Visualisation of Viable Renal Cells in Donor Kidneys during Normothermic Machine Perfusion

Redesigning an IPK system to fit inside an IVIS scanner

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Preface

This is the report about the internship in the first year of the master Biomedical Engineering, Medical Device Design at the University of Groningen. Commissioned by Prof. Dr. Leuvenink of the Department Surgical, Sector Transplantation and organ quality, a perfusion machine for an isolated rat kidney was redesigned to fit inside an IVIS scanner. The full design process is described in this report.

Because this internship took place during the lockdown due to COVID-19, the lab was not very accessible. I did not go to the lab until the end of the third week. During the fourth week I saw the IVIS scanner for the first time in real live. This meant that I had to design a machine based on an existing machine which fit inside another machine, without seeing them first. It made the design process more complicated and little things that you would notice in real life only became apparent to me when I showed up with the prototype.

The project was fairly big for this kind of internship. The internship was only ten weeks. This meant that, among other things, the workplace could not be asked to build the base. The manufacturing would take too long, which meant to device could not be tested as much.

Because I do not have the tools to build a prototype like this, I asked my sister to help. We have spent many nights coming up with ideas and tweaking the design to allow for all the tubes and wires. Having her to help, made building the prototype a lot of fun!

Working in the surgical lab and testing the device in the animal lab of the UMCG was very exciting. I was lucky to get this internship, and I enjoyed it greatly thanks to everybody that helped me through the process.

Analysis phase

Introduction

The amount of kidney patients increases every year. Over the last 10 years, in the Netherlands, the number went from 2000 to 2500 patients per million population [1]. The number of new patients per year has remained the same, but due to the immense shortage of transplantable kidneys the total number is growing [1, 2, 3, 4]. In the end stage of renal disease, transplantation is the best treatment. Patients that get a transplant instead of dialysis have a better quality of life, less health care costs and a better survival rate [3]. To slim the difference between the supply and demand, kidneys of donation after circulatory death (DCD) and extended criteria donors (ECD) are used more and more often [5, 6]. (ECD are donors over the age of 60 or donors aged 50-59 with two comorbidities [7])

To preserve the kidneys during the transplantation, static cold storage perfusion (SCS) is mostly used. During SCS a kidney is stored in ice until it is transplanted [8]. This gives a satisfying transplant outcome when using after brain dead donors (DBD) [9]. Before transplanting, the kidneys are judged based on donor-risk-factor and appearance [2]. The quality assessment of DCD and EDC kidneys is harder compared to DBD kidneys. Many of these higher risk kidneys show poor functionality after transplantation [2, 5, 6], which could have been prevented with a better selection process.

An alternative to SCS is normothermic machine perfusion (NMP). During NMP the kidneys are perfused with warm oxygenated bloodlike substance called perfusate. The perfusate can remove toxic products, provide energy substrates and it can support functions for reconditioning and repair [6, 9]. This helps the renal cells to become active just as they would after transplantation [2, 9]. During NMP the normal physiological status of an organ can be preserved [9]. This Leads to improved organ quality and an elongated time until transplantation. It can also help with the quality assessment by evaluating the urine production and the perfusion characteristics of the kidney, for instance resistance [2, 6, 10]. Because the predictability of the kidney on NMP increases, the number of kidneys that fail after transplantation decreases [2, 6].

Unfortunately, kidneys from deceased donors can have a delayed reaction [2, 7, 11], not showing good perfusion characteristics right away. This could either mean delayed graft function or primary nonfunction [11]. Primary nonfunction entails a permanent loss of kidney function right after transplantation. It severely lowers the chance of survival for a patient [12]. Due to the chance of experiencing primary nonfunction the protocol for higher risk kidneys is very strict and perfectly good functioning kidneys often get discarded [2, 11].

The potential idea is to test if the kidneys are suitable for transplantation with the administration of near-infrared fluorescent nanoparticles to the perfusate during perfusion. If the kidney is functional, the kidney processes the perfusate and the nanoparticles will be spread through the entire organ. After administering the nanoparticles, a scan is made with the IVIS scanner, which is a device that can measure fluorescence. In this scan the difference in fluorescence between a healthy and a slightly damaged kidney can clearly be seen. When the nanoparticles are charged with mRNA, the functional kidney has shown to transcribe the strands. This is a biological process which indicates cellular activity and thus a functioning kidney [2].

Problem definition

The immense shortage of donor kidneys has led to the use of DCD and ECD kidneys. These donor kidneys are harder to assess compared to DBD kidneys, which causes them to be more prone to experiencing post-transplantation failure [2, 5, 6]. Using NMP instead of SCS does help with the quality assessment of all donor kidneys. The reason being that not only the donor-risk-factor and appearance of the kidney are considered, but also the actual renal function of the donor kidney. Even with using the NMP the assessment can still be hard due to delayed graft function. To get an even better estimation of the behaviour of the kidney after transplantation, near infrared-fluorescent nanoparticles can be used. By administering the nanoparticles to the perfusate during NMP, the function of the kidney can be shown by an IVIS scanner [2].

Fluorescence scanning is preferred over other optical imaging techniques because the optical radiation used to excite the nanoparticles is non-ionizing [13, 14]. Consequently, the radiation causes no harm and is minimally invasive [14]. One of the downsides to this technique is: it does not have a deep penetration depth [14]. This is partly caused by autofluorescence, which is the fluorescence of natural substances when excited with light. Autofluorescence is stronger when working at a smaller wavelength [15]. By using near-infrared light this problem is partly solved. Near infrared wavelengths (700-1100nm) are above the absorption region of blood (<600nm) and below the absorption region of water (>1500nm) [14]. Thus, when using near-infrared nanoparticles the effect of autofluorescence is least noticeable [13, 14, 16]. Even when using near infrared-light, the penetration depth will still be limited. For this project this will not be a problem, since a rat kidney is very thin and small. When the technique is used on human kidneys, which are bigger and thicker, it might be wise to look into an adapted version of this fluorescence technique.

The benefit of using nanoparticles is that they are so small, they can be adsorbed into the tissue alongside other molecules [13]. Therefore, it can be used to show the absorption behaviour of the tissue.

The reaction of the kidneys to the nanoparticles and mRNA-strands has only been recorded while the organs were removed from the perfusion machine, rinsed, and put on a piece of paper. To make scans while the organs are still being perfused, the perfusion machine needs to be inside the IVIS scanner. At the moment all the perfusion machines are too big to fit inside the scanner. This leads to the assignment: to redesign the perfusion machine to be smaller so it can fit inside an IVIS scanner [2].

Isolated Perfused Kidney (IPK) system

The IPK system signifies an ex-vivo model of a whole kidney perfused through the renal artery with a synthetic oxygenated medium, the perfusate. The system contains an adjustable pump that regulates the flow of the medium and a canula that collects the urine from the ureter. The urine and perfusate can be analysed to study the renal function without the influence of external tissues. The system is often used as a research tool to study drug regulation [17, 18].

An IPK systems consist of a perfusate reservoir, a roller pump, a heat exchanger, filters, an oxygenator, overflow valves, a sample port, a flow meter, a manometer, a thermometer, a bubble trap, and an organ chamber [4, 17, 18, 19]. The IPK system normally holds a pressure of 70-90 mmHg and a flowrate of 40ml/min during perfusion [4, 18, 19].

The oxygenator can be described as the artificial lungs the system. It provides gas exchange without bubble formation [20]. The oxygenator works with moistened pressurized carbogen (95% oxygen and 5% carbon) [17, 18, 19, 21].

The oxygen level needs to be regulated to prevent hyperoxia or hypoxia in the kidney, respectively too high levels and insufficient levels of oxygen in the tissue. Normally carbogen is used, because hyperoxia is known to restrict arteria and reduce blood flow. Hypoxia is known to do exactly the opposite. Therefore, carbon dioxide prevents vasoconstriction due to oxygen [22, 23]. When hyperoxia persists, more and more oxygen radicals are formed. Radicals will react with anything and can therefore be very harmful for the tissue [24]. When hypoxia persists, the cells begin to use the anaerobic pathways to generate the needed energy. Lactic acid is produced, and the cell environment becomes more acidic. If this happens for a prolonged amount of time it is called acidosis, which can cause cell death [25]. Both hyperoxia or hypoxia are toxic for the kidney tissue if they present themselves for an extended time and will lead to tissue scarring [26].

A schematic version of the system can be seen in Figure 1. It works as follows: The kidney is placed in the organ chamber on a piece of nylon mesh. The mesh prevents loose tissue from entering the perfusate reservoir. The chamber is kept at 37^{o}C [19]. The perfusate is pumped from the reservoir through the pump, through the heat exchanger (which is not in the scheme) [4] and through the filters. It flows through the oxygenator where it is aerated. Attached to the oxygenator are a pressure relief tube and a control valve with a perfusate sample port that controls the level of aerated perfusate in the system [19]. The sample port can essentially be anywhere in the system, except next to the pressure sensor. Taking a sample would otherwise mess with pressure measurement [4]. The perfusate can flow back to the reservoir via the control valve in case of too much flow. The aerated perfusate is pumped through the flow meter. It passes a manometer and a flow control valve. In case of too much pressure or flow, the valve can be adjusted and the perfusate will flow back to the reservoir [19, 27]. From the flow meter the perfusate passes a bubble trap. This is a three-way stopcock. If any bubbles have formed in the system, they will rise to the top part of the stopcock instead of flowing down into the kidney [21, 28]. After passing the bubble trap the perfusate flows into renal artery of the kidney and back through the mesh into the reservoir. A urine collection tube is attached to the ureter apart from the reservoir [19, 27].

A problem with the normal organ chambers is that the perfusate can leak a bit around the kidney and the reservoir is below the kidney. The nanoparticles are in the perfusate, which means that if the kidney is scanned while in the organ chamber, there will be a lot of noise in the scans. The organ chamber should be redesigned to minimize the noise from loose nanoparticles.

There are some differences between the schematic version of the IPK system used in reference [19], Figure 1, and the real life version which is made in the UMCG lab, Figure 2. The schematic version shows filters, which are not in the real-life version. They are not in the system, because the perfusate can be filtered before it is put into the IPK system, and the filter give extra resistance which can cause problems [4]. For this project, an added problem is that the filters could potentially filter out the nanoparticles in the perfusate. This would result in less or no activity showing in the scans and would therefore negate the goal of the project.

Another difference is the presence of syringe connections in the real-life version. When the flow in a certain tube/part of the system is occluded, a syringe can be attached to the connection. The syringe is than used to put extra pressure on the occlusion which helps it come loose. These connections are not in the schematic version, which could mean two things: the connections are either not in the system or they are not in the schematic version. Schematic drawings do not always include all the details, to make the picture clearer. Even so, the connections are only in the real-life system for safety reasons. The system could function normally without them. The risk of having to rebuild a part of the system is just higher without them.

The schematic version does not have an oxygen sensor. This sensor is optional since the oxygen level can be tested via the samples taken from the sample port. Measuring the oxygen level of the perfusate is done to show if the kidney is metabolically active. A pH sensor could be used as an oxygen sensor since the pH becomes higher during hyperoxia and lower during hypoxia due to the production of excess protons [25].

The schematic version is an open system, the real-life version is a closed system. A closed system means that the renal vein is canulated. This entails that all the perfusate coming out of the kidney goes directly through a tube to the reservoir. In an open system the perfusate streams out of the kidney and is collected below the organ chamber. The closed system was used in the real-life system because the pressure in the vein was measured. That is also why there are two pressure sensors in the real-life version instead of one. For this project it is not needed to take this second pressure measurement, which means that both open- and closed systems are viable for the project. A disadvantage of a closed system is that it can be very challenging to cannulate the small vein of a rat kidney. On the other hand, an advantage of the system is that it does mostly eliminate the chance of perfusate nanoparticles leaking around the kidney during scanning.

The heat exchanger is absent in the schematic version. Instead of using heat exchanger the system is fully enclosed in a plexiglass chamber, which is kept at 37°C.

The real-life version has two bubble traps instead of one. It has one on the heat exchanger and one just before the organ chamber. It does not matter how many bubble traps are in the system, as long as it is certain that bubbles will not reach the kidney. This would damage the kidney severely.

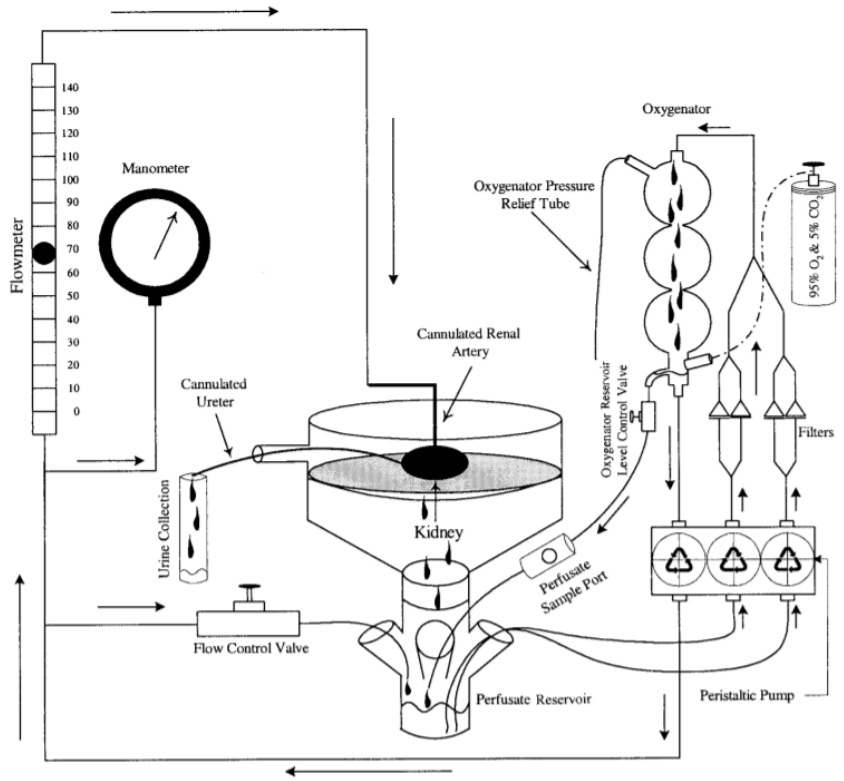


Figure 1. A schematic drawing of an IPK system [14].

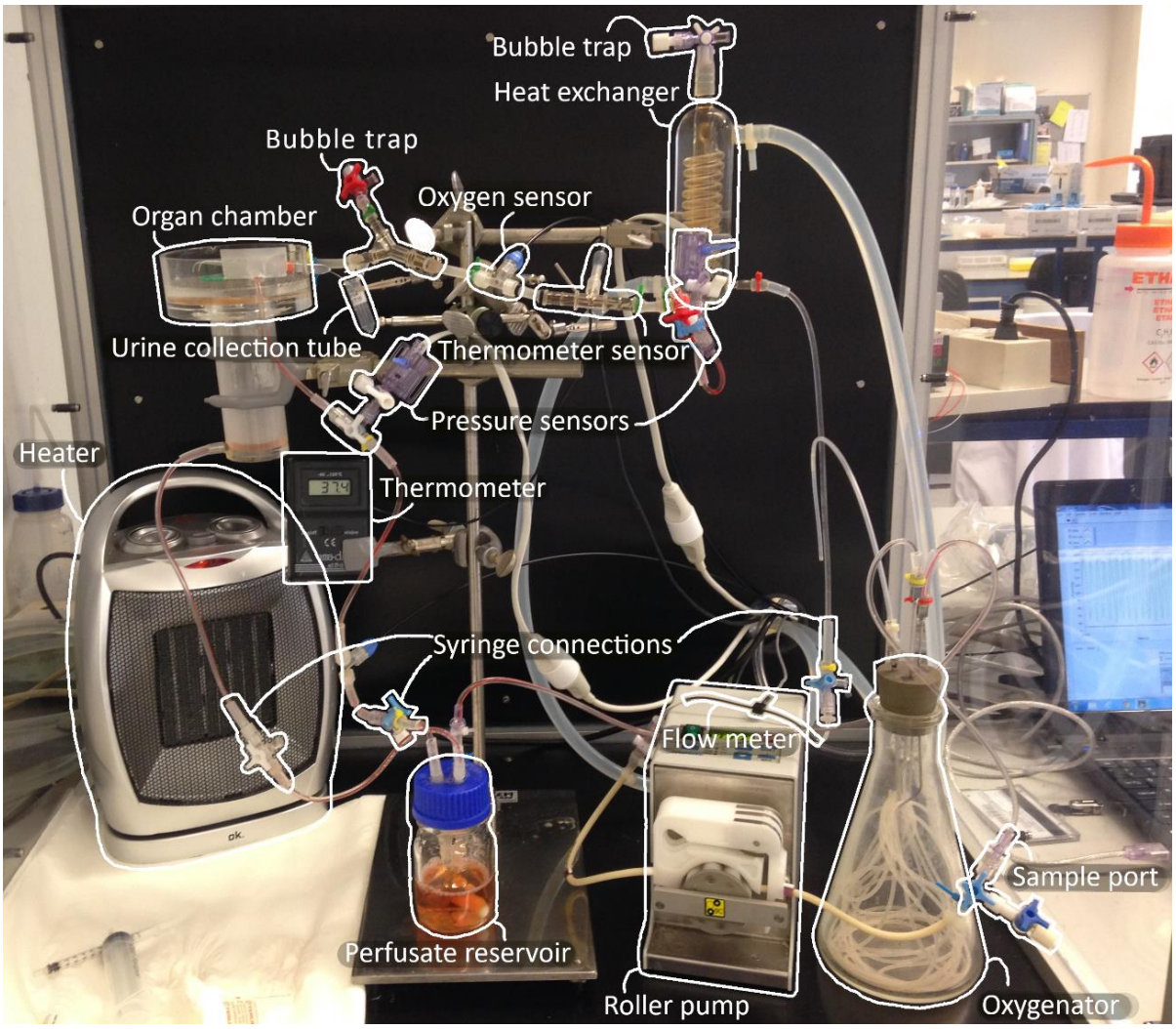


Figure 2. The IPK system in the surgical lab of the UMCG.

IVIS scanner

The name IVIS stands for In Vivo Imaging System. It is a high throughput fluorescence and bioluminescence imaging system for animal models [29, 30]. It can measure fluorescent receptors between the blue and near infrared wavelengths. Different wavelengths can be chosen to be scanned by using different filters. The IVIS scanner has the feature that it can scan from the top or from the bottom of the animal model [1].

The chamber is a low background imaging chamber, which keeps light from leaking in. Consequently, if the scanner is closed during scanning, this will result in cleaner scans. The scans will always show some noise due to, for instance, hair, intestinal phosphorescence or autofluorescence [16]. The scanner gets the best signal when the tissue that needs to be scanned is as bare as possible. The more layers of tissue or material on top of it the specimen, the weaker the signal gets [16].

The chamber has a built-in anaesthesia- and a heating system, to keep animal models warm and anesthetized during scanning [29]. In other words, the oxygen flow can be regulated from the outside with a flowmeter through the gas anaesthesia inlet and outlet ports and the temperature inside the scanner can be set to 37°C. This heating system makes the use of a heater obsolete. The IVIS scanner is equipped with an oxygen tube, so the oxygenator in the new system will most likely not be fed with carbogen but oxygen [4]. The tubes for the oxygen are attached to the IVIS scanner with a special kind of connection to stop light from leaking in [16]. If the 100% oxygen causes hyperoxia, a pH buffer can be used to counteract the effect and reduce cell death. Just like carbon dioxide does when using carbogen [25].

The IVIS scanner that is used in this project is an IVIS100. This is a slightly older model. The inside measurements are 38 x 40 x 35 cm (which is smaller than the newer models). The bottom of the inside of the scanner is adjustable in height. There is no possibility for any sort of cable to pass from the inside to the outside. A normal scan using this device usually takes 90-120 min. It is not a continuous scan, because that would lead to too much data. Instead, every 5 min. a scan will be taken. In between the scans the chamber can be opened if needed. It cannot be open for long periods because the temperature inside the chamber would decrease too much.

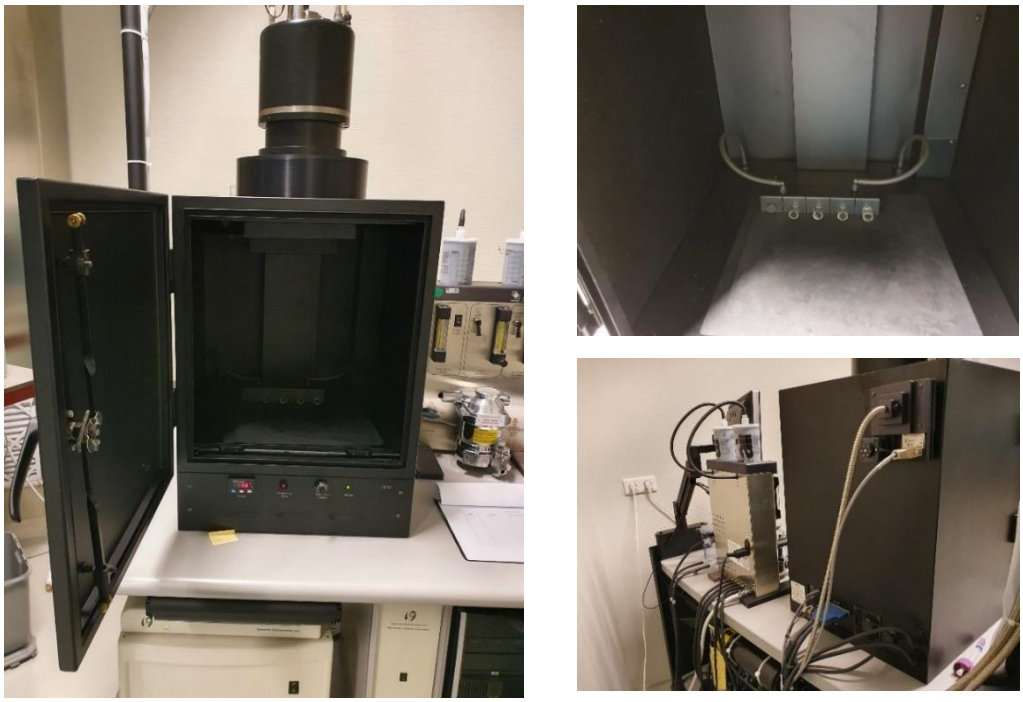


Figure 3. The IVIS100. The IVIS scanner that will be used for the project.

Market research

Full system

There are already some Normothermic kidney perfusion machines on the market. The company Organ Assist sells the product "Kidney assist", see Figure 4. It consists of a pump unit and a thermo unit with flow, temperature, pressure, and level sensors. This perfusion machine does everything that is needed to be able to keep the kidneys preserved. Two problems with this system are that it is meant for human kidneys, which are bigger and need more flow, and the dimensions of the device are 112 x 92,5 x 62,5 cm, which is too big [10].

Figure 4. The kidney assist. A normothermic kidney perfusion machine made by Organ Assist [9]

There are also IPK systems with all the needed features (mentioned in the IPK system section) on the market. For instance, the "Radnoti Isolated Rodent Liver/Kidney Apparatus", as seen in Figure 5 [31]. The problem with this system is still that it is too big to fit inside an IVIS scanner.

Figure 5. The Radnoti Isolated Rodent Liver/Kidney Apparatus. An IPK system made by AD administrations [21].

Another example of a fully equipped IPK system is the "Universal Perfusion System (UP-100)", see Figure 6. It can be used for a liver, kidney, heart, and a mesenteric bed of small rodents [32]. It already looks a lot smaller than the Radnoti, which is a big improvement, but it has a lot of cables running from and to it. The IPK system that is needed for the IVIS scanner must be wireless.

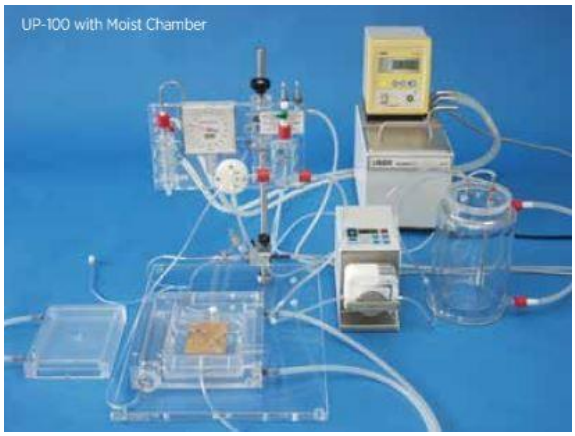


Figure 6. The Universal Perfusion System (UP-100). An IPK system made by Harvard Apparatus [22].

Since there is not a full system already on the market that satisfies all the requirements, the device needs to be designed from scratch. The separate parts, like the pump and the heat exchanger that fit the requirements could be available to buy. Therefore, the market research was continued for the separate parts. The bubble trap and other small coupling parts are already small enough to potentially be used in the new device.

Pump

The “Perel, Mini Peristaltic Pump” is very small pump that could be considered for the device, see Figure 7. Its size is 65 x 40 x 40 mm, it can pump up to 39ml/min, it runs on 6V, and its weight is 95g. The energy source could be attached by soldering it to the solder lip. The maximum flow is just 1 ml below the required maximum flow, which should not be a big problem. But the other problematic thing about this pump is: the instructions say that it cannot come in contact with food products [33]. Perfusate is not a food product, but, just like food, it should not become toxic. Otherwise, the kidney will fail.

Figure 7. The “Perel Mini Peristaltic Pump”. A small peristaltic pump made by the company Perel.

The “CMP-32B, mini liquid pump” is another example of a very small pump, see Figure 8. The technical features do not specify what kind of pump it is, but the characteristics meet the requirements. The pump has a maximum flow of 50ml/min, the size is 17,5 x 26,5 x 45 mm and it needs an energy source of 3V. [34]

Figure 8. The “CMP-32B, mini liquid pump”. A small pump made by the company Alldoo.

The “Gikfun 12V DC Peristatltic Pump” is intended for aquarium use, but does meet all the standards, see Figure 9. It has a maximum flowrate of 100 ml/min, runs on an energy source of 12V and 39,5 x 36,6 x 63.2 mm [35].

Figure 9. The “Gikfun 12V DC Peristatltic Pump”. A small peristatic pump made by the company Gikfun [27].

Stakeholder analysis

To determine the size of the problem and the consequences for each party involved, a stakeholder analysis is done. This analysis can be seen in Table 1.

Table 1. The stakeholder analysis.

Stakeholders	**Characteristics**	**Expectations**	**Potentials and deficiencies**	**Implications and conclusions for the project**
Donors	People who gave consent on their donor codicil.	More of the donor organs are put to good use.	The donor-risk-factor is too high, or the kidney is too damaged to salvage the organ with NMP. Not only DBD but also DCD and EDC donor organs can be used.	Kidneys that would normally be discarded can be used to test the device.
Patients	Dysfunctional kidneys require dialysis and thus decrease the quality of life severely. The longer on the waitlist, the lower the life expectancy.	More available kidneys of the same quality. Less complications after the transplantation. A transplanted kidney reduces the needed medical care and increases the quality of life.	Must have compatibility with the donor organ. An increased availability of kidneys could save a lot of lives.	Patients with transplanted kidneys on which the device is used can help show the viability of the device.
Family of patient	The emotional and financial pressure of having a sick family member can be enormous.	Family member will be healthier.	Emotional support can get patients through difficult times.	Can support the patients. To help them have a quick recovery.
Surgeons	Hard to assess the quality of a DCD and EDC donor kidney before transplantation can lead to complications after the transplantation.	Better assessment of the DCD and EDC donor kidneys.	A healthier kidney is easier to transplant. The kidneys become easier to assess, which will lead to less failed kidneys.	Can test the device and compare it to the normal standard.
Society	Prefer cheap healthcare with a high quality. More kidney patients who are not suitable for work can be a burden on the healthy people of society.	Less kidney patients, means cheaper healthcare and less workload.	Healthcare cannot get more expensive.	Can pay for healthcare.
Insurance	Provides health care as cheap as possible.	Less dialysis patients will result in lower costs for treatment.	Does not want to fund the device if the benefits do not out way the cost.	Critical to subsidize the new system.
Industry	Always want to bring innovative products to the market.	Making profit of off a new product.	A good proposal must be in place, otherwise nobody might want to consider developing the product.	Manufacturers can help develop the new system.

Cause-effect diagram

To visualize the underlying problem, a cause-effect diagram is made. As can be seen in Figure 10, kidney failure has a lot of problematic effects. If a problem in the top of the diagram is solved, all the following effects will also be solved.

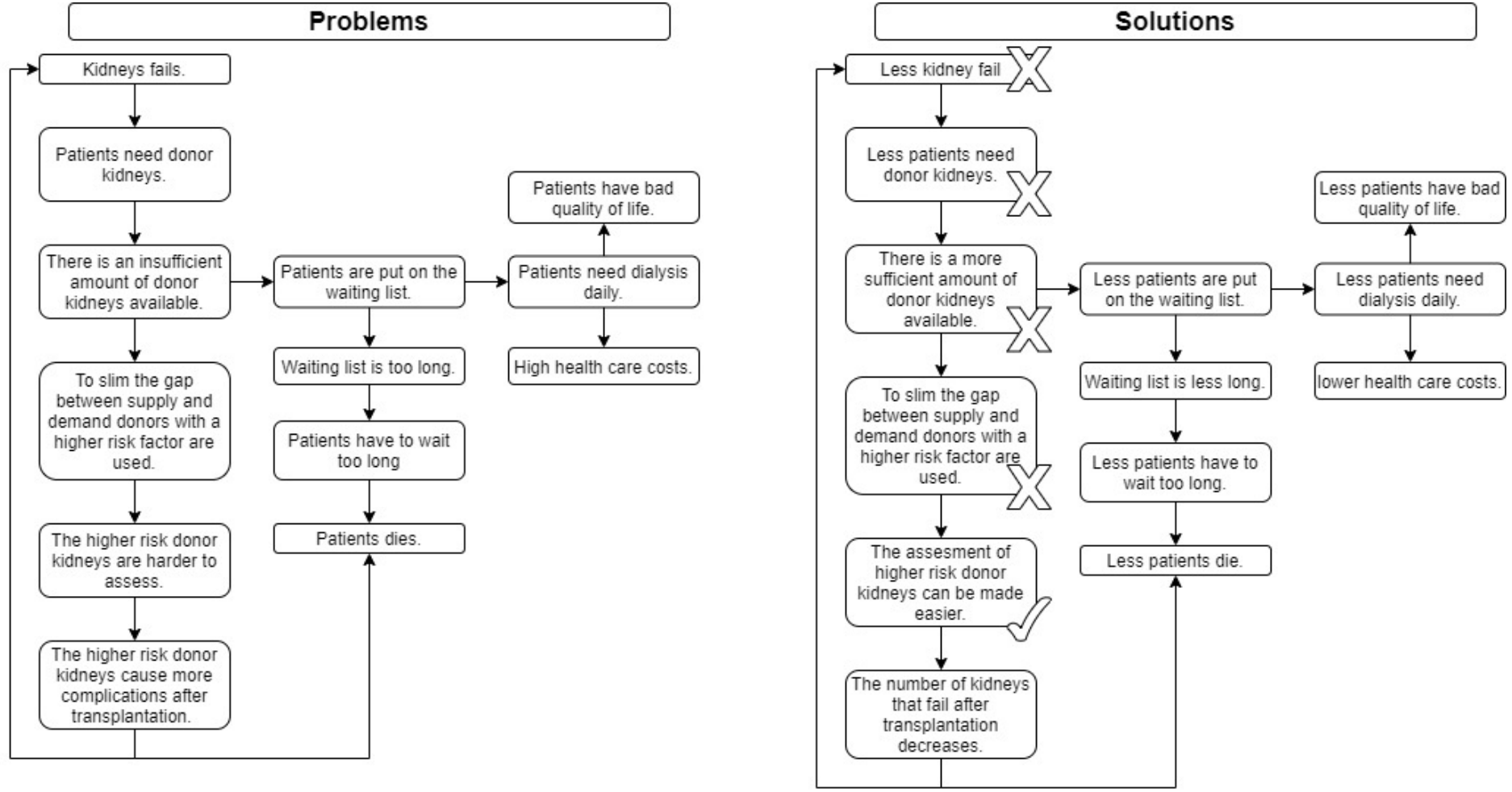


Figure 10. The cause-effect diagram. (right) Problems with their effects. (left) Solutions with their effects.

Goal of the product

As can be seen in Figure 10, there are many ways to tackle the problem of the shortage of donor kidneys: the kidneys can stop failing, the patients can stop needing donor kidneys, more DCD and EDC donor kidneys can be used to slim the gap between supply and demand and the assessment of higher risk donor kidneys can be made easier.

Unfortunately, we do not yet live in a time where we can stop kidneys from failing. This makes solving the first problem unrealistic. In the foreseeable future we must expect that the amount of kidney failures will not significantly decrease.

Patients can also not stop needing donor kidneys. As said in the problem description, the alternative, dialysis, not only has a higher health insurance cost but it also lowers the life expectancy and the quality of life. A kidney transplant is the best treatment a patient can receive at the moment.

The only way to use more DCD and EDC donor kidneys is to lower the restrictive protocol. These kidneys already have a higher risk of failing. Lowering the restrictions will only increase this chance. More patients would loop back to the beginning of the cause-effect diagram: “Kidneys fail”. This strategy would maybe temporarily solve the problem, but many patients will end up with the same symptoms as before or worse.

The first problem that can realistically be solved is “Higher risk donor kidneys are harder to assess”. By making the assessment easier, less kidneys will fail after transplantation and more kidneys can be used. Because the cause-effect diagram is actually a cause-effect loop, solving the assessment problem will partly fix the top problem as well. As can be seen in the solutions part of the cause effect diagram: If less kidneys fail after transplantation, less kidneys fail overall. This means less patients need a donor kidney, the donor kidney supply will be less insufficient, the waiting list will not be as long, less patients will die, and more patients will have a better quality of life.

As mentioned in the problem description NMP can be used to make the assessment easier. The perfusion characteristics can be used to review the quality of the organ. Unfortunately, some kidneys have a delayed graft function and NMP alone is not reliable enough when using EDC and DCD kidneys. A solution for this is already devised: administering nanoparticles (potentially) charged with mRNA during NMP and scanning it with an optical scanner. If a scan is made, a healthy kidney will be more fluorescent compared to a slightly damaged kidney. Still delayed graft function will cause the kidney to show fluorescence later. A scan that is timed incorrectly will not show fluorescence. A healthy kidney would still be discarded. To compensate the potential of unfortunate timing, a live scan can be made. To do this a IPK system should be inside the IVIS scanner at the moment of scanning.

To summarize, the best way to try and solve the problem of the donor kidney shortage is to make the assessment of the kidneys easier. The first step in doing so is scanning a rat kidney perfused with nanoparticles during NMP. Which leads to the goal of the product: to perfuse a rat kidney while inside an IVIS scanner.

Design assignment

To realise the goal, a device must be designed that can pump warm oxygenated bloodlike substance through the kidney and it should collect the urine output in a separate container. The system cannot let any bubbles reach the kidney and perfusate should be clean. The flow and pressure should be consistent and regulated to fit the “needs” of the specific kidney. The device should work wireless and the only assistance it can receive during the scan is through computerized commands. The kidney is attached to the device before entering the IVIS scanner and it is removed from the device after exiting the scanner. The rat kidney must be perfused for the duration of a scan, which is 90-120min.

Demarcation

The device that must be designed is meant for perfusing a rat kidney while inside the IVIS100 scanner. It must keep the kidney in a perfused state for the duration of a scan. The base idea of the IPK system used by the UMCG (described earlier) should be used. The device is meant to be used in either the animal testing lab of the UMCG or the lab of the partner university in Denmark, Aarhus University.

Program of requirements and wishes

Function requirements

- The device must be able to regulate the pressure to be between 70 and 90 mmHg.
- The device must be able to regulate the flowrate to be between 20 and 40ml/min.
- The device must be able to regulate the temperature to be between 36,5^{o}C and 37,5^{o}C.
- The device must be able to oxygenate the perfusate.
- The device should only need computerized assistance during the scanning period.

Safety requirements

- The device must not let air bubbles enter the perfused organ.
- The device must be safe to use. With clear instructions a trained professional should be able to use the device without getting injured or damaging the organ.
- It must be possible to disinfect the device.
- The device must be stable. Under no circumstances should it be able to fall.

Economic requirements

- The device cannot cost more than 5.000 euros.

Ergonomic requirements

- The portable part of the device must be wireless.
- One person must be able to place the device in the IVIS scanner. While a kidney is perfused the portable part of the device cannot be heavier than 10kg or it should have a structure to help the person get the device into to IVIS scanner.

Size requirements

- The device must fit inside the IVIS scanner. The portable part of the device cannot be bigger than 38 x 40 x 35 cm.

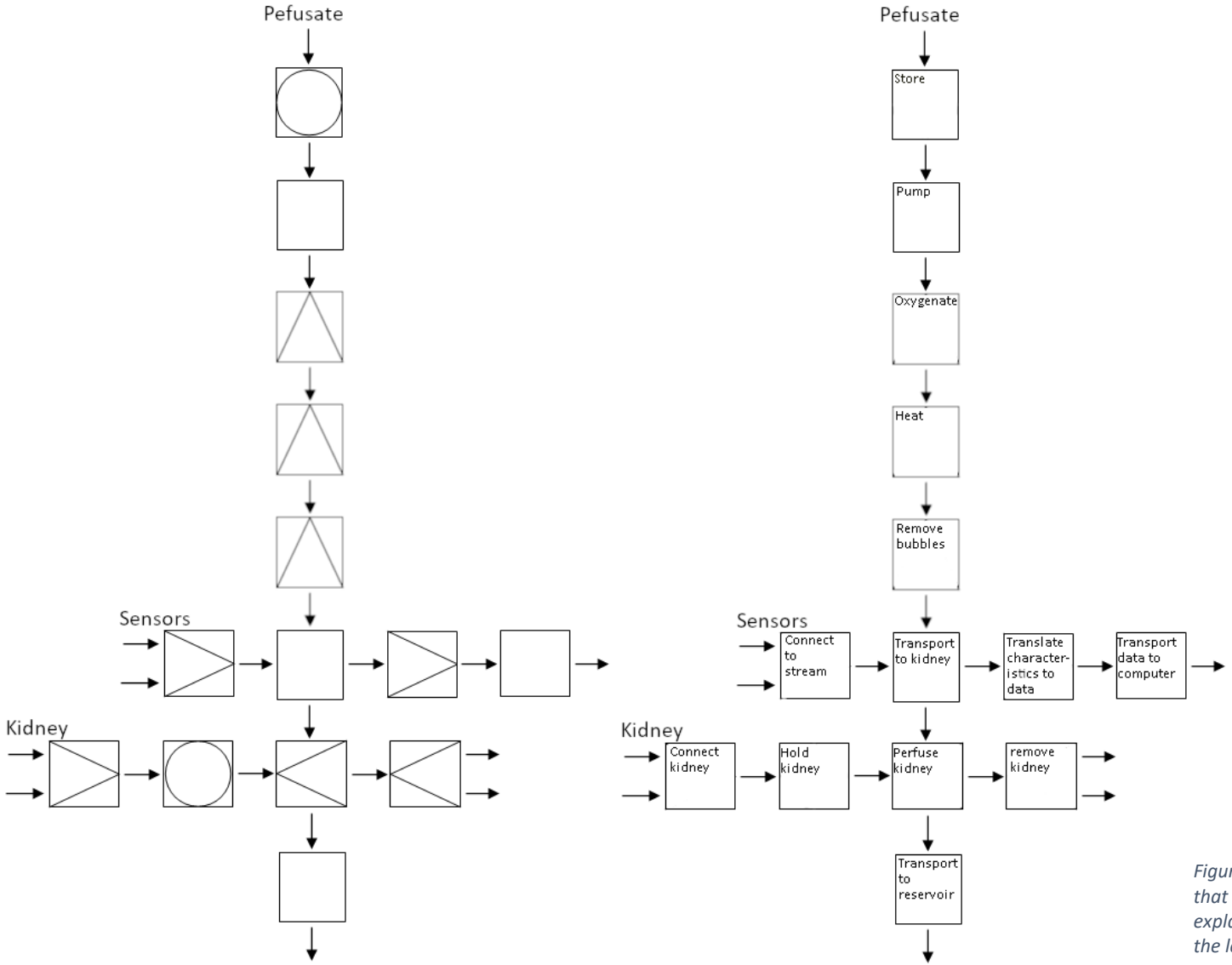
Time requirements

- The battery life must be at least as long as the time it takes to do a full scan, which is max. 120 min.
- The device must have a lifetime of at least 10 years.

User wishes

- The device should be low maintenance. Preferably only once a year parts need to be replaced.
- The device should be made of durable materials.
- Nanoparticles under and around the kidney should be avoided as much as possible to reduce noise in the scans.

Function analysis



To identify the base functions the device must have to function properly, a function analysis is done. All the separate functions are divided into groups which are signified by a unique block. The blocks are aligned in a scheme to show the interaction between the functions. See Figure 11 for the function analysis of the device that needs to be designed. These functions are, in fact, the same as a normal IPK system and need to be preserved in the new design.

Figure 11. (left) Function analysis of the device that needs to be designed. (right) The explained version of the function analysis on the left.

Synthesis phase 1

Morphological scheme

To make the design process of the device easier, a morphological scheme is made. The basic function of the device each get a row in the scheme. During a brainstorm session, multiple solutions are though up for each function. The morphological scheme can be seen in Figure 12.

Transport perfusate	Tilted	Closed system	Longer vein with funnel	Funnel/ coffee filter	Leaking rope	Porous layer
Organ storage	Hammock	Bars	Mesh	Planck	Floating	Inbetween water bags
Pressure generator	Centrifugal	Peristaltic	Gravity	Piston	Membrane	
Oxygen sensor	Oxygen sensor	Sample port	Ph sensor			
Energy source	Batteries	Generator				
Base	Box	Box with boxes	Slots	Cardrige	Cage	
Perfusate storage	Funnel	Flask	Bag	Sponge	Box	Dubble bottom
Oxygenator	Membrane	Thin tubing	Sheat	Combination of oxygenator and heat exchanger	Combination of oxygenator and pump	
Heat exchanger-substance	Water	Air	Fire			
Heat exchanger shape	Pipe outside pipe	Pipe inside pipe	Heating element	Combination of oxygenator and heat exchanger		
Connection to computer	SD-cart	Wifi	Bluetooth	Laptop in chamber		
Attachment	Snap buttons	Buttons	Velcro	Slide studs	Magnets	Screws
	Click buckle	Edge clip	Crocodile clip	Clamps		
Pressure regulation	Pressure relieve valve	Sensor feedback	Computerized			

Figure 12. Morphological scheme.

To make the preconcepts the solutions are stringed together. The morphological scheme with the paths for each concept can be seen in Figure 13.

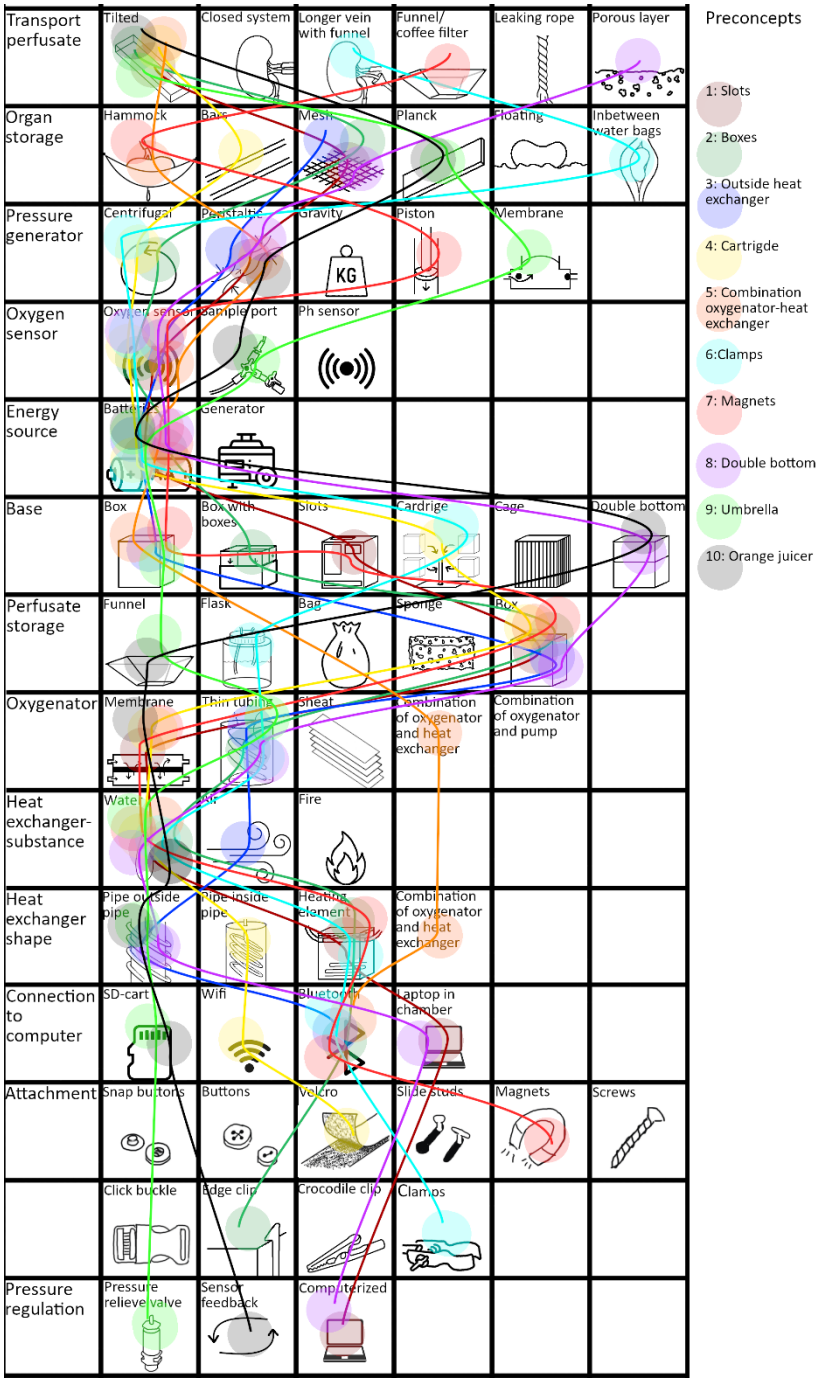


Figure 13. Morphological scheme with paths for each concept.

Preconcepts

Preconcept 1: Slots

The first preconcept consists of a box with five slots. The first slot contains the organ chamber. Below the organ chamber is a tilted surface which lets the perfusate flow the reservoir in slot two. The roller pump above the reservoir in slot two pumps the perfusate to the heat exchanger and oxygenator in slot three. After the heat exchanger and oxygenator, the perfusate is pumped through the sensors in slot four and back to the kidney in the organ chamber. The sensors in slot four are connect to the laptop in slot 5. The laptop saves the collected data. If the pressure or flow is too high or too low the laptop gives the command to the pump to pump harder or slower.

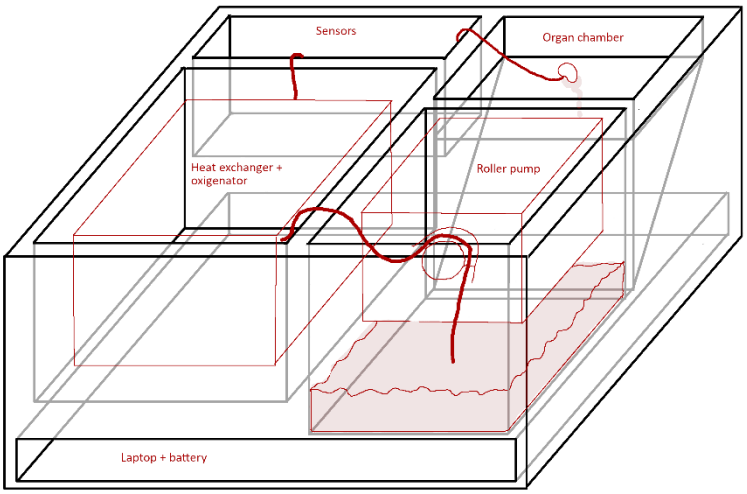


Figure 14. Preconcept 1: Slots

Preconcept 2: Boxes

The second preconcept consists of a box with smaller boxes in it. The boxes can individually be taken out to make the assembly and for cleaning the perfusion machine. The box with the organ chamber is very shallow. Below the organ chamber is the box that contains the reservoir. To reduce the amount of noise in the scan, due to the nanoparticles in the reservoir, the mesh in the organ chamber is made of aluminium. The system uses a centrifugal pump. The water heater has a reservoir with a heating element below the pump and oxygenator. The system works with a Bluetooth device that sends the data gathered from the sensors to a computer outside of the IVIS chamber. The tube that enters the organ chamber is attached to a bubble trap to eliminate bubbles in the perfusate.

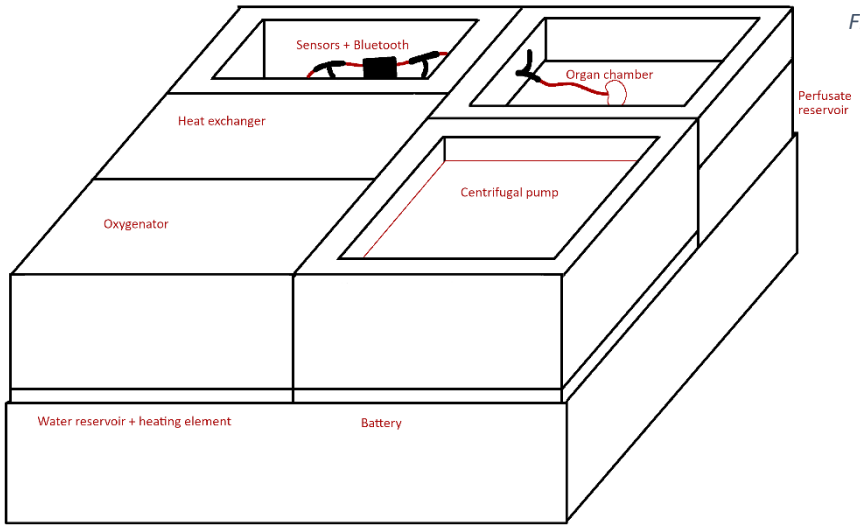


Figure 15. Preconcept 2: Boxes

Preconcept 3: Outside heat exchanger

The third preconcept consists of a closed loop system in a box. The organ chamber is the same as in the original design of the UMCG setup. To reduce the noise due to nanoparticles in the reservoir, an aluminium foil mesh is used. The perfusate is pumped by a roller pump through the oxygenator, the sensors and back to the organ chamber. The oxygen is heated outside of the chamber by heating the tube the gas flows through. Because the chamber and the oxygen are always at 37°C the perfusate will not cool down.

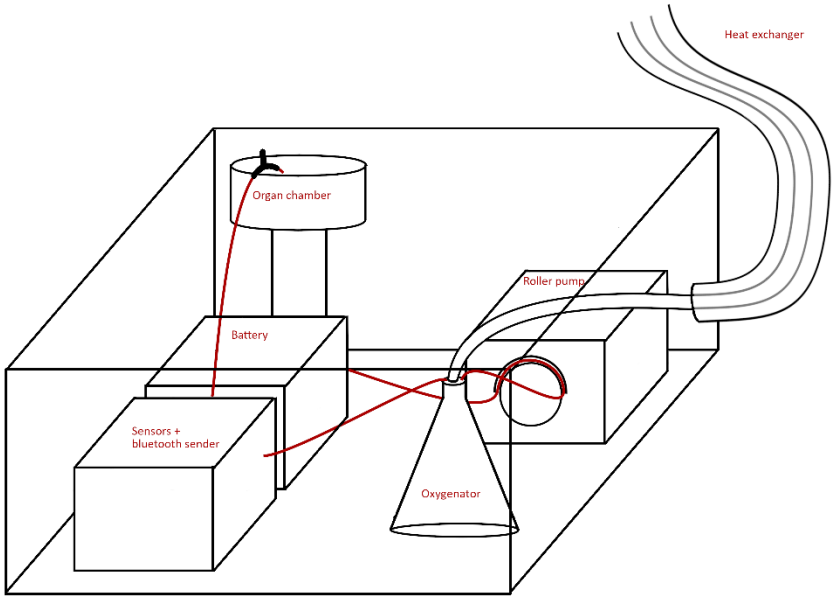


Figure 16. Preconcept 3: Outside heat exchanger.

Preconcept 4: Cartridge

The fourth preconcept consists of a plate with a Velcro wall in the middle. All the individual pieces of the system also contain a Velcro wall. They can be stuck to the right place on the wall during assembly. The organ chamber consists of two bars on which the kidney can rest. Underneath the bars is a slide through which the perfusate can reach the reservoir. Because of the slide, the reservoir does not have to be directly beneath the kidney. The system works with a centrifugal pump which pumps the perfusate through the heat exchanger and oxygenator and through the sensors back to the kidney. The data is transferred to a computer outside the chamber with the use of Wi-Fi.

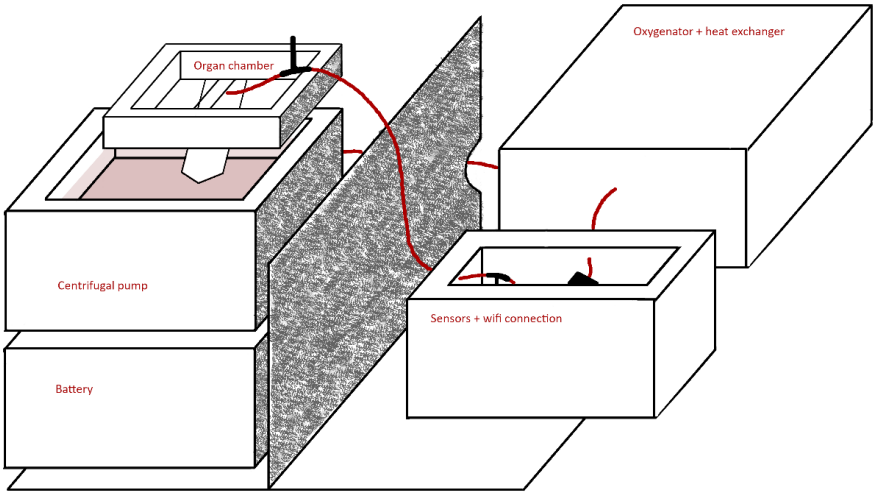


Figure 17. Preconcept 4: Cartridge.

Preconcept 5: Combination oxygenator-heat exchanger

The fifth preconcept is a box containing two reservoirs. One for water and one for perfusate. The water is heated by a heating element. The heated water and the oxygen from outside the chamber are both pumped through the oxygenator heat exchanger combination [36]. The perfusate is pumped through the oxygenator heat exchanger combination, through the sensors and bubble trap and then through the kidney. The kidney lays on a water bag with a curved top the bottom of the water bag is made of aluminium to reduce de noise from the reservoir underneath. The perfusate leaking out of the kidney drips down into the reservoir where it is reused.

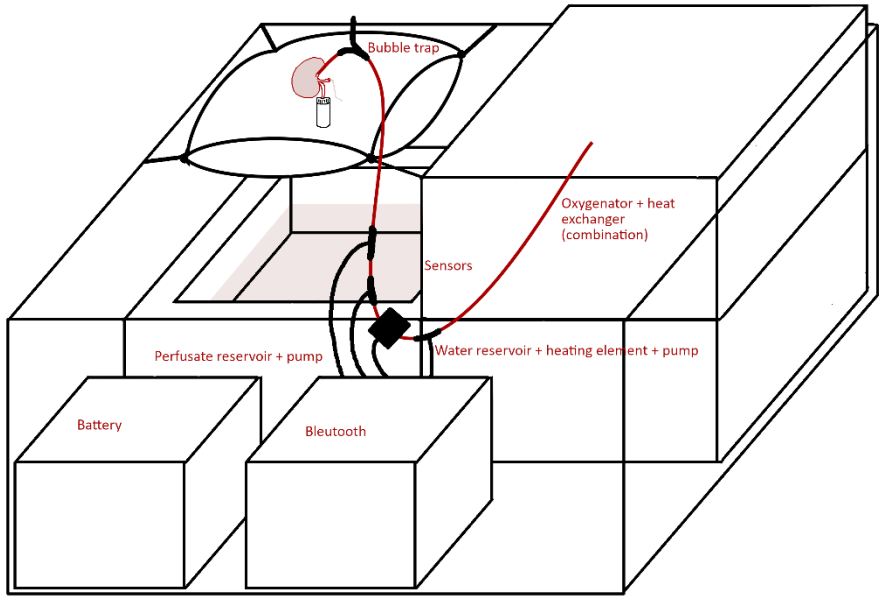


Figure 18. Preconcept 5: Combination oxygenator-heat exchanger.

Preconcept 6: Clamps

The sixth preconcept consist of a base plate with a rod in the middle. Attached to the rod are clamps which can hold the individual pieces of the IPK system. The organ chamber is made from a folded over water sack. The kidney is placed in the fold. The water in the sack is kept warm by the water from the water heater. The perfusate is pumped, with a centrifugal pump, through the oxygenator, the heat exchanger, the sensors and back to the organ chamber. Before it reaches the kidney, it passes a bubble trap. The data from the sensors is transferred via Bluetooth to a computer outside the IVIS chamber.

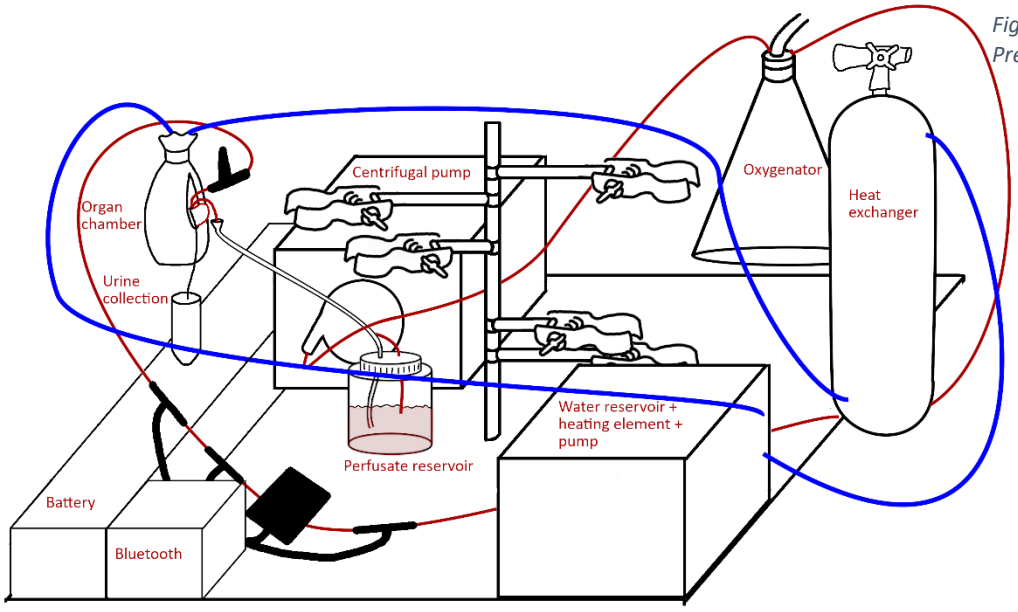


Figure 19. Preconcept 6: Clamps

Preconcept 7: Magnets

The seventh preconcept consist of a metal box. All the components have magnets, with which they can be stuck to the right place in the box. The organ chamber has a funnel shape. The perfusate can leak through the bottom of the funnel into the reservoir. This can be done with a small tube, to offset the reservoir relative to the organ chamber. This will, again, reduce the noise in the scan due to nanoparticles in the perfusate. The system uses a piston pump.

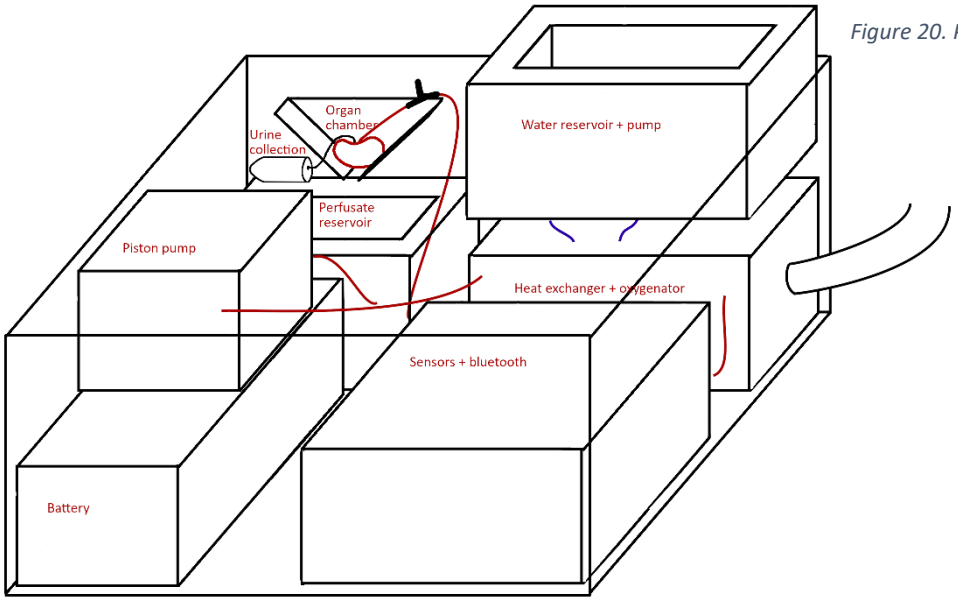


Figure 20. Preconcept 7: Magnets.

Preconcept 8: Double bottom

The eight preconcept consists of a box with an aluminium plate halfway up. Underneath the plate are the reservoir, the roller pump, the oxygenator, and the battery. On top of the layer are the sensors, the laptop, and the organ chamber. The plate reduces the noise in the scan due to perfusate. The bottom of the organ chamber is made from three layers of aluminium mesh. This gives the perfusate the chance to drip into the reservoir while also reducing the noise in the scans. The oxygen is heated before entering the IVIS chamber, by heating the tube outside of the chamber just as preconcept 3. Because the chamber, the oxygen and the perfusate are at 37°C, a heating system in the chamber is not necessary before entering the organ chamber the perfusate passes a bubble trap.

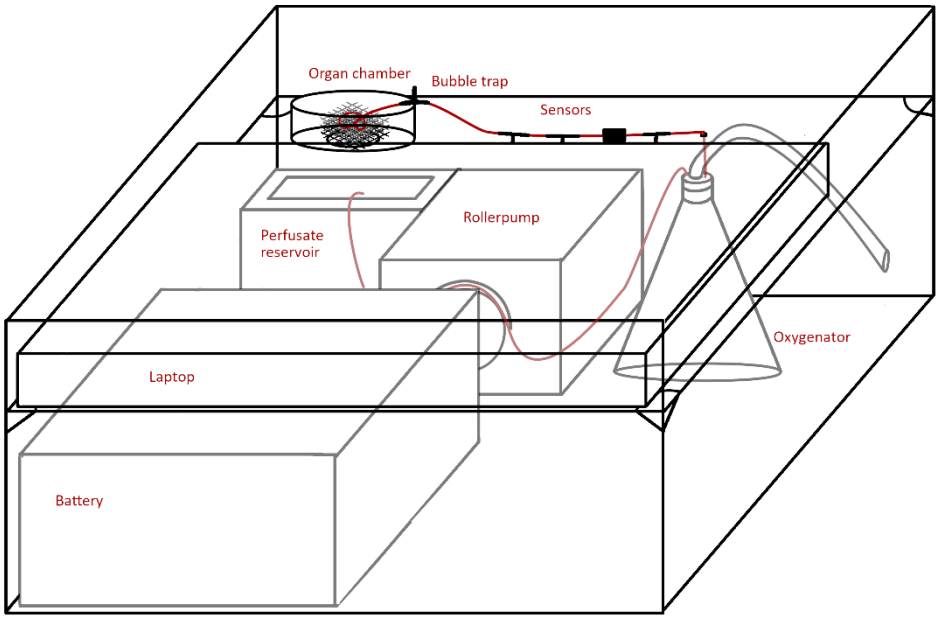


Figure 21. Preconcept 8 Double bottom.

Preconcept 9: Umbrella

The nineth preconcept has a special organ chamber to prevent noise as much as possible. This organ chamber is shaped like an umbrella with a dish underneath. The kidney lays on the top of the umbrella. The perfusate seeps out of the kidney and flows of off the umbrella into the dish. The dish is tapered so that the perfusate flows underneath the umbrella. The umbrella is made from aluminium so the scanner cannot pick up the signal from the nanoparticles in the perfusate.
The system uses a membrane pump. The sensor data stored on a SD-cart. If the pressure gets too high the pressure relieve valve will give in. This will ruin the experiment, but not the system. Heated oxygen is used, like in preconcept 3, to keep the perfusate at the right temperature. The oxygen level of the perfusate can be tested by opening the chamber in between scans and taking a sample via the sample port.

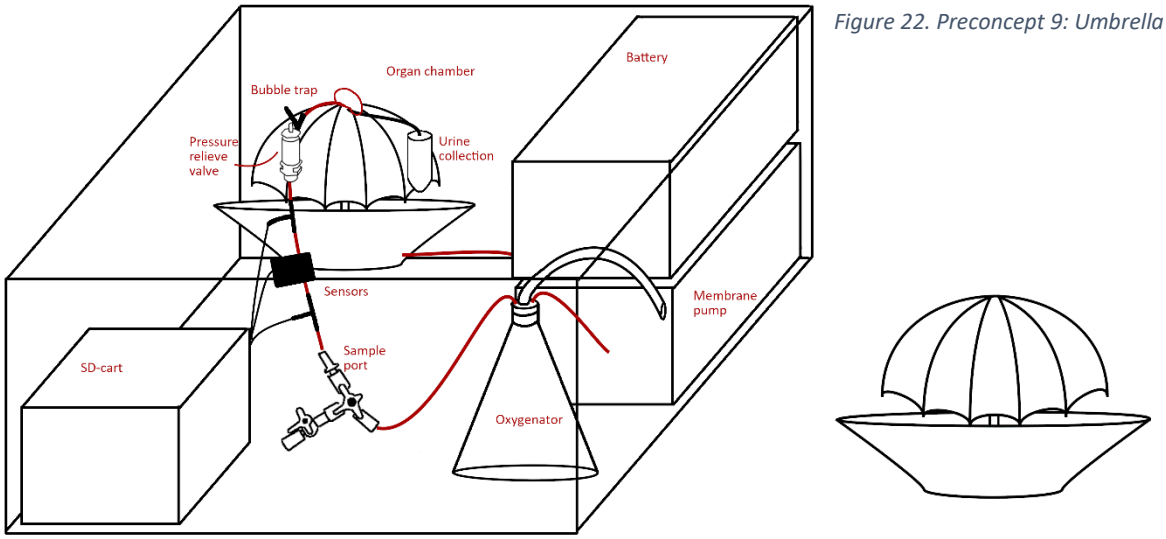


Figure 22. Preconcept 9: Umbrella

Preconcept 10: Orange juicer

The tenth preconcept also has a special organ chamber to prevent noise as much as possible. This organ chamber is shaped like an orange juicer. The top is flatter, but it has groves that lead the perfusate easily down the slope. The perfusate reservoir is a ring around the bottom of the chamber. This reservoir will show like a ring around the kidney in the scans. The system uses a roller pump. The sensor data is saved on a SD-cart. The pressure sensor is in a feedback loop with the roller pump. If the pressure gets too high the roller pump will pump less. Heated oxygen is used, like in preconcept 3. The oxygen level of the perfusate can be tested just like in preconcept 9.

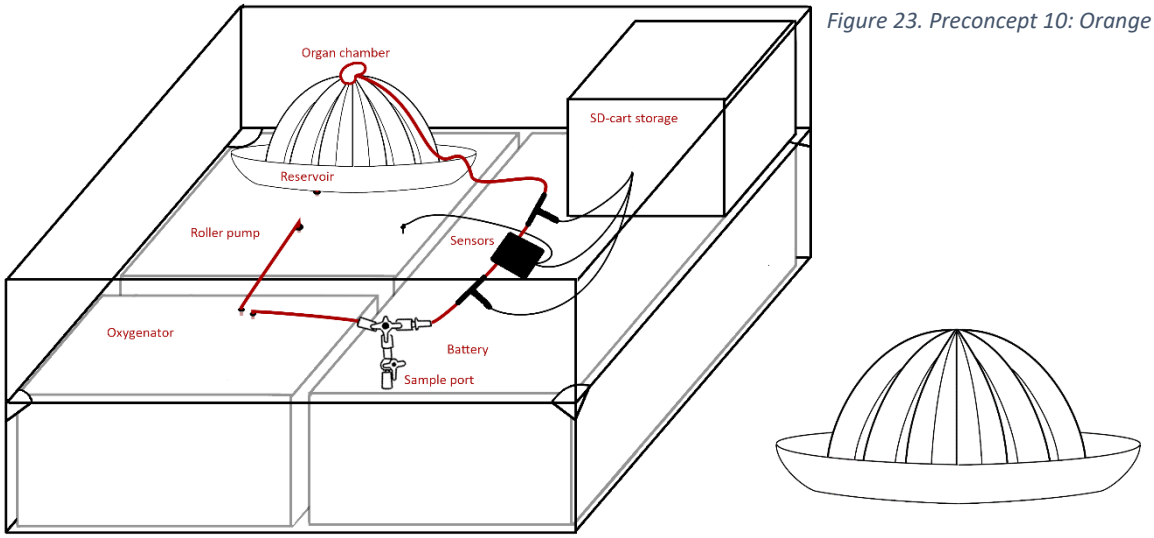


Figure 23. Preconcept 10: Orange juicer.

Preconcept selection

To choose the best preconcept, the concepts are scored based on criteria and weight factors. The preconcept with the highest score is the best concept for this project. The criteria are based on the program of requirements and wishes. The requirements were reduced to the eight most important criteria. To many criteria would clutter the results and make the selection procedure needlessly difficult.

Criteria

1. The device can perfuse a rat kidney for the duration of a scan.
2. The device can regulate the pressure in the system.
3. The device can measure the oxygen saturation, temperature, pressure, and flowrate of the perfusate.
4. The IVIS chamber is closed as much as possible.
5. The device reduces noise in the scans, due to nanoparticles outside the kidney, as much as possible.
6. The device can be regulated and read out with computerized assistance outside the IVIS chamber.
7. The device is easy to place in the IVIS scanner.
8. The device is cheap.

Weigh factors

The weighing factors are based the opinion of every stakeholder. The criteria that are more important will weigh more. To calculate the weight factors the Analytic Hierarchy Process (AHP) is used [37]. Of the seven stakeholders the most important six are considered. Manly because the family of the patients is a part of society and only six stakeholders fit in the AHP excel document. The six stakeholders are: donors, patients, surgeons, society, insurance, and industry. The criteria are scored for every stakeholder and averaged via an iteration process. The results can be seen in Figure

Matrix	Perfuses kidney	Pressure regulation	Measurements	Chamber closed	Reduce noise	Computerized assistance	Easy to manouver	Cheap	normalized principal Eigenvector
Perfuses kidney	1	2	4 2/5	5 2/5	4 1/4	4 2/5	6	6 1/8	37,0%
Pressure regulation	1/2	1	3 1/3	4 1/4	2 8/9	3	4 3/4	5	21,7%
Measurements	2/9	2/7	1	1 5/8	3/5	2/3	2 1/7	2 1/4	7,6%
Chamber closed	1/5	1/4	5/8	1	2/5	3/7	1 5/8	1 1/2	5,7%
Reduce noise	1/4	1/3	1 2/3	2 4/7	1	1 1/4	2 4/7	2	9,5%
Computerized assistance	2/9	1/3	1 1/2	2 2/7	4/5	1	2 2/7	1 8/9	8,7%
Easy to manouver	1/6	1/5	1/2	5/8	2/5	3/7	1	1 4/9	5,0%
Cheap	1/6	1/5	4/9	2/3	1/2	1/2	2/3	1	4,9%

Figure 24. The AHP results. The matrix shows the importance of every criterion in comparison to each other. The normalized principle eigenvector are the final weigh factors.

24.

Scoring table

All concepts are given scores between 1 and 5 for each of the criteria. This can be seen in Table 2.

*Table 2. Scoring table. In the row "Total * WF" the final score for each concept can be seen.*

		Preconcepts									
Criteria	**WF**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	37	5	5	5	5	5	5	5	5	5	5
2	22	5	3	2	3	2	3	3	5	3	5
3	8	5	5	5	5	5	5	5	5	5	5
4	6	5	5	5	5	5	5	5	5	3	3
5	10	3	3	3	4	5	4	4	5	5	4
6	9	5	3	3	3	3	3	3	5	1	1
7	5	3	3	1	3	3	1	3	4	2	4
8	5	3	3	4	3	2	4	3	3	3	3
	Total	34	30	28	31	30	30	31	37	27	30
	Total * WF	470	408	381	418	401	413	418	495	393	437

As can be seen in the scoring table, preconcept 8: Double bottom had the highest score (green), then preconcept 1: Slots (yellow) and in third place was preconcept 10: Orange juicer (orange). The concepts that scored high were mainly the concepts with a laptop inside of the chamber. Both preconcept 1 and 8 have this feature. Another aspect that scored high was the double bottom. Both preconcept 8 and 10 have an aluminium plate in the middle of the box to reduce the noise due to nanoparticles in the perfusate and to make more space for the assembly. Preconcept 8 and 10 also both use heated oxygen to prevent the perfusate from cooling down, instead of using a heat exchanger inside of the chamber.

Some preconcept had one feature that scored high, like the organ chamber of preconcept 5, but other important parts of the system were lacking. Because of this preconcept 5 ended up with a proportionately lower score than other concepts. This shows that the scoring system is slightly skewed. Only systems of which all features are great, score high. In synthesis phase 2 some higher scoring features of "losing" concepts can still be considered for the final concept.

Synthesis phase 2

To know if every preconcept is viable in the limited space, the individual components that make up the IPK system assembly were measured. Only the once already available in the surgical lab of the UMCG could be measured. If necessary, other components can be bought. But if the available components can be used, it would be simpler. This would leave more time to focus on the rest of the device.

Table 3. The available, potential components for the device with their measurements.

Component	**Size (cm)**
Powerbank/Battery	16 x 23 x 5
Roller pump	19 x 13 x 10
Oxygenator flask	19 x 10 (diameter)
Medos oxygenator	13 x 9 (diameter)
Heat exchanger	15 x 3,5 (diameter)
Heat exchanger water reservoir plus heating element	17 x 31,5 x 8
Small laptop	29,5 x 21 x 2,5
Controller Controller with adaptors connected	26,6 x 19,7 x 9,5 32,6 x 31,7 x 9,5

The powerbank that is available is the Xtrom, AL490, AC Power Bank PRO 41.600. It is a lithium-ion battery with 149,76Wh (41.600 mAh) when it is fully charged. It has two AC outputs of 220 V, two USB outputs of 5V and an USB-c output of 5V.

The available roller pump is an Ismatec Reglo Digital roller pump. it needs an AC output with 100 – 230V and the power consumption is 75W. It can pump up to 68ml/min [38].

The oxygenator flask that is used in the current system is a standard 500ml flask.

The medos oxygenator has a microporous hollow fibre membrane [39]. It is the smallest one Medos sells, and it is made for the hart-long surgery of neonatal patients.

The heat exchanger is a Radnot heat exchanger. It needs a large flow of warm water. This is pumped from the top to the bottom.

The heat exchanger reservoir is a Julabo water bath. It can keep water at a steady temperature with 0.15^{o}C accuracy. It needs an AC output of 220V [40].

The available laptop is an Acer aspire R3 series laptop. It uses 19V and a current of 2.37A, which means that it consumes 45W on average. Other laptops are available in the surgical lab if this laptop is not strong enough.

The available controller is made by the technicians of the surgical lab to perfuse pig kidneys. It has more power than is needed for the rat setup. The pump, pressure sensor, temperature sensor and, if necessary, the flow sensor connect to it. It has a USB data connector that can potentially connect to a laptop. Lastly, it has a power input. A picture of the controller without top on can be seen in Figure 25.

The current setup uses an adapter connected to a laptop. This laptop can read out the sensors with a program called Labview, and it can control the pump. This Labview program is an executable, which

means it cannot be changed to support a new setup. If a different pump or different sensors are used, new control software needs to be written.

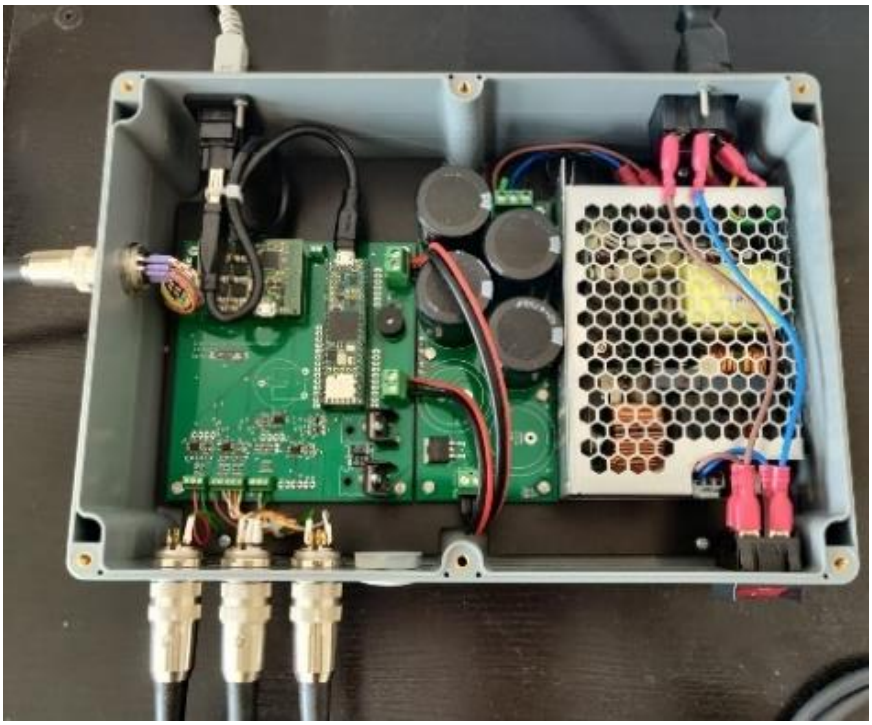


Figure 25. The controller made for the perfusion of pig kidneys without the top on.

Preconcept 1: Slots

To visualize if the components will fit, all the sizes are drawn into the figure from synthesis phase one. For preconcept 1 this can be seen in Figure 26. The red lines are the maximum sizes the new device can have. Only the bigger components are included. Some components are drawn inside each other, because they were drawn like that in synthesis phase 1. Of course, they cannot actually be inside each other, so the measurements will be added up to see the total sizes. For instance, the pump and the reservoir for the heat exchanger are drawn inside the heat exchanger.
Looking at the height of the design, if the components on the side of the heat exchanger (left side) are added up, the height would come to 35,5 cm (2,5 + 8 + 10 + 15). If the oxygenator is also on top of the heat exchanger pump this height would be 39,5 cm (2,5 + 8 + 10 + 19). In this design there is material in between the components as well. This would add even more height. As the red bar indicates, the height cannot exceed 35 cm, which means this design is too high.
Looking at the width of the design, the laptop must be turned with its side facing the front, otherwise the powerbank and the laptop would not fit next to each other. The roller pump and the heating reservoir do fit nicely next to each other.
Looking at the length of the design, the heat exchanger reservoir is quite long. It has a length of 31,5 cm. Behind the reservoir are the sensors. A big reservoir would not leave much space for these sensors.

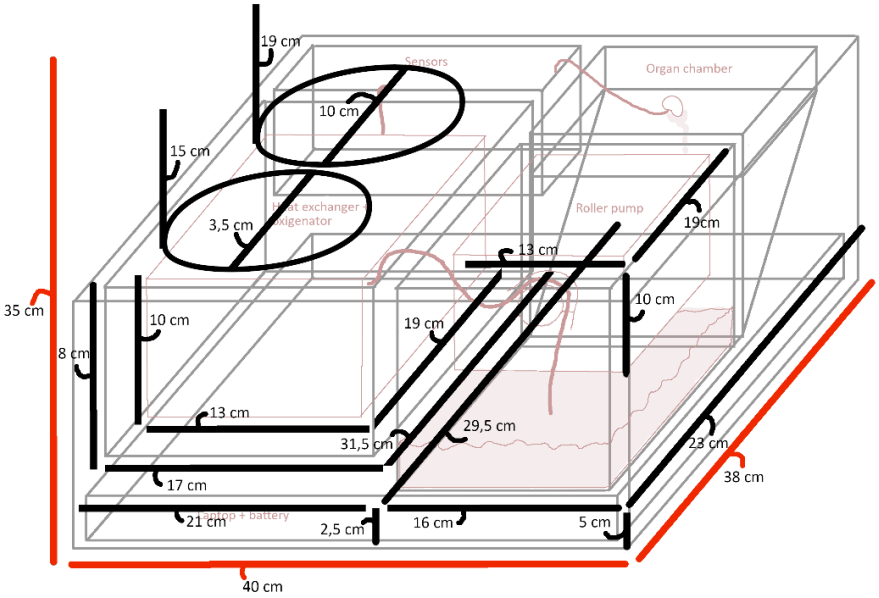


Figure 26. Preconcept 1: Slots, with the measurements for all the mayor components.

Benefits:

Everything has its own place with support. It does not stand on top of each other. The laptop inside the chamber allows for good communication with the system, both to read out the system and to regulate the characteristics of the perfusate flow.

Disadvantages:

This design is too big for the IVIS scanner chamber. Even without calculating room for all the tubes and the sensors the size exceeds the required size. If this design were to be chosen multiple things need to be changed for it to work. For instance, the heat exchanger should be changed to the same heat exchanger that the other two winning preconcepts use. This would eliminate a very big portion of the equipment inside the chamber.
The heat exchanger pump, the roller pump, and the laptop (if it does not have sufficient battery for two hours of use) need electricity. This would be a lot to ask of one powerbank. The powerbank only has two AC outlets, and for this setup at least three outlets are required.

Preconcept 8: Double bottom

Figure 27 shows the measurements of preconcept 8.
Looking at the height of the design, the tallest component below the double bottom is the oxygenator flask with 19cm. The full height would than come to 21,5 cm (19 + 2,5) or higher if the organ chamber is higher than 2,5 cm. Even so this would fit with room to spare.
Looking at the width of the design, the roller pump and oxygenator together are 23cm (13 + 10) wide. The perfusate reservoir cannot exceed 17cm (40 – 23). The reservoir does not have to be this wide, which means that the design satisfies the width requirement as well.
Looking at the length, the organ chamber cannot be bigger than 17cm (38 – 21) in length. Otherwise, it will not fit behind the laptop. The organ chamber does not have to be that big, so the design will fit. The powerbank and the roller pump fit spacious behind each other as well.

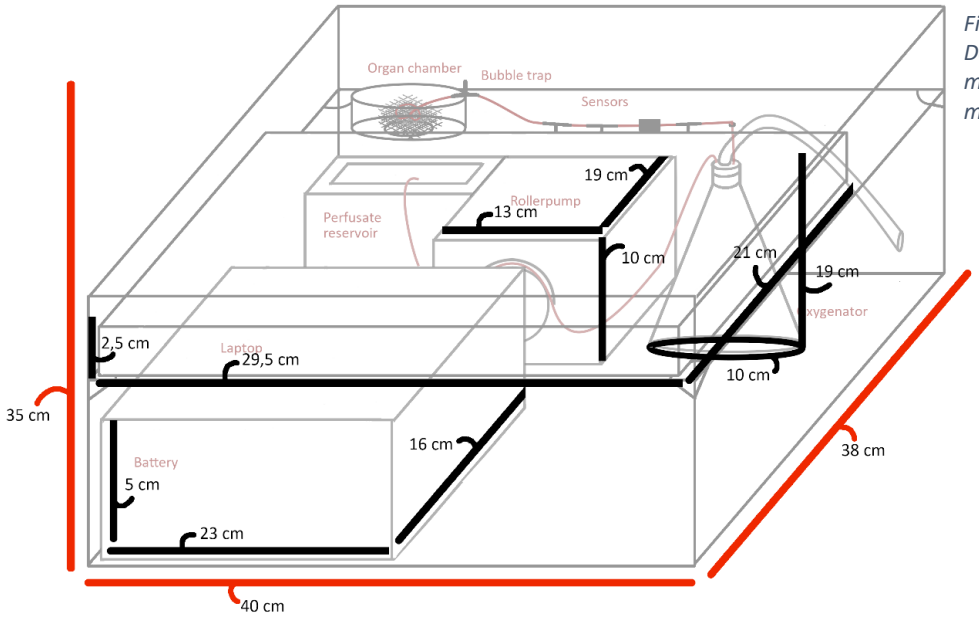


Figure 27. Preconcept 8: Double bottom, with the measurements for all the mayor components.

Benefits:

The three meshes and the double bottom reduce the noise due to nanoparticles in the perfusate outside of the kidney. Everything fits very nicely inside the measurements of the IVIS scanner. The laptop allows for a good communication with the device.

Disadvantages:

The double bottom takes up extra space because it must be above the tallest component in the bottom of the device. As mentioned earlier, there is enough room to use this double bottom. Both the pump and the laptop need electricity. The laptop has its own battery, but that must be strong enough to run for two hours without a charger or it must be hooked up to the powerbank as well.

Preconcept 10: Orange juicer

Figure 28 shows the measurements of preconcept 10.
Looking at the height of the design, the tallest component below the double bottom is, again, the oxygenator flask of 19cm. This means the SD-cart storage and the organ chamber cannot exceed a height of 16cm (35 – 19). Which is plenty enough room for both items.
Looking at the width of the design, the roller pump and the powerbank combine to be the widest point in the system. There widths add up to 29cm (16 + 13) in total. This will thus fit with room to spare.
Looking at the length of the design, the longest part of the system is the length of the roller pump and oxygenator added up. Together they have a length of 29cm (10 + 19). Since the length can be 38cm this will fit, again, with room to spare.

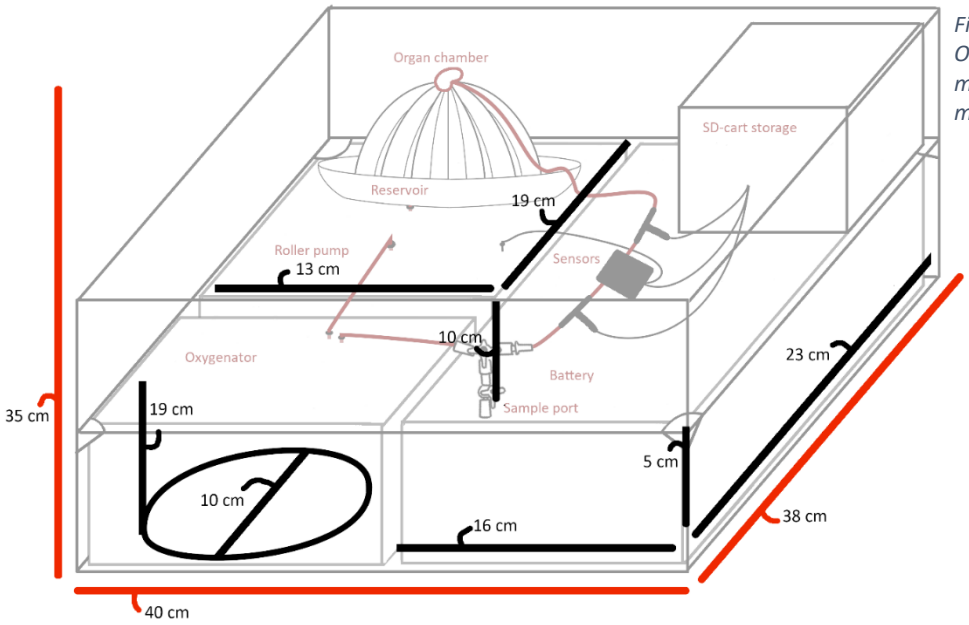


Figure 28. Preconcept 10: Orange juicer, with the measurements for all the mayor components.

Benefits:

The device can be very small. Instead of 40 x 38 x 35 it can be about 29 x 29 x 25. There is a direct feedback loop from the pressure sensor to the roller pump. This would mean that when the pressure rises the pump will react almost instantaneous. The noise from the nanoparticles in the perfusate outside of the kidney is moved away from the kidney, which will make the scans better readable. Only the roller pump needs electricity, which means the powerbank will be able to keep the system running for a longer period of time.

Disadvantages:

The IVIS chamber must be opened to take a sample via the sample port. This is the only way to check the saturation of oxygen in the perfusate. There is no computerized link to the system, so it cannot be regulated from outside the chamber. The data can only be read out afterwards, when the SD-cart is taken out of the system. The noise from the nanoparticles in the perfusate outside of the kidney is not reduced, but it is moved away from the kidney. There is a chance that even though the noise is moved, the scan will still be very noise due to the ring reservoir.

Extra considerations

As mentioned in synthesis phase 1, the scoring system is slightly skewed. Because of this some higher scoring parts of a concept can get lost in the low score of the rest of the system. To recover them, the individual scores for each criterion are important.

Besides preconcept 8, preconcepts 5 and 9 also scored high on criterium 5: The device reduces noise in the scans, due to nanoparticles outside the kidney, as much as possible. The organ chambers of these two concepts are also designed to keep as much perfusate out of the scans. If the three meshes of concept 8 do not work, a more rounded shape, like the bubble or the umbrella, can be considered.

The available controller could be used instead of the laptop. In principle it does the same: all the sensors and the pump are connected and if a Bluetooth device is connected the controller could send the data to a computer outside the chamber. De controller that is now used is too big, it is not wireless and has too much power for the rat kidney perfusion. It is made for the DP2 pump, which is a fairly large pump for this system. If another pump is used the control program must be rewritten. If a controller is used, a new one should be made that has less power and is less big.

All the winning preconcepts use a roller pump. Even though, the concepts with the centrifugal pump scored higher on average on the second criterium: The device can regulate the pressure in the system. Two of the three winning concepts scored high on the second criteria because they function with a laptop inside the chamber. The laptop solves the disadvantages of a roller pump by giving enough feedback. Unlike a roller pump, a centrifugal pump will never push through if the pressure suddenly becomes higher. Without a laptop a centrifugal pump would be necessary. With a laptop and feedback, it is less necessary to have a centrifugal pump, but it can still be considered.

If the powerbank were to run out in the middle of the experiment it can be exchanged by another fairly easily. The chamber can be opened in between scans. The only problem at the moment is that the roller pump is now directly fed by the powerbank itself. If the powerbank were to be exchanged during the experiment the pump would stop pumping for a moment. This could ruin the scan and the experiment. If the pump were attached to the laptop and the laptop to the powerbank, the pump would run on the battery of the laptop. The powerbank could then be changed during the experiment. This is only a problem if the powerbank is not strong enough to support the laptop or controller and the pump for two hours.

Another solution to exchanging the powerbank could be to charge it while it operates in between scans. To test if this was possible the powerbank was hooked up to the pump and charged at the same time. It could charge and support other electrical devices at the same time, however, it charges very slowly. Slower that the pump depletes it.

The available roller pump uses 75W per hour. If the powerbank is fully charged it has a capacity of 41.600mAh. For an outlet of 220V the power bank has 9020W (41,6 * 220). This means the roller pump can easily run for two hours when hooked up to this powerbank.

The Gikfun pump, mentioned in the analysis phase, uses 12V and a current of 80mA [35]. This means the pump would use 9,6W per hour (12 * 0,8). So, the powerbank would be able to keep the system running for far longer than a scan.

A piston pump uses more watts than the roller pump with the same flowrate. The Ismatec Reglo-CPF Digital uses 140W per hour [38]. With this pump the system could still function with the powerbank for a long time.

An example of a centrifugal pump is the “DC 5V small transparent submersible Impeller Centrifugal brushless water pump” sold by Ebay. It can pump up to 90ml/min. It needs a power source of 5V and a current of 0.23A [41]. This means it needs 1.15W per hour. So, this pump could also support the system for a long time on the available powerbank.

the Medos Deltastream DP2 pomp, mentioned earlier, is a bigger centrifugal pump which is used by the surgical lab to perfuse pig and human kidneys. The flow of this pump is between 0 and 8L/min [42]. This flow is high for the IPK-system. The benefit of using this pump is that the available controller has a control program for this particular pump. If the combination of this pump and the controller would be used, the controller does not have to be changed much.

Looking at all the power demands of the pumps, the small centrifugal pump and the small roller pump are most frugal. Both pumps, and the piston pump, would need a different control program on the laptop then is used right now, since the control program currently in use is written for the bigger roller pump. The powerbank is not new, and the maximum capacity might have been decreased over the years. To see if the bigger roller pump could still be supported for two hours, the combination of the available powerbank and the roller pump was tested. After two hours of pumping at the highest flowrate the pump was still running and the display of the powerbank showed 96% capacity. Thus, the powerbank is still functioning properly, and the roller pump is still a viable option. With this much capacity left the laptop could potentially be charged by the powerbank as well. Since all the winning concepts did have a roller pump and because of the time limit on the internship, the bigger roller pump will be used. Making a new controller or control program would take too long.

All the concepts work with either a Bluetooth or other wireless connection from inside of the IVIS chamber to a device outside the chamber. The housing of the scanner is al metal. There is a chance such a connection is not possible because the chamber could act like a faraday cage. This means that most of the communication is blocked by the structure of the chamber. To test if the preconcepts are viable a Bluetooth box was used. The Bluetooth box is a JBL flip 4, which uses Bluetooth version 4.2. The Bluetooth box was put into the chamber and while the chamber was closed a mobile phone outside the chamber was connected to the box. The box linked immediately and played music while the chamber was closed. This experiment showed that it is easily possible to communicate via Bluetooth and other wireless forms of communication with a device inside the chamber. Thus, the preconcept communication is still viable.

Since there is a time limit on the internship and making a new controller for the roller pump will take up too much time, the adapter and laptop setup that is used in the IPK system right now will be used in the new device. The laptop will be linked to a laptop outside of the chamber.

Preconcept selection

Scoring the preconcepts will be done in the same way as in synthesis phase 1. The weight factors and criteria will be the same. The only difference is the newfound knowledge about the three concepts. The results of the selection can be seen in Table 4.

Table 4. Scoring table for the top three concepts.

Criteria	WF	Preconcepts		
		1	8	10
1	37	5	5	5
2	22	5	5	5
3	8	5	5	5
4	6	5	5	3
5	10	2	5	3
6	9	5	5	1
7	5	1	4	4
8	5	3	3	3
Total		31	37	29
Total * WF		450	495	427

As can be seen in Table 4, the top three highest scoring concepts are still scoring in the same order as they did in synthesis phase 1. Both the second and third place got a lower score than before. The second place, preconcept 1, got a lower score because it does not fit inside the chamber. The third place, preconcept 10, got a lower score because it does not reduce noise as much as other ideas do. The score from the highest scoring concept, preconcept 8, stayed the same. Therefore, preconcept 8 will be further detailed in synthesis phase 3.

The preconcepts were presented to the stakeholders. They agreed with the conclusions and the choice of the winning concept.

Synthesis phase 3

Concept

The concept that was selected is concept 8: Double Bottom. A 3D drawing was made to work the idea out in more detail. This drawing can be seen in Figure 29. After receiving feedback, a problem with the design came to light. The IVIS scanner has a camera in the middle of the ceiling of the chamber. To scan the organ, it should be near the middle of the chamber as well. In this design the organ chamber cannot simply be moved to the middle of the chamber since the laptop is in that place. To make room for the organ chamber, the laptop can be moved to the bottom of the design, just as in preconcept 1, an extra slot/double bottom can be made to facilitate this. If this makes the device to high, a different glass container can be used for the oxygenator. At the moment the oxygenator is the highest object underneath the double bottom and determines the height of the double bottom. If the container is exchanged the double bottom could be lowered to the height of the second highest object.

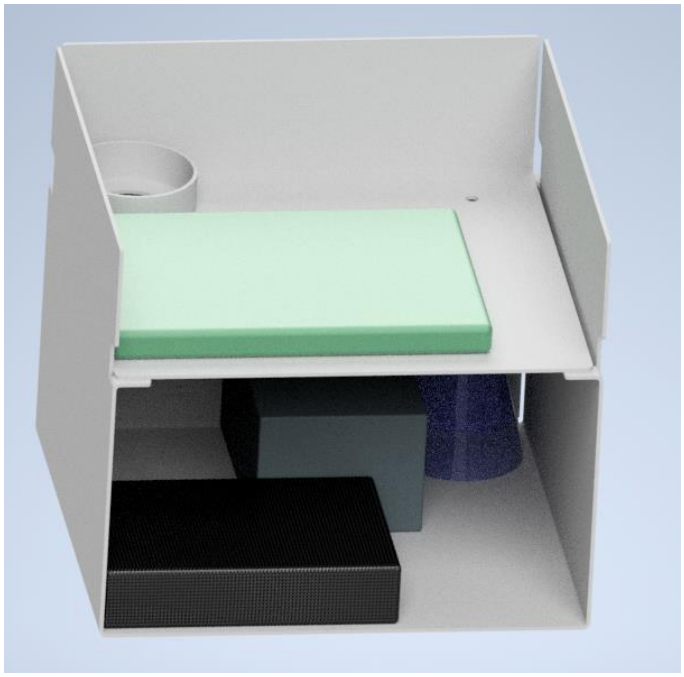


Figure 29. The 3D drawing of the first draft of the device.

Final concept

The final concept can be seen in Figure 30. It consists of a casing with two double bottoms in it. The first one is at a height of 4cm and the second one at a height of 21cm from the bottom of the casing. The height of the second double bottom is dependent on the width of the battery. In earlier designs this was not the case, because the battery was not on its side. But, since the organ chamber is transferred to the middle of the design, the perfusate reservoir also must be in the middle of the design. The roller pump must be turned 90 degrees to make room for the reservoir. Because the roller pump is turned, the battery can only fit if it is tilted on its side. The battery is 16cm in width, which means the second double bottom must be at least 17 cm above the other double bottom. That is why it is placed at 21cm (17 + 4) from the bottom of the design. The double bottoms rest on flanges bend from the casing. Underneath the first double bottom is room for a laptop. There are holes in this lower double bottom to feed through any cables for electricity and information from the sensors and to the pump, just like in the previous version of the design. On top of the lower double bottom are the powerbank, the roller pump, the oxygenator, the perfusate reservoir, most of the cables and the adapter for the pressure sensor and the oxygen sensor. As mentioned earlier, the reservoir is in the middle, underneath the organ chamber, the other objects are around it. On top of the second double bottom are the sensors and the organ chamber. The organ chamber is made of a standing up, circular edge with a hole in the middle. Three loose rings with mesh are stacked on top of each other on the inside of the organ chamber ring. In the right back corner of the second double bottom is a small hole through which the tube with the oxygenated perfusate is fed. On the left back side of the second double bottom are to bigger holes to feed the cables of the sensors through. The laptop inside the chamber will communicate with a computer outside the chamber. This way the data can be monitored and, if needed, the pump can be controlled. The oxygen will be warmed by running the tube, through which it enters the chamber, through warm water. For more pictures of the final design see the appendix, Figure 42.

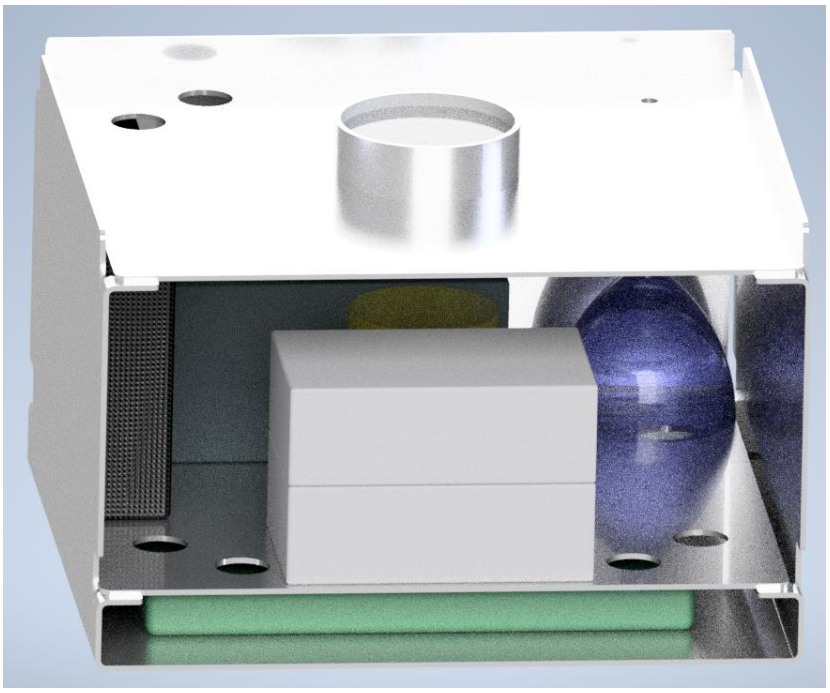


Figure 30. The final concept, with the bigger objects draw in.

Material selection

Controller

The controller that is used is a laptop with the programs Labview and Teamviewer. The adapters of the pressure- and oxygen sensors and the pump will be linked to this laptop via USB and controlled by the Labview program. The laptop that is used is the Acer aspire R3 series laptop already mentioned in synthesis 2. The benefits of this laptop are the size, and it is foldable, which means that the screen can rotated 360° until it touches the back of the laptop. This means that the laptop does not have to be closed when placed inside the device and will therefore not go into sleep mode.

Wireless connection

A second computer, located outside of the chamber, will be used to control the laptop inside the chamber. This computer also has the program Teamviewer, which helps it link to the laptop inside the chamber via Wifi-connection. The second computer used for this project is an "Acer Aspire 5336 Series", but it could be any computer that can link to Wifi and on which Teamviewer can be installed.

Pump

The pump that is going to be used is the available roller pump is an Ismatec Reglo Digital roller pump, already mentioned in synthesis phase 2.

A rs232 to USB converter needs to be used to connect the pump to the laptop, since the laptop does not have a rs232 inlet.

Battery

The battery that is going to be used is the available "Xtrom, AL490, AC Power Bank PRO 41.600", already mentioned in synthesis phase 2.

Reservoir

The reservoir is going to be a small flask with a lid. In the lid are two holes through which the inlet- and outlet tubes can be fed.

Connections between components

PE tubing is used for all the tubing in between the components. If silicone were to be used the perfusate would lose a lot of oxygen, because silicone is more permeable to gasses than PE.

The connections used at the ends of the tubes are Lure lock connections. The main three used are the female- and male hose barb connectors and the three-way valve.

All the connections are secured with small tie wraps.

Oxygenator gas

Carbogen will be used as the gas to oxygenate the perfusate. As mentioned in the analysis phase, a buffer like carbon dioxide is needed to prevent tissue damage. There is no outlet of carbogen in the CDP near the IVIS scanner, which means a gas canister will be used.

Oxygenator flask

The oxygen flask will be made of glass. Glass reduces the oxygen loss due to permeability of the container. The shape of the flask does not matter.

Oxygenator tubing

The tubing in the oxygenator will be made of very thin, permeable material. Approximately 2m to 3m of silicone tubing is used inside the oxygenator flask to oxygenate the perfusate. The tubing that is used is "Rubber BV, Python Silicone tube, 1706".

Sensors

The pressure sensor that will be used is the “Edwards Lifesciences TruWave pressure transducer PX600F”. Which are already used for many setups in the surgical lab. An adapter is needed to use these pressure sensors. This adapter is made by the workspace in the UMCG and for this setup one of them is already available in the lab.

As the oxygen saturation sensor, the “PreSens Fibox 4” sensor will be used. This is a sensor that can measure the dissolved oxygen in a liquid instead of measuring the oxygen in carriers, like red blood cells. The IPK system uses perfusate instead of blood to perfuse the kidney. Perfusate has no oxygen carriers. The sensors come with their own adapter.

The thermometer that is used is the “Ama-digit digital thermometer ad 15th, -40 - +120°C”. This thermometer can only be read from the display.

Heat exchanger

The heat exchanger that is used is the Julabo water bath mentioned in synthesis phase 2. The tube through which the carbogen runs, runs through this bath. This way, the carbogen is heated before entering the chamber.

Heat exchanger tubing

The tubes through which the carbogen runs will be made from polyethylene (PE). Just as for the tubing in between components, the main reason for this is less oxygen loss due to permeability of the material.

Casing

The casing will be made from a material that is sturdy enough to hold the weight of all the devices. The IVIS scanner must not be able to scan through the material, to reduce any noise from the perfusate underneath the double bottom. Since it should also be easy to clean and not to heavy, opaque Plexiglas, aluminium and stainless steel are suitable options.

Organ chamber

The meshes in the organ chamber will be made from a fine mesh. Not to fine, the perfusate should be able to run through it. But it should be fine enough to reduce the noise from the organ chamber as much as possible. It will be made from aluminium or stainless steel since these metals will block the noise very well and they will not rust over time.

Cable management

Tie wraps will be used to make bundles out of all the power cords. This will make the device more accessible.

Cost analysis

To make an overview of the total cost of the device a cost analysis is done. All the separate components are priced, as well as the man hours the assembly and crafting separate pieces will take.

*Table 5. The cost analysis. * The Acer Aspire R3 series laptop is not sold anymore. Six years ago, it was ± €600. ** The Acer Aspire 5336 series laptop is not sold anymore. Similar laptops are sold by Cool blue for €270,00 [43]. Because the Acer Aspire 5336 is an older model the price was estimated to be €200,00. *** The adapters are made by the workplace of the UMCG. This price is an estimation. **** 5cm for heating the carbogen (see next page for the calculation) and the rest of the length to connect the carbogen tank to the IVIS chamber ***** The organ chamber has a diameter of approximately 10cm. which means it has a surface of 0,0079m2. Three meshes are needed, so the total price will be (16,50/0,75)*0,0079*3 ≈ 0,5184.*

Component	**Needed quantity**	**Price per**	**Total price**
Laptop	1	± €100,00* per piece	€100,00
Second computer	1	± €200,00** per piece	€200,00
Roller pump	1	€3.190,77 per piece [44]	€3.190,77
rs232 to USB converter	1	€9,95 per piece [45]	€9,95
Powerbank	1	€270,00 per piece [46]	€270,00
Reservoir	1	$16.99 per 3 pieces [47]	€4,66
Tubing	Max. 5m	€173,79 per 100m [48]	€8,69
Lure Lock male connections	5	€20,09 per 10 pieces [49]	€10,05
Lure Lock female connections	2	€48,49 per 100 pieces [50]	€0,97
Lure Lock three-way valves	3	€1,04 per piece [51]	€3,12
Bubble trap Y-piece	1	€61,20 per 10 pieces [52]	€6,12
Tie wraps (small)	Max. 30	€0,99 per 100 pieces [53]	€0,30
Oxygenator flask	1	€7,15 per piece [54]	€7,15
Oxygenator flask rubber cork	1	€23,39 per 20 pieces [55]	€1,17
Oxygenator tubing	Max. 3m	€0,73 per meter [56]	€2,19
Pressure sensor	1	$10,00 per piece [57]	€8,27
Adapter pressure sensor	1	± €1500,00*** per piece [28]	€1500,00
Thermometer	1	€57,57 per piece [58]	€50,75
Oxygen saturation sensor	1	€50,00 per piece [59]	€50,00
Heat exchanger water bath	1	€1651,00 per piece [59]	€1651,00
Heat exchanger PE tube	Max. 3m****	€96,73 per 50m [48]	€5,80
Casing	1	± €325 per piece [60]	€325
Organ chamber meshes	3	€16,50 per 0,75m^2 [61]	€0,52*****
Tie wraps (big)	Max. 30	€2,99 per 100 pieces [62]	€0,90
		Total components	€7407,38
Manufacturing costs			
Man hours assembly	± 6 hours	€50,00 per hour	€300,00
Man hours + Plasma cutter + Metal bending machine	± 0,5 hours	€100,00 per hour	€50,00
Man hours + Lathe + Welding machine	± 0,5 hours	€60,00 per hour	€30,00
		Total manufacturing costs	€380,00
		Total	€7787,38

As can be seen in Table 5, the total cost of manufacturing the device will be €7787,38. This high price is mostly determined by the roller pump, the adapter for the pressure sensor and the heat exchanger water bath.

Calculation for heating the carbogen

The carbogen needs to be heated before entering the IVIS chamber, as already mentioned in synthesis phase 1 and 2. To approximate how much tube at least needs to go through the water bath to heat the carbogen to 37°C, the formulas for specific heat and for thermal conductivity are used:

$$(1) \qquad Q = m * C * \Delta T \ [63]$$

$$Q = Heat\ in\ J$$
$$m = Mass\ in\ kg$$
$$C = \ Specific\ heat\ in\ \frac{J}{kg * K}$$
$$\Delta T = T_2 - T_1 = Temperature\ difference\ in\ K$$

$$(2) \qquad P = \frac{\lambda * A * \Delta T}{d} \ [64]$$

$$P = Power\ in\ W$$
$$\lambda = \ \ thermal\ conductivity\ coefficient\ in \frac{W}{K * m}$$
$$A = the\ surface\ area\ of\ the\ tube\ in\ m^2$$
$$\Delta T = T_{water} - T_{gas} \ = Temperature\ difference\ in\ K$$
$$d = Wall\ thicknes\ of\ the\ tube\ in\ m$$

It is assumed that the flow of carbogen will be 100ml/min during the perfusion. This is higher than actually used during perfusion [28]. Which is 100/60 = 1,667 ml/sec. Which is 1,667/10^6=1,667*10^{-6} m^3. That is the volume per second which is needed to perfuse the kidney. The density of oxygen at room temperature is 1,331 kg/m^3 [65]. The mass is thus, 1,331*1,667*10^{-6} = 2,218*10^{-6} kg/s.

The specific heat of oxygen is 0,918 J/(g*m) [66]. But the specific heat asked for in the formula is in J/(kg*m). So, C will be 0,918*1000 = 918 J/kg*m

The carbogen in the tank is room temperature, which is 20°C. When the gas leaves the tank, it cools off a lot, because of the sudden pressure difference. A reduction valve on the tank prevents the carbogen from cooling too much. Because of this valve, the pressure difference between the outside and inside of the tank is only 0,5 bar instead of 200 bar. But since there is still a pressure difference the carbogen will not be 20°C when it enters the tube. It will cool off a couple of degrees. It is assumed that it will cool 4°C, which makes T_1 16°C = 289,15K.

T_2 needs to be 37°C = 310,15K, to keep the kidney at body temperature during perfusion.

The thermal conductivity coefficient of Low-density PE is 0,33 W/(m*K) [67].

The surface area of the tube, the circumference of the tube is needed. To calculate the circumference the diameter is needed. The average of the inner- and outer diameter is taken for this calculation, which are respectively 10mm = 0,1m and 14mm = 0,14m [48]. That means the circumference is ((0,1*0,14)/2)*π = 0,0377m. The surface area will then be the circumference times the length of the tube. But since the length is the only unknown, this cannot be calculated yet.

T_{water} is 37°C so the oxygen will not accidentally overheat. 37°C = 310,15K

For T_{gas} the average temperature of the carbogen will be taken, which is (37°C + 16°C)/2 = 26,5°C = 299,65K.

The wall thickness is 4mm = 0,04m [48].

The mass is in kg/s which means the energy will be in J/s = W. So, formula (1) will give an answer in W. Formulas (1) and (2) can then be equated, which will give the formula:

$$m(per\ sec) * C * \Delta T = \frac{\lambda * A * \Delta T}{d}$$

$$2{,}218 * 10^{-6} * 918 * (310{,}15 - 289{,}15) = \frac{0{,}33 * 0{,}0377 * L * (310{,}15 - 299{,}65)}{0{,}04}$$

$$L = 0{,}013m$$

Only 1,3cm is needed to heat the oxygen. This seem like a very short amount of tube, but a flow of only 100ml/min is very low and therefore easy to heat up. To be on the save side and to allow for a larger flow if needed, 5cm tube can be used.

Stress analysis

A stress analysis was done to simulate the behaviour of the casing during the scans. The different analyses can be seen in Figure 31 up to and including Figure 34. Figure 31 shows the analysis of the lower double bottom if it was made from aluminium. Figure 32 shows the analysis of the double bottom if it was made from stainless steel. Only the lower double bottom is shown because it is weaker due to the many holes, and it has to carry more than the top double bottom. This entails that if the lower double bottom is sturdy enough, the top double bottom will automatically be sturdy enough as well. As can be seen in both figures, the Von Mises stress will never get high enough to let the double bottom break. But both displacement figures show that the double bottom will be bend. The aluminium will bend more than the stainless steel. The maximum displacement of the aluminium is 0,661 mm and that of stainless steel is 0,2416mm. The minimum displacement is zero for both cases. This means that the total displacement is not even a mm in total in both cases. The design will be safe to use as both aluminium and stainless steel.

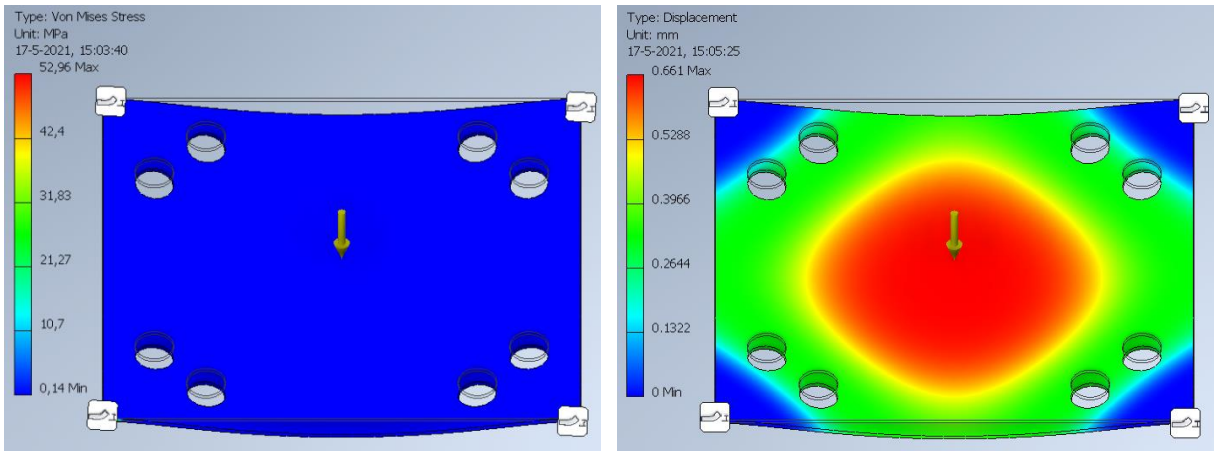


Figure 31. Stress analysis. Von Mises (left). Displacement (right). 60N. Aluminium.

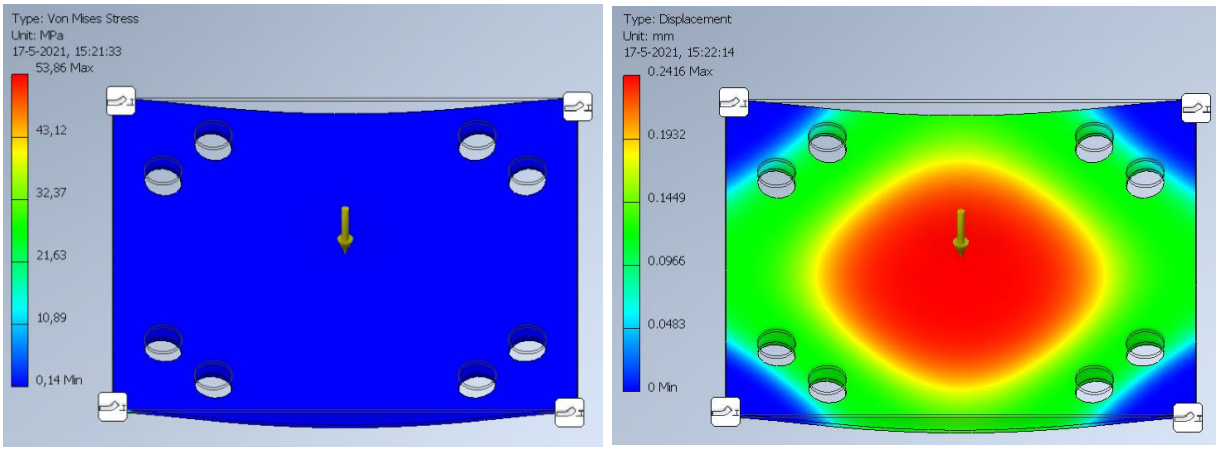


Figure 32. Stress analysis. 60N. Stainless steel. Von Mises (left). Displacement (right).

Figure 33 and Figure 34 show the different stress analyses of the casing. Respectively, they show the behaviour of the aluminium and the stainless steel versions of the casing. Again the Von Mises stress will not be big enough to let the casing break of. The displacement analyses do show some red parts. The aluminium version has a maxumim displacement of 0.1101mm, for the stainless steel version this is 0.04028mm. Because the bottom is fixed, just like the corners of the double bottom are, is the minimum displacement for both cases is 0mm. The total displacement is therefore respectively 0.11mm and 0.04mm. This means both materials are viable.

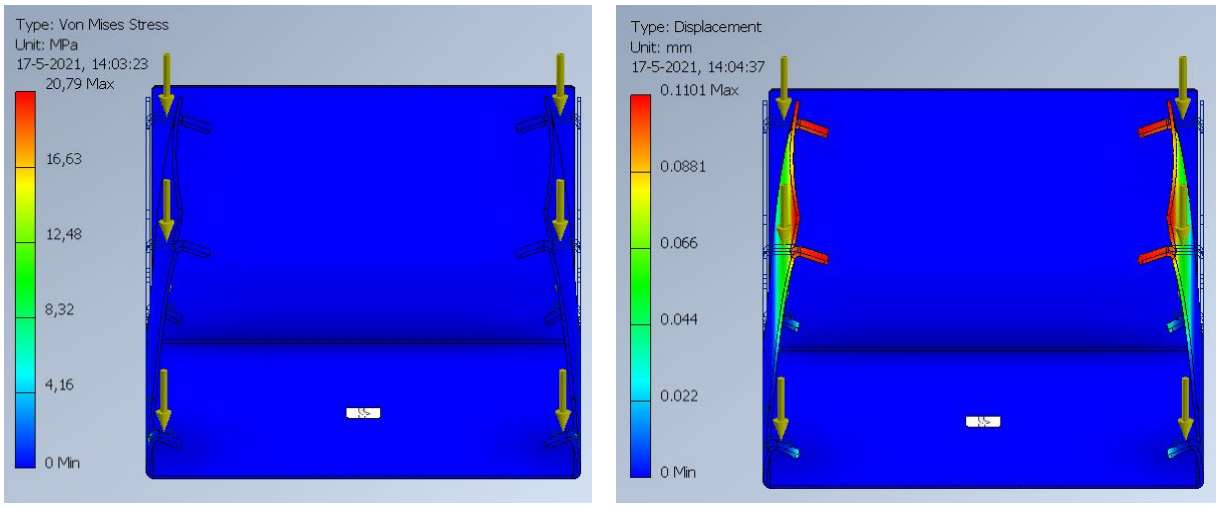


Figure 33. Stress analysis. 60N below, 20N above. Aluminium. Von Mises (left). Displacement (right).

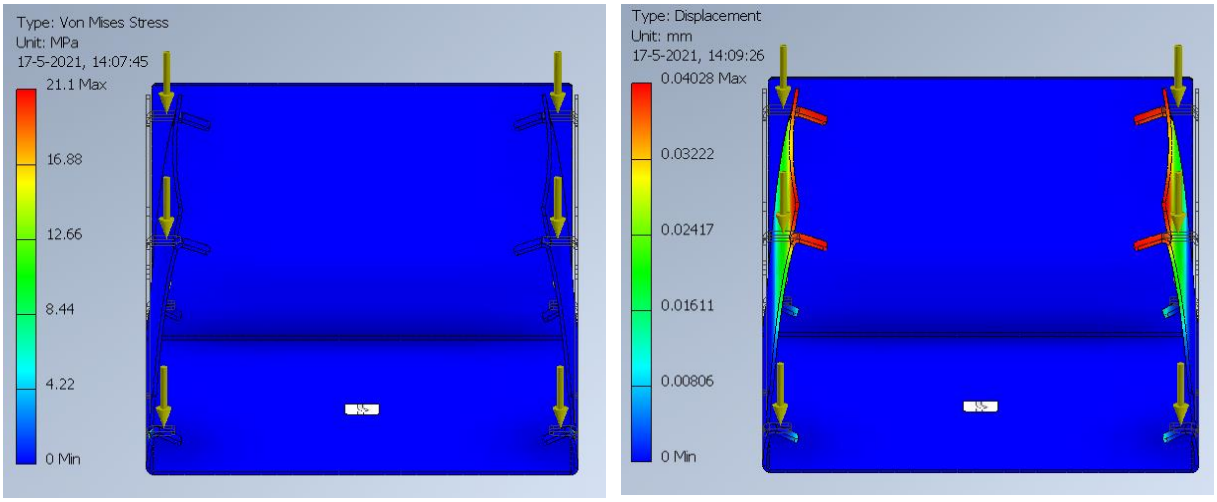


Figure 34. Stress analysis. 60N below, 20N above. Stainless steel. Von Mises (left). Displacement (right).

Technical drawings

Not many technical drawings are needed for this project since most of the components are already complete devices. The casing does need some technical drawings since it needs to be plasma cut, bend into place, drilled and welded. For these procedures, every separate component needs a drawing of the flat pattern plus a picture of the folded result. These drawings should include all the measurements and angles of the part. This means that both double bottoms and the casing need separate drawings. For plasma cutting the parts, another drawing needs to be made with the flat patterns of all the parts on one sheet. This drawing only needs the bend angles and the diameter of every hole. The computer attached to the plasma cutter can find the other measurements. All these drawings can be found in the appendix in Figure 44 to Figure 47.

Risk analysis

A risk analysis is done to show the biggest risks of the device. These risks can then be solved if they are too big. For every part of the device the individual risks are written down in a scheme. Every risk is scored based on three factors: the failure probability, the severeness of failure and the probability of not detecting the failure in time. In Table 6 all the individual scores for these factors and their explanation can be seen. A risk gets a score for each of the factors. To calculate the total score the three scores are multiplied. If the total score of a risk is higher than 20, the risk is too high. In this case the device should either be changed, or the risk plan should include a solution to lower the risk. The result of the risk analysis can be seen in Table 7.

Table 6. The scores for every factor with their explanation [68].

	Failure probability	**Severeness of failure**	**Probability of not detecting in time**
1	Very low (once per 20 years)	Possibly not detected (no costs)	Failure is immediately detected
2	Low (once per 10 years)	Low (repair costs below € 50)	Failure is detected within an hour
3	Less low (once every 5 years)	Less severe (repair + consequence costs below €100)	Failure is detected within a day
4	Below average	Below average (overall costs below €1000)	Failure is detected within a week
5	Average	Average (overall costs above €1000)	Failure is detected within a month
6	Above average	Above average (costs above €1000 and/or slightly wounded)	Failure is detected within a year
7	Rather high	Rather serious (costs above €10.000 and/or slightly up to severe wounded)	Failure is detected at routine inspection
8	High	High (costs above €10.000 and/or one person dead)	Failure is detected at intensive inspection
9	Very high	Very high (costs above €100.000 and/or several deaths)	Failure is detected only with specialized equipment
10	Sure	Catastrophic (costs above €1.000.000 and/or more than 10 deaths)	Failure is not detected in time

*Table 7. The risk analysis. P = The failure probability, S = The severeness of the failure, D = The probability of not detecting the failure in time, T = The total score = P*S*D. In the status column, the status of the risk is given. Ok = T < 20, Not ok = T > 20.*

#	Component	Reason of failure	Effect of failure	Score				Status
				P	S	D	T	
1	Battery	Wear	Run empty during the test	4	4	1	16	Ok
			Stop working all together	2	4	1	8	Ok
2	Casing	Wear	Break and the double bottom will fall on the equipment below	1	4	2	8	Ok
			Break and form sharp edges	1	6	3	18	Ok
3	Pump	Wear	Stop working	3	4	1	12	Ok
			Leak	4	4	1	16	Ok
			Lower pressure	2	3	1	6	Ok
		Wrong regulation	Too high pressure	3	4	1	12	Ok
			Lower pressure	3	3	1	9	Ok
4	Double bottom	Wear	The equipment on top of the double bottom could fall on the equipment below.	1	4	2	8	Ok
5	Oxygenator	Rough with the casing	Break	3	6	1	18	Ok
		Wear of the cork	Leakage	2	2	4	16	Ok
6	Sensors	Wear	Wrong data	3	4	3	36	Not ok
			Stop working	2	4	1	8	Ok
		Wrong calibration	Wrong data	3	4	3	36	Not ok
7	Heat exchanger	Wear	Break and the oxygen will be cold. This will cool down the kidney.	2	4	1	8	Ok
8	Laptop	Wear	Stop working	3	4	1	12	Ok
		Wrong calibration	Wrong data and wrong feedback	3	4	3	36	Not ok
9	Organ chamber	Wear	Meshes could break	2	4	2	16	Ok
			The noise from the nanoparticles is less reduced.	3	2	2	12	Ok
10	Bubble trap	Wear	Bubbles in the kidney	2	4	1	8	Ok

Risk plan

As can be seen in Table 7, there are 3 risks too large to ignore. All three of them are related to the sensors giving of wrong data. The biggest problem is that it will not be immediately detected, like for instance complete failure of the sensors would. Multiple experiments could be done before these failures are discovered, which would render their results meaningless. Two of the risks are that the calibration of the sensors or the laptop are wrong. The other one is that the sensors do not function properly altogether. If the sensors are tested before every experiment the failure could be detected earlier. This would lower all three risks.

There are two risks that are not too high but come close to the borderline. They both have a total score of 18. The main reason they share this score is because people using the device could get slightly hurt if these risks were to occur. In the first risk an operator could hurt him-/herself on the sharp edges of the broken casing. In the second risk the operator could hurt him-/herself on the sharp edges of the broken glass container of the oxygenator. Therefore, it is wise to be cautious while using the device.

Test procedure

According to the "Guidance on Classification Rules for in vitro Diagnostic Medical Devices under Regulation (EU) 2017/746" the device can be classified as class A, the first out of four risk-based classes [69, 70]. The reason being that rule 5a states: "Products for general laboratory use, accessories which possess no critical characteristics, buffer solutions, washing solutions, and general culture media and histological stains, intended by the manufacturer to make them suitable for in vitro diagnostic procedures relating to a specific examination" all belong to class A [69]. In short, the device is categorized as "laboratory equipment intended for in vitro diagnostic testing" and therefore has a low risk factor [71]. Low risk factor products can be tested under own supervision.

Because the system is a testing setup it is hard to say how it must be tested. Other setups in literature do not show a test procedure either. Testing setups are meant to test if the setup works, which is basically also testing if it is safe to use. The system that the new device uses is almost completely copied from the IPK system the UMCG already uses, which was mentioned in the analysis phase. This system was tested by trying to achieve the same flow as other systems in literature already showed [28]. This means, to test the new device it would simply have to show the same values as the other system did.

One important part of testing if the new device is safe, is testing if it is cleanable. Everything that cannot be cleaned should not be used more than once. To test this the device can be used once and cleaned right after. If the individual parts are scanned the IVIS will show parts that are still dirty and can therefore only be used once.

Prototype

Almost all the components for the prototype are already available in the lab. The only components that needed to be acquired/fabricated before the prototype could be assembled were the casing and the organ chamber. After asking for advise at among others the workshop of the UMCG, it was decided to make the prototype out of plastic from hardware store and other stores. The double bottom was made underneath the base instead of inside the base, because all the available boxes did not have the height to make the three layers inside the box. Another reason was that all the available boxes were also slightly trapezoid shaped, this made the bottom of the box too small to fit the laptop.

All the materials that were needed for both components were ordered of off amazon and bought at IKEA, Hornbach, Action and Praxis. The casing was made from a combination of a plastic box from IKEA, a thin plastic plate, nuts and bolts, a thin PVC pipe and rubber grips. Because the box was too long and the design has one open side, the box was sawn through at 37cm from the side. It was made a little shorter than the maximum length stated in the requirements to make sure the device easily fits inside the scanner. A hole was made in the middle of the lid. The hole was made to fit the ridge on the bottom of the bowls that are meant to act as the organ chamber. Two holes with a diameter of 2cm were made above the place where the battery will go. These holes are meant to feed the wires from the sensors through to the laptop. They were made above the battery, because this will result in the least amount of noise from the fluorescent nanoparticles underneath the lid. One more hole was made in the lid at 6,5 cm from the sides, above the place where the oxygenator will go. This hole is meant for the tube coming out of the oxygenator. Eight holes with a diameter of 2cm were made in the bottom of the box. All at 4 and 8cm form the sides. These holes are meant to feed all the wires to and from the laptop. Four more holes were made in the corners of the bottom of the box, with a diameter of 5mm. These are meant for the screws to fit through. The thin plate was cut exactly as big as the lid of the box (which is the biggest part in width and length of the box). The four holes in the bottom of the box were copied on the plate. With a bigger, round drill the holes were made cone shaped. This ensures that the screws will fall into the plate instead of sticking out. This will make the device more stable and helps prevent the workspace from getting scratched. From the thin PVC pipe, four pieces of 3cm were sawed. The screws were pushed through the plate, through the PVC pipe, through the bottom of the box, through a washer and then screwed tight with a nut. The pipe acts as a spacer and will keep the plate and the bottom of the box 3cm apart. Four little grips were stuck in the corners on the bottom of the thin plate. They keep the base from sliding during the assembly of the device. After cutting or sawing any part of the base, it was sanded to get rid of any sharp edges.

The organ chamber on top is made from multiple plastic bowls from IKEA, silicone sealant, a small funnel and meshes. Two of the bowls were cut into four rings with a width of 1cm. Two rings that fit inside each other form one ring with the mesh in between. To fit the mesh, it was bend into shape and cut with normal scissors. The mesh was kept in place by gluing the two rings together with silicone sealant. Another bowl was used as a base. A hole was made in the bottom of this base bowl, which is 8mm smaller than the diameter of the funnel (5mm for the rim, so 3mm smaller than the funnel itself). The bowl was heated up over the stove. Once at melting temperature the bowl was pushed over a glass bottleneck. This gave the bowl a more sloping bottom and made the funnel fit better. After melting the bowl multiple times over the glass bottle, the funnel was attached to the bowl with silicone sealant. The rings can be stacked on top of each other inside the base bowl to form the complete organ chamber design. The first version of the prototype can be seen in Figure 35. More pictures can be seen in the appendix in Figure 48.

Figure 35. The first version of the prototype with the organ chamber in place and the three rings of the organ chamber displayed in front of it.

All the equipment was installed in the prototype. Small lure lock connectors and multiple different tubes, mostly made from PE, were used to connect the funnel on the organ chamber to the reservoir, the reservoir through the pump to the oxygenator, the oxygenator to the sensors and the sensors. The pressure sensor was linked to the corresponding adapter. The adapter was linked with a USB-connection to the laptop. The power cords of the laptop and the pump were attached to the battery. The pump was also attached to the laptop with a rs232 to USB converter. Since the pump does not have another connector that fits the laptop. This full setup can be seen in Figure 36. More pictures can be seen in the appendix in Figure 49.

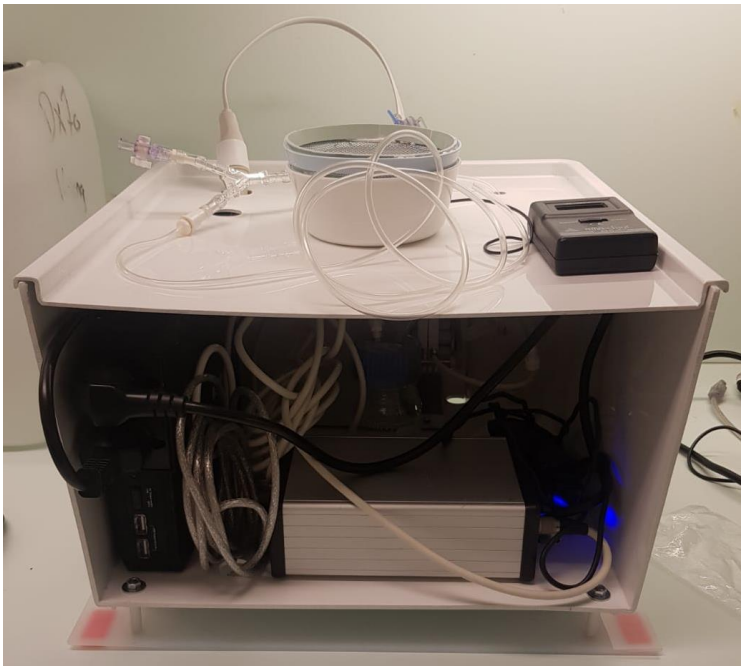


Figure 36. The first version of the prototype with all the equipment installed.

The first round of corrections to the prototype

After fitting all the equipment in the base for the first time a couple of problems became apparent. These were all small problems that were quick to fix. The power plug of the pressure sensor was 1mm wider than the two holes in the lid of the base. To fix this, one of the holes was made slightly bigger with a power saw.

The small Acer laptop can just fit underneath the base, but the bigger Acer laptops that are used in the lab are 1mm too high to fit underneath. To make it possible to use the bigger laptop and to make it easier to fit the smaller laptop, extra spacers were made. The spacers are 1cm longer than the one used in the first version. They fit the screws one size larger than the once used earlier.

The funnel in the organ chamber came lose after applying to much pressure during the instalment. To fix it and make it stronger than before, it was cleaned, and the plastic bowl was heated up again. To shape the heated bowl, the funnel was pushed into it instead of the glass bottle. Afterwards the funnel fit the bowl better and they were glued together again.

The kidney needs to lay fixed inside the device. The organ chamber used in the UMCG version, mentioned in the analysis phase, had a small metal tube through the side. The inside of the metal tube could be connected to the small tube that is attached to the artery of the kidney, the outside of the metal tube could be attached to the tubing system coming from the sensors. This was replicated in the top ring of the organ chamber. A small hole was drilled, small enough to fit the little pipe snugly. The small pipe was pushed through and the tube coming from the sensors was connected to this small pipe.

The cables were a mess, as can be seen in Figure 36. Because of this the second layer of the device was not accessible. To solve this tie wraps were used to bind the cables in neat bundles.

There was no hole for the oxygen tube to go from outside the base directly to the oxygenator. The tube now must go around the whole base, which is inconvenient. To fix this a hole of 2cm was made in the upper right corner of the backside of the base.

As before, after something was cut or drilled the edges were sanded to get rid of any sharp edges. The prototype base after all the corrections mentioned above can be seen in Figure 37. More pictures can be seen in the appendix in Figure 50.

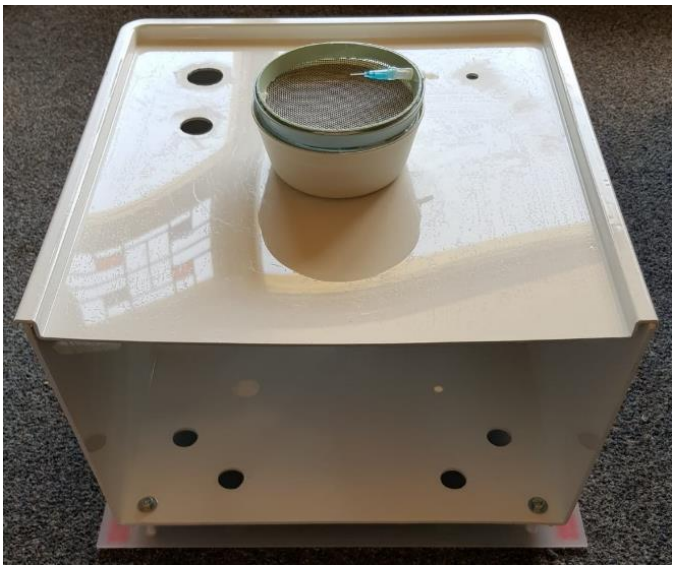


Figure 37. The prototype after the first round of corrections.

The second round of corrections to the prototype

For the prototype only the pressure sensor and the thermometer are included. The Presens sensors do fit in the system, but the short amount of time left in the internship did not allow for the instalment of the sensors.

All the connections of tube-to-plastic were secured with a small tie wraps. The liquid circuit was tested, before the rest of the equipment was built into the prototype. This was done to make sure the circuit was watertight. Not all the equipment is waterproof, so if the circuit is not watertight the equipment might break from water damage.

LabVIEW was installed on the laptop, alongside a driver for the adapter. Teamviewer was installed on the laptop and on the computer (in this case also a laptop) that stays outside of the scanner. This program makes it possible to control the laptop inside the chamber from a distance, which means the pump can be controlled and the sensors can be read out. This system was tested before building the small laptop into the prototype.

The hole in the top ring, for the connection to the kidney, was tilted upwards. This made the connection too high. The kidney would float above the mesh instead of laying on it. Another hole, which is tilted more downwards, was made on the opposite side.

The top ring needed two more holes at a 90-degree angle from the first one to make room for the drain of the ureter. There should be two holes to allow both left and right kidneys to be attached to the device. These holes were also tilted more downwards than the first one.

The prototype after the second round of corrections, with all the equipment installed, can be seen in Figure 38. More pictures can be seen in the appendix in Figure 51.

Figure 38. The prototype after the second round of corrections, with all the equipment installed.

The third round of corrections to the prototype

The prototype was tested inside the IVIS scanner. A photo of the prototype inside the IVIS scanner can be seen in the appendix in Figure 52. The setup worked, but a couple of problems again came to light. The device fits exactly with 1cm to spare. There is less room than anticipated, because there is a lock on the door that protrudes the edges of the door by more than a centimetre (for a photo see

the appendix, Figure 53). The base plate was made only 37 x 37cm to make it easier to place the device in the scanner, therefore the device still fits.

The device must be placed a little to the left because the back of the chamber has an indent on the right side. The scan still shows the full organ chamber, it is just not in the middle of the scan. Because it is a prototype, and it is functional it is not that big of a problem.

The scanner has a small connector for the gas tubes (see Figure 39), which is hard to remove. It is attached to two flexible tubes, so it can be moved a bit. When it lays on the bottom of the scanner, the device does not fit inside the scanner depth wise. To fit the device the connector must be lifted onto the bottom plate, which can be done by putting your arm carefully over the device and lifting the connector. This is quite tricky to do, but the device fits if the connector is on the bottom plate.

Figure 39. The gas tube attachment in the back of the IVIS chamber.

The Wi-Fi-connection in the CDP is bad. Both the laptop inside the scanner and the computer outside the scanner could not steadily connect to it. This meant that the laptop inside the chamber could not be taken over and the pump could not be controlled. The Wi-fi connection needs to be steady because If one of laptops loses connection during a test, the pump would stop and the attached kidney could get damaged. This problem was fixed by making a hotspot with a mobile phone. While using the hotspot the connection worked flawlessly.

To test if the meshes stop the fluorescent signals from passing through the Caliper XFM-2 Fluorescent Phantom was used. This light source consists of small sticks that light up when scanned with the IVIS. Sticks for the wavelengths 605nm, 680nm and 750nm were used, because those are the wavelengths come closest to the wavelength of the near infrared nanoparticles that will be used when the real kidneys are tested. Two scans were made, one with the meshes and one without the meshes as a control. The sticks either did not work or the scanner did not work, because the sticks did not show up in both pictures. The scan did reveal the autofluorescence of the organ chamber. The chamber is white which makes it reflect the light. To fix this the inside of the organ chamber was painted black. This was done by first sanding the surface, spraying it with a primer and then spraying it with black paint, to make sure the paint would not flake.

The top ring is not laying securely on the organ chamber. By having a loose laying ring, the risk of moving the kidney to much while carrying the device to the IVIS scanner is too high. The other two rings fall inside of the organ chamber, which makes them secure, but the top ring is laying on top of the edge of the second ring. To make the top of the organ chamber higher, one of the other bowls from Ikea was used. First the bottom of the bowl was removed, then the remaining ring was cut up into five equal pieces. (Five were cut instead of four to make room in between the panels. This makes sure that the tubes coming in and out of the top ring can fit through the gaps in between the panels easily.) Four of these pieces were sanded to get rid of any rough edges and to make the inside

surface was rough. The outside of the organ chamber was sanded as well. The pieces were glued to the outside of the organ chamber, with one edge sticking out 1cm above the top of the organ chamber.

The bubble trap must always be upright. If the bubble trap is not upright the bubbles will not float out of the perfusate and they will reach the kidney. To make sure the bubble trap was upright, a screw with a little loop at the end was screwed into the lid of the device. It was put on the left side of the organ chamber. With a tie wrap the bubble trap was attached to the loop.

The final version of the prototype can be seen in Figure 40. For more pictures of the final prototype see the appendix, Figure 54. A close-up of the secured bubble trap and the improved organ chamber can be seen in the appendix, Figure 55.

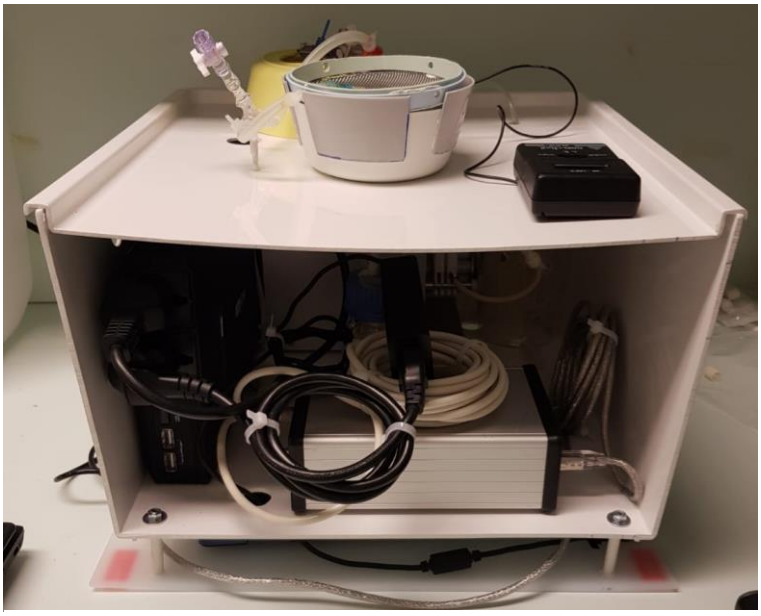


Figure 40. The final version of the prototype.

Test

To test the meshes again, a fluid was used. This is better than just a light source, because it mimics the interaction between the perfusate and the meshes better. There were multiple options for fluorescent fluids, for instance: Indocyanine green (ICG), M-Cherry and Acridine Orange (AO). ICG and M-Cherry are both in the near infrared side of the fluorescence spectrum [72, 73], with a wavelength higher than 800 [74]. AO has a wavelength in between 500 and 600nm [75]. The IVIS scanner can pick up wavelengths of 430 to 850 nm [30]. Which means all the fluorescent fluids can be used, if the near infrared fluids do not have a wavelength bigger than 850nm. AO was used because it was available.

Before testing with the fluorescent fluid, a control scan was taken. This can be seen in the top two pictures of Figure 41. After the control was taken, 100ml of a concentration of 10^{-5}M of AO was poured on top of the meshes, which flowed into the reservoir beneath the organ chamber. This concentration is based on what was found in literature [76, 77, 78]. Before the pump was started, a second scan was made. This scan can be seen in the middle two pictures of Figure 41. After scanning for a second time the pump was started and the fluid filled the whole system. The last scan was made after the outlet, to which the kidney will be attached, started spilling the fluid. The pump was stopped again before the scan was made.

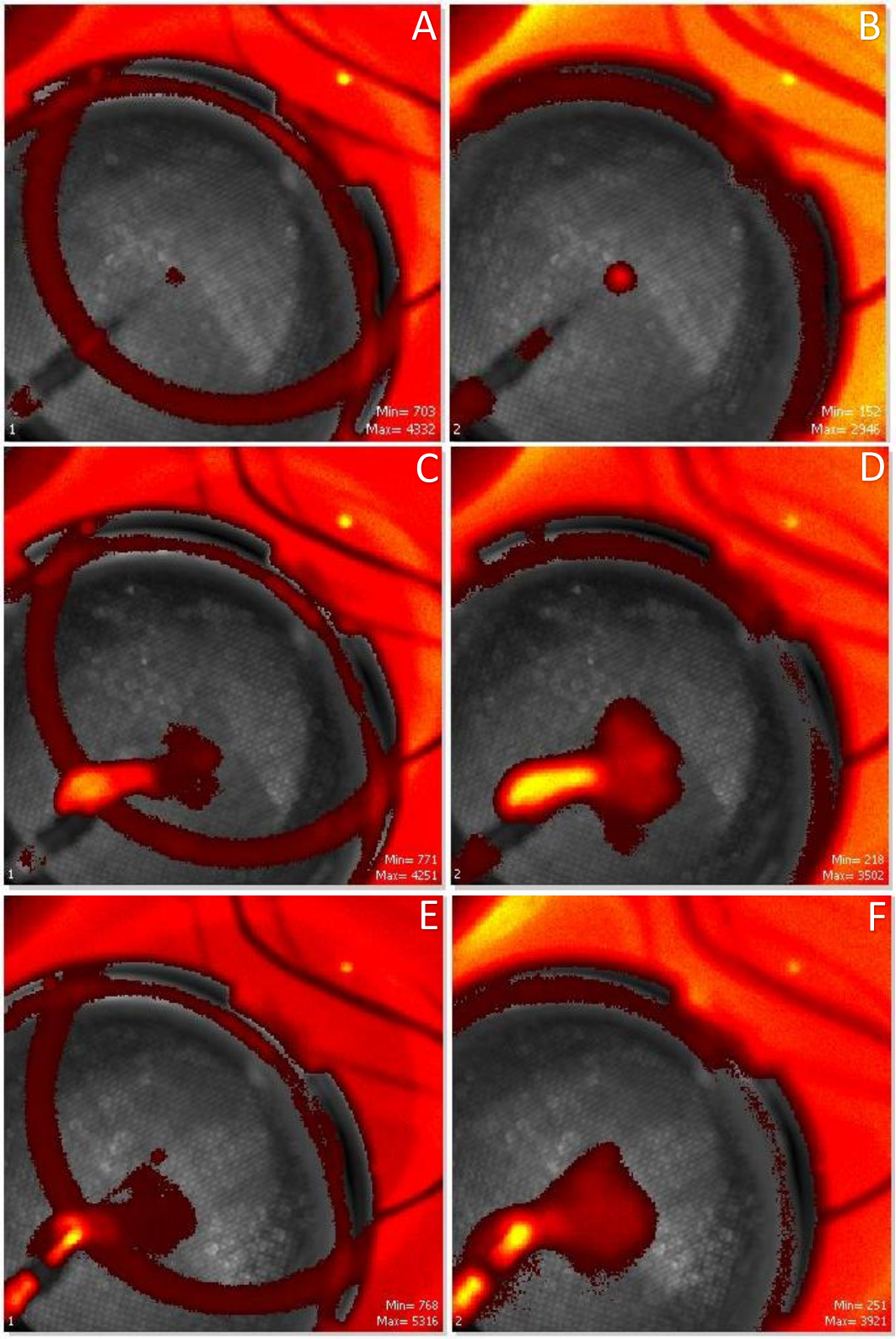


Figure 41. Scans of the IVIS scanner. (A & B) Control, without AO. (D & C) AO was poured through the meshes, but not yet run through the system. (E & F) AO was fully run through the system.

The half circle in pictures A, C and E is made by the scanner itself. The IVIS scanner compensates the light balance to the amount of fluorescence that is in the picture. This can clearly be seen when comparing pictures B and F of Figure 41. The background in these pictures is a completely different colour, even though nothing has changed to the background in between scans. In pictures A and B all the autofluorescence of the prototype can be seen. In pictures C and D, the fluorescence of the AO that was poured into the meshes can be seen. The meshes hold some of the fluid due to surface tension. This is the fluorescence that is seen in pictures C and D. The reservoir was almost full during the second scan, but it cannot be seen. Picture E and F show the device after pumping the AO through the whole system. Again, the fluid that is held in the meshes due to surface tension can be seen. It is less bright compared to pictures C and D. The brightest part in the pictures now is the tube which will be connected to the kidney.

Discussion

In the analysis phase the goal of the product was stated as: “to perfuse a rat kidney while inside an IVIS scanner.” The prototype fulfils this goal. It fits inside the IVIS scanner, and it can perfuse a rat kidney while inside without having to open the chamber during the scans. Many things can be improved on the prototype, but the base of the device is in place and works.

One requirement, stated in the analysis phase, is not met by the design. This is the economic requirement: “The device cannot cost more than 5.000 euro’s”. In hindsight, this requirement was not stated correctly. The machines, like the pump, the adapter for the pressure sensors and the heat exchanger water bath, which are in the original UMCG setup and are indispensable, already cost more than the requirements states. The weighting factors, in the selection process in synthesis phase 1, show that this is the least important requirement. Of course, this does not solve the problem, but it does diminish the size of the problem. The prototype does fit the requirements since most of the components were already available in the lab. The total costs were only €62,01.

All the other requirements are met. The wishes are mostly fulfilled as well: the device is made from durable material and the noise from the nanoparticles around the kidney is avoided as much as possible. The device is not very low maintenance because it has so many different delicate components. Probably, more than once a year maintenance should be done, to keep the device functioning.

From the results in Figure 41 can be concluded that the meshes do stop all the noise coming from the reservoir. However, they do create some noise themselves by holding the liquid. This might create a haze around the kidney. But as the last scan showed, the signal from the lingering liquid is not as strong as from the tubes, because the meshes only hold a thin film compared to the amount of liquid in the tubes. To see if the haze is too distracting, the device should be tested with a real kidney. This would be the next step in the development process of the device.

Recommendations for a future prototype

To read of the temperature of the perfusate, the door of the IVIS must be opened. Which means that, to regulate the heat of the water bath, through which the oxygen tube runs, the door should be opened. In between scans the door can be opened, and the function requirements “The device should only need computerized assistance during the scanning period.” and “The device must be able to regulate the temperature to be between 36,5^{o}C and 37,5^{o}C.” are still met. It would still be preferred if the temperature could also be regulated from outside the chamber. This means for a next prototype, a thermometer that can be read out by the laptop should be considered.

The next prototype should have a slightly off-centre organ chamber. As mentioned earlier, in the section on the prototype, the device must be placed in the IVIS scanner chamber a little to the left. This is because of the indent in the right back side. If the organ chamber is a little to the right of the centre of the lid, the scans would show the organ chamber in the middle of the scan.

The next prototype should be slightly smaller in width and length. This will make it easier to lift the device into the scanner and it would eliminate the tricky manoeuvre of having to put the gas tube connector on the base plate.

The scans showed that the white parts of the device have a great deal of autofluorescence. A next prototype should be matt black, like the inside of the organ chamber, to cancel this effect.

If more sensors are added, the Labview program will not be enough to control and read out the device. Either a new program should be written, or multiple programs should be used.

Conclusion

The prototype is a solution for the problem stated in the problem description in the analysis phase: “At the moment all the perfusion machines are too big to fit inside the scanner.” The goal: “to perfuse a rat kidney while inside an IVIS scanner.” can be achieved by using the prototype.

All the functional-, safety-, ergonomic-, size- and time requirements are fulfilled. Only the economic requirement is not met, which was (according to the Analytic Hierarchy Process) the least important requirement. Two out of three wishes are taken into account.

The future perspective for this project is to test the device with a real rat kidney, mainly to see how the machine holds up, and to see if the meshes of the organ chamber do not hold to much liquid which can be too bright compared to the fluorescence of the kidney. The device could benefit from some changes, but the final prototype of this internship is a viable option for further testing of rat kidneys.

References

[1] Nefrovisie, “RENINE annual report 2019,” 2019. [Online]. Available: https://www.nefrovisie.nl/wp-content/uploads/2021/01/Jaarrapport_Renine19.pdf#:~:text=3.-,Renal%20replacement%20therapy%3A%20prevalence%20and%20incidence,the%20Netherlands%20(Figure%203.2)..

[2] P. B. J. P. J. K. P. M. P. P. C. M. P. L. V. M. P. O. B. Prof. Henri Leuvenink, *Visualisation of Viable Renal Cells in Donor Kidneys during Normothermic Machine Perfusion.,* Dutch kidney foundation, application form innovation grant, 2019.

[3] L. Y. P. T. A. &. M. M. L. Lee, “Living Kidney Donation: Strategies to Increase the Donor Pool,” *Surgical Clinics of North America,* p. 37–47, 2019.

[4] P. Ottens, Interviewee, [Interview]. 12 04 2021.

[5] E. R. B. L. I. I. K. S. A. S. B. M. A. F. R. G. T. W. G. C. W. L. T. S. J. S. W. E. d. P. L. H. M. A. L. R. V. D. T. A. E. H. S. A. N. Thompson, “Novel delivery of cellular therapy to reduce ischemia reperfusion injury in kidney transplantation,” *American Journal of Transplantation,* p. 1402–1414, May 2020.

[6] E. I. J. N. &. M. R. C. Rijkse, “Machine perfusion in abdominal organ transplantation: Current use in the Netherlands,” *World Journal of Transplantation,* p. 15–28, 2020.

[7] H. M. Y. B. A. K. B. K. B. U. &. S. P. Mundt, “Optimized donor management and organ preservation before kidney transplantation,” *Transplant International,* p. 974–984, 2016.

[8] S. J. F. R. S. M. J. A. G. G. M. T. D. &. W. C. H. Tingle, “Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation,” *Cochrane Database of Systematic Reviews,* 2019.

[9] A. &. H. J. Weissenbacher, “Normothermic machine perfusion of the kidney,” *Current Opinion in Organ Transplantation,* p. 571–576, 2017.

[10] Organ assist , “Kidney assist brochure,” [Online]. Available: https://www.organ-assist.nl/products/kidney-assist/.

[11] E. R. E. S. B. V. R. Q. A. C. F. R.-S. M. J. C. &. B. M. Miñambres, “Strict selection criteria in uncontrolled donation after circulatory death provide excellent long-term kidney graft survival,” *Clinical Transplantation,* pp. 1-8, 2020.

[12] R. L. Z. P. S. D. P. K. R. G. R. L. D. F. R. V. C. S. &. K. A. Parasuraman, “Primary Nonfunction of Renal Allograft Secondary to Acute Oxalate Nephropathy,” *Case Reports in Transplantation,* p. 1–4, 2011.

[13] E. H. G. H. D. J. Antonio Benayas, Near Infrared-Emitting Nanoparticles for Biomedical Applications, Cham, Switzerland: Springer, 2020.

[14] E. ˙. A. a. J. H. Adair, “Near infrared imaging with nanoparticles,” *John Wiley & Sons, Inc.,* vol. September/october, no. Volume 2, pp. 461-477, 2010.

[15] F. Rost, Encyclopedia of Spectroscopy and Spectrometry (Third Edition), Academic Press, 2017.

[16] C. V. d. Kolk, Interviewee, [Interview]. 13 04 2021.

[17] D. Taft, “The Isolated Perfused Rat Kidney Model: A Useful Tool for Drug Discovery and Development,” *Current Drug Discovery Technologies,* p. 97–111, 2006.

[18] H. H. C. B. P. A. &. L. K. M. Chang, “Isolated perfused rat kidney: a technical update.,” *Experimental Animals,* vol. 62, no. 1, p. 19–23, 2013.

[19] C. S. G. R. J. K. A. R. &. S. D. E. Lepsy, “Effects of organic anion, organic cation, and dipeptide transport inhibitors on cefdinir in the isolated perfused rat kidney,” *Antimicrobial Agents and Chemotherapy,* p. 689–696, 2003.

[20] E. E.Chabanon, Comprehensive Membrane Science and Engineering (Second Edition), Elsevier Science, 2017.

[21] R. H. Bowman, “Gluconeogenesis in the isolated perfused rat kidney,” *Journal of Biological Chemistry,* p. 1604–1612, 1970.

[22] E. o. O. a. C. B. o. C. H. i. Humans, “Hélène Kergoat; Caroline Faucher,” *Investigative Ophthalmology & Visual Science,* vol. November, no. Volume 40, pp. 2906-2911, 1999.

[23] M. H. B. J. K. J. Å. Mellström, “Effect of Hyperoxia and Hypoxia on Subcutaneous Tissue Gases and pH,” *European surgical research,* vol. 31, p. 333–339, 1999.

[24] P. N. Clifford Deutschman, Evidence-Based Practice of Critical Care (third edition), Elsevier, 2019.

[25] W. R. M. J. C. Y. D. B. M. W. &. R. E. B. David A. Brown, “Hypoxic Cell Death is Reduced by pH Buffering in a Model of Engineered Heart Tissue,” *Artificial Cells, Blood Substitutes, and Biotechnology,* vol. juli, pp. 94-113, 2009.

[26] W. P. J. F. R. P. a. M. P. K. Daniela Patinha, “Cooperative Oxygen Sensing by the Kidney and Carotid Body in Blood Pressure Control,” *Frontiers of physiology,* vol. October, no. Volume 8, pp. 7-19, 2017.

[27] J. M. R. B. D. &. K. H. A. Nishiitsutsuji-Uwo, “Metabolic activities of the isolated perfused rat kidney,” *The Biochemical Journal,* p. 852–862, 1967.

[28] H. Leuvenink, Interviewee, *Prof. Dr..* [Interview]. 07 04 2021.

[29] MD Anderson Cancer Center, “IVIS 100,” [Online]. Available: https://www.mdanderson.org/research/research-resources/core-facilities/small-animal-imaging-facility/instrumentation/ivis-100_.html .

[30] Perkin Elmer, “IVIS Spectrum In Vivo Imaging System,” [Online]. Available: https://www.perkinelmer.com/nl/product/ivis-instrument-spectrum-120v-andor-c-124262 .

[31] AD indstruments, “In-vitro Liver/Kidney Perfusion Chamber,” [Online]. Available: https://www.adinstruments.com/products/vitro-liverkidney-perfusion-chamber.

[32] HA (Harvard Apparatus), “Universal Perfusion System (UP-100),” [Online]. Available: https://www.harvardapparatus.com/universal-perfusion-system-up-100.html .

[33] Perel, “Perel Mini Peristaltische Pomp - 6 V,” discountoffice.nl, [Online]. Available: https://discountoffice.nl/p/mini-peristaltische-pomp-6-v/?gclid=Cj0KCQjwsLWDBhCmARIsAPSL3_01iPMv9nrJvMmOmn8sfo73g2GLhgW7CCijSY0Thgu3ND3kQQ9w-mEaAsb4EALw_wcB. [Accessed 13 04 2021].

[34] Alldoo, “Products,” www.alldoo.com, [Online]. Available: https://www.alldoo.com/product/micropump-CMP32-mini-membrane-water-liquid-pumps.html. [Accessed 13 04 2021].

[35] Gikfun, “Gikfun 12V DC Dosing Pump Peristaltic Dosing Head with Connector For Arduino Aquarium Lab Analytic Diy AE1207,” www.amazon.com, [Online]. Available: https://www.amazon.com/Gikfun-Peristaltic-Connector-Aquarium-Analytic/dp/B01IUVHB8E/ref=sr_1_3?dchild=1&keywords=Peristaltic+Pump&qid=1618325134&sr=8-3#productDetails. [Accessed 13 04 2021].

[36] Getinge, “QUADROX: a member of Oxygenators,” 20 maart 2017. [Online]. Available: https://www.youtube.com/watch?v=8d4cX6niuiU.

[37] C. C. A.-N. 3. S. License., *AHP template,* 171 Second Street, Suite 300, San Francisco, California, 94105, USA..

[38] Ismatec, “REGLO Digital,” Ismatec, [Online]. Available: http://www.ismatec.com/int_e/pumps/t_reglo/reglo_digital.htm. [Accessed 29 4 2021].

[39] Medos, “Medos Oxgenators and Reservoirs,” Medos, [Online]. Available: https://www.trytech.co.jp/en/products/cardiac-surgery/medos.html. [Accessed 29 4 2021].

[40] Julabo, “Waterbaden,” Julabo, [Online]. Available: https://www.julabo.com/nl/producten/waterbaden. [Accessed 29 4 2021].

[41] Ebay, “DC 5V Small Transparent Submersible Impeller Centrifugal Brushless Water Pump,” [Online]. Available: https://www.ebay.com/itm/193520451346. [Accessed 6 5 2021].

[42] Medos, *medos Broschüre Deltastream System,* Stolberg, Germany: Medos, 2011.

[43] Coolblue, “Acer Aspire,” Acer, [Online]. Available: https://www.coolblue.nl/acer/acer-aspire?cmt=c_a%2Ccp_11694444188%2Ca_112569409566%2Ct_kwd-16816120%2Cn_g%2Cd_c&gclid=Cj0KCQjw8IaGBhCHARIsAGIRRYooyJ_ZlM4oXW28CltnbmJO-K57NSRgG1KN0hHLUx-SZbQ1hWkKFx4aAutrEALw_wcB&sorteren=laagste-prijs&pagina=1. [Accessed 10 6 2021].

[44] Cole-Parmer, “Ismatec Reglo ICC Dreikanalige Digital-Peristaltikpumpe mit 8 Rollen,” Ismatec, [Online]. Available: https://www.coleparmer.de/i/ismatec-reglo-icc-digital-peristaltic-pump-3-channel-8-roller-100-to-240-vac/7800170. [Accessed 13 05 2021].

[45] Kabelshop.nl, “USB naar RS232 | Nedis | 0.9 meter (USB A 2.0, D-sub 9 pins, Busgevoed),” Nedis, [Online]. Available: https://www.kabelshop.nl/Nedis-USB-naar-RS232-Nedis-0-9-meter-USB-A-2-0-D-sub-9-pins-Busgevoed-CCGW60852BK09-i21017-t1439709.html?gclid=Cj0KCQjwh_eFBhDZARIsALHjIKdOu1J1xuwSWAGOvBqS5INmnQxPLb9Sb9ptArTmqM0GbOB1BcQQ5IcaAvaOEALw_wcB. [Accessed 7 6 2021].

[46] cameranu.nl, “Xtorm AC Power Bank Pro 41.600 (2x230v),” Xtorm, [Online]. Available: https://www.cameranu.nl/nl/p2473785/xtorm-ac-power-bank-pro-41-600-2x230v-?bgid=39342-AGI-126786628068-ASI-407741247791-2473785&gclid=CjwKCAjw-e2EBhAhEiwAJI5jg5deDv-SWYFuSgStfLKlCwYmo9u-JPjRfnzYuhrgHiJW7gGM-W0pbhoCFwkQAvD_BwE. [Accessed 13 05 2021].

[47] Alexnld, “100/ 250/ 500ML GLASS REAGENT BOTTLE BOROSILICATE CAPACITY BOTTLE BLUE SCREW CAP,” Alexnld, [Online]. Available: https://alexnld.com/product/100-250-500ml-glass-reagent-bottle-borosilicate-capacity-bottle-blue-screw-cap/. [Accessed 13 05 2021].

[48] Rubber BV, “Polyethyleenslang,” Rubber BV, [Online]. Available: https://www.rubberbv.nl/artikelen-webshop/slangen/polyethyleenslang. [Accessed 17 05 2021].

[49] Grandado.com, “Pak Van 10X1/8 Inch Barb Mannelijke Luer Tapered Fitting (Polyprop) luer Lock Tapered Connector,” Vmatic, [Online]. Available: https://nl.grandado.com/products/pak-van-10x1-8-inch-barb-mannelijke-luer-tapered-fitting-polyprop-luer-lock-tapered-connector?variant=UHJvZHVjdFZhcmlhbnQ6MjI5ODMzOTA. [Accessed 9 6 2021].

[50] Grandado.com, “100 Stks/partij 1/8 Inch Barb Vrouwelijke Luer Tapered Spuit Fitting (Polyprop) Luer Lock Tapered Connector,” Vmatic, [Online]. Available: https://nl.grandado.com/products/100-stks-partij-1-8-inch-barb-vrouwelijke-luer-tapered-spuit-fitting-polyprop-luer-lock-tapered-connector?variant=UHJvZHVjdFZhcmlhbnQ6MjI1MTMwMjE. [Accessed 9 6 2021].

[51] Merkala, “Driewegkraan luer-lock per stuk,” Merkala, [Online]. Available: https://www.merkala.nl/driewegkraan-luer-lock-per-stuk.html. [Accessed 9 6 2021].

[52] Nine Life, “Oxygen Supply Tubing Y Adapter - Set of TEN (10),” SP Medical, [Online]. Available: https://www.ninelife.nl/products/oxygen-supply-tubing-y-adapter-set-of-ten-10?gclid=Cj0KCQjw8IaGBhCHARIsAGIRRYqply7q0K6ASi1rOGt7E_l8m5pk2VWtr0X6ZXyphJmJgrWgsYTjVfUaArHzEALw_wcB. [Accessed 10 6 2021].

[53] Alle kabels, “Kabelbinder - Tie-Wrap,” Alle kabels, [Online]. Available: https://www.allekabels.nl/kabelbinder/32/660/kabelbinder-tie-wrap.html?gclid=Cj0KCQjwzYGGBhCTARIsAHdMTQxNqx6_GyX_hzZSSU_f6wiVwNjsNXMDENsJEUsfcuLihlB9bMYBIFQaAlbOEALw_wcB. [Accessed 9 6 2021].

[54] Glazen-en-potten.nl, “Erlenmeyer 500ml nauwe hals borosilicaat met afgeronde rand,” Glazen-en-potten.nl, [Online]. Available: https://www.glazen-en-potten.nl/erlenmeyer-500ml-nauwe-hals-borosilicaat-met-afgeronde-rand-263.html. [Accessed 14 05 2021].

[55] Grandado.com, “20 stks/partij 000 #-4 # Rubber stopper, erlenmeyer plug flessenstop, reageerbuis stopper,” Grandado.com, [Online]. Available: https://nl.grandado.com/products/20-stks-partij-000-4-rubber-stopper-erlenmeyer-plug-flessenstop-reageerbuis-stopper?gclid=CjwKCAjwqIiFBhAHEiwANg9szlm0TfF2hwhhWaFgqqxpHp8M97iiCHbytgbopX-rhqYVDcZsZMH-3xoCBGQQAvD_BwE&variant=UHJvZHVjdFZhcmlhbnQ6MTk4NjQ0NjU2. [Accessed 14 05 2021].

[56] Rubberbv, “python siliconslang,” Rubberbv, [Online]. Available: https://www.rubberbv.nl/artikelen-webshop/slangen/silicon/python-siliconslang. [Accessed 17 05 2021].

[57] eSutures.com, “PX284: EDWARDS TRUWAVE DPT PX600F PRESSURE MONITORING SET 84",” Edwards Lifesciences, [Online]. Available: https://www.esutures.com/product/0-in-date/93-edwards/885-pressure-monitoring/46237391-edwards-truwave-dpt-px600f-pressure-monitoring-set-84-PX284/. [Accessed 13 05 2021].

[58] BOOM, “Ama-digit digitale thermometer ad 15 th, -40 - +120°C,” Amarell, [Online]. Available: https://www.boomlab.nl/product/29568100+amarell-ama-digit-digitale-thermometer-ad-15-th-40-1200c-29568100. [Accessed 7 6 2021].

[59] J. Zwaagstra, Interviewee, *Chirurg.* [Interview]. 28 05 2021.

[60] E. H. Bos, Interviewee, *Research intstrumentmaker.* [Interview]. 17 05 2021.

[61] deminiatuurwinkel.nl, “HomeAluminium landschapsbouw gaas 100x75cm,” Noch, [Online]. Available: https://www.deminiaturenwinkel.nl/aluminium-landschapsbouw-gaas-100x75cm-noch.html?source=googlebase. [Accessed 14 05 2021].

[62] Alle kabels, “Kabelbinder,” Alle kabels, [Online]. Available: https://www.allekabels.nl/kabelbinder/32/1289032/kabelbinder.html. [Accessed 9 6 2021].

[63] natuurkundeuitgelegd.nl, “Soortelijke warmte,” natuurkundeuitgelegd.nl, [Online]. Available: https://natuurkundeuitgelegd.nl/videolessen.php?video=soortelijkewarmte#:~:text=Met%20Q%3Dc*m*delta%20T%20reken%20je%20uit,massa%20van%20de%20verbrande%20brandstof).. [Accessed 11 6 2021].

[64] natuurkundeuitgelegd.nl, natuurkundeuitgelegd.nl, [Online]. Available: https://natuurkundeuitgelegd.nl/videolessen.php?video=warmtegeleiding. [Accessed 11 6 2021].

[65] The Engineering Toolbox, “Densities and molecular weights of some common gases - acetylene, air, methane, nitrogen, oxygen and others,” The Engineering Toolbox, [Online]. Available: https://www.engineeringtoolbox.com/gas-density-d_158.html. [Accessed 11 6 2021].

[66] The Engineering Toolbox, “Specific heat of Oxygen Gas - O2 - at temperatures ranging 175 - 6000 K,” The Engineering Toolbox, [Online]. Available: https://www.engineeringtoolbox.com/oxygen-d_978.html. [Accessed 11 6 2021].

[67] The Engineering Toolbox, “Thermal conductivity of plastics,” The Engineering Toolbox, [Online]. Available: https://www.engineeringtoolbox.com/thermal-conductivity-plastics-d_1786.html. [Accessed 11 6 2021].

[68] E. v. d. H. G.J. Verkerke, reader Ontwerpen 2, version 2.0, Groningen, 2011.

[69] Medical Device Coordination Group, *Guidance on Classification Rules for in vitro Diagnostic Medical Devices under Regulation (EU) 2017/746,* 2017.

[70] Emergo, “European CE Marking for In Vitro Diagnostic (IVD) devices,” Emergo, [Online]. Available: https://www.emergobyul.com/services/europe/europe-ivd-registration. [Accessed 10 5 2021].

[71] RIVM, *In Vitro Diagnostic Medical Devices,* Bilthoven, 2006.

[72] M. B. A. V. E. M. B. P. G. &. V. V. V. Daria M. Shcherbakova, “Bright monomeric near-infrared fluorescent proteins as tags and biosensors for multiscale imaging,” *Nature communications,* 19 8 2016.

[73] M. T. Z. |. W. L. Olszewski, “Indocyanine green near-infrared lymphangiography for evaluation of effectiveness of edema fluid flow under therapeutic compression,” *Biophotonics,* 18 7 2018.

[74] SHIMADZU, “Near-Infrared Region Measurement and Related Considerations Part 1,” SHIMADZU, [Online]. Available: https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/tips-ftir/nir1.html. [Accessed 7 6 2021].

[75] ThermoFisher, “Acridine Orange,” Invitrogen, [Online]. Available: https://www.thermofisher.com/order/catalog/product/A1301#/A1301. [Accessed 7 6 2021].

[76] J. B. L. a. D. M. G. 0. P. R. B. CundaN*, “Polyelectrolyte Complexes, 1 The Effect of pH and Ionic Strength on the Stoichiometryof Model Polycation - Polyanion Complexes,” *Die Makromolekulare Chemi,* no. 180, pp. 2913-2922, 1979.

[77] Y. M. Tomoko Komiyama, “Concentration Depolarization of the Fluorescence and Phosphorescence of Acridine Orange Cations,” *Bulletin of the Chemical Society of Japan,* vol. 49, no. 4, pp. 864-867, 1976.

[78] T. T. a. S. O. Yoshinori MOR1YAMA, “Acridine Orange as a Fluorescent Probe for Lysosomal Proton Pump,” *Journal of Biochemistry,* vol. 94, no. 4, pp. 1333-1336, 1982.

[79] Conrad, “Heraeus Nexensos W-SZK(0) PT100 Printplaat-temperatuursensor -20 tot +100 °C 100 Ω 3850 ppm/K,” Heraeus Nexensos. [Online]. [Accessed 13 05 2021].

[80] Ubuy, “RSN Lab 25x Male luer Lock 1/16" 1.6mm PP Hose Barb,” RSN Lab, [Online]. Available: https://www.ubuy.co.nl/en/catalog/product/view/id/30533988/s/rsn-lab-25x-male-luer-lock-116-1-6mm-pp-hose-barb. [Accessed 9 6 2021].

Appendix

Final concept

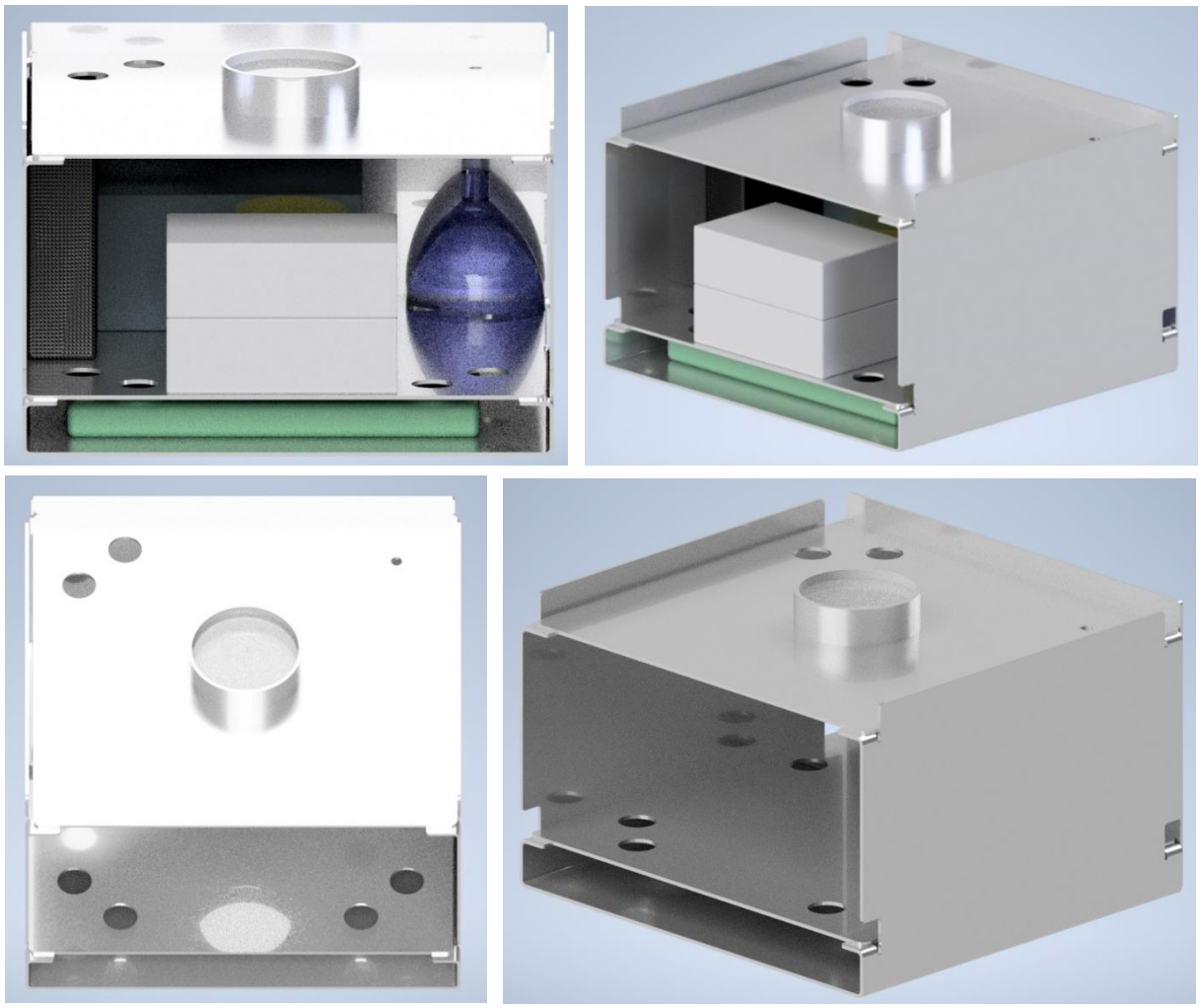


Figure 42. (Top) The final design with the big components drawn in. (Bottom) The final design of the casing.

Technical drawings

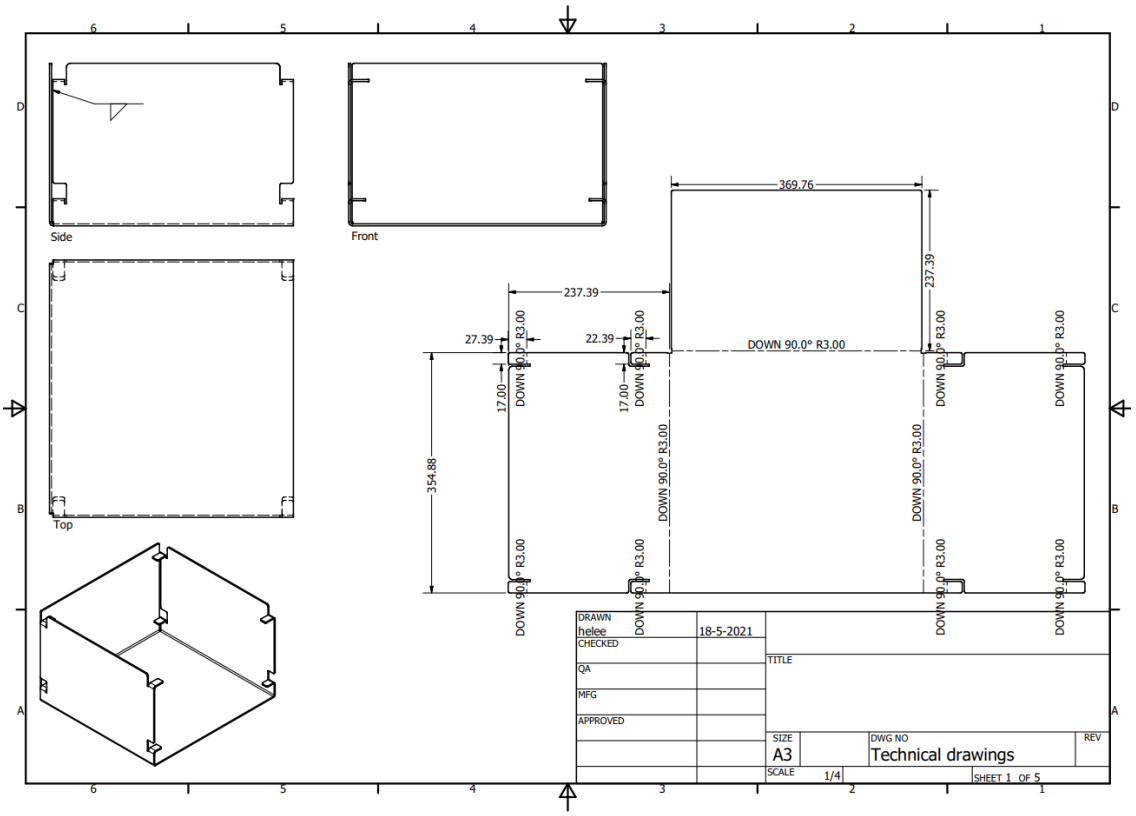


Figure 44. Technical drawing of the base of the casing.

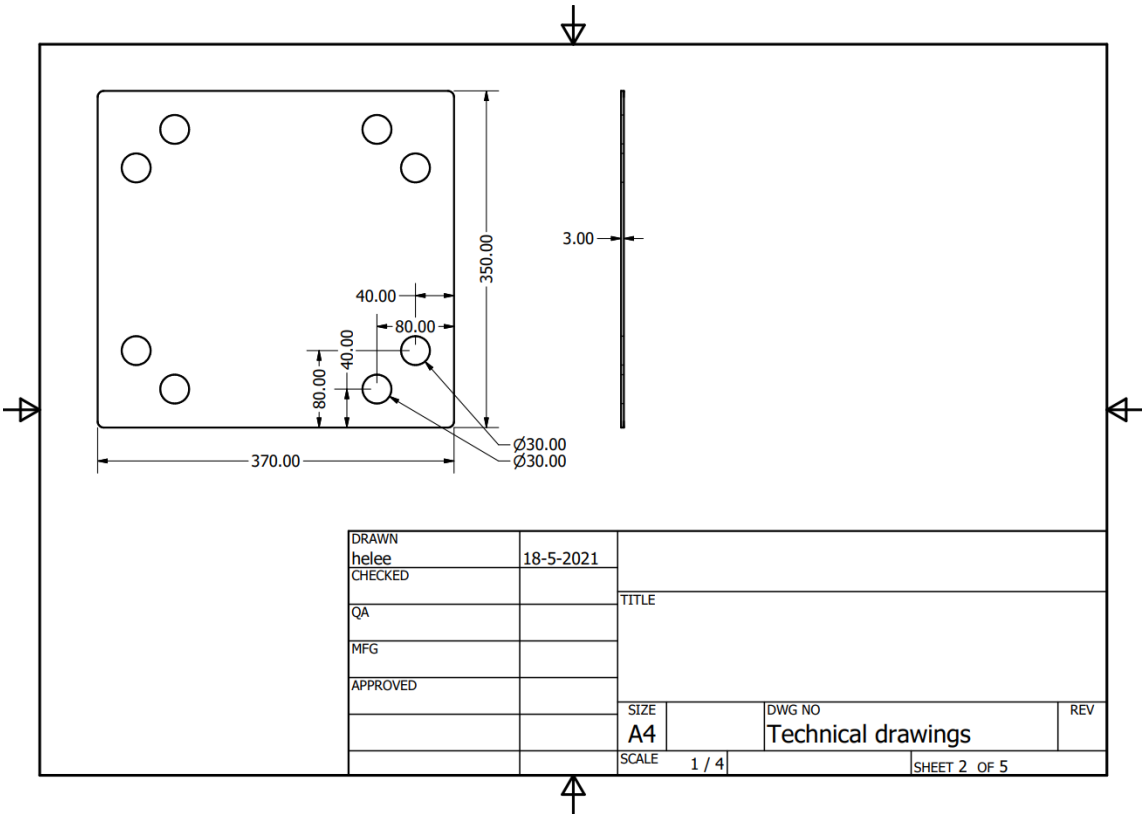


Figure 43. Technical drawing of the lower double bottom

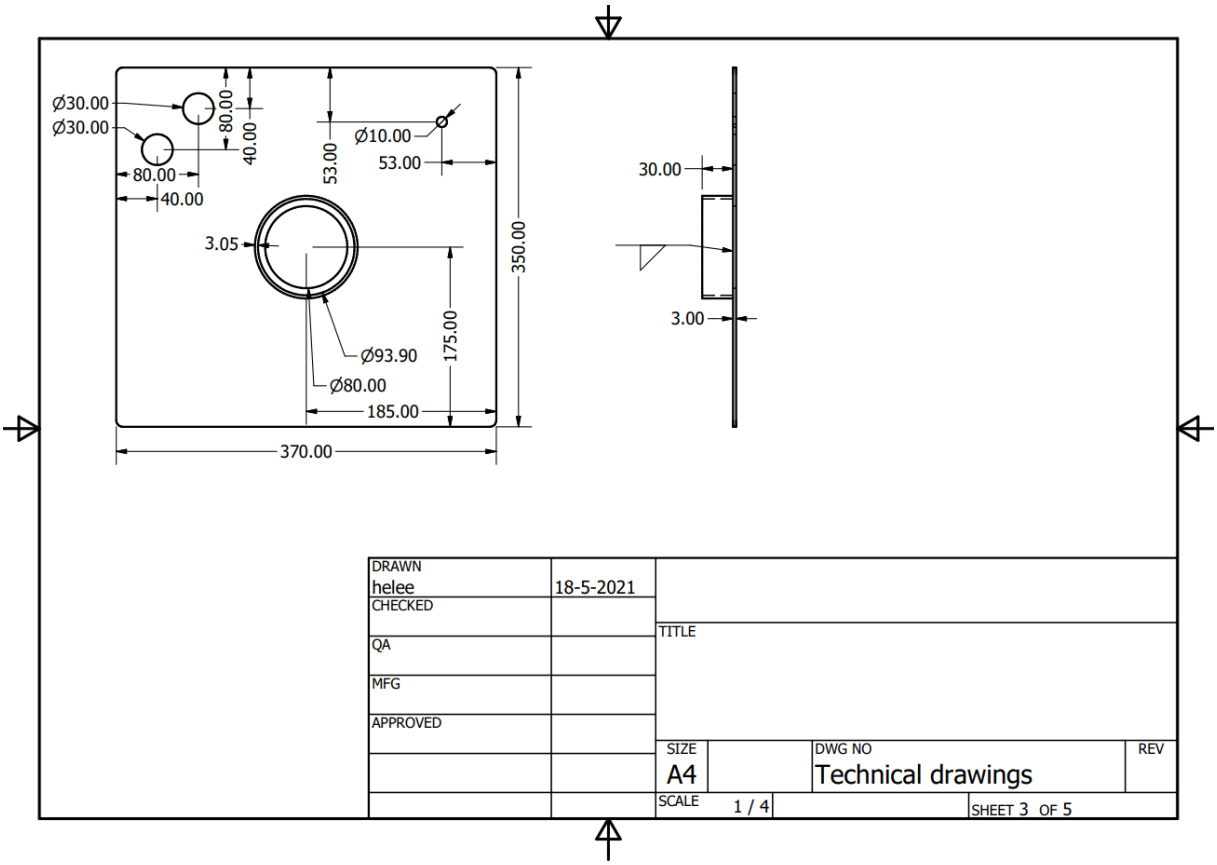


Figure 45. Technical drawing of the upper double bottom.

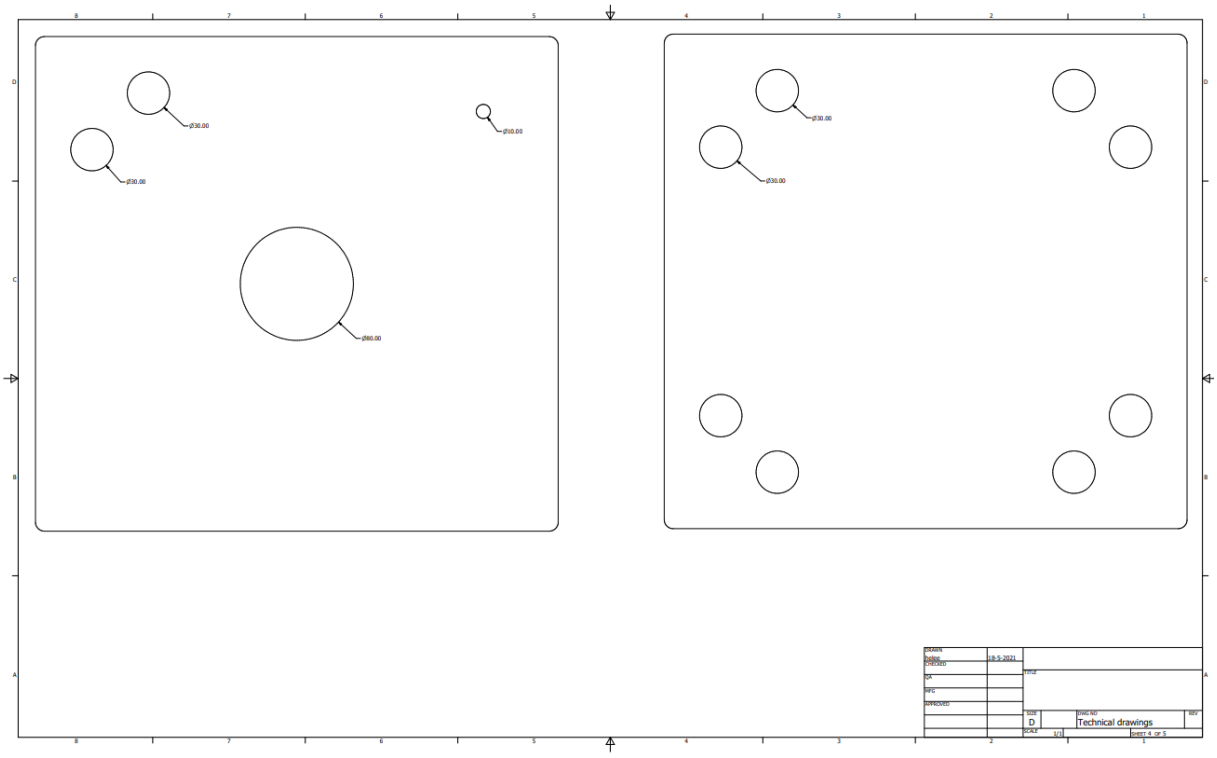


Figure 46. Flatpatterns of the double bottoms for the plasma cutter.

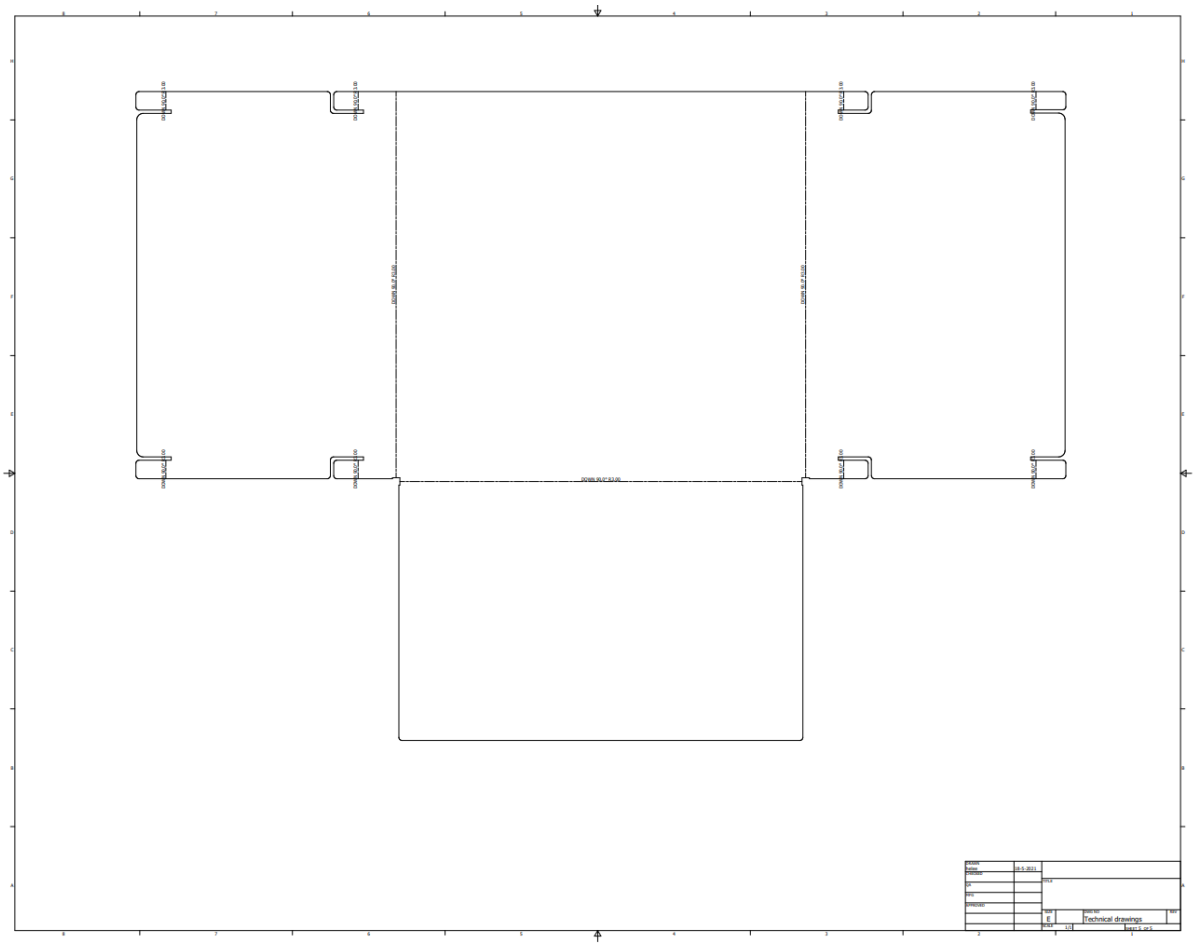


Figure 47. Flatpattern of the casing for the plasma cutter.

Prototype

Figure 48. The first version of the prototype. (Top left) The rings of the organ chamber are shown separate from each other. (Top right and bottom right) fully assembled casing and organ chamber. (Bottom left) Casing without the organ chamber.

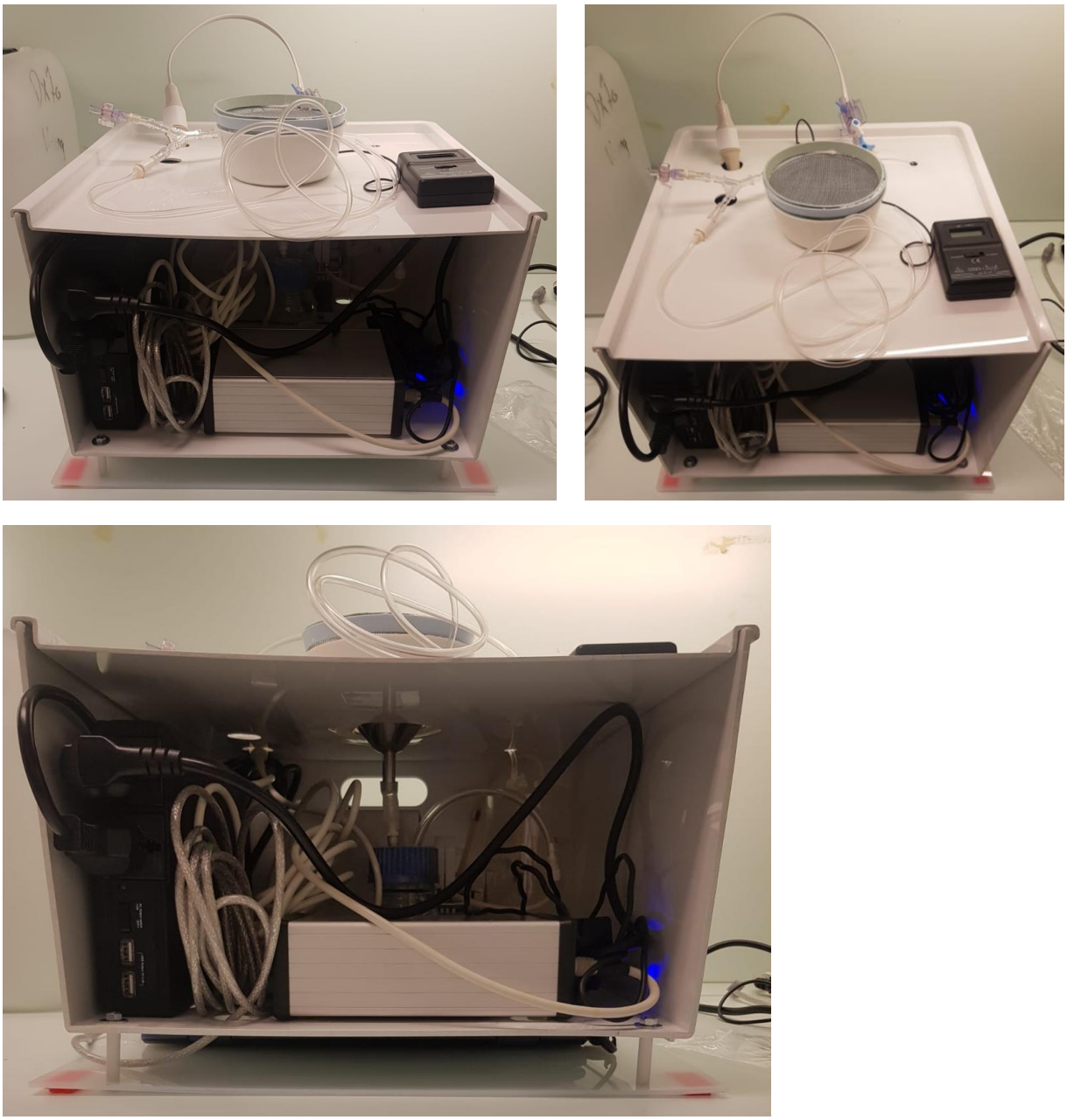


Figure 49. The first version of the prototype with all the equipment installed.

Figure 50. The prototype base after the first round of corrections.

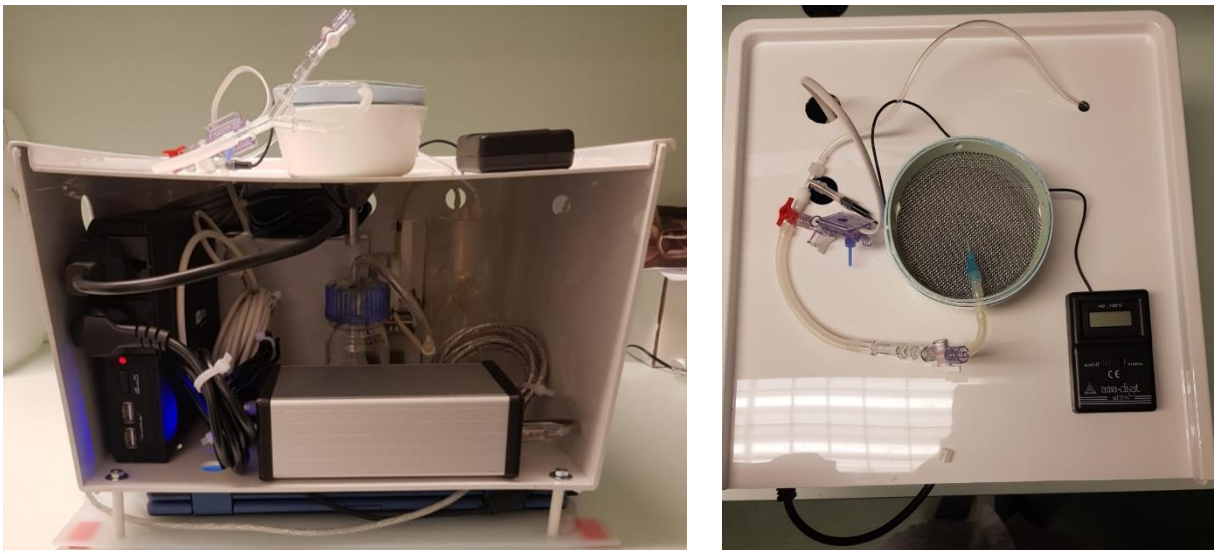


Figure 51. The prototype after the second round of corrections with all the equipment installed.

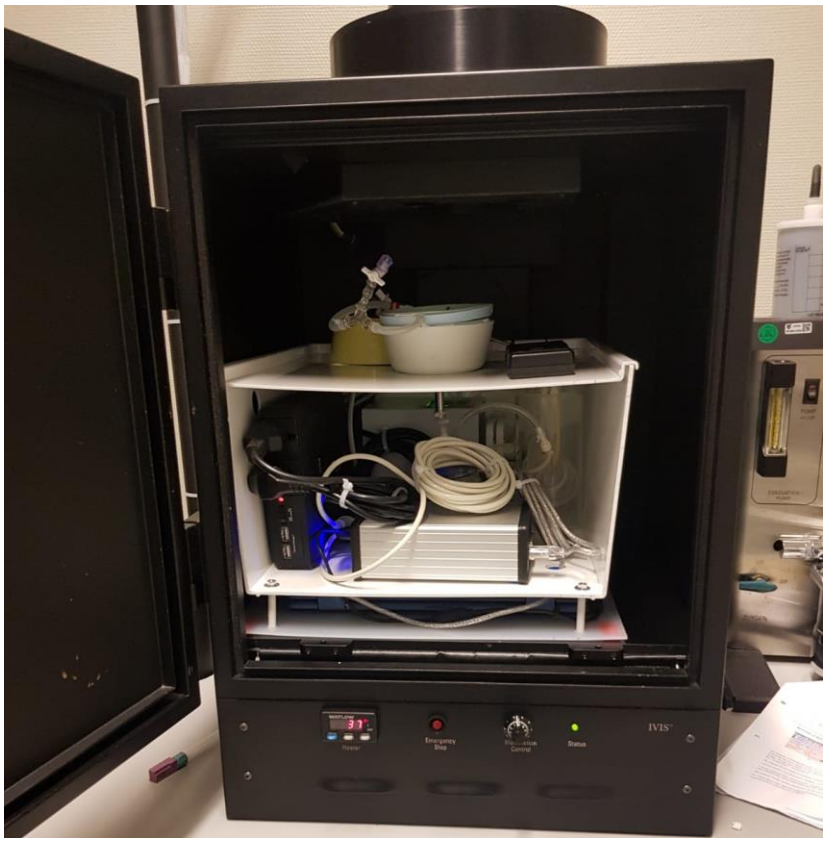


Figure 52. The prototype inside the IVIS scanner.

Figure 53. The door of the IVIS with the protruding lock.

Figure 54. The final version of the prototype.

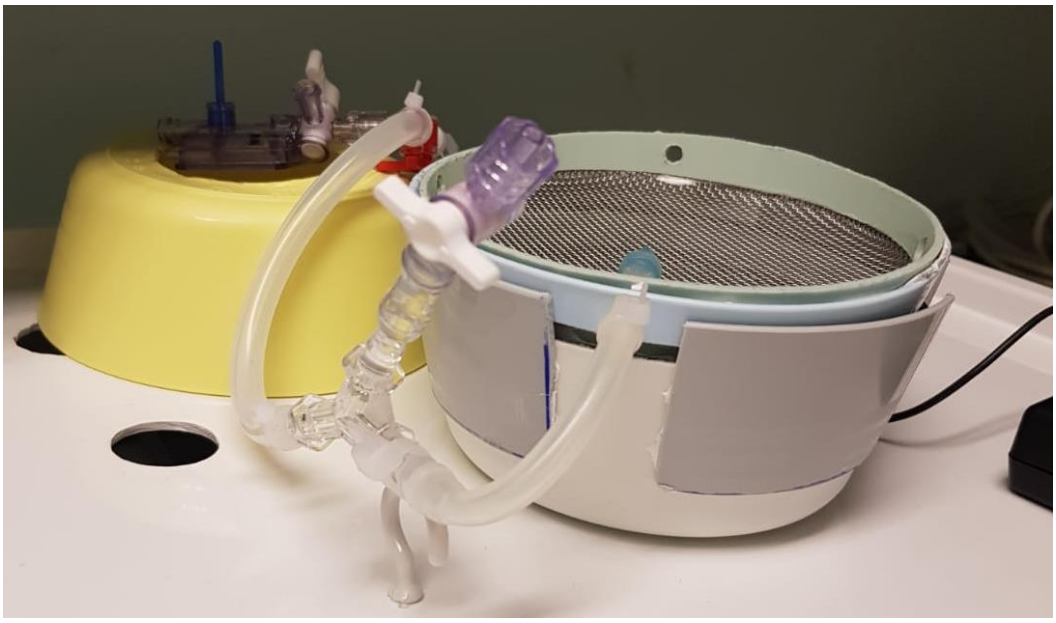


Figure 55. A close-up of the secured bubble trap and the improved organ chamber.