

# Temperature controlled Microbioreactorsystems

By  
Kevin Marczyk

Under supervision of  
Martin Velthuis, MSc  
prof. dr. G.J.W. (Gert-Jan) Euverink

GRADUATION REPORT

Submitted to  
Hanze University of Applied Science Groningen

in partial fulfilment of the requirements  
for the degree of

Fulltime Honours Bachelor Advanced Sensor Applications

19/06/2016

## **Abstract**

### *Temperature controlled Microbioreactorsystems*

By  
Kevin Marczyk

The report concerns research and development of a non-invasive automated temperature control system for bioreactors on a microscale. The aim of the project was to develop a system that could apply a temperature gradient to a standardized 96 well microtiterplate.

The developed system uses a thermographic camera to determine the temperature of the wells of a microtiterplate, this sensor data is then used to calculate an output to the temperature control system. This control system consists of 2 Peltier elements which are able to heat an aluminium heatsink located underneath the microtiterplate and keep the wells at a stable temperature.

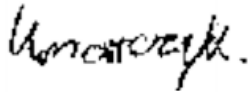
Further research can be performed on the design of a lid for the microtiterplates which is able to let through all near infrared light to limit disturbances and contaminations. A recommendation is to replace the infrared camera by separate infrared thermometers due to their smaller size.

## Declaration

I hereby certify that this report constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the report describes original work that has not previously been presented for the award of any other degree of any institution.

Signed,



Kevin Marczyk

## Acknowledgements

I would like to express my gratitude to my supervisors Martin Velthuis (MSc) and Prof. Dr. Gert-Jan Euverink from the university of Groningen for allowing me to work on this project. Furthermore, I would like to express my thanks to Adam Wexler from research institute Wetsus for providing his knowledge and sharing his expertise.

## Table of contents

1	Rationale.....	10
2	Situational & Theoretical analysis .....	12
2.1	Temperature monitoring.....	12
2.2	Methods of temperature determination .....	14
2.3	Methods of temperature control .....	16
3	Conceptual model.....	22
3.1	Temperature monitoring.....	22
3.2	Temperature control .....	25
3.3	Thermal conductivity of polystyrene.....	26
3.4	Proposed setup.....	28
3.4.1	Temperature control .....	29
3.4.2	Circuit design .....	30
3.4.3	Selection of the Peltier element.....	31
3.4.4	Aluminium heatsink.....	31
4	Research design.....	32
5	Performance evaluation .....	33
5.1	Accuracy & Hold stability.....	33
5.2	Disturbance rejection .....	35
5.3	Rise time .....	36
5.4	Overshoot.....	37
5.5	Maximum temperature difference over the microtiterplate.....	38
6	Conclusion .....	40
7	Recommendations.....	42
8	Bibliography.....	43
9	Appendices .....	46
9.1	A: MATLAB Code.....	47
9.2	B: Images of the constructed set up.....	59
9.3	C: Accuracy and Stability at 45 degrees Celsius.....	63
9.4	D: Confidence interval calculations about Accuracy and Stability at 30, 37, and 50 degrees Celsius.....	66

## List of Figures

Figure 1 – A small scale benchtop fermenter used for cell culture, bacteria or fungi for example [24].	10
Figure 2 - A large scale 90 liter bioreactor used for cellulosic ethanol research [25].	10
Figure 3 - Polystyrene infrared spectrogram [4].	12
Figure 4 - The transmission spectrum of polypropylene [5].	13
Figure 5 - The transmission spectrum of polycarbonate [5].	13
Figure 6 - Well positions of a 96 well microtiterplate [9]. Measurements are in Milimeters/(Inches).	15
Figure 7 - Two mirrors deflecting a beam on an X, Y plane [26].	17
Figure 8 - The absorption spectrum of water [27].	18
Figure 9 – The absorption coefficient of water for different wavelengths of electromagnetic radiation [25].	19
Figure 10 - Intensity of the electromagnetic radiation in a single well when using a 337.5 Watt IR source. ....	20
Figure 11 – BIO-RAD’s CFX Connect™ Real-Time PCR Detection System [15].	21
Figure 12 – A Peltier element heating up an aluminium heatsink, the temperature of which the infrared camera is unable to determine. However, when adding water on top of the heatsink the temperature becomes apparent.	21
Figure 13 - An example image from the Arduino compatible infrared camera [23].	23
Figure 14 - Typical FOV of Melexis MLX90614xCI [34].	23
Figure 15 - Accuracy of MLX90614 [34]. ‘To’ is the object temperature, ‘Ta’ is the sensor temperature. The situation in which the sensor would be used is outlined in black.	24
Figure 16 - Use of multiple MLX90614 devices in SMBus network [21].	24
Figure 17 – A microtiterplate that had heated up to the maximum temperature possible. The well at Spot 9 is filled with tap water, the remaining wells are empty.	26
Figure 18 –A sample of the initial test results displaying the temperature throughout the wall of a well. A 12 Watt Peltier element is located underneath an aluminium heatsink, the microtiterplate is positioned overtop. The well at Spot 7 and Spot 8 is filled with water, while the well at Spot 9 is empty. Each image shows the microtiterplate at a different temperature, the results show that there is a difference in temperature throughout the well, about 1 degree Celsius in the best case scenario, in the worst case a temperature difference of 3 degrees Celsius was found.	27
Figure 19 - An example setup of a microbioreactor system. The green cone represents the field of view of the infrared camera. Note that the temperature gradient plate located below the microtiterplate is not depicted here.	28
Figure 20 – The separate parts required for assembly of the setup.	28
Figure 21 - A flowchart describing how the system will perform.	29
Figure 22 -The graphical user interface allows the user to connect to the camera and make sure that the measurement points are aligned to the wells of the microtiterplate.	30
Figure 23 – Circuit schematics. Instead of 1 Peltier element, the final design has 4 Peltier elements connected in the same manner.	30
Figure 24 – A thermal gradient over a microtiterplate, the leftmost 2 columns will be set at a maximum temperature, while the rightmost 2 columns will be set at a minimum temperature [15].	31
Figure 26 – The temperature over time for three different temperatures, 30 degrees Celsius, 50 degrees Celsius, and a nominal temperature of 37 degrees Celsius.	33
Figure 27 – The error distribution for a temperature of 30 degrees Celsius.	33
Figure 25 - The error distribution for a temperature of 37 degrees Celsius.	33
Figure 28 - The error distribution for a temperature of 50 degrees Celsius.	34

Figure 29 - A graph depicting the temperature of the hot side of the microtiterplate over time. In this case it is demonstrated how the system is able to handle disturbances. In this example, a gust of colder air cooled down the wells of the microtiterplate which was temperature controlled to be at 45 degrees Celsius. The system reacts quickly, however some overshoot occurs because the heat needs to travel from the Peltier element, to the aluminium heatsink and through a layer of polystyrene. ....	35
Figure 30 - Temperature of the system over time while heating up to a goal temperature of 45 degrees Celsius. At 400 seconds the goal temperature was reached, however due to overshoot the temperature of the system was stable at 500 seconds. ....	36
Figure 31 - The temperature of the hot side of the microtiterplate logged over time with different goal temperatures. ....	37
Figure 32 - The temperature difference between the hot and cold side in a graph while the system is set at 50 degrees Celsius. The y axis represents the difference in temperature between the hot and cold side of the microtiterplate. The overshoot can be found in this graph as the 'bump' towards the right within the red rectangle. After the overshoot the difference between the cold and hot side is lowered by about 2 degrees, which can also be concluded from the data in the red rectangle. This results in a usable temperature gradient. ....	38
Figure 33 - The temperature difference between the hot and cold side in a graph while the system is set at 45 degrees Celsius. The y axis represents the difference in temperature between the hot and cold side of the microtiterplate. After stabilizing at a goal temperature the temperature difference over the microtiterplate will go down. ....	39
Figure 34 - The temperature of the hot side of the microtiterplate while the system has a goal temperature of 45 degrees Celsius. ....	64
Figure 35 - A bell curve depicting the relative occurrences of temperature errors when the system is stable at 45 degrees Celsius. The mean error is -0.05 and the dataset has a standard deviation of 0.10. ....	64





## 1 Rationale

The goal of this chapter is to introduce the reader to the topic at hand and provide background information related to the research that will be done. It also includes the research question with the supporting sub-questions.

Bioreactors are commonly used to support a biologically active environment; such is needed when growing cells for example. Organisms inside a bioreactor are often submerged in a medium. The bioreactor devices affect the growth of the organisms by controlling the important factors such as temperature, available nutrients, pH and gasses which are possibly dissolved in the medium. For this reason, bioreactors are often closed off, this allows for more precise control over the substance inside since no outside factors are able to influence the substance inside [1].



*Figure 2 - A large scale 90 liter bioreactor used for cellulosic ethanol research [39].*



*Figure 1 – A small scale benchtop fermenter used for cell culture, bacteria or fungi for example [38].*

The reason for this research is that it is currently relatively expensive to have lots of bioreactors for microbiological screening, there is a need for smaller, cheaper bioreactors. These bioreactors take up quite some space compared to the microtiterplates which are widely used by microbiologists nowadays. The plates can contain up to 96 wells, each with a working volume of 200 to 250 microliters, and are relatively cheap to manufacture [2]. Using these microtiterplates for microbiological screening comes with some negative aspects as well, for example there is no temperature monitoring or control [2]. With only 200 to 250 microliters of volume to work with, it is not possible to have an invasive heating solution, since that would reduce the amount the well is able to support.

Currently microbiologists do not measure the temperature and pH of each well, since they are not used as fermenters yet. Investment costs are too high for a large number of fermenters. The process of fermenting takes a long time and is quite intensive when managing more than a few, which makes it more expensive.

The desired situation is an automated system which can measure and control the temperature of each well individually using a non-invasive method (for example image analysis using an infrared camera) and a proportional–integral–derivative (PID) control system. In this way you would have control over multiple smaller fermenters, without the effort that it would usually require when using larger fermenters. By controlling the temperature of each well microbiologists are able to create a temperature gradient and compare the fermentation process at different temperatures. The UG has requested that the system is able to keep the wells at a stable temperature between 30 degrees Celsius and 50 degrees Celsius depending on the user. When the system is stable at a temperature the temperature cannot differ more than 0.5 degrees Celsius from the target temperature. The rise time of the system was not specified; the only requirement was that the bacteria must be able to survive. Individual control of the temperature of the wells would be ideal, since this would give the user more control and would allow for 96 different cultures at 96 temperatures in a single microtiterplate.

These smaller fermenters will require less space and can be handled easily by a single biologist. They are cheap to produce, but should not compromise control and reliability. From these requirements of the UG and the information on microtiterplates we can find our research question to be:

- *How can the temperature of the substances in the different wells of a 96-well microtiterplate using an automated non-invasive method?*

#### Sub-questions

- *How do we measure the temperature of these compartments using a non-invasive method?*
- *What method of non-invasive heating would allow for transfer of heat energy without killing bacteria growing in the wells?*
- *What would a possible 'microbioreactor' setup look like?*
  - *What would be the performance of such a system?*
    - *Accuracy*
    - *Hold stability*
    - *Response time*
    - *Maximum temperature difference over the microtiterplate*
    - *Disturbance rejection*

## 2 Situational & Theoretical analysis

This chapter evaluates the current situation and the desired situation by the University of Groningen (UG). Different temperature determination and control methods will be compared and their suitability for the application will be evaluated.

Stakeholder are most importantly the biologists who will be working with the 96 well microtiterplates. When they are able to monitor and control the temperature easily more people would use it. In this situation the UG made one of the requirements to keep the amount of wires going from and to the microtiterplate to a minimum because of the fact that the microtiterplates should be able to be swapped at an instant. Sensors such as a thermistor needs 2 wires, 1 common ground, and you would need 96 of them to measure each individual well in a microtiterplate. This would result in almost 200 wires. Currently the temperature of individual wells is not monitored.

### 2.1 Temperature monitoring

The 96 well microtiterplates from BRAND are made of polystyrene [3]. These microplates are currently used at the host organization; therefore, the research will focus on a solution compatible with the BRAND microtiterplates.

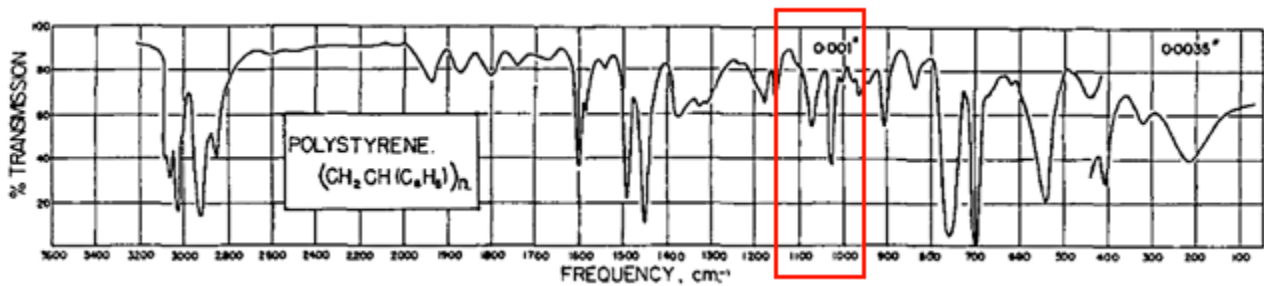


Figure 3 - Polystyrene infrared spectrogram [4].

A method for non-invasive temperature monitoring could be using the heat energy which is radiating from the substance in the well [5]. Wien's Displacement Law, equation ( 1 ), determines at which frequency most thermal radiation will be emitted by the object or substance.

$$\lambda = \frac{b}{T} \quad (1)$$

Where T is the absolute temperature in kelvin, b is the constant of proportionality ( $2.8977729 \times 10^{-3} \text{ m K}$ ) and  $\lambda$  is the peak wavelength. The temperature of the compartments will be kept warmer than 30 degrees Celsius and will not exceed 50 degrees Celsius. This means that the peak frequency for the radiated infrared light is  $10.056 \mu\text{m}$  at 15 degrees Celsius and  $8.967 \mu\text{m}$  at 50 degrees Celsius. This equates to  $994 \text{ cm}^{-1}$  at 15 degrees Celsius and  $1115 \text{ cm}^{-1}$  at 50 degrees Celsius (converting from  $\mu\text{m}$  to  $\text{cm}^{-1}$  is done by dividing 10000 by the amount of micron).

Looking at Figure 3, we can see that in the range of  $994 \text{ cm}^{-1}$  to  $1115 \text{ cm}^{-1}$   $\mu\text{m}$  some infrared light is absorbed by the polystyrene case. Two major peaks are present, one where the transmission dips slightly below 40%. A possible 60% absorption of the infrared light emitted will have quite an influence on measurements.

In an optimal use case the material of which the microtiterplates are made would be completely transparent for infrared light.

The microplates are sometimes also made of polycarbonate or polypropylene, these 2 materials do not offer any advantage over polystyrene when comparing the infrared absorption spectrum [6].

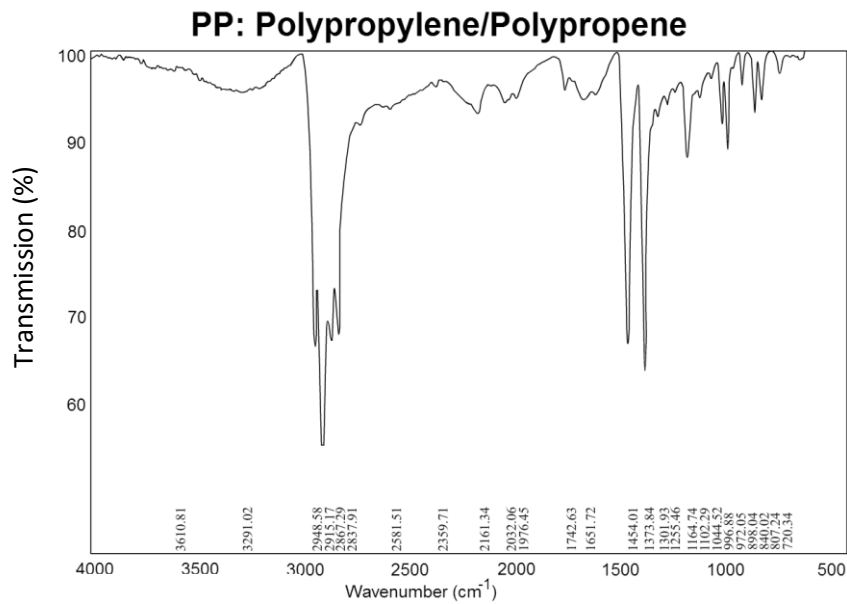


Figure 4 - The transmission spectrum of polypropylene [6].

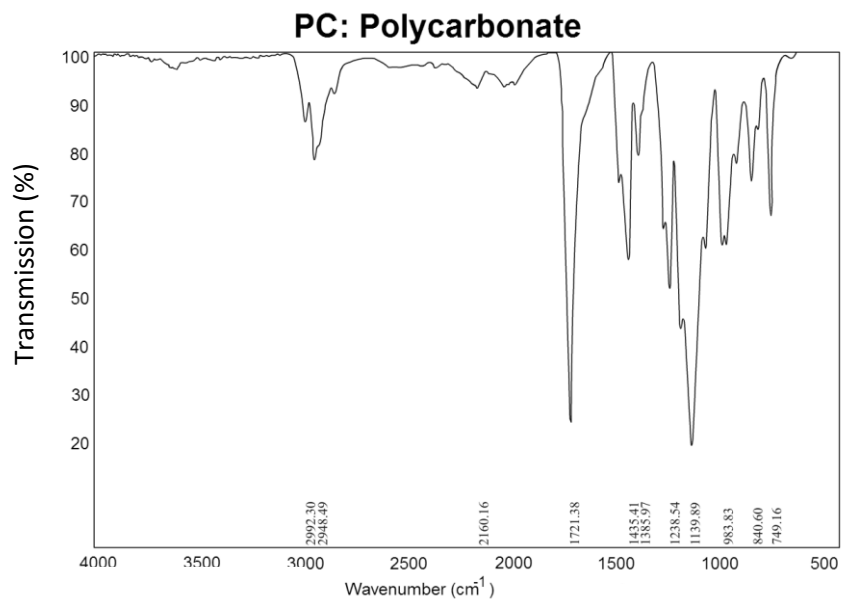


Figure 5 - The transmission spectrum of polycarbonate [6].

## 2.2 Methods of temperature determination

This chapter discusses the different possible method which could be used to determine the temperature of the substance inside the wells of a microtiterplate. It is important that the measurement does not affect the substance in any way, when using invasive temperature measurement methods, we reduce the amount of substance which is able to fit inside the well, since these are only able to contain 200 to 250 microliters non-invasive measurement methods are preferred.

Non-invasive temperature measurement methods work in the same way generally; objects emit infrared energy which is picked up by sensors. Different sensors could be used for this application:

- A single infrared thermometer [5]
- 96 separate infrared temperature sensors [5]
- An infrared camera [7]

When compared to the other temperature monitoring solutions, an infrared camera would seem optimal in this situation to determine the temperature of 96 well of a microtiterplate in a short time and in a non-invasive method. Infrared cameras are basically like regular cameras but meant for a different part of the electromagnetic spectrum. All objects emit some infrared radiation which can be picked up by the camera. From the amount of infrared radiation we can determine the temperature of the object.

One of the limitations of an infrared camera is that it is only able to detect the temperature of the surface. Since we are dealing with a small volume, 200 to 250 microliters [3], the surface temperature might not be noticeably different from the temperature in the middle of the liquid, it has to be tested whether this is true in our situation. Another drawback which has a chance to influence the measurements is the reflectivity of infrared light from other sources on the 96 well microtiterplates. A way to reduce this is by making an “infrared-proof” case around the setup, in this way it is not possible for infrared light from outside sources to reach the camera.

Emissivity of an object is a measure of its ability to radiate infrared energy compared to a perfect black body. It is not accounted for when using an infrared camera, this means that it cannot distinguish between different materials [8]. In order to get a more accurate reading, the emissivity of the material should be taken into consideration.

Another non-invasive method would be to use an infrared laser thermometer [5]; this would mean that we need a lot of mechanical movement to point the laser to the right well. Mechanical movement means that determining the temperature of every well takes more time and the system needs more maintenance over time. Infrared cameras are relatively expensive compared to thermistors and loads of wiring, compared to the infrared camera used now, even 96 separate infrared temperature sensors would be significantly cheaper. The infrared camera used by the UG costs 12000 euros [2], while separate infrared temperature sensors could be obtained for about 10 to 15 euro per unit. Each well in a 96 well microtiterplates has a diameter of 9 mm [9], this gives some additional restrictions to the specifications of an infrared temperature sensor, specifically the size, which raises the cost.

## Temperature control

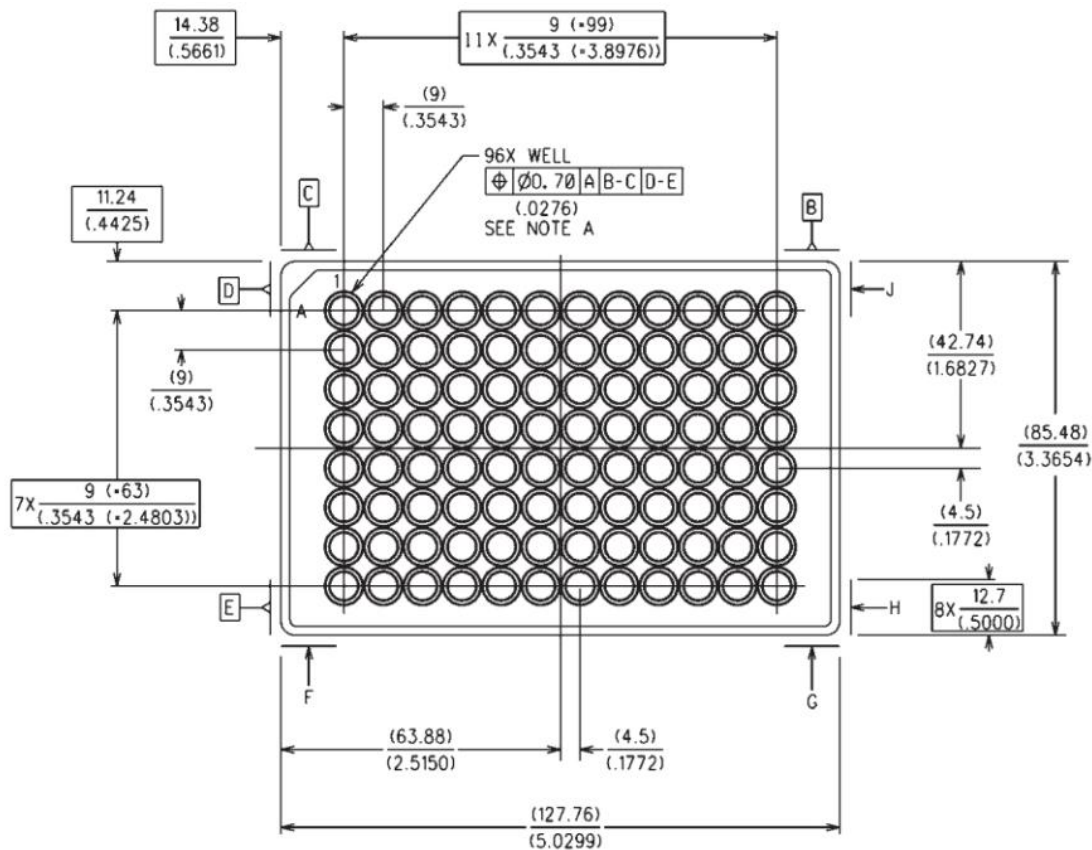


Figure 6 - Well positions of a 96 well microtiterplate [9]. Measurements are in Millimeters/(Inches).

Each well in the microtiterplate has a working volume of 200 to 250 microliters. The substance in the well is only exposed to the outside at the top. 230 microliters equates to about 7 mm of liquid.

The substance in the well will most often contain water with a specific heat capacity of  $4.186 \text{ J g}^{-1} \text{ K}^{-1}$ , which is a relatively high specific heat capacity. 250 microliters of water would equate to 0.250 grams of water, which would mean that every well would need 1.047 J to increase the temperature by 1 degree Celsius.

## 2.3 Methods of temperature control

When discussing non-invasive temperature control methods, we assume the fact that the heating element is not in direct contact with the substance in the well of the microtiterplate. A couple of possible non-invasive heating methods are as follows.

1. Induction heating [10]
2. Laser heating
  - Infrared laser with a black object in the substance [11]
  - Infrared laser to heat the water directly [12]
3. Infrared pattern projection [13]
4. Microwave phased array [13] [14]
5. Heated air or water, also known as convection is technically not non-contact, however in this case non-contact is defined as “not in contact with the substance inside the well of the microtiterplate”. [15]
6. Conductance using a temperature gradient plate [15]

Each method of temperature control will be explained and its pros and cons will be compared.

### 1. Induction heating

Induction heating will only work when there is an electrically conducting object inside the wells of the microtiterplate. It is not possible to wrap a coil around each individual well, since they are located so close together that there is no excess space left (Figure 6) [9].

Induction heating works by inducing eddy currents, heat energy will be generated due to the currents [10]. This method would be similar to how an induction cooktop operates; this time it would be on a smaller scale.

To control each well individually we would need 96 separate coils, which are small enough to fit under each well. This method of heating would be efficient since the electrically conducting object inside the well will heat up directly and will transfer the heat to the substance in the well. When ‘activating’ a coil the surrounding coils will also experience the change in the magnetic field, causing them to generate electrical energy as well, this phenomenon is known as *crosstalk* and will need to be accounted for [16].

The main advantage of using induction to control the temperature is the fact that it is safe (for the operator) to use when compared to a laser [10]. The power which is needed to heat up the wells will be used efficiently. Though the cost will go up since 96 small coils will be needed to control the temperature of the wells individually.

A big disadvantage when using induction heating, is that an object reduces the amount of substance the well is able to hold, however when making this object small it will create a ‘hotspot’. At this location in the well all the heat energy will be concentrated and might deviate a lot from the average, or desired, temperature in the well, causing stress for surrounding bacteria which might even die. To prevent this from occurring, heating the substance must be done slowly and gradually which is easier to be done when the object is slightly larger, since its heat capacity will go up. By including a small object in the well, this method is technically not non-intrusive. Not all metals will work well, they are limited by their compatibility with induction; they need to be ferromagnetic [10]. Another constraint is that the metal might influence the substance, caused by rusting for example; these factors limit the suitable materials.



## 2. Laser heating

A laser emits a beam of coherent monochromatic light, meaning that it only emits light at a certain wavelength [17]. Since the beam is not divergent nor convergent, it is able to be focussed on a tight spot. A laser keeps adding energy to a ray of light, which makes it a concentration of energy [18]. The laser should be a relatively high powered laser and at a wavelength where the water absorbs most, if possible all, of the energy emitted by the laser.

However, even if 100% is absorbed by the water, or an object in the substance, it would still possibly take quite a while to heat up. Because these wells contain a small amount of volume, the substance will lose heat quite rapidly, so short bursts of energy are needed to maintain the temperature.

The requirement set by the UG is to maintain the temperature within 0.5 degrees Celsius of the desired temperature, so whenever the temperature has reached to lower limit, it would need about 1 Joule of energy to heat back up by 0.5 degrees Celsius. A 100 mW laser could supply 100 mJ per second to a single well, meaning that it would need about 10 seconds to get the temperature back to the desired temperature. However, in this situation we assume that the substance does not lose any heat energy to the environment while heating up.

Directing the laser to the right well could be done by the use of a 2 axis mirror galvanometer or two single axis galvanometers as can be seen in Figure 7. By controlling the X and Y axis one is able to direct the beam to any of the 96 wells.

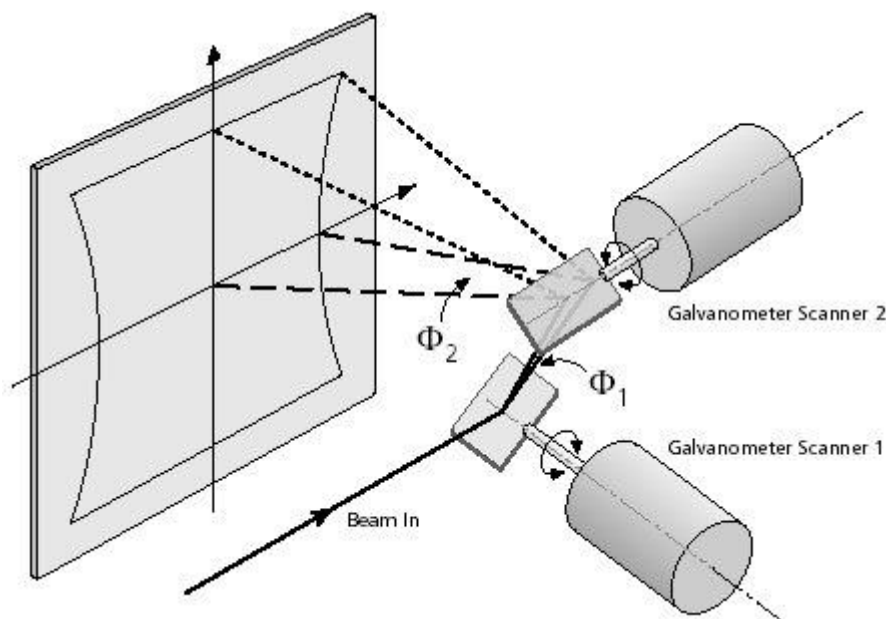


Figure 7 - Two mirrors deflecting a beam on an X, Y plane [40].



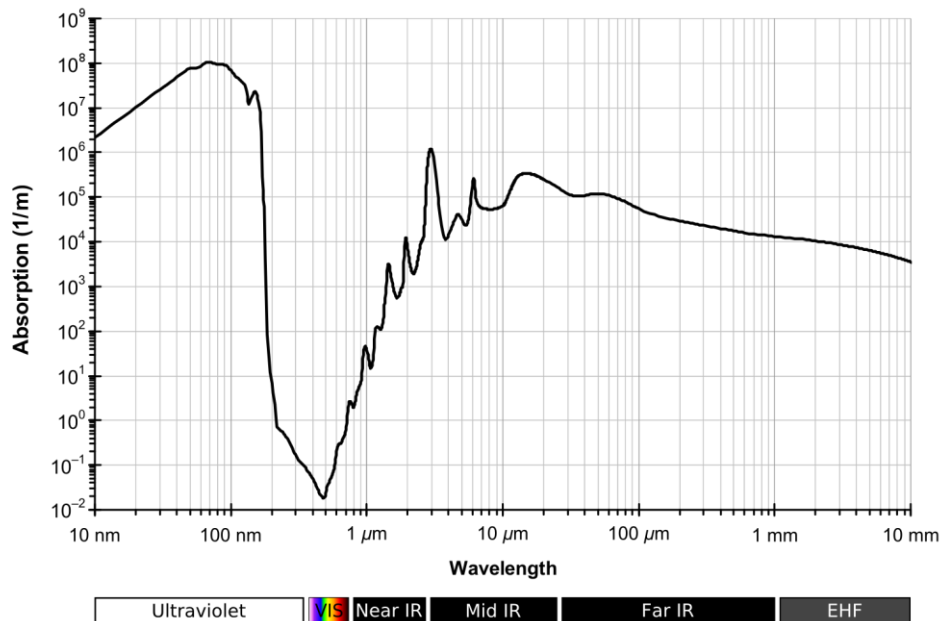


Figure 8 - The absorption spectrum of water [41].

Penetration of the water will be dependent on the wavelength used. Infrared radiation ranges from 700 nm to 1 mm (430 THz – 300 GHz) [19]. It is not desired to heat the top micron of the substance, if this is the case one of two problems will occur:

1. The infrared camera will determine that the substance in the well has reached the desired temperature and therefore the heating process will be stopped. In this case the rest of the substance in the well is still below the desired temperature.
2. The infrared heating process has to continue for quite some time to make sure that the heat has evenly distributed throughout the substance in the well, this could cause a 'hotspot' at the top few microns which could in turn kill the bacteria.

Preferably a wavelength and intensity should be used which is able to penetrate 7 mm of water, since this will allow for more water to aid in the absorption of heat energy from the infrared radiation. Ultraviolet is not suitable since this will kill the bacteria. From Figure 8 we can conclude that water becomes absorbing over the near infrared region, which ranges from 0.78  $\mu\text{m}$  to 3  $\mu\text{m}$  [20]. An appropriate wavelength for absorption could be 1450 nm, at this wavelength the absorption by water increases significantly [21].

Another method to heat up the substance in the well with a laser is by adding a small object which is able to absorb the energy from the laser being beamed on it. This reduces the amount which is able to fit inside the well, since there is an additional object located inside. The technique does not have to be dropped immediately because of this, since the object could be shaped so it would be flat and be located at the bottom of the well.

Penetration depth of infrared light in water will be discussed in method 3.

When using a laser to heat the substance inside one of the wells of a microtiterplate certain safety concerns are involved. One cannot determine if an infrared laser is turned on or off with the naked eye and a laser powerful enough to heat water can cause permanent damage to one's retina. Unfortunately, due to these safety concerns this method will not be considered for an end result, it could however be used for a proof of principle for non-invasive heating.

### 3. Infrared pattern projection

Texas Instruments digital light processing (DLP) chipsets are often found in projectors [22]. This method is essentially the same working principle as heating using a laser. However, in this case we can use a light bulb which is able to emit near infrared light. To control which wells need heat energy one would use the DLP chipset to project a pattern overtop the microtiterplate.

Philips sells numerous infrared incandescent light bulbs, designed for applying heat in a non-invasive manner [23]. The most powerful which is available is able to output 375 Watt, 90% of which is emitted as infrared heat energy [23]. This results in 337.5 Watt of infrared heat energy.

This 337.5 Watt needs to be focussed at the microtiterplate, which has a surface area of 109.21 cm<sup>2</sup> [9]. From this information we are able to find out what the intensity of the electromagnetic radiation is at the surface of the microtiterplate using equation ( 2 ).

$$F(x_0) = \frac{337.5}{8.548 * 12.776} = 3.09 \text{ W/cm}^2 \quad (2)$$

To find out how this energy is absorbed by water equation ( 3 ) and equation ( 4 ) can be used.

$$F(x) = F(x_0)e^{-a(x-x_0)} \quad (3)$$

$$a = \frac{4\pi f k}{c} \quad (4)$$

Where  $a$  is the absorption coefficient,  $k$  is the extinction coefficient,  $c$  is the speed of light in m/s,  $f$  is the frequency of the electromagnetic radiation which is being used. These coefficients for water are  $k = 0.00012487$  and  $\alpha = 10.822 \text{ cm}^{-1}$  [24]. 9 shows that the absorption coefficient changes when using different wavelengths, at 1450 nm the absorption coefficient peaks.

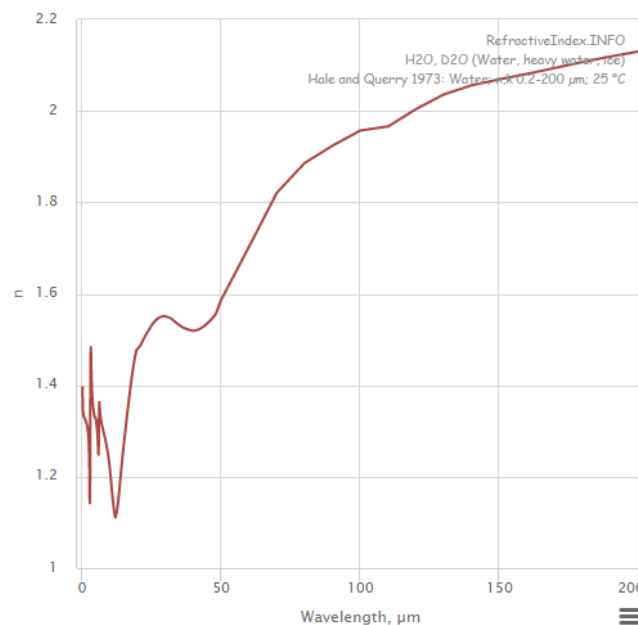


Figure 9 – The absorption coefficient of water for different wavelengths of electromagnetic radiation [25].

Plotting  $F(x)$  shows how the intensity changes throughout a sample, as can be seen in Figure 10. For each wavelength the shape of this graph will generally be the same.

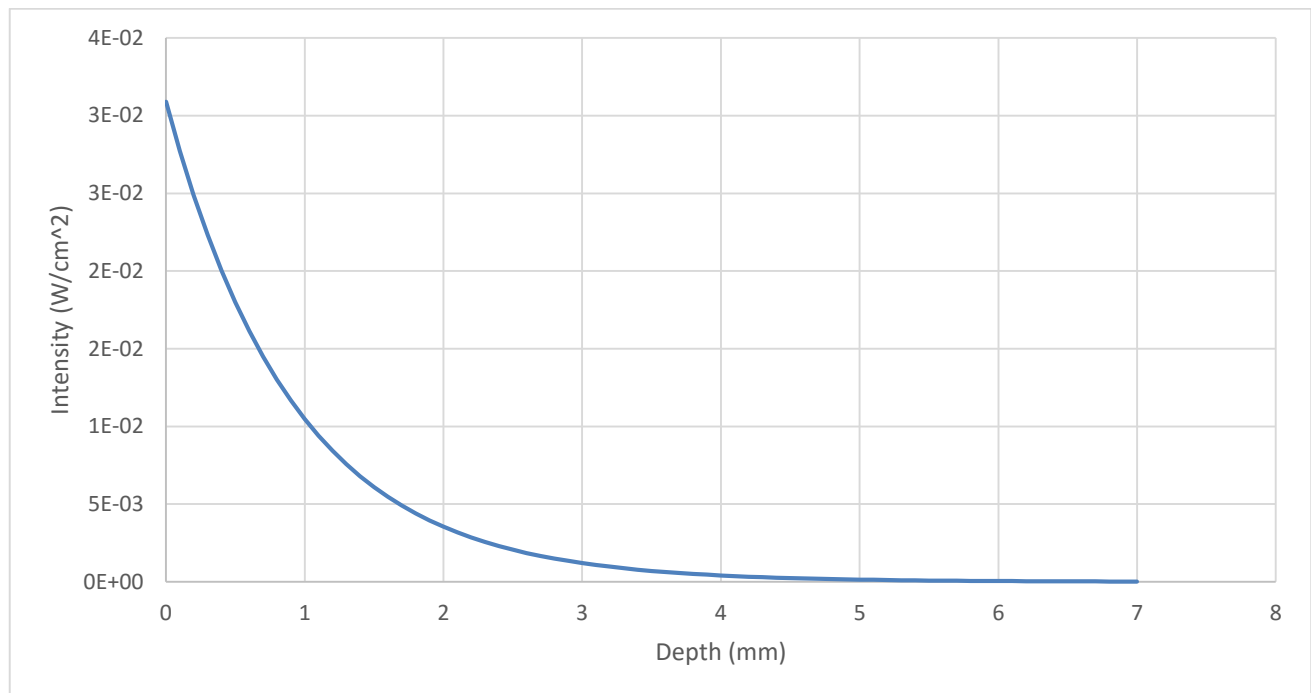


Figure 10 - Intensity of the electromagnetic radiation in a single well when using a 337.5 Watt IR source.

Figure 10 shows that the top 3 mm will absorb most of the electromagnetic radiation. Causing the bottom few millimetres to heat up less. Since heat rises, the bottom few millimetres will not naturally heat up, so mixing is required to evenly distribute the heat throughout a sample.

Choosing a different frequency to use for the infrared bulb could positively affect the penetration depth, since absorbance of the heat energy by the water will be lowered. This will however create the need for a bulb with a higher wattage.

#### 4. Microwave phased array

An array of microwave emitters overtop the microtiterplate could allow for constructive interference at the right points in the well, causing those points to heat up [13] [14]. A downside to using this method is that hotspots are inherent to it, meaning large temperature deviations throughout the sample will occur; these could quite possibly kill the surrounding bacteria.

#### 5. Convection

Using warm water or air to heat up the wells does not allow for individual temperature control of the substance in the wells since the microtiterplate will be partially submerged. This essentially will give the same result as an incubation chamber. It can however, allow you to control the temperature of a row or column of wells when you have multiple streams of water/air. Water has a relatively high specific heat, which will slow down the temperature control process since more energy is required to be transferred to affect the temperature of the water. Another factor will be that keeping the water at a high temperature will allow it to evaporate quicker, which means that this method will need regular maintenance.

## 6. Conductance using a temperature gradient plate

Temperature gradient plate have been around for a while; they have also been used to control the temperature of microtiterplates. In these cases, the temperature gradient plate will not determine the temperature of the substances in the wells of the microtiterplate.

Polystyrene is known and used for its incredibly low thermal conductance, especially in expanded form (0.033 W/m K [26]). The polystyrene variant used for the production of microtiterplates is also known as “crystal” polystyrene and has a slightly higher thermal conductance (0.1 W/m K to 0.13 W/m K [27]). This makes it more likely for conduction to be suitable.

To create a temperature gradient an aluminium plate will be positioned below the microtiterplate to act as a heatsink. Using multiple peltier elements one is able to heat up one side of the aluminium plate more than the other, the thermal energy will transfer throughout the aluminium plate quickly since aluminium has a relatively high thermal conductivity (205.0 W/m K [26]). In theory a copper plate would work better, since copper has a higher heat conductivity (385.0 W/m K [26]), however aluminium has the added benefit of reflecting infrared energy, as can be observed in Figure 12. In this way the infrared camera only picks up the microtiterplate, the heatsink located below will not interfere with the measurements. This aluminium plate can be made small enough by a CNC milling machine so it sits tightly under the microtiterplate and as close to the wells as possible.

This method of creating a temperature gradient on a microtiterplate by using Peltier elements has been proven to work well. BIO-RAD produces and sells a CFX Connect™ Real-Time PCR Detection System, which uses the same method to control the temperature of the microtiterplate [28]. They were able to create a maximum temperature difference over the plate of 24 degrees Celsius. In Figure 11 this device is shown; note that in this case, the microtiterplate is enclosed to minimise heat losses and temperature deviations from external sources.



Figure 11 – BIO-RAD's CFX Connect™ Real-Time PCR Detection System [28].

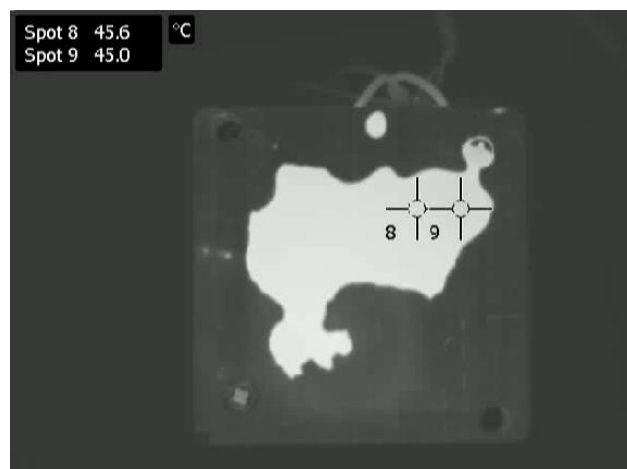


Figure 12 – A Peltier element heating up an aluminium heatsink, the temperature of which the infrared camera is unable to determine. However, when adding water on top of the heatsink the temperature becomes apparent.

### 3 Conceptual model

#### 3.1 Temperature monitoring

Temperature monitoring is a causal problem, since the researcher is trying to find a solution to the problem of not being able to monitor and control the temperature of the well of the microtiterplates. One of the most important factors/constraints are the non-invasive measurement and control of the temperature. Another important factor is that we have to deal with 96 wells, all of which need to be kept at a temperature desired by the microbiologist.

Using an infrared camera to determine the temperatures would be the optimal method. Since we want to measure the temperature of all the different wells at the same time.

A FLIR (Forward looking infrared) A320 [29] infrared camera is available at the UG. The image analysis will have to be done on a separate computing device. The infrared image has a relatively small size of 320 by 240 pixels. The image processing will be doable on a device such as a laptop [29].

The field of view of the A320 camera is 25° by 18.8° with a minimal focus distance of 0.4 m [29]. Using trigonometry, equations ( 5 ), ( 6 ), ( 7 ) and ( 8 ), we can find out that at a distance of 40 cm we can determine the temperature of an area about 17.7 cm by 13.2 cm, a smaller area would not be in focus and would therefore result in a lower accuracy.

$$\tan\left(\frac{25}{2}\right) = \frac{x}{0.4} \quad (5)$$

$$x = \tan\left(\frac{25}{2}\right) * 0.4 = 0.177 \text{ m} \quad (6)$$

$$\tan\left(\frac{18.8}{2}\right) = \frac{x}{0.4} \quad (7)$$

$$x = \tan\left(\frac{18.8}{2}\right) * 0.4 = 0.132 \text{ m} \quad (8)$$

This would give us an area of about 234 cm<sup>2</sup>. A resolution of 320 by 240 pixels would result in about 328 pixels per cm<sup>2</sup>.

From Figure 6 we can conclude that the 96 wells on a microtiterplate are positioned in an area of 72 mm by 108 mm. The field of view of the FLIR A320 is sufficient for this use. The FLIR A320 is able to measure certain spots, however there are a maximum of 10 measuring spots when using the camera's software, FLIR IR Monitor (v 2.0.11.0). Using colour analysis of the video feed should allow us to determine the temperature of any spot in view.

If the material of which the 96 well microtiterplates is made (polystyrene) does not work well with infrared, a different material needs to be chosen, which might not be readily available. In that case the microtiterplate needs to be manufactured specifically for this purpose. A different construction method where the microtiterplates would have thinner walls could work, however all plates have to meet certain standards which can no longer be reached when you are changing dimensions.

There are also Arduino compatible infrared camera modules, such as the 'FLiR Dev Kit'. These have significantly lower specifications than the industrial infrared cameras but could be suitable for this project. The resolution of this camera is only 80 by 60 pixels, which would result in a significantly lower pixel density of the image. However, one would not compromise on the thermal sensitivity, which is still 50 mK. The camera itself is fixed focus, which is not a problem in a when it is positioned in a fixed set up where the microtiterplate is always in the same position, and it compensates for the environment temperature [30].



Figure 13 - An example image from the Arduino compatible infrared camera [37].

An alternative solution would be to buy, for example, 96 Amphenol ZTP-135SR sensors. These infrared temperature sensors use a TO-46 package which has a maximum diameter of 5.84 mm at most [31] [32]. These will fit next to each other when located on the bottom of the 96 well microtiterplates, the accuracy for these sensors would have to be evaluated. An advantage of using 96 separate sensors is that you do not need a separate computing device for image processing and analysis which is needed when using an infrared camera. You could do the data analysis on a microcontroller which requires about 5 Watt, compared to the FLiR A320 which could use up to 24 Watt and additionally needs a computer which would run continuously.

If the accuracy of the alternative infrared temperature sensors is sufficient, they will still cost less than an infrared camera, but in this situation the infrared camera, approx. 12000 euros [2], is already in house and ready for use [33]. The UG prefers not to buy new equipment in this situation if the current equipment fits the requirements.

Another sensor which would fit the requirements quite well is the Melexis MLX90614 series of infrared heat sensors, specifically the Melexis MLX90614xCI. This version has a narrow field of view which can be seen in Figure 14; this fits the application quite well.

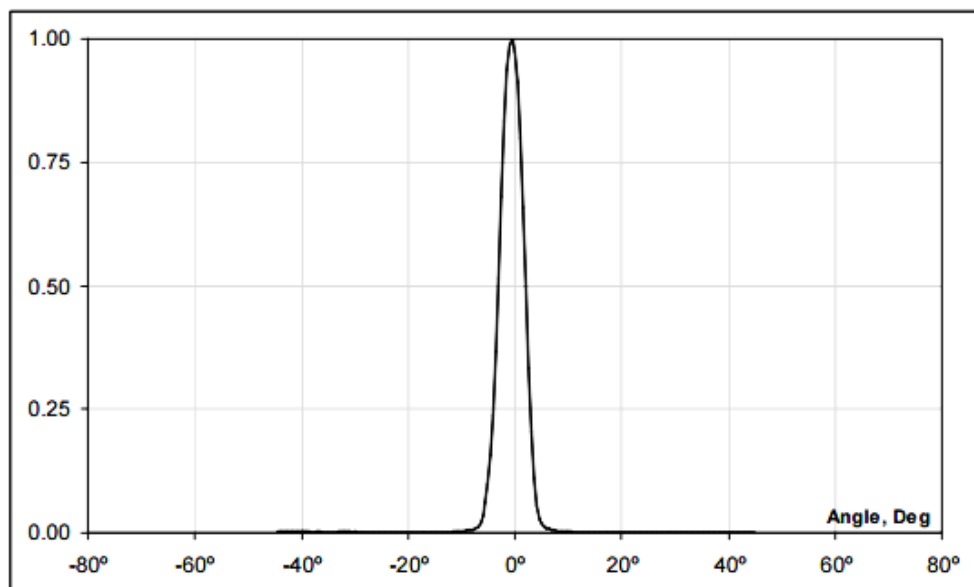


Figure 14 - Typical FOV of Melexis MLX90614xCI [34].

The sensor also fits the requirement for a maximum error of 0.5 degrees Celsius, as can be depicted from Figure 15. The sensor would be used in situations where the temperature of the substance in the well could vary from 15 to 50 degrees Celsius. The system would be set up in a lab where the temperature would be around 20 degrees Celsius.

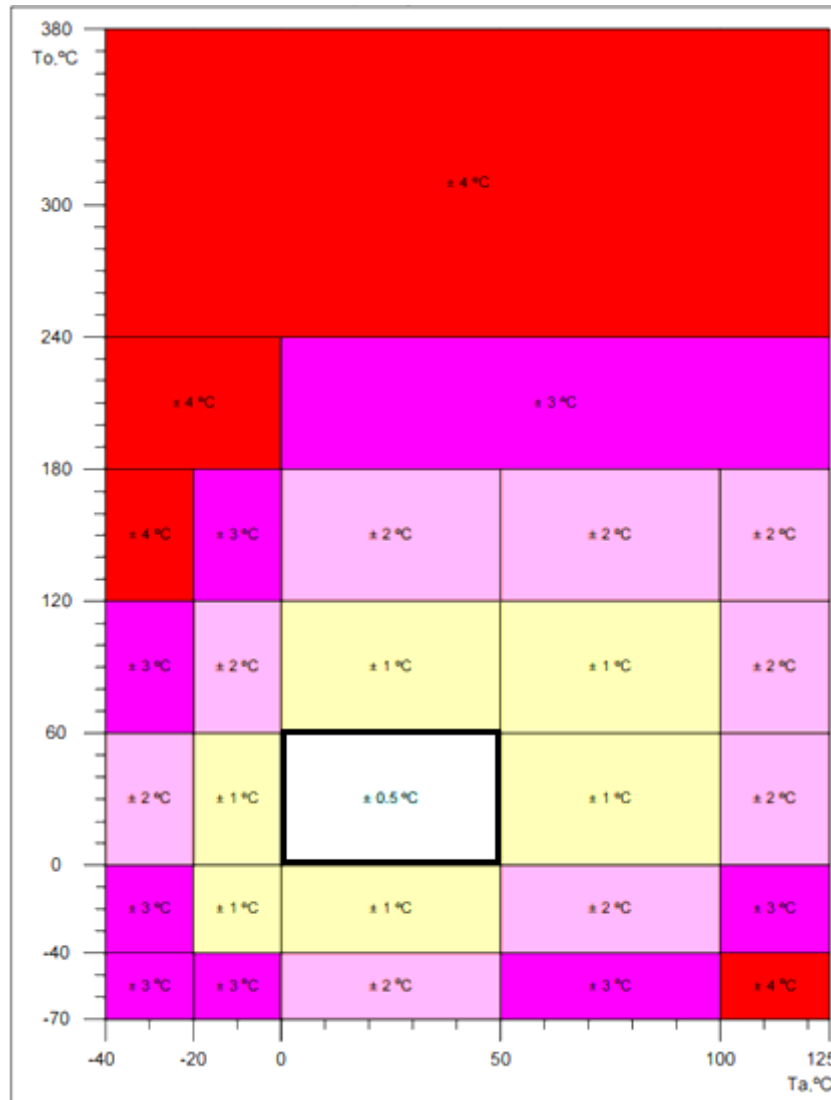


Figure 15 - Accuracy of MLX90614 [34]. 'To' is the object temperature, 'Ta' is the sensor temperature. The situation in which the sensor would be used is outlined in black.

An advantage of using these sensors is that it is possible to read out 127 of them over 2 common wires (Figure 16). They are small enough to fit next to each other over each well, while an infrared camera would need a minimum focus distance, the sensors could be located closer to the microtiterplate, making the system quite a bit more compact [29] [34]. This would also allow the system to be enclosed to minimise heat loss and reduce outside factors which could influence the temperature.

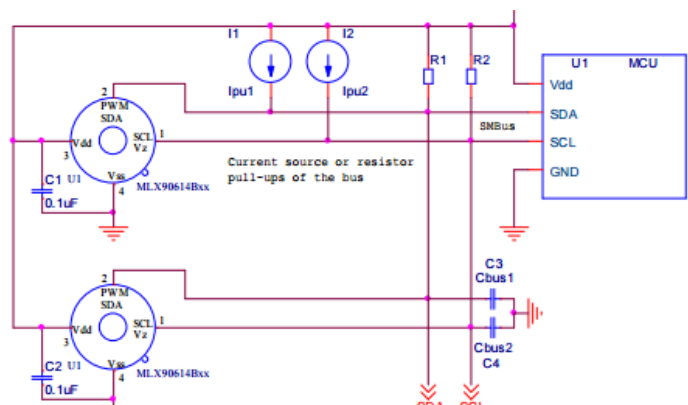


Figure 16 - Use of multiple MLX90614 devices in SMBus network [34].

### 3.2 Temperature control

Controlling the temperature without a large amount of wires which need to be placed in the 96 wells of the microtiterplates is preferred by the UG. Because of this, non-invasive heating solutions will be evaluated to see if they could be used.

For the control of the temperature a laser was proposed, since this method would be non-invasive. Using a laser comes with its own constraints, since you would need some mechanical movement to direct the laser to the right well. This mechanical movement could be on the laser itself, but could also be 2 mirrors each rotating on a different axis, also known as Galvo mirrors. In this way we could direct the laser to any of the 96 wells in the microtiterplates. Using a laser which is powerful enough to heat a relatively small amount of water also comes with certain safety concerns which is why this method will not be considered for an end result.

A method of keeping the different compartments at a certain temperature would require an extra solid object in the compartment. The outsides of the compartment should let infrared light go through, while the object in the compartment should absorb the infrared light and consequently release heat energy to the substance. Including an object in the well is technically not non-invasive and will therefore not be considered for an end result.

Projecting an infrared pattern on top of the microtiterplate looks promising in theory, however this method is dropped due to time constraints. A suitable solution for the time that is available is the conductance, this method will need optimization since the microtiterplate is not able to be enclosed due to the fact that the temperature of the wells will be determined by an infrared camera located 40 cm from the microtiterplate.



### 3.3 Thermal conductivity of polystyrene

How well the heat of the Peltier elements is able to penetrate the bottom of the microtiterplate will need to be evaluated, since it is only 1 mm of polystyrene and there are other products which are made to control the temperature of a microtiterplate using the same technique no complications are anticipated at this point.

A test has been done on the penetration of heat energy through 1 mm of polystyrene. A 12 Watt Peltier element heats up a heatsink, which in turn heats up the bottom of a microtiterplate. Figure 12 displayed the maximum temperature of the system is about 45 degrees Celsius. When positioning the microtiterplate overtop the heatsink, the surrounding air will be unable to contact the heatsink. In this way the microtiterplate will prevent some heat loss, raising the temperature to about 53.4 degrees Celsius. This can be seen in Figure 17.

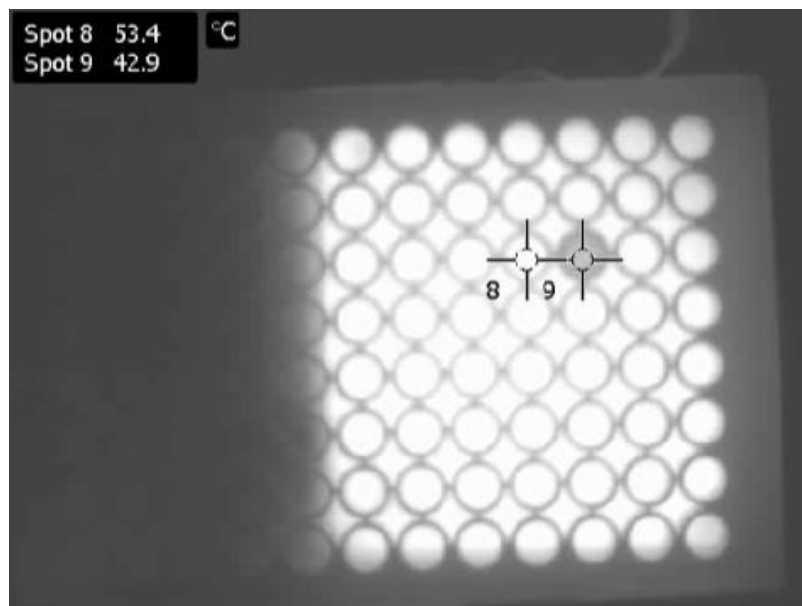


Figure 17 – A microtiterplate that had heated up to the maximum temperature possible. The well at Spot 9 is filled with tap water, the remaining wells are empty.

How uniform the temperature is distributed throughout a well when heating using this technique will be relatively the same as the uniformity of the temperature of the well itself. This can be seen in Figure 18. This is the outside surface temperature of the well, due to the fact that water has a relatively high thermal conductivity, the temperature of the water can be estimated by knowing the temperature of the outside surface of the wells, the temperature of the water is expected to be more uniform because of the high conductivity of heat of water.

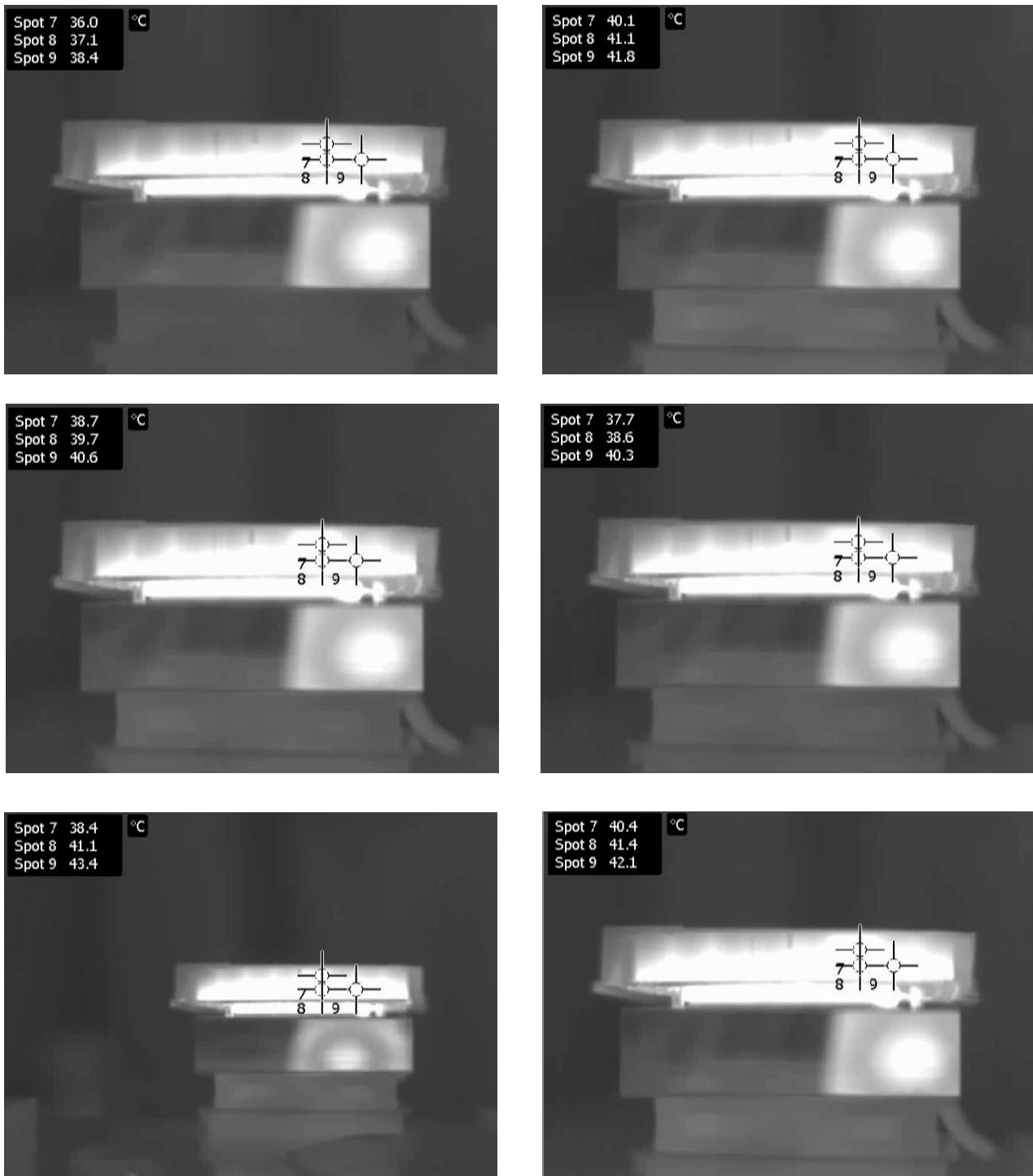


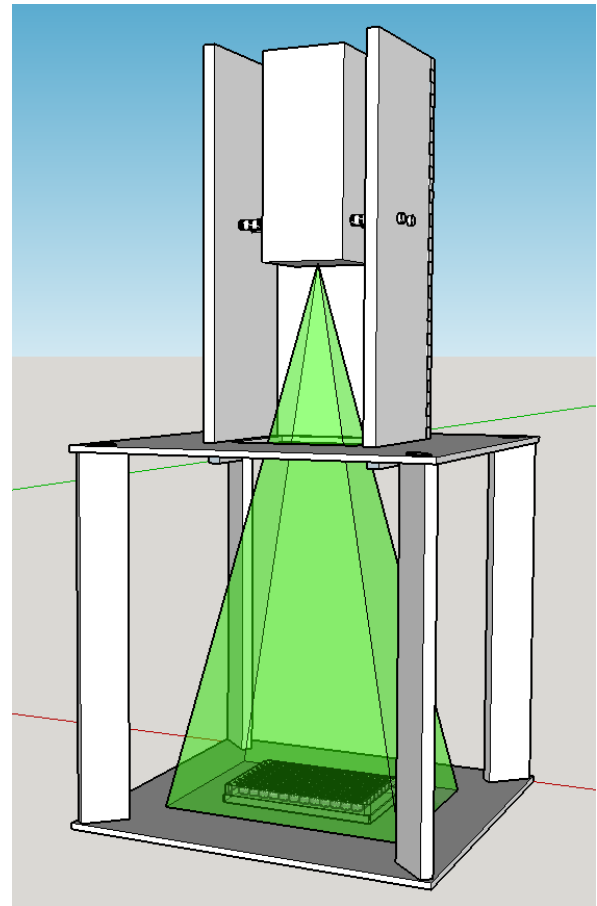
Figure 18 –A sample of the initial test results displaying the temperature throughout the wall of a well. A 12 Watt Peltier element is located underneath an aluminium heatsink, the microtiterplate is positioned overtop. The well at Spot 7 and Spot 8 is filled with water, while the well at Spot 9 is empty. Each image shows the microtiterplate at a different temperature, the results show that there is a difference in temperature throughout the well, about 1 degree Celsius in the best case scenario, in the worst case a temperature difference of 3 degrees Celsius was found.

### 3.4 Proposed setup

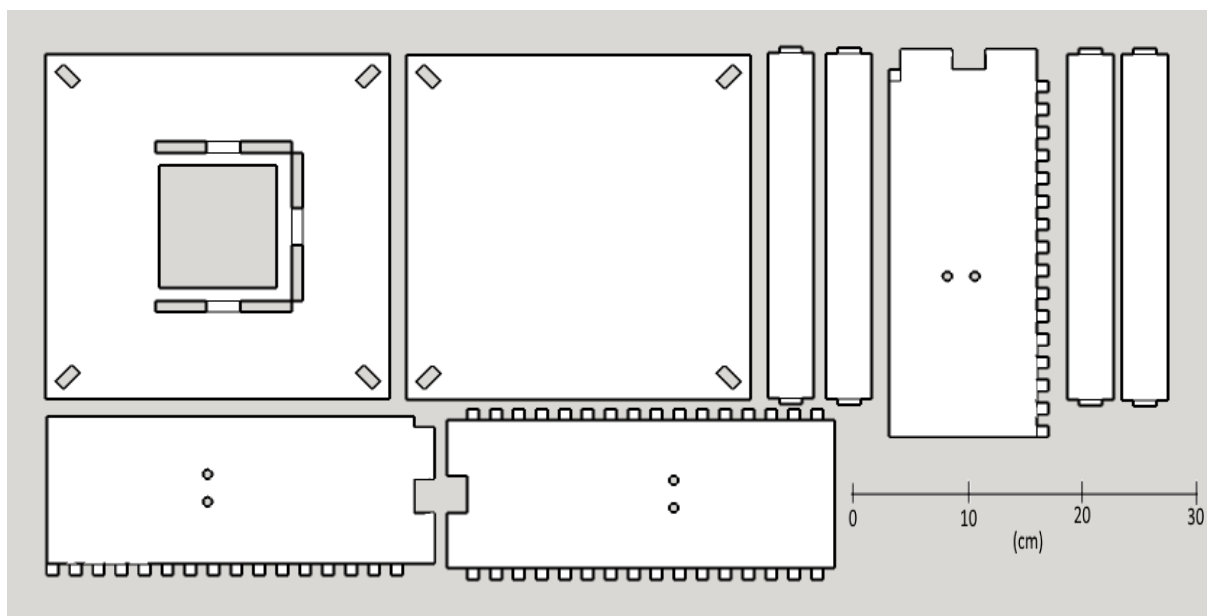
How a possible microbioreactor setup could look like is portrayed in Figure 19. The infrared camera will be located overtop the microtiterplate at a distance of 45 cm, since it needs a minimum focus distance of 40 cm. This does make the setup quite a bit bulkier than it could have been if 96 separate infrared temperature sensors would have been selected. Since the camera has quite a bit of weight to it, it needs to be stable, which is why the setup will be positioned on a 30 cm by 30 cm plate and the camera will be secured to the side using the possible mounting options. Figure 20 displays the parts required to construct the setup.

The setup was designed to be laser cut from sheets of Plexiglas. Different parts are able to be interlocked which makes the setup easy to construct and deconstruct. All parts are 0.8 cm thick. To secure the camera to the plate M4 screws can be used. The camera needs to be overtop the microtiterplate; setting the exact measurements points can be done using software.

Nevertheless, to make sure that one does not need to set exact measurement points every time the setup will be used, the camera must be in the same position each time the setup is constructed.



*Figure 19 - An example setup of a microbioreactor system. The green cone represents the field of view of the infrared camera. Note that the temperature gradient plate located below the microtiterplate is not depicted here.*



*Figure 20 – The separate parts required for assembly of the setup.*

### 3.4.1 Temperature control

After the temperature of the wells is determined, the difference between the desired and the current temperature will determine the signal to the Peltier element. A MATLAB program will be able to send a PWM signal to an Arduino Mega, which is in turn connected to the Peltier elements via a circuit of transistors.

Figure 21 describes how the system will perform, during initialization the graphical user interface (GUI) will be set up, MATLAB will connect to the Arduino and will check if any software is missing.

The user still has to, manually, discover and connect to the right camera. This step will not be automated since it can be that the user has multiple cameras connected.

The video data of the camera will be streamed over the network to the host computer (Figure 22) where the user will be able to visually inspect the location of the measurement points. If the locations are not correct adjustments could be made accordingly.

After this task has been performed the average temperature of a row of wells will be calculated and the output will be used to determine the duty cycle of the PWM signal.

The user has set 2 goal temperatures, one for the cold side, and another one for the warm side of the microtiterplate. Using a PID controller will allow for stable temperature control, the output of the PID controller will be a PWM signal which will control the voltage over the Peltier elements. The MATLAB program has 2 separate PID controllers for this task, one for the cold side and one for the warm side.

The MATLAB code can be found in Appendix A and is meant to be used in MATLAB r2015b with the FLIR Atlas SDK (v. 3.1.16014.2001)

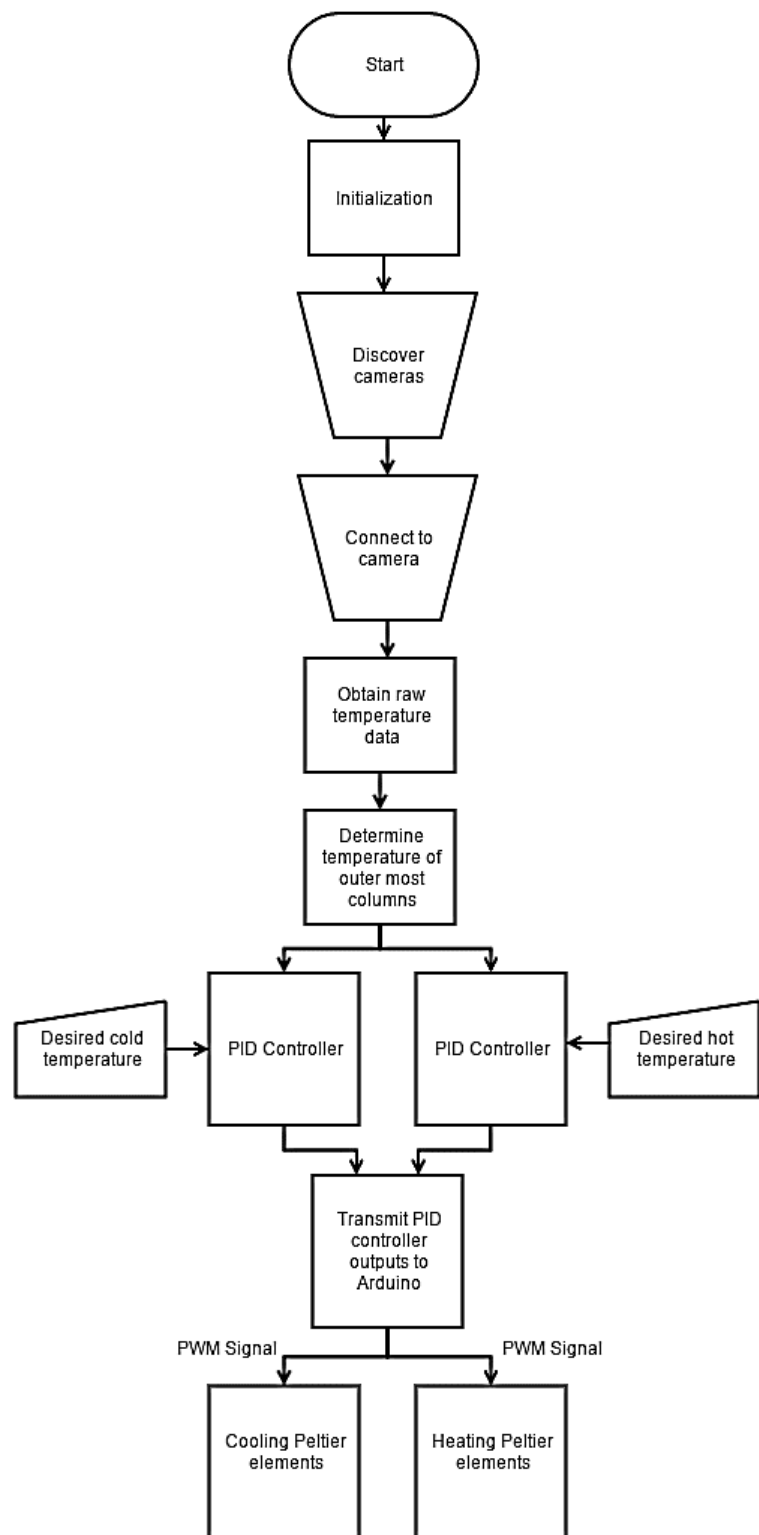


Figure 21 - A flowchart describing how the system will perform.

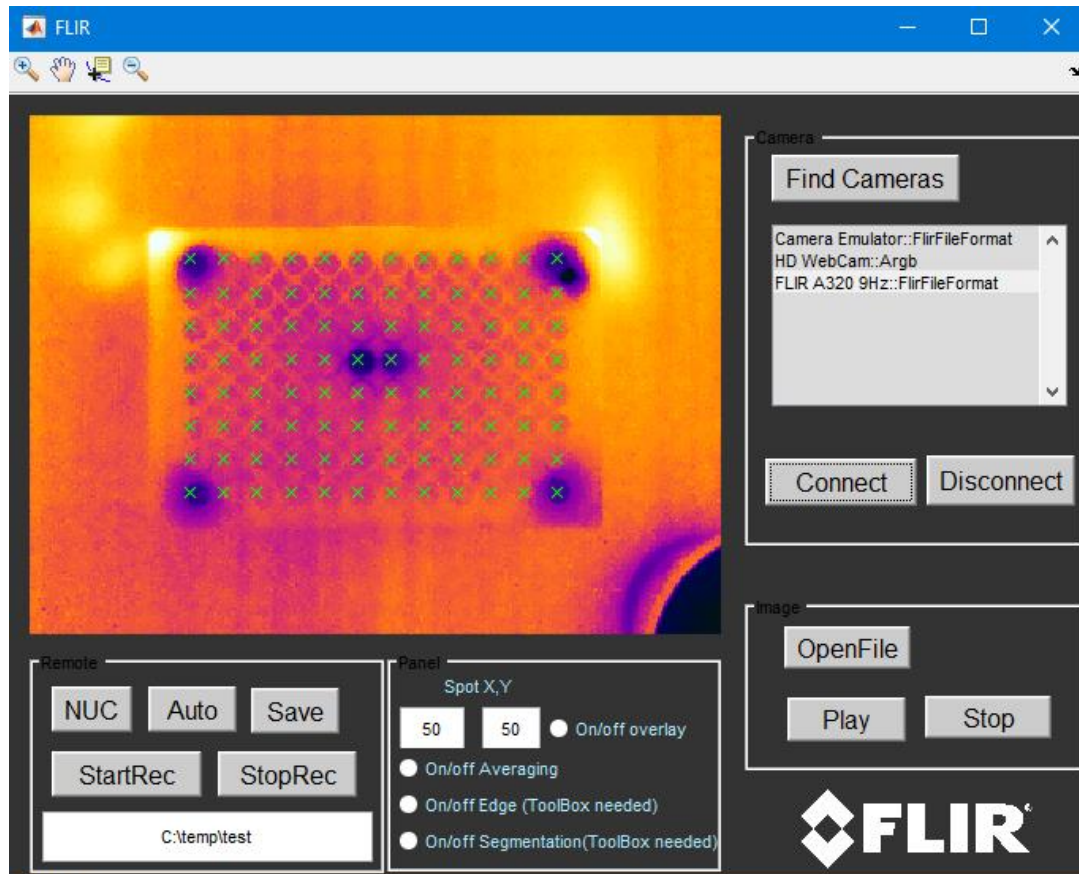


Figure 22 -The graphical user interface allows the user to connect to the camera and make sure that the measurement points are aligned to the wells of the microtiterplate.

### 3.4.2 Circuit design

One of the constraints of using the camera is that MATLAB is required since it is the program that is able to communicate with the infrared camera and the Arduino simultaneously. MATLAB can utilize the PWM pins on the Arduino to control the voltage over the individual Peltier elements.

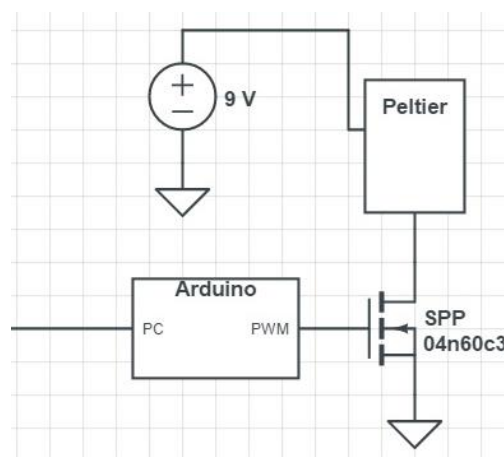
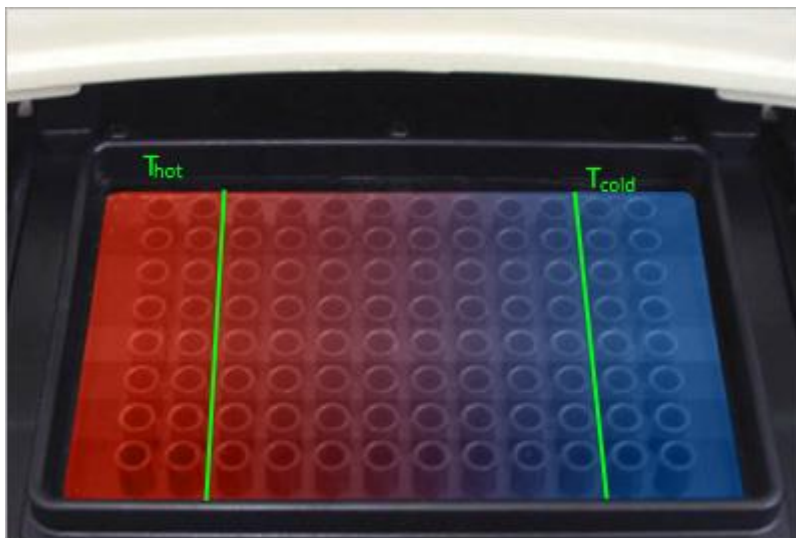


Figure 23 – Circuit schematics. Instead of 1 Peltier element, the final design has 4 Peltier elements connected in the same manner.

### 3.4.3 Selection of the Peltier element

The Peltier element selection process started with setting additional requirements such as size, energy output, and maximum temperature difference of the element. Initial experiments have shown that a single Peltier element of 12 Watts was unable to keep a well filled with water at a temperature of 50 degrees Celsius (Figure 17). A requirement for the minimum and maximum temperature difference over the microtiterplate was not specified by the UG.

To produce the largest temperature difference over the microtiterplate multiple Peltier elements would be used. At one side of the heatsink, the Peltier elements would input heat energy into the heatsink, while on the other side of they would extract heat energy from the heatsink. This would result in the largest possible temperature difference over the microtiterplate.



*Figure 24 – A thermal gradient over a microtiterplate, the leftmost 2 columns will be set at a maximum temperature, while the rightmost 2 columns will be set at a minimum temperature [28].*

An ET-063-10-13 from European Thermodynamics Limited has been selected for use in the system due to the fact that it fits the requirements, a significant factor is the fact that it has a size of 30 mm by 15 mm [35]. 17.9 Watts will be more than enough when using 2 of these Peltier elements next to each other, essentially creating a single larger more powerful Peltier element with a size of 60 mm by 15 mm, which will allow one to set the temperature of 2 columns of wells of the microtiterplate. At the other side of the microtiterplate 2 column will be cooled, leaving 8 columns of wells for the temperatures gradient as can be seen in Figure 24.

### 3.4.4 Aluminium heatsink

An aluminium heatsink was CNC milled at the workshop at Nijenborgh 4, UG. Aluminium was selected because of its reflective properties (Figure 12). This method will ensure that the camera will pick up the temperature of the wells and not the temperature of the heatsink located underneath the wells. The heatsink has been modified so that it fits microtiterplates from all brands used by the UG. An image of this heatsink can be found in Appendix B.

## 4 Research design

The most important aspect for the UG is the monitoring of the temperature of all 96 wells of the microtiterplate. If it is not possible to determine the temperature of the wells of the microtiterplates, it will not be possible to control the temperature of each individual one.

First I will be focussing on the infrared camera, the most important aspect since it is the first aspect of controlling the temperature. The following questions will be answered by experiments.

- Can the infrared camera determine the temperature of the substance inside the compartment?
  - o Put liquids with 2 different temperatures in 2 compartments, measure using the infrared camera.
- Does it display the temperature of the compartment itself?
  - o Same as above.

These 2 questions are a proof of principle, and it does seem from previous research (Figure 17) that the infrared camera is able to determine the temperature of the substance, in this case water, inside the wells.

The following questions are there to quantify the performance of such a system;

- What is the response time of such a system?
- What is the maximum temperature difference possible over the microtiterplate?
  - o Without active cooling
- Can the system handle disturbances?
- How stable can the system keep the wells at a certain temperature?

An optimal material for the microtiterplates would be one that does not affect infrared light at all, it should pass through without being absorbed or reflected. From theoretical research it is concluded that the material of which the microtiterplates is made interferes with infrared measurements.



## 5 Performance evaluation

In the performance evaluation chapter different aspects of the developed system will be evaluated. The aspects that are evaluated will include information about the answers to the research questions and sub-questions.

### 5.1 Accuracy & Hold stability

The accuracy and stability are evaluated for 3 different goal temperatures, the lower limit (30 degrees Celsius), the upper limit (50 degrees Celsius), and a nominal temperature of 37 degrees Celsius. 37 Degrees Celsius is temperature used for the optimum growth of *Escherichia coli* [36]. *Escherichia coli* is found in the human body, which also has temperature of 37 degrees Celsius. Appendix C evaluates the accuracy and hold stability of the system at a temperature of 45 degrees Celsius.

The temperature was considered 'stable' when the temperature had reached the target temperature within an error margin of 0.5 degrees Celsius after the overshoot.

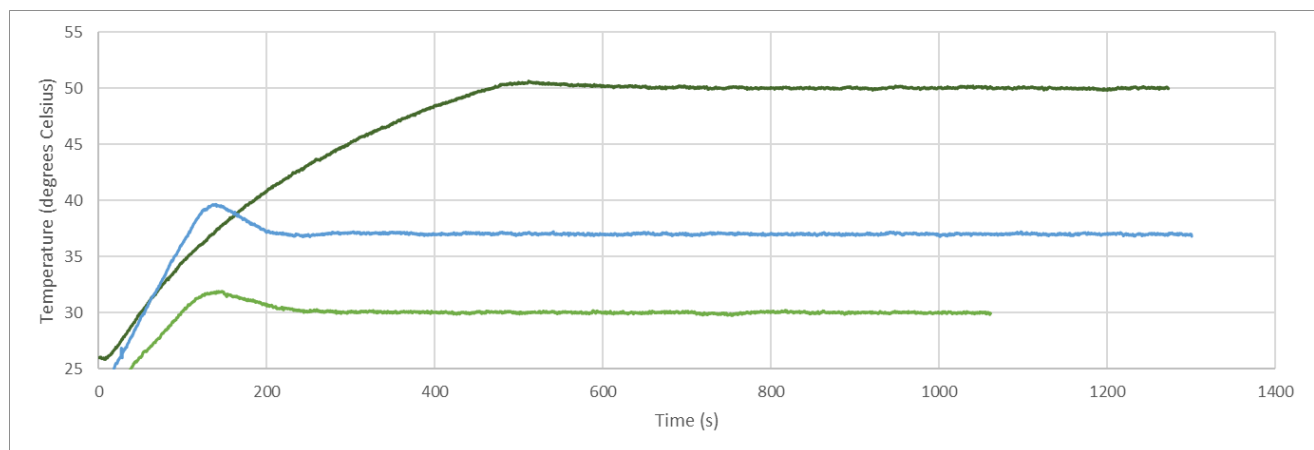


Figure 25 – The temperature over time for three different temperatures, 30 degrees Celsius, 50 degrees Celsius, and a nominal temperature of 37 degrees Celsius.

To evaluate the accuracy and hold stability of the system at these temperatures, a bell curve can be plotted and the confidence interval can be calculated.

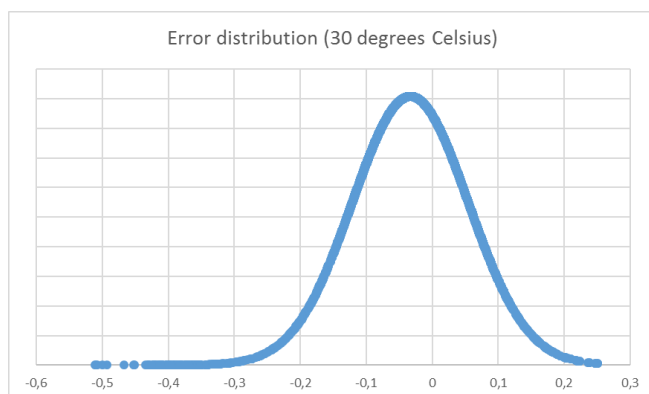


Figure 26 – The error distribution for a temperature of 30 degrees Celsius.

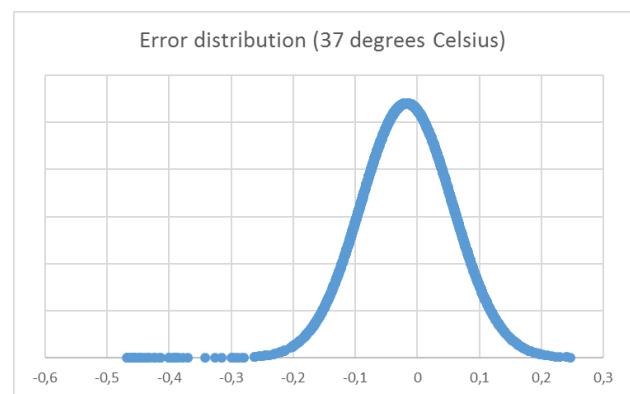
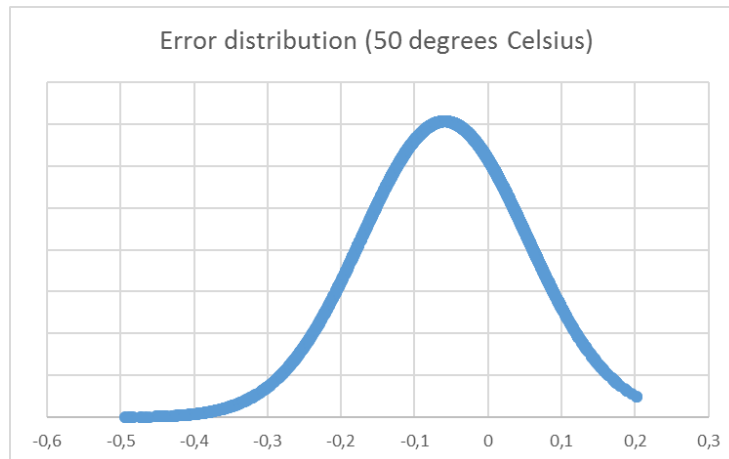


Figure 27 - The error distribution for a temperature of 37 degrees Celsius.





*Figure 28 - The error distribution for a temperature of 50 degrees Celsius.*

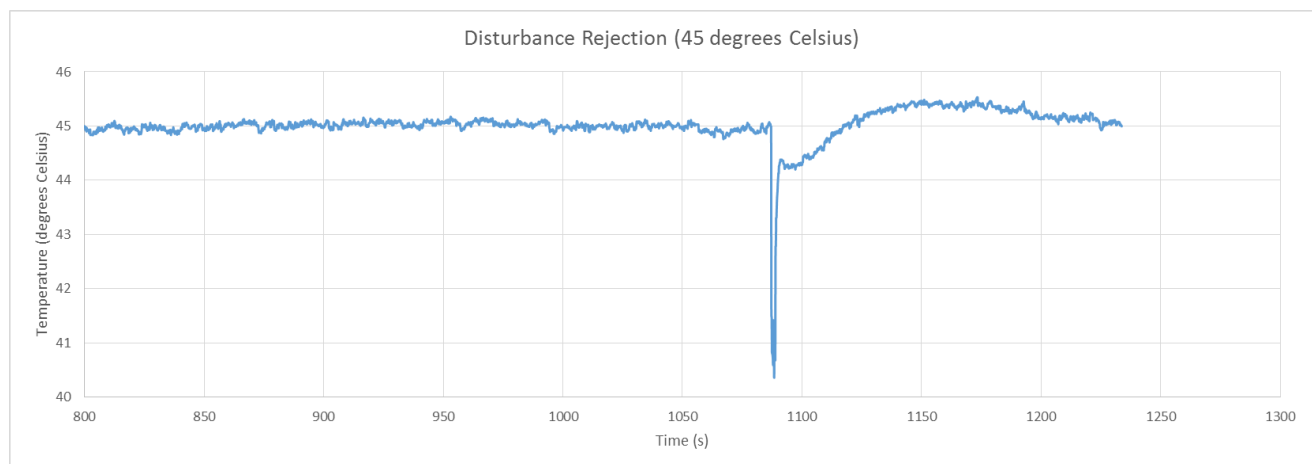
In each of these three scenarios I am 100% confident that the true absolute error will be smaller than 0.5 degrees Celsius. Furthermore, I am 97 % confident that the true absolute error will be smaller than 0.2 degrees Celsius for a goal temperature of 30 degrees Celsius, I am 99 % confident that the true absolute error will be smaller than 0.2 degrees Celsius for a goal temperature of 37 degrees Celsius and I am 88 % confident that the true absolute error will be smaller than 0.2 degrees Celsius for a goal temperature of 50 degrees Celsius.

Appendix D contains specific calculations about the confidence interval.

## 5.2 Disturbance rejection

Since the setup is completely open on all 4 sides, disturbances, such as a gust of colder air from a door opening, can happen. Figure 29 shows how the system is able to handle disturbances. In this case a gust of colder air cooled down the wells of the microtiterplate by almost a degree. The system quickly increases the output due to the fact that a relatively big change in temperature over a short period of time happened. This increases the derivative factor of the PID loop significantly and causes an increase of the duty cycle of the PWM signal. An overshoot occurs due to the increasing integral factor of the PID control loop and the delay in response, after 100 seconds the temperature is stable at 45 degrees Celsius again.

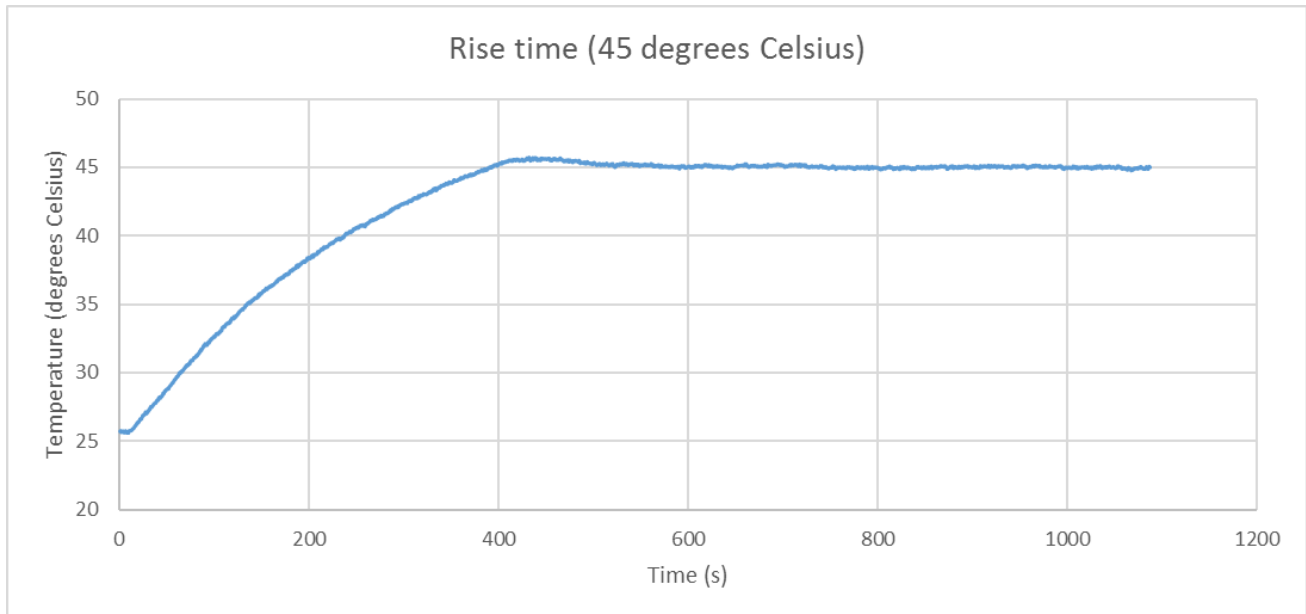
The disturbance rejection was tested at a relatively high temperature since the disturbance would cause the temperature of the system to drop more at a higher temperature.



*Figure 29 - A graph depicting the temperature of the hot side of the microtiterplate over time. In this case it is demonstrated how the system is able to handle disturbances. In this example, a gust of colder air cooled down the wells of the microtiterplate which was temperature controlled to be at 45 degrees Celsius. The system reacts quickly, however some overshoot occurs because the heat needs to travel from the Peltier element, to the aluminium heatsink and through a layer of polystyrene.*

### 5.3 Rise time

The rise time of the system was not specified by the UG, one of the important matters is that the bacteria inside the wells must be able to survive. The system was calibrated so even at its maximum output the Peltier elements will not heat up too quickly. From room temperature to 45 degrees takes about 400 seconds, or about 6 and a half minutes. Since the system loses more heat energy to the environment at higher temperatures the temperature increase will take longer at higher temperatures.



*Figure 30 - Temperature of the system over time while heating up to a goal temperature of 45 degrees Celsius. At 400 seconds the goal temperature was reached, however due to overshoot the temperature of the system was stable at 500 seconds.*

## 5.4 Overshoot

Overshoot is inherent to this PID controller, because of the relatively slow reaction time, since the heat needs to be absorbed by the aluminium heatsink located under the microtiterplate and needs to penetrate a layer of polystyrene, which is known and used for its ability to barely conduct any heat.

Nevertheless, the derivative part of a PID controller can be tuned to reduce the overshoot, this can however reduce the stability of a PID controller. Since the hold stability is more important for the UG and the overshoot is manageable at common temperatures, such as 37 degrees Celsius, stability will not be sacrificed for a reduced overshoot. The rise time and overshoot can be found in Figure 31.

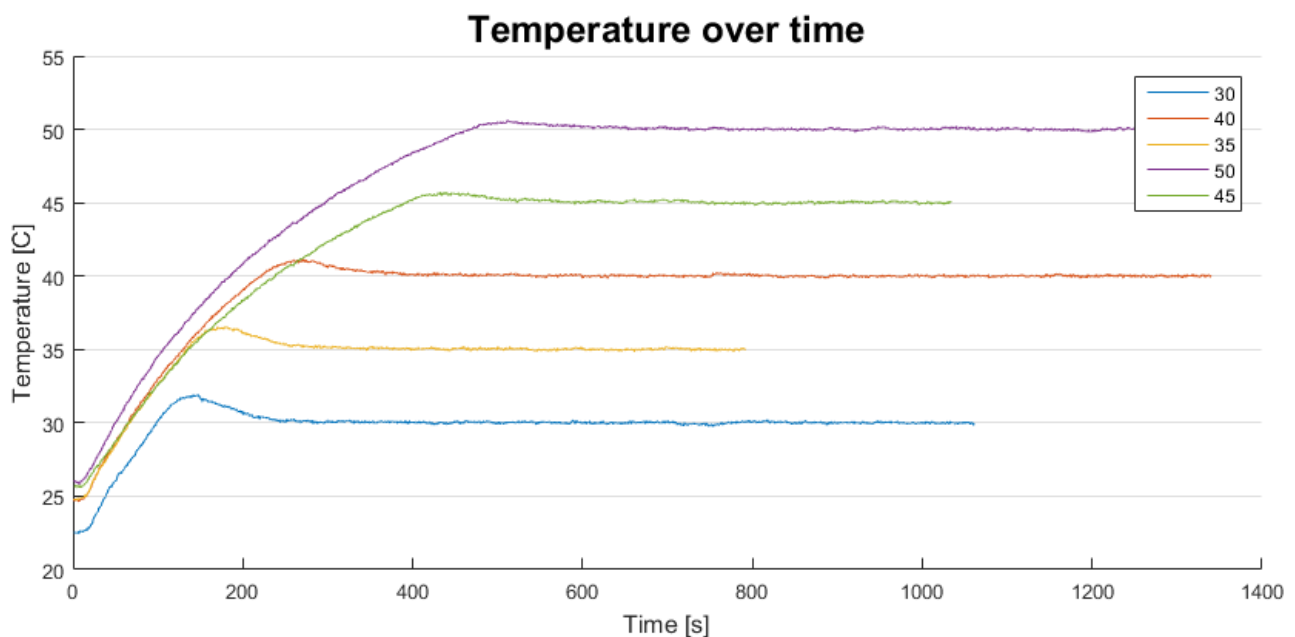


Figure 31 - The temperature of the hot side of the microtiterplate logged over time with different goal temperatures.

## 5.5 Maximum temperature difference over the microtiterplate.

Currently the system is performing with only the 'heating' Peltier elements, the aluminium heatsink located under the microtiterplate has a large surface area and is suspended in the air, causing it to lose a lot of heat energy to the environment. For this purpose, the temperature difference over the microtiterplate is already sufficient for the UG, however a controlled

### Temperature difference while stable

In this case the goal temperature of the system was set at 50 degrees Celsius. Since aluminium is a great conductor of heat the temperature difference over the heatsink should become lower when the system is stable. This test was performed to determine how much this temperature difference could lower in a 'worst case' scenario. From Figure 32 we can conclude that the maximum temperature difference over the microtiterplate would be 6 degrees Celsius.

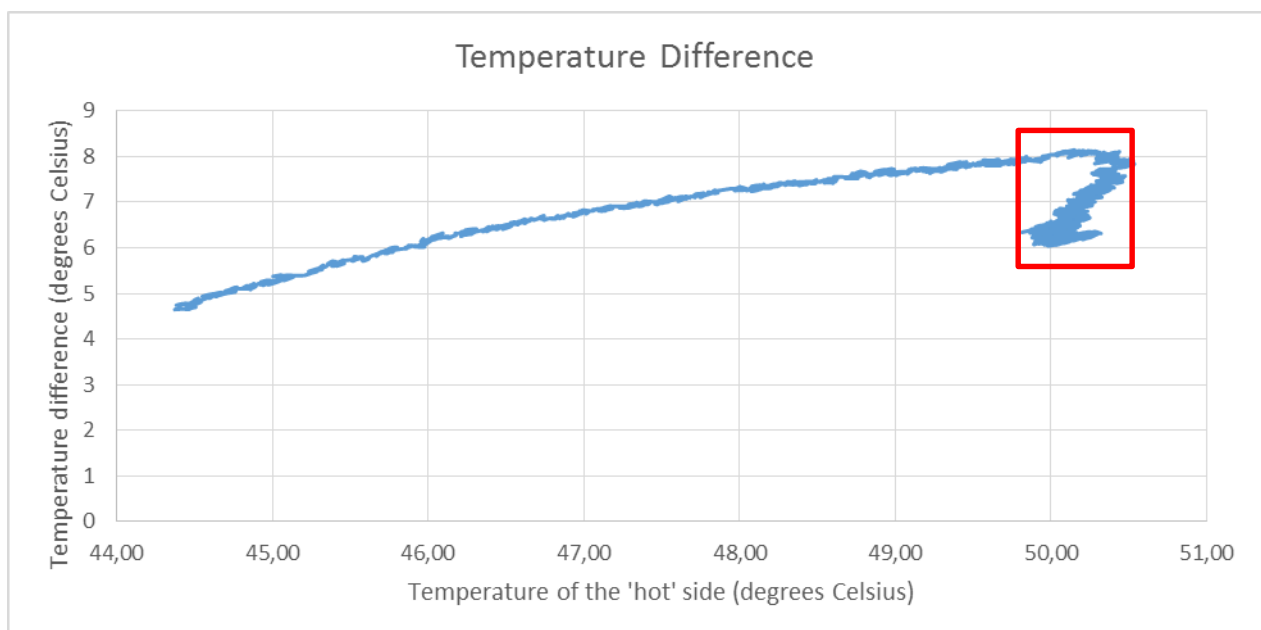
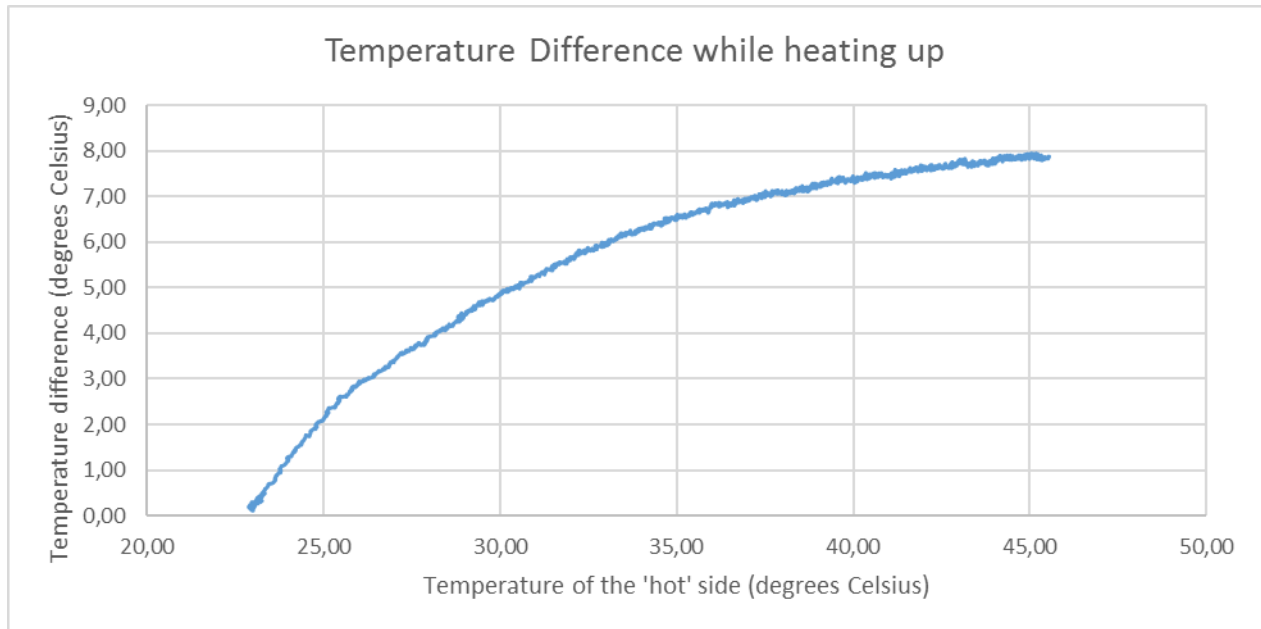


Figure 32 - The temperature difference between the hot and cold side in a graph while the system is set at 50 degrees Celsius. The y axis represents the difference in temperature between the hot and cold side of the microtiterplate. The overshoot can be found in this graph as the 'bump' towards the right within the red rectangle. After the overshoot the difference between the cold and hot side is lowered by about 2 degrees, which can also be concluded from the data in the red rectangle. This results in a usable temperature gradient.

## Temperature difference while heating up

Figure 33 illustrates the difference in temperature over the microtiterplate while heating up, this is meant as an indication for the temperature difference over the microtiterplate the user can expect.



*Figure 33 - The temperature difference between the hot and cold side in a graph while the system is set at 45 degrees Celsius. The y axis represents the difference in temperature between the hot and cold side of the microtiterplate. After stabilizing at a goal temperature the temperature difference over the microtiterplate will go down.*

## 6 Conclusion

In this chapter the answers the research question and the supporting sub-questions will be discussed. To answer the research question, the supporting sub-questions will be answered first.

- *How do we measure the temperature of these compartments using a non-invasive method?*

Theoretical research shows that measuring the infrared heat energy emitted by the wells is an optimal non-invasive method. By using the FLIR A320 infrared camera we are able to determine the temperature of each individual well with an accuracy of 50 mK.

- *What method of non-invasive heating would allow for transfer of heat energy without killing bacteria growing in the wells?*

Conductance using an aluminium heatsink and 2 Peltier elements will not kill the bacteria. The goal temperature of the system is still met relatively quickly. A PID controller allows for a stable temperature of the system.

- *What would a possible 'microbioreactor' setup look like?*

An example setup of a temperature controlled microbioreactorsystems can be found in Appendix B and in Figure 19. A limitation of using the FLIR A320 infrared camera is that it has a minimum focus distance, making the setup taller. Using individual infrared temperature sensors would result in a smaller setup without losing performance.

- *What would be the performance of such a system?*

- *Accuracy & Hold stability*

The system developed is able to meet the requirement of an accuracy and stability of 0.5 degrees at all temperatures. For a nominal temperature of 37 degrees Celsius I am 99% confident that the true absolute error will be smaller than 0.2 degrees Celsius.

- *Response time*

There was not a required response time set by the UG, however, the system is able to reach a stable temperature of 50 degrees Celsius in 10 minutes.

- *Maximum temperature difference over the microtiterplate*

Since there is no active cooling, the temperature difference over the microtiterplate is dependent on the temperature of the 'hot' side of the microtiterplate. At a temperature of 50 degrees Celsius the 'cold' side would be about 44 degrees Celsius. This temperature differences lowers as the goal temperature of the 'hot' side is lowered.

- *Disturbance rejection*

The system developed is completely open on all 4 sides, but it is able to cope with short disturbances such as a gust of colder air (Figure 29). In one of the worst cases it would take approximately 100 seconds to get to a stable situation again.

The answer to the research question;

***How do we control the temperature of the substances in the different wells of a 96-well microtiterplate using an automated non-invasive method?***

By combining the FLIR A320 infrared camera with a PID control system connected to 2 Peltier elements located underneath an aluminium heatsink, we are able to control the temperature of the hot side of the microtiterplate. Since the heatsink is suspended in the air, it loses the heat energy, which applies a temperature gradient over the microtiterplate. The temperature of the wells cannot be controlled individually using this system. An active cooling element on the 'cold' side of the microtiterplate is not available in this system, nonetheless it can allow for more control and a greater temperature difference over the microtiterplate.



## 7 Recommendations

The infrared camera which was available at the UG met the requirements for this system, however separate infrared thermometers are recommended because of the following:

- The camera has an accuracy of 50 mK, which is not needed for this use case. It also has a high resolution sensor, capable of measuring thousands of separate temperatures at the same time. The microtiterplates currently used have 96 wells, the infrared camera becomes more useful with microtiterplates which have more, smaller wells. An alternative option is to place multiple 96 well microtiterplates underneath the infrared camera, which might need to be placed further away. To prevent the setup from becoming too tall, aluminium plates could be used to reflect the infrared light emitted by the substances in the wells of the microtiterplates.
- Separate infrared thermometers would result in a lower cost system which could perform equally, or even better than the current system due to the fact that the microtiterplate could be enclosed in a small box, reducing the heat loss and possible external disturbances. Using separate infrared thermometer would allow the system to function completely on the Arduino, eliminating the requirement of a separate PC and lowering the power usage.

Recommendations for further research are as follows:

- To distribute the heat energy throughout the substance inside the wells, the content inside each well should be stirred constantly.
- For a final design, a PCB should be created which is able to handle the amount of amperes required.
- Active temperature control of the 'cold' side with active cooling of those 2 Peltier elements allows for more control by the user. The current system fulfils the requirements set by the UG, a temperature gradient is created over the microtiterplate.
- To prevent external bacteria from contaminated the samples, the microtiterplate must be closed off. Since the microtiterplates are manufactured with certain standards, it would be possible to create a universal cap which could fit microtiterplates from different brands. This cap would ideally let through all electromagnetic radiation in the infrared spectrum. Closing of the microtiterplates would also prevent thermal disturbances and limit evaporation of the samples.

## 8 Bibliography

- [1] S. V. Labs, "Bioreactor Basics," Sakshat, 2011. [Online]. Available: <http://iitd.vlab.co.in/?sub=63&brch=177&sim=647&cnt=1>. [Accessed 15 06 2016].
- [2] p. d. G. Euverink, Interviewee, [Interview]. 08 02 2016.
- [3] BRAND, "Technical Data Sheets," [Online]. Available: <http://www.brand.de/en/products/life-science-products/brandplatesr-microplates/technical-data-sheets/>. [Accessed 12 02 2016].
- [4] C. Y. L. a. S. KRIMM, "Infrared Spectra of High Polymers. VI.," 1958. [Online]. Available: <http://onlinelibrary.wiley.com.proxy.hanze.nl/doi/10.1002/pol.1958.1202711520/full>. [Accessed 19 02 2016].
- [5] OMEGA, "Infrared Thermometer," OMEGA, 2016. [Online]. Available: <http://www.omega.com/prodinfo/infraredthermometer.html>. [Accessed 15 6 2016].
- [6] Doitpoms, "Example Infrared Spectra," [Online]. Available: <http://www.doitpoms.ac.uk/tlplib/artefact/flash/infrared.swf>. [Accessed 2016 2 15].
- [7] FLIR, "How Does an IR Camera Work," FLIR, 2016. [Online]. Available: <http://www.flir.com/corporate/display/?id=41523>. [Accessed 15 06 2016].
- [8] MIKRON, "Table of emissivity of various surfaces," [Online]. Available: [http://www-eng.lbl.gov/~dw/projects/DW4229\\_LHC\\_detector\\_analysis/calculations/emissivity2.pdf](http://www-eng.lbl.gov/~dw/projects/DW4229_LHC_detector_analysis/calculations/emissivity2.pdf). [Accessed 15 02 2016].
- [9] SLAS, "Well positions for microplates," 13 10 2011. [Online]. Available: [http://www.slas.org/default/assets/File/ANSI\\_SLAS\\_4-2004\\_WellPositions.pdf](http://www.slas.org/default/assets/File/ANSI_SLAS_4-2004_WellPositions.pdf). [Accessed 11 02 2016].
- [10] GH-Group, "What is induction heating?," GH-Group, 2016. [Online]. Available: [http://www.gh-ia.com/induction\\_heating.html](http://www.gh-ia.com/induction_heating.html). [Accessed 15 06 2016].
- [11] TWI, "What is laser heating?," TWI, 2016. [Online]. Available: <http://www.twi-global.com/technical-knowledge/faqs/process-faqs/faq-what-is-laser-heating/>. [Accessed 15 06 2016].
- [12] A. Takamizawa, S. Kajimoto, J. Hobley, K. Hatanaka, K. Ohtab and H. Fukumura, "Explosive boiling of water after pulsed IR laser heating," Advance Article, 2002.
- [13] A. Wexler, Interviewee, *Interview with A. Wexler*. [Interview]. 6 4 2016.
- [14] S. TECH, "How does the microwave oven work?," SCI TECH, 2016. [Online]. Available: [http://scitech.web.cern.ch/scitech/TopTech/01/MicroWaveOven/microwave\\_2.shtml](http://scitech.web.cern.ch/scitech/TopTech/01/MicroWaveOven/microwave_2.shtml). [Accessed 15 6 2016].

- [15] hyperphysics, "Heat Transfer," hyperphysics, 2016. [Online]. Available: <http://hyperphysics.phy-astr.gsu.edu/hbase/thermo/heatra.html>. [Accessed 15 6 2016].
- [16] C. Bauer, "Inductive Cross-Talk during Alternating Current Measurements," Quantum Design.
- [17] O. Dictionary, "laser," Oxford Dictionary, 2016. [Online]. Available: <http://www.oxforddictionaries.com/definition/english/laser>. [Accessed 15 6 2016].
- [18] C. Woodford, "Lasers," ExplainThatStuff, 21 05 2016. [Online]. Available: <http://www.explainthatstuff.com/lasers.html>. [Accessed 15 06 2016].
- [19] W. M. Haynes, CRC Handbook of Chemistry and Physics, 92nd ed., CRC Press, 2011.
- [20] ISO, *ISO 20473:2007 - Optics and photonics -- Spectral bands*, 2015.
- [21] J. Byrnes, Unexploded Ordnance Detection and Mitigation., Springer, 2009.
- [22] T. Instruments, "TI DLP," Texas Instruments, 2016. [Online]. Available: <http://www.ti.com/lstds/ti/dlp-technology/dlp-technology-home.page>. [Accessed 15 6 2016].
- [23] Philips, "InfraRed Industrial Heat Incandescent," 2016. [Online]. Available: <http://www.lighting.philips.com/main/prof/lamps/special-lamps/infrared/infrared-industrial-heat-incandescent>. [Accessed 19 04 2016].
- [24] H. a. Querry, Water, 1973.
- [25] R. I. Database, "Refractive Index Database," 2008-2016. [Online]. Available: <http://refractiveindex.info/?shelf=main&book=H2O&page=Hale>. [Accessed 19 04 2016].
- [26] hyperphysics, "Thermal Conductivity," [Online]. Available: <http://hyperphysics.phy-astr.gsu.edu/hbase/tables/thrcn.html>. [Accessed 21 04 2016].
- [27] goodfellow, "Polystyrene," 2008-2016. [Online]. Available: <http://www.goodfellow.com/E/Polystyrene.html>. [Accessed 21 04 2016].
- [28] BIO-RAD, "CFX Connect™ Real-Time PCR Detection System," [Online]. Available: [http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin\\_6102.pdf](http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6102.pdf). [Accessed 21 04 2016].
- [29] FLIR, "FLIR A320," 2016. [Online]. Available: [http://www.flir.com/uploadedFiles/Thermography\\_APAC/Products/Product\\_Literture/090722%20A320%20datasheet\\_eng.pdf](http://www.flir.com/uploadedFiles/Thermography_APAC/Products/Product_Literture/090722%20A320%20datasheet_eng.pdf). [Accessed 09 02 2016].
- [30] Sparkfun, "FLiR Dev Kit," 21 01 2015. [Online]. Available: <https://www.sparkfun.com/products/13233>. [Accessed 01 03 2016].

- [31] Central, "Package Details - TO-46," 2008 06 2008. [Online]. Available: <https://www.centalsemi.com/PDFS/CASE/TO-46PD.PDF>. [Accessed 11 02 2016].
- [32] Amphenol, "ZTP-135SR Thermometrics Thermopile IR Sensor," 2014. [Online]. Available: <http://www.farnell.com/datasheets/1855965.pdf>. [Accessed 11 02 2016].
- [33] Farnell, "AMPHENOL ADVANCED SENSORS ZTP-135SR THERMOPILE IR SENSOR, -20 DEGREE TO +100DEG, TO-46," 2016. [Online]. Available: <http://nl.farnell.com/amphenol-advanced-sensors/ztp-135sr/thermopile-ir-sensor-20-degree/dp/2506255>. [Accessed 11 02 2016].
- [34] Melexis, "MLX90614 family," 29 06 2015. [Online]. Available: <http://www.mouser.com/ds/2/734/901090614-706680.pdf>. [Accessed 11 03 2016].
- [35] E. T. Limited, "ET-063-10-13," [Online]. Available: <http://docs-europe.electrocomponents.com/webdocs/144a/0900766b8144a9b3.pdf>. [Accessed 04 05 2016].
- [36] Upinder Fotadar Dr., "Growth of Escherichia coli at elevated temperatures," *Journal of Bacteriology*, vol. 45, no. 5, 2007.
- [37] NPOOLE, "FLIR Lepton Hookup Guide," 26 03 2015. [Online]. Available: <https://learn.sparkfun.com/tutorials/flir-lepton-hookup-guide>. [Accessed 01 03 2016].
- [38] LambdaInstruments, "Benchtop Bioreactor and Fermentor," [Online]. Available: <http://lambda-instruments.in/index.php/products/bioreactor-fermentor-overview>. [Accessed 30 03 2016].
- [39] U. D. o. Energy, "Image Gallery," 2008. [Online]. Available: <http://genomics.energy.gov/>. [Accessed 30 03 2016].
- [40] zamisel, "Laser scanners," [Online]. Available: <http://www.zamisel.com/SSpostavka2.html>. [Accessed 13 04 2016].
- [41] SAP, "Optical Absorption of Water Compendium," 1998. [Online]. Available: <http://omlc.org/spectra/water/abs/index.html>. [Accessed 15 04 2016].

## 9 Appendices

## **Appendix A**

### **9.1 MATLAB Code**

```
function varargout = GUITest(varargin)
% GUITEST MATLAB code for GUITest.fig
%   GUITEST, by itself, creates a new GUITEST or raises the existing
%   singleton*.
%
%   H = GUITEST returns the handle to a new GUITEST or the handle to
%   the existing singleton*.
%
%   GUITEST('CALLBACK',hObject,eventData,handles,...) calls the local
%   function named CALLBACK in GUITEST.M with the given input arguments.
%
%   GUITEST('Property','Value',...) creates a new GUITEST or raises the
%   existing singleton*. Starting from the left, property value pairs are
%   applied to the GUI before GUITest_OpeningFcn gets called. An
%   unrecognized property name or invalid value makes property application
%   stop. All inputs are passed to GUITest_OpeningFcn via varargin.
%
%   *See GUI Options on GUIDE's Tools menu. Choose "GUI allows only one
%   instance to run (singleton)".
%
% See also: GUIDE, GUIDATA, GUIHANDLES

% Edit the above text to modify the response to help GUITest

% Created by GUIDE v2.5 13-Oct-2015 16:24:34

% Last Modified by Kevin Marczyk

% Begin initialization code - DO NOT EDIT

gui_Singleton = 1;
gui_State = struct('gui_Name',    mfilename, ...
    'gui_Singleton', gui_Singleton, ...
    'gui_OpeningFcn', @GUITest_OpeningFcn, ...
    'gui_OutputFcn', @GUITest_OutputFcn, ...
    'gui_LayoutFcn', [] , ...
    'gui_Callback', []);
if nargin && ischar(varargin{1})
    gui_State.gui_Callback = str2func(varargin{1});
end

if nargout
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end

% End initialization code - DO NOT EDIT

% --- Executes just before GUITest is made visible.
```

```
function GUITest_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to GUITest (see VARARGIN)

% Choose default command line output for GUITest
handles.output = hObject;

% Update handles structure
guidata(hObject, handles);
fig = imread('flirLogo.png');
axes(handles.axes2);
imshow(fig);

hasIPT = license('test', 'image_toolbox');
if ~hasIPT
    % User does not have the toolbox installed.
    message = sprintf('Sorry, but you do not seem to have the Matlab Image Processing Toolbox.\nYou will not
be able to run the Edge or Segmentation filter.\nContinue anyway?');
    reply = questdlg(message, 'Toolbox missing', 'Yes', 'No', 'Yes');
    if strcmpi(reply, 'No')
        % User said No, so exit.
        return;
    end
end

% UIWAIT makes GUITest wait for user response (see UIRESUME)
% uiwait(handles.figure1);

% --- Outputs from this function are returned to the command line.
function varargout = GUITest_OutputFcn(hObject, eventdata, handles)
% varargout  cell array for returning output args (see VARARGOUT);
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Get default command line output from handles structure
varargout{1} = handles.output;

% --- Executes on button press in pushbutton1.
function pushbutton1_Callback(hObject, eventdata, handles)
% hObject    handle to pushbutton1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

%AddAssembly Flir.Atlas.Live.dll
atPath = getenv('FLIR_Atlas_MATLAB');
```



```

atLive = strcat(atPath,'Flir.Atlas.Live.dll');
asmInfo = NET.addAssembly(atLive);
%init camera discovery
test = Flir.Atlas.Live.Discovery.Discovery;
% search for cameras for 10 seconds
disc = test.Start(10);
% put the result in a list box
for i =0:disc.Count-1
    s1 = strcat(char(disc.Item(i).Name),':');
    s2 = strcat(s1,char(disc.Item(i).SelectedStreamingFormat));
    str{i+1} = s2;
end
set(handles.listbox1,'string',str);
handles.disc = disc;
guidata(hObject,handles)

% --- Executes on selection change in listbox1.
function listbox1_Callback(hObject, eventdata, handles)
% hObject    handle to listbox1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: contents = cellstr(get(hObject,'String')) returns listbox1 contents as cell array
%    contents{get(hObject,'Value')} returns selected item from listbox1
index_selected = get(hObject,'Value');

% --- Executes during object creation, after setting all properties.
function listbox1_CreateFcn(hObject, eventdata, handles)
% hObject    handle to listbox1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: listbox controls usually have a white background on Windows.
%    See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

% --- Executes on button press in pushbuttonConnect.
function pushbuttonConnect_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonConnect (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
index_selected = get(handles.listbox1,'Value');
disc = handles.disc;
% check if it is FlirFileFormat or mpeg streaming
if(strcmp(char(disc.Item(index_selected-1).SelectedStreamingFormat),'FlirFileFormat'))
    %It is FlirFileFormat init a ThermalCamera
    ImStream = Flir.Atlas.Live.Device.ThermalCamera(true);
    ImStream.Connect(disc.Item(index_selected-1));
    %save the stream

```

```
handles.ImStream = ImStream;
handles.stop = 1;
guidata(hObject,handles)
%set the Iron palette
pal = ImStream.ThermalImage.PaletteManager;
ImStream.ThermalImage.Palette = pal.Iron;
%read x y position
x = str2num(get(handles.edit4,'String'));
y = str2num(get(handles.edit3,'String'));
oldX = x;
oldY = y;
%add spot

%Change stuff here
spot = ImStream.ThermalImage.Measurements.Add(System.Drawing.Point(x, y));
pause(1);

%Set up PID controller values
%OPTIMIZATION IS DONE HERE
a = arduino;
referenceHot = 40;           % Desired hot temperature
referenceCold = 30;         % Desired cold temperature
ypHot = 0;                   % init response
ypCold = 0;                  % init response
KpHot = 30;                   % proportional constant
KpCold = 0;                   % proportional constant
KiHot = 0.5;                  % Integral constant
KiCold = 0;                   % Integral constant
KdHot = 5;                    % derivative constant
KdCold = 0;                   % derivative constant

previous_errorHot = 0;
previous_errorCold = 0;
integralHot = 0;
integralCold = 0;
dtHot = 1;
dtCold = 0.1;
%tic
drawn = 0;
ImStream.ThermalImage.Measurements.Clear
while handles.stop
    %get the colorized image
    img = ImStream.ThermalImage.ImageArray;
    %convert to Matlab type
    X = uint8(img);
    axes(handles.axes1);

    %Set Filter1 on or off
    if get(handles.radiobutton2, 'value')
        X = Filter1(X);
    end
end
```

```
if get(handles radiobutton3, 'value')
    X = Filter2(X);
end
if get(handles radiobutton4, 'value')
    X = Filter3(X);
end
%show image with Matlab imshow
imshow(X);
Temperatures = zeros(1,96);
i = 1;
%show overlay for spot (for full speed set it to off in gui)
hold off;

tic;
ImStream.ThermalImage.Measurements.Count;
if(ImStream.ThermalImage.Measurements.Count == 0)
    offsetX = 75;
    offsetY = 67;
    for c = 0:7
        for r = 0:11
            b = offsetX + 168/11*r;
            v = offsetY + 107/7*c;
            plot(b, v, 'x', 5, 5, 'Color', 'green');
            ImStream.ThermalImage.Measurements.Add(System.Drawing.Point(b, v));
            i = i+1;
        end
    end
end
end

%uncomment to see locations to place wells
%    hold on
%        for c = 0:7
%            for r = 0:11
%                b = offsetX + 168/11*r;
%                v = offsetY + 107/7*c;
%                plot(b, v, 'x', 5, 5, 'Color', 'green');
%            end
%        end
%    hold off

for i = 0 : 95
    spot = ImStream.ThermalImage.Measurements.Item(i);
    Temperatures(i + 1) = spot.Value.Value;
end

drawnow
handles = guidata(hObject);

%Read temps from spots and write them to a file
time = toc;
%    for i = 1:96
```

```
%      fprintf('%d: %f %f\n', i, Temperatures(i), time);
%      fid = fopen('C:\Users\kmarc\Desktop\temperatures.txt','a'); %Open matlab as administrator
%      fprintf(fid,'%d: %f %f\n', i, Temperatures(i), time);
%      fclose(fid);
%      end

%Calculate PID controller output Hot
ypHot =
(Temperatures(12)+Temperatures(24)+Temperatures(36)+Temperatures(48)+Temperatures(60)+Temperatures(72)+Temperatures(84)+Temperatures(96))/8;

errorHot = referenceHot-ypHot;          % update error
integralHot = integralHot + errorHot*time; % integral term
%limit integral
if (integralHot > 100/KiHot)
    integralHot = 100/KiHot;
elseif integralHot < -100/KiHot
    integralHot = -100/KiHot;
end
derivativeHot = (errorHot-previous_errorHot)/time; % derivative term
uHot = KpHot*errorHot+KiHot*integralHot+KdHot*derivativeHot; % action of control
DutyCycleHot = uHot/100;
if DutyCycleHot < 0
    DutyCycleHot = 0;
elseif DutyCycleHot > 1
    DutyCycleHot = 1;
end
writePWMDutyCycle(a,'D2',DutyCycleHot);
writePWMDutyCycle(a,'D3',DutyCycleHot);
previous_errorHot = errorHot;

%Calculate PID controller output Cold
ypCold =
(Temperatures(1)+Temperatures(13)+Temperatures(25)+Temperatures(37)+Temperatures(49)+Temperatures(61)+Temperatures(73)+Temperatures(85))/8;
errorCold = referenceCold-ypCold;          % update error
integralCold = integralCold + errorCold*dtCold; % integral term
%limit integral
if (integralCold > 100/KiCold)
    integralCold = 100/KiCold;
elseif integralCold < -100/KiCold
    integralCold = -100/KiCold;
end
derivativeCold = (errorCold-previous_errorCold)/dtCold; % derivative term
uCold = KpCold*errorCold+KiCold*integralCold+KdCold*derivativeCold; % action of control
DutyCycleCold = uCold/255;
if DutyCycleCold < 0
    DutyCycleCold = 0;
elseif DutyCycleCold > 0.4
```

```
DutyCycleCold = 0.4;
end
writePWMDutyCycle(a,'D4',DutyCycleCold);
writePWMDutyCycle(a,'D5',DutyCycleCold);
previous_errorCold = errorCold;

%Print hot temp, cold temp, dutycyclehot
clc
fprintf('Hot: %.2f\t Cold: %.2f\t DCH: %.2f\n', ypHot, ypCold, DutyCycleHot)
fprintf('P: %.2f\t I: %.2f\t D: %.2f\n', KpHot*errorHot, integralHot*KiHot, derivativeHot*KdHot)

%print to file
fid = fopen('C:\Users\kmarc\Desktop\temperatures.txt','a'); %Open matlab as administrator
fprintf(fid,'%f\t%f\t%f\n', ypHot, ypCold, time);
fclose(fid);
end
else
%mpeg stream
ImStream = Flir.Atlas.Live.Device.VideoOverlayCamera(true);
%connect
ImStream.Connect(disc.Item(index_selected-1));
handles.ImStream = ImStream;
handles.stop = 1;
guidata(hObject,handles)
pause(1);
while handles.stop
% get the Image
img = ImStream.VisualImage.ImageArray;
X = uint8(img);
axes(handles.axes1);
imshow(X,[]);
drawnow
handles = guidata(hObject);
end
end
ImStream = handles.ImStream;
ImStream.Disconnect();
ImStream.Dispose();

% --- Executes on button press in pushbuttonDisconnect.
function pushbuttonDisconnect_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonDisconnect (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
handles.stop = 0;
fclose all;
guidata(hObject,handles);

% --- Executes on button press in pushbuttonNUC.
function pushbuttonNUC_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonNUC (see GCBO)
```

```
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
ImStream = handles.ImStream;
ImStream.RemoteControl.CameraAction.Nuc;

% --- Executes on button press in pushbuttonAuto.
function pushbuttonAuto_Callback(hObject, eventdata, handles)
% hObject handle to pushbuttonAuto (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
ImStream = handles.ImStream;
ImStream.RemoteControl.Focus.Mode(Flir.Atlas.Live.Remote.FocusMode.Auto);

% --- Executes on button press in pushbuttonSave.
function pushbuttonSave_Callback(hObject, eventdata, handles)
% hObject handle to pushbuttonSave (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
ImStream = handles.ImStream;
%read the input string
editString = get(handles.edit2,'string');
edit = strcat(editString, '.jpg');
%save snapshot
ImStream.GetImage().EnterLock();
ImStream.ThermalImage.SaveSnapshot(edit,ImStream.ThermalImage.Image);
ImStream.GetImage().ExitLock();

% --- Executes on button press in pushbuttonOpen.
function pushbuttonOpen_Callback(hObject, eventdata, handles)
% hObject handle to pushbuttonOpen (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
[filename path] = uigetfile;
s1 = strcat(path,filename);
%Load Flir Atlas
atPath = getenv('FLIR_Atlas_MATLAB');
atImage = strcat(atPath,'Flir.Atlas.Image.dll');
asmInfo = NET.addAssembly(atImage);
%open a thermal image
file = Flir.Atlas.Image.ThermalImageFile;
file.Open(s1);
axes(handles.axes1);
%Get the image as an array
img = file.ImageArray;
%convert to Matlab type
X = uint8(img);
%Use matlabs imshow to show the image
imshow(X);
%close file
file.Close;
handles.Filename = s1;
```

```
guidata(hObject,handles);

% --- Executes on button press in pushbuttonPlay.
function pushbuttonPlay_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonPlay (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
%Load Flir Atlas
atPath = getenv('FLIR_Atlas_MATLAB');
atImage = strcat(atPath,'Flir.Atlas.Image.dll');
asmInfo = NET.addAssembly(atImage);
%start a playback player
file = Flir.Atlas.Image.ThermalImageFile(handles.Filename);
seq = file.ThermalSequencePlayer();
%number of frames
seq.Count
%loop the sequence
seq.Loop = 1;
seq.Play;
handles.Loop = 1;
handles.seq = seq;
guidata(hObject,handles);
while handles.Loop
    %get the image in signal values
    img = file.ImageProcessing.GetPixelsArray;
    X = double(img);
    pause(0.0001);%let matlab render the image
    axes(handles.axes1);
    imshow(X,[]);
    handles = guidata(hObject);

end

% --- Executes on button press in pushbuttonStop.
function pushbuttonStop_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonStop (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
seq = handles.seq;
seq.Stop;
handles.Loop = 0;
guidata(hObject,handles);

% --- Executes on button press in pushbuttonStartRec.
function pushbuttonStartRec_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonStartRec (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
ImStream = handles.ImStream;
editString = get(handles.edit2,'string');
edit = strcat(editString,'.seq');
```

```
%start recording
ImStream.Recorder.Start(edit);
% --- Executes on button press in pushbuttonStopRec.
function pushbuttonStopRec_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonStopRec (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
ImStream = handles.ImStream;
%stop recording
ImStream.Recorder.Stop;

function edit2_Callback(hObject, eventdata, handles)
% hObject    handle to edit2 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of edit2 as text
%        str2double(get(hObject,'String')) returns contents of edit2 as a double
handles.edit = get(hObject,'string');
guidata(hObject,handles);

% --- Executes during object creation, after setting all properties.
function edit2_CreateFcn(hObject, eventdata, handles)
% hObject    handle to edit2 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%        See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function edit3_Callback(hObject, eventdata, handles)
% hObject    handle to edit3 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of edit3 as text
%        str2double(get(hObject,'String')) returns contents of edit3 as a double

% --- Executes during object creation, after setting all properties.
function edit3_CreateFcn(hObject, eventdata, handles)
% hObject    handle to edit3 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%        See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
```



end

```
function edit4_Callback(hObject, eventdata, handles)
% hObject    handle to edit4 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of edit4 as text
%        str2double(get(hObject,'String')) returns contents of edit4 as a double
```

```
% --- Executes during object creation, after setting all properties.
function edit4_CreateFcn(hObject, eventdata, handles)
% hObject    handle to edit4 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called
```

```
% Hint: edit controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end
```

```
% --- Executes on button press in radiobutton1.
function radiobutton1_Callback(hObject, eventdata, handles)
% hObject    handle to radiobutton1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
```

```
% Hint: get(hObject,'Value') returns toggle state of radiobutton1
```

```
% --- Executes on button press in radiobutton2.
function radiobutton2_Callback(hObject, eventdata, handles)
% hObject    handle to radiobutton2 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
```

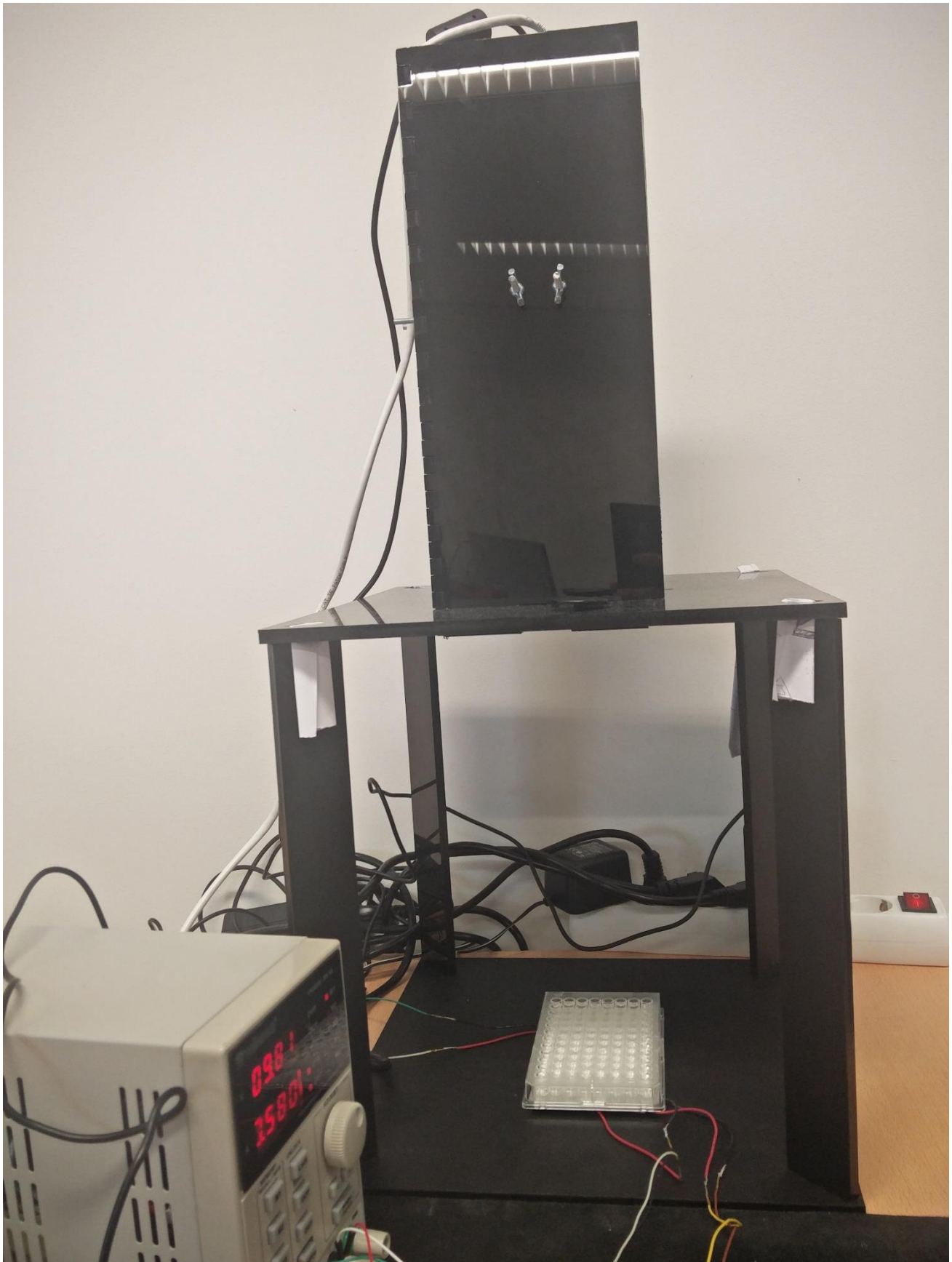
```
% Hint: get(hObject,'Value') returns toggle state of radiobutton2
```

```
% --- Executes on button press in radiobutton3.
function radiobutton3_Callback(hObject, eventdata, handles)
% hObject    handle to radiobutton3 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% Hint: get(hObject,'Value') returns toggle state of radiobutton3
% --- Executes on button press in radiobutton4.
function radiobutton4_Callback(hObject, eventdata, handles)
% hObject    handle to radiobutton4 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% Hint: get(hObject,'Value') returns toggle state of radiobutton4
```

## **Appendix B**

### **9.2 Images of the constructed set up**









## **Appendix C**

### **9.3 Accuracy and Stability at 45 degrees Celsius**

The accuracy and stability are evaluated when the system was at a stable temperature of 45 degrees Celsius (after initial overshoot). The temperature was considered 'stable' when the temperature had reached the target temperature within an error margin of 0.5 degrees Celsius after the overshoot. The error was defined as the difference between the measured temperature and the desired temperature.

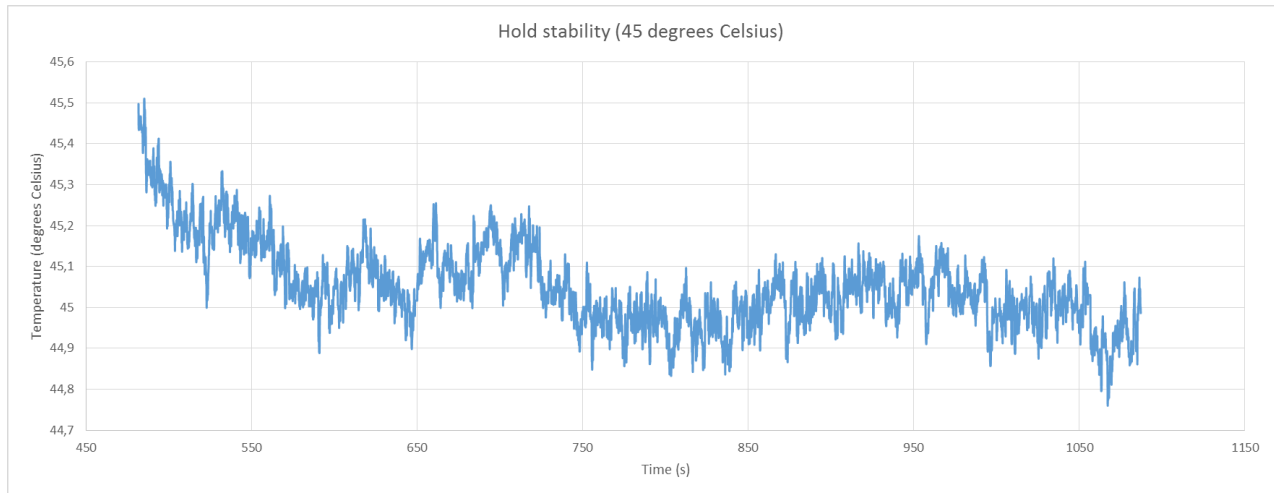


Figure 34 - The temperature of the hot side of the microtiterplate while the system has a goal temperature of 45 degrees Celsius.

Figure 35 shows that when the target temperature of 45 degrees is reached and the system is stable, the error will barely exceed more than half a degree. From Figure 34 we can conclude that this occurs at the start, and might still be part of the overshoot.

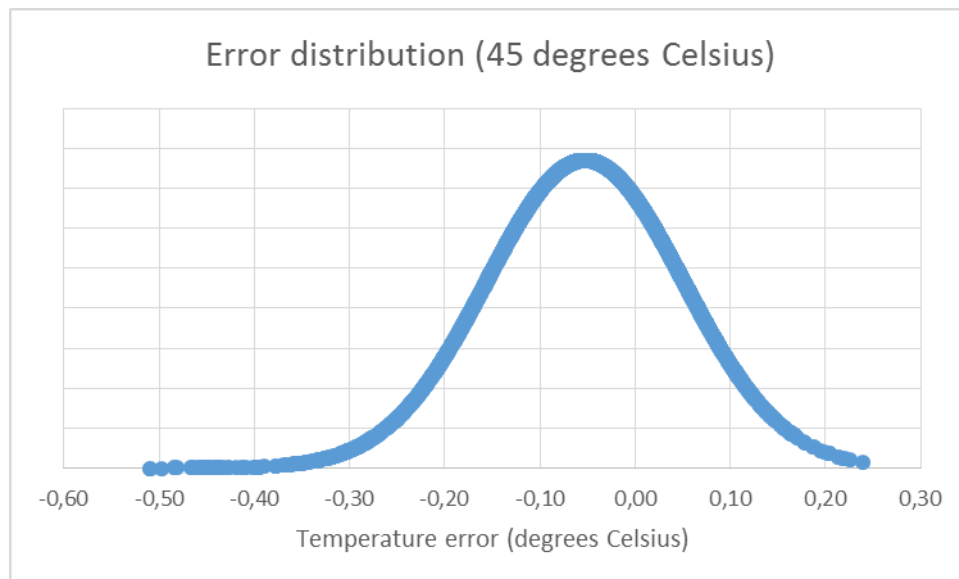


Figure 35 - A bell curve depicting the relative occurrences of temperature errors when the system is stable at 45 degrees Celsius. The mean error is -0.05 and the dataset has a standard deviation of 0.10.

Calculating the probability that the absolute error is always smaller than 0.5 degrees Celsius can be done as follows.

$$Z_1 = \frac{X_1 - \mu}{\sigma} = \frac{-0.5 - -0.05}{0.1035} = -4.3478$$

$$Z_2 = \frac{X_2 - \mu}{\sigma} = \frac{0.5 - -0.05}{0.1035} = 5.314$$

From these Z values we get:

$$\begin{aligned}\Pr(-0.5 \leq X \leq 0.5) &= \Pr\left(\frac{-0.5 - -0.05}{0.1035} \leq Z \leq \frac{0.5 - -0.05}{0.1035}\right) = \Pr(-4.3478 \leq Z \leq 5.314) \\ &= 1 - 0 = 1\end{aligned}$$

From this dataset we can conclude that when the system is stable at 45 degrees Celsius, the absolute error will never be greater than 0.5 degrees Celsius. Therefore, I am 100% certain that the true absolute error will not be greater than 0.5 degrees Celsius when the system is stable at 45 degrees Celsius.

Recalculating the probability for a maximum absolute error of 0.2 degrees Celsius results in a probability of 0.92. Therefore, I am 92 percent certain that the true absolute error will not be larger than 0.2 degrees Celsius when the system is stable at 45 degrees Celsius.



## Appendix D

### 9.4 Confidence interval calculations about Accuracy and Stability at 30, 37, and 50 degrees Celsius

Goal Temperature (degrees Celsius)	Allowed Error (degrees Celsius)	Z values	Calculation	Confidence interval
<b>30</b>	0.5	Z1	$\frac{-0.5 - -0.033}{0.088} = -6.07$	100 %
		Z2	$\frac{0.5 - -0.033}{0.088} = 5.31$	
	0.2	Z1	$\frac{-0.2 - -0.033}{0.088} = -2.66$	97 %
		Z2	$\frac{0.2 - -0.033}{0.088} = 1.90$	
<b>37</b>	0.5	Z1	$\frac{-0.5 - -0.0017}{0.074} = -7.01$	100 %
		Z2	$\frac{0.5 - -0.0017}{0.074} = 6.54$	
	0.2	Z1	$\frac{-0.2 - -0.0017}{0.074} = -2.94$	99%
		Z2	$\frac{0.2 - -0.0017}{0.074} = 2.48$	
<b>50</b>	0.5	Z1	$\frac{-0.5 - -0.059}{0.11} = -4.95$	100 %
		Z2	$\frac{0.5 - -0.059}{0.11} = 3.90$	
	0.2	Z1	$\frac{-0.2 - -0.059}{0.11} = -2.29$	88 %
		Z2	$\frac{0.2 - -0.059}{0.11} = 1.25$	