure is then to extract the mean of this area over time.

8.7.2 Test

The testing images for this process, is a time-lapse of a complete incubation phase. Here images are taken of the sample, with a frequency of one image per three minutes. The system will focus on an already known positive well, well 46, thereby providing a positive result to the system. On figure 8.19 the sample at hand can be seen, as well as the well of interest.

Figure 8.19: The sample and the well for analyzing.
8.7.3 Results

The results on figure 8.20 show that after approximately 18 hours, the growth of the Coliform bacteria starts, in this well. Thereby the system successfully analysis a complete incubation phase of a water sample. Proving that the created method works.

![Graph of incubation phase](image)

**Figure 8.20:** A graph of the incubation phase, monitoring well nr. 46.
Part V

Closure
Chapter 9

Conclusion

This chapter will conclude on the findings in the report. These will be compared to the problem statement in section 2.1, by comparing the system results with the requirements in section 2.3.

The problem statement for this project is:

Is it possible to create a vision based system to automatically detect Quanti-Tray/2000 and analyze the water samples within?

As a start of the process, an analysis of the problem at hand, was conducted. This analysis introduced the procedure of manually analyzing the Colilert results. Here the Quanti-Tray and Quanti-Tray/2000 was introduced, and the potential problems regarding localization appeared. The conceptual idea from Amphi-Bac was introduced, a focus area was chosen, the vision solution for the concept. Then started the prototype build, this included a physical model and a software solution. The physical model’s task was to capture a good image of the Quanti-Tray/2000, despite a reflective surface. For the software a series of different methods was created and combined to detect and analyze the Quanti-Tray/2000. Involving both localizing the Quanti-tray, mapping coordinates and analyzing each well.

The results from the module tests, showed both promising results, but also the limitation of the prototype. Looking at the results with the requirements, section 2.3, in mind, then the prototype did not meet the requirements. A lot of miss located Quanti-Tray/2000, due to miss placement and therefore not inside the camera FOV. A trend of non centered mapping occurred. However the system was able to track the progress of color change in wells. Therefore the set requirements is not meet.
Chapter 10

Discussion

The purpose of this chapter is to reflect and highlight issues in the project. This will especially be concerning the prototype and the requirement testing.

The prototype build in the progress of this report faced some limitations. For a fast building process, the whole setup, became very prototype-like. And with no machining tools available at Amphi-Bac, it took its form. Some of the improvements that could have been made to it: This illumination setup, did not serve its purpose of giving a shadow free illumination, this could be due to less that proper placement, but a new lighting setup could limited the shadows and highlight found on the images. Alternatively a tray of the Quanti-Tray/2000 could be used, only exposing the top of the wells. Making both localizing and analysis easier. A properly mounted solution would not be a accepting to outside interference. Building the cardboard made the prototype very light, making it vulnerable to moments of the incubation cambers door.

Even though the system did not meet all the requirements, it still managed to read and entire incubation phase, and detect a change in color. Using a better lighting setup, could yield an increase in area of interest of the smallest wells. This is due to the limitation of reflection and highlighting, providing the additional area for the mapping.

If building a new prototype for this project, then the solution would be based on custom lighting. The camera was more than sufficient, however more professional cameras could provide a mode true to live color.
Chapter 11

Bibliography


Part VI

Appendix
Appendix A

Quanti-Tray MPN
### IDEXX 51-Well Quanti-Tray® MPN Table

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IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

09-63234-00
Appendix B

Quanti-Tray/2000 MPN
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**IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)**
Appendix C

Locate wells

Figure C.1: Image nr. 1
Figure C.2: Image nr. 2
Figure C.3: Image nr. 3
Figure C.4: Image nr. 4
Figure C.5: Image nr. 6
Figure C.6: Image nr. 7