UNIVERSITAT DE BARCELONA

Schallware

*Master Thesis* – ***Master in Innovation and Entrepreneurship in Biomedical Engineering***

# Development of a monitorization method for the acquisition of 3D ultrasound volumes on in-vivo mouse models for cancer evaluation in brain and abdominal tumors & its market viability assessment

Barcelona, 13th January 2020
Author: Umi Mir Kato
Supervisor: Gernot Jehle
Tutor: Roser Sala

## Abstract

In the cancer research, non-invasive imaging techniques are widely used in tumor mouse models in preclinical studies, in order to understand cancer evolution and to find new treatments. However, most current techniques for orthotopic tumor tracking during treatment testing are either too expensive or time-consuming. Within this context, this master thesis project was developed in Schallware GmbH with the collaboration of Experimental Pharmacology & Oncology (EPO) GmbH. Its main objective was to develop a new product based on ultrasound (US) mouse scans *in vivo* with a robotic arm in order to obtain 3D volumes of brain and pancreatic tumors, and a posterior assessment of its market viability. With some experiments, we concluded that our solution allowed the monitorization of tumor growth as quantitative readout of therapeutic efficacies in mouse studies. Results confirmed that our product achieved fast and accurate results for pancreatic tumors, but that it still needs improvements in order to precisely find the brain tumor in the US image. Therefore, even though the product is the fastest solution for EPO so far and Schallware has a good target market, it is not ready to be sold in the market yet.

# Index

## List of Figures

## List of Tables

# 1. Introduction and Objectives

This master thesis project was developed in Schallware GmbH, a company founded in 2001 in Berlin, who specialize in US simulations and 3D representations of the patients for internal medicine, obstetrics and gynecology and cardiology. Schallware is one of the few US simulation companies in the world which obtains 3D volumes with real images. The acquisition of the images is done by scanning real patients while the doctor moves a transducer through the part of the tissue of interest and then, all the images obtained are recorded at the same time. All these images can then be seen and simulated on a screen when a probe simulator goes through the same tissue of interest but on the mannequin (see Figure 1).


**Figure 2.** Schallware ultrasound simulator [1].

In addition to Schallware, this project was developed with the collaboration of the Experimental Pharmacology & Oncology (EPO) GmbH. They supported the project by providing the required mouse models, following the German ethical rules of animal care and maintenance (*Tierschutzgesetz,* [2]). The EPO GmbH is an internationally working Contract Research Organization (CRO) from Berlin that has more than 20 years of experience in the field of oncology research as well as pharmacology related services. While working with mouse models, they realized that they could not track the orthotopic tumor growth using a standard US imaging technique, so they made contact with Schallware in order to find a solution. Schallware decided to help EPO by improving the innovative approach using a robotic arm for scanning the mice *in vivo*. This was the point when the present master thesis project was proposed.

The main aim of this project is to develop an innovative method of monitorization and measurement of the orthotopic cancer growth *in vivo* in mouse models within short periods using US images and to assess its market viability. In order to achieve this, the small-scaled objectives are:

- To optimize and decide the best parameters for the images acquisition. In order to do so, all parameters of the US device and different probes will be tested on a small mouse phantom specially built for these attempts before trying it on real *in vivo* mouse models.
- To set up the robotic arm so that it can automatically drive the probe through the mouse body in order to obtain several US images.
- To obtain images from healthy mice, brain and pancreatic tumors in order to adapt the robotic arm to any situation.

- To post-process and to segment the tumors from the images for the determination of a final volume using the Schallware Simulator.
- To evaluate the possible applications and the viability of the new method in the market.

All in all, the project will allow an *in vivo* monitoring of cancer during therapy as this new method obtains 3D US volumes in alive anesthetized mouse models for intracranial brain tumors (glioblastoma) and orthotopic pancreatic tumors. With this, Schallware's final objective is to sell the new product/method to pharmaceutic companies as well as to implement the robotic arm for US images acquisition. As for EPO's aim, this CRO would like to get access to a faster and more accurate process for tracking the tumor volume particularly for orthotopic tumor models.

### 1.1. Schallware Business Overview

Schallware is a company which specialize in ultrasound simulators. The Schallware Simulator is based on clinical ultrasound data recorded from the scans of real patients. This offers pathological findings and variants of anatomical textures and structures, and virtual models of animated heart, fetus or abdomen for continuous scanning around organs, to better understand the human's anatomy and dynamics. As the aim of the simulator is to offer individual training to whoever needs to learn US imaging, it also contains instructions (step-by-step tutorials with virtual model and real cases), questions and answers (Q&A) to evaluate guidelines (department specific competences) and courses to perform real examinations of a selection of patients [1]. The company is divided in two main sections: Research & Development (where the software and new ideas are developed), and US images acquisitions in hospitals. Hospitals such as the UKE (Universitätsklinikum Hamburg-Eppendorf) from Hamburg for pediatric US images or the MHH (Medizinische Hochschule Hannover) from Hannover for internal medicine US images. This master thesis project is developed in the R&D section of the company.

Schallware sells the simulators worldwide and its market is mainly focused on university hospitals, pharmacologic companies and health-related congresses. Regarding its competitors, they are divided in three types:

a) The ones who only use phantoms, so they need a real US device with its transducers in order to test image visualization of different organs: SimuLab, etc.
b) The ones who only work with virtual images and mannequins: CAE, SonoSim, Virtamed, BluePhantom, etc.
c) The ones who only work with real data scans and with or without mannequins: MedaPhor, ScanTrainer, etc.

Nevertheless, none of them provide a combination of real and virtual data with dummies in the same product. Thus, Schallware is a very innovative company in the market of ultrasound simulators.

In order to be successful with any company, taking into account the market trends, being sure to fulfill the demand is crucial. In this context, first of all, the ultrasound device market is expected to grow during the next few years – it was valued at €4.72 billion in 2018 and is expected to generate around €7.3 billion by 2025 [3]. Due to the increase in the invasive imaging procedures, the rising demand for ultrasound technologies and growing investments and funds for upgrading devices. Nevertheless, there is something which hampers and slows down this growth: the lack of experienced ultrasound

technologists [4]. The proposed solution is to use a robotic arm in order to scan the mouse, and therefore it can help the ultrasound device market to grow even faster. From the clinical point of view, Schallware would like to implement the use of the robotic arm to hold and move the transducer for image acquisitions from human patients as it is more precise and accurate than when it is done manually by a doctor – a steady hand movement is essential to avoid any acquisition error and to get more resolution. If Schallware improves the acquisition methods, it can be stronger than the competitors. Regarding the medical simulation market, it is expected to reach a value of €3.6 billion in 2024 [5].

### 1.2. Background

In the tumor research field, it is still important and useful to work with mouse models in order to investigate tumor biology and to develop new therapies for human cancer. Many oncology researchers believe that animal research is essential to understand how cancers develop and behave within a whole organism, and how to treat them effectively [6]. Mouse models are able to accurately reflect many aspects of human tumor physiology such as angiogenesis, tumor-stromal interaction or hormone dependency [7]. Measurements of changes in the tumor size by diagnostic images are a standard method for monitoring responses to anticancer therapies [8].

EPO is a research institute that works for different pharmaceutic companies testing and making preclinical studies with new drugs or therapies for cancer or other illnesses. Its current procedure for tumor evaluation with a certain drug treatment is as follows: they usually buy NMRI (Naval Medical Research Institute) nu/nu (nude) immunodeficient mice to JanVier labs, a French laboratory specialized in rodent research models [9]. When the mice are 6-8 weeks old, they are divided in two groups, ones with only tumor and others with tumor and drug treatment. Tumors come from cell line derived (CDX) or patient derived xenografts (PDX) and are orthotopically transplanted. Afterwards, the treatment to be tested is applied to only one group of mice. Normally, the study finishes after 8 weeks from the day of transplantation. However, especially for orthotopic tumor models, *in vivo* measurements can be expensive and time consuming. Therefore, measurements throughout the study are not always possible and have to be made after sacrificing animals. Within these limitations, there are different ways to evaluate treatment outcome:

a) All animals are sacrificed at the same time: once the first animal shows signs of health impairment, all mice are sacrificed. All tumors had the same time to grow (until the day of sacrifice) and one can compare the tumor size of the control group to the treatment group. This is called tumor growth inhibition (TGI or T/C), which is a commonly used parameter in that kind of studies.
   If the tumors within the groups grew homogeneously that would be completely fine; but if one mouse had a tumor that was growing faster or at a crucial place within the organ of interest that made it to get sick earlier, one have to sacrifice all animals because of this outlier. One cannot check if tumors have similar sizes and grow at the same position without a method to monitor tumor growth *in vivo.* The issue is that, in the rest of mice, the tumors then maybe are still quite small and differences between control and treatment are not as pronounced as they could be if they had waited longer and went on with the treatment.
b) Animals are sacrificed individually: one avoids sacrificing animals too early due to an outlier, but all tumors will have different amount of time to grow. Therefore comparison of tumor sizes is not possible, but by comparing survival of control

and treatment groups, beneficial treatment effects on Overall Survival (OS) can be analyzed.

Using the first option, one does not get data about OS, but there is no data about growth inhibition (TGI or T/C) if one follows the second option. Both setups have different outcomes: TGI, T/C or OS. If one can check the tumor size regularly via US images, it would be possible to get more data from one study: how individual tumor grows (relative tumor volume, RTV), calculation of TGI to analyze which time of treatment is the most beneficial, and survival time (OS).

There are currently several limitations to the use of US in tumor monitoring. First, in the case of abdominal tumors, one can scan them using US images, but this usually takes a lot work and time. As for the brain tumors, they have not been able to use the US device as the skull reflects the US waves making mirroring artifacts and blocking the observation of anything below the bone. Without a scan, it is not possible to know if the mice already had a tumor before the transplantation, making statistics less powerful. To avoid these problems in most of the studies, the tumor is transplanted subcutaneously, growing as a tumor nodule under the skin – this is called an ectopic tumor because it is transplanted to another organ different from the origin. One can easily measure the tumor size with a caliper several times every week while the animal is alive (they measure two axes and calculate/interpolate the volume from that). These results are quite accurate and exact, but it is an even more artificial system as the tumor then is not surrounded by the tissue of the original tumor context, so results might not entirely reflect the actual biological and clinical situation.

These weaknesses show a need of tracking orthotopic tumors during treatment testing. For brain tumors, it would be very helpful to check the tumor growth during the study without the need of sacrificing the animals. The process of segmentation of the tumor is tedious and not efficient. Secondly, all the tumors could be orthotopic (transplanted to the same organ of its origin), so conditions would be closer to the original ones in the patient and the results would be closer to the results that they would get if they could test the drug in the patient. Measuring the tumor throughout the study allows to get more data on different parameters of tumor growth and treatment outcome from just one study (T/C, RTV, OS) and to achieve stronger statistics as it is possible to sort out mice that had no tumors from the beginning. In addition, the number of animals used, and the time spent in a study would be reduced while the amount of data from a study would be increased. If the study time is reduced, it is possible to perform more studies and to test more treatments, and therefore the probability of finding an effective cancer treatment increases.

### 1.3. State of the Art

In this section, the status of the different non-invasive imaging techniques to monitor mouse tumor models will be reviewed. Significant progresses have been done so far in the field. These progresses include the improvement of specialized hardware for small animal imaging methodologies as well as the development of new imaging techniques during the last few years [7]. The most important imaging techniques currently used in cancer evaluation in small animals are the following ones: Micro-Positron Emission Tomography (micro-PET), Micro-Single Photon Emission Computed Tomography (micro-SPECT), Micro-Magnetic Resonance Imaging (micro-MRI), Micro-Computed Tomography (micro-CT), Bioluminescence and US imaging. Nevertheless, none of them have used the combination of a robotic arm with any simulator to track the tumors for volumes obtaining.

Micro-PET is a nuclear imaging technique that can measure different biological processes, such as quantitative measure of tumor cell metabolism, and make longitudinal calculations of animal models. It has a high resolution and sensitivity and high degree of versality due to the variety of probes and strategies combining other techniques [10]. It also allows non-invasive, real-time tracking of the tumor in vivo [11]. However, the radioisotopes used for this technique generally have a short life, the resolution is quite low, and the noise can easily be increased with an unincorporated substrate [7].

Micro-SPECT uses radioisotopes with longer half-lives than in PET and can provide 3-dimensional spatial distributions of ɣ-ray-emitting radionuclide imaging agents or therapeutics [12]. The combination of this technique with the micro-CT has been able to resolve the intratumoral dispersion pattern and quantify the infection percentage in solid tumors in small animals [13]. The disadvantage of this technique is its low resolution, its high price and that is between 10-100-fold less sensitive than micro-PET.

Micro-MRI would be the best solution for determination of the tumor growth as it is able to provide both high-resolution anatomical information and functional measurements of tumor physiology [7]. It is a non-ionizing technique with full three-dimensional capabilities, excellent soft-tissue contrast, and high spatial resolution. Some studies have been able to quantitatively track tumor development and progression in lungs [14], in the lymphatic system [15], in the brain [16], etc. However, the temporal resolution is much slower than for US – the scans normally last 3-10 min –, so it is much more susceptible to patient motion, which is an issue for the volume obtention [17]. The cost of MRI scanners is relatively high. The price for the systems for animal imaging between 1.5-T and 14-T are in a range from 900.000€ to 5.5M€.

Micro-CT usually provides high-resolution anatomic information, especially morphological detections of tumors and metastases in lung and bones. It can be used either on its own or in conjunction with lower-resolution functional imaging modalities, such as micro-PET or micro-SPECT. Nevertheless, its limitations are based on the associated radiation dose and relatively poor soft tissue contrast [18].

Bioluminescence is also an excellent approach in order to evaluate the tumor growth in vivo, but there is a need of genetically modified mouse models as it is necessary to implant the luciferase gene. It is not possible to calculate the total volume either. With the light intensity, the only thing they can evaluate is how the tumor has changed regarding the size. It has low anatomic resolution, and the light emission is prone to attenuation with increased tissue depth [7].

The US is a non-ionizing and non-invasive, relatively inexpensive and easily portable imaging technique which provides high spatial and temporal resolution. Owing to such a high imaging frame-rate, it is fast and has a real-time imaging capability. One weakness of the technique can be the relatively poor soft-tissue contrast or the fact that gas and bone do not allow the ultrasound waves to go through them, so certain organs are more difficult to be imaged [17, 19, 20]. Microbubbles can be used for contrast enhancement to avoid poor vision of some organs and Doppler-based modes can determine the velocity of a moving tissue, normally blood. Thus, EPO decided that US imaging is the best alternative for them so far but still needs proper improvements.

Some researchers have already tried to use the ultrasound device to track the tumors in mouse models *in vivo* [21, 22, 23, 24, 25, 26]. However, obtaining good images for brain tumors is especially difficult because the skull reflects the US waves and we see nothing

below the skull. One of the solutions that they have implemented was to use head implants [27] with miniaturized ultrasound probe or making a skull window, but both are invasive methods, so EPO is not allowed to use them. As for the abdominal tumors, it is easier to use US devices for tracking them as there are only soft-tissue organs [28]. One of the things that can disturb the image acquisition is the air inside the bowels. 3D volumes have only been made for prostate cancer in mouse model [29].

It is noticeable to say that FujiFilm VisualSonics has a set of imaging systems called VEVO which are scientific US devices for preclinical studies. They are mainly used in cancer imaging for translation research as they can characterize tumor tissue *in vivo*, non-invasively, detect lesions early, monitor tumor development and assess the response to therapy. They also provide 3D volume reconstructions from B-mode images (see Figure 2) and accurately quantify volume of orthotopic and subcutaneous tumors longitudinally [30]. S-sharp company has also a US preclinical imaging system called Prospect, which is useful for real-time information in small animals, but it is not able to provide volumes. Thus, FujiFilm VisualSonics is, in practice, the only competitor in the market. At the EPO GmbH, the VEVO 2100 model is used for tracking abdominal tumors, but yet the procedure of volumes determination is still time consuming.

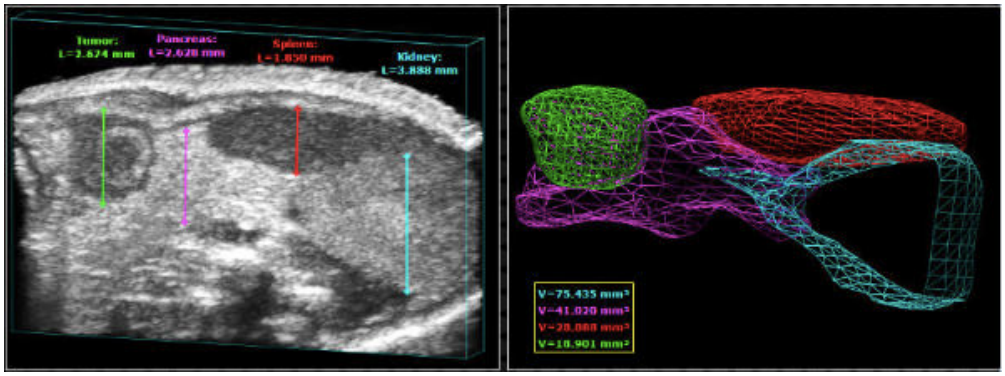


**Figure 2.** *Left:* B-mode image of the spleen, kidney, pancreas and orthotopic pancreatic tumor in a mouse. *Right:* 3D volume reconstruction. [30].

Combining the advantages from the use of a robotic arm for an accurate US scan recording and the tools provided by the Schallware Simulator for the segmentation and volume determination allowed to develop a new method which only has one competitor in the market.

### 1.4. Market Analysis

Cancer is the second leading cause of death globally. In 2018, there were 17 million new cases of cancer worldwide, where 8.8 million (52%) of these were in males and 8.2 million (48%) in females. This resulted in 9.6 million deaths. Furthermore, the World age-standardized incidence rate item indicates that there are 204.7 new cancer cases for every 100.000 men, and 175.6 for every 1.000.000 females worldwide; and the cancer incidence rates are projected to increase by 62% for 2030. The most common types of cancer are lung, female breast, bowel and prostate cancers, which together represent 43% of all new worldwide cases [31].

The economic impact of cancer is large. In high-income regions, 15% of the social welfare system and 20% of health systems expenses go towards cancer care. In the EU, productivity costs due to premature cancer-related mortality fall €42.6 billion/year and lost working days cost €9.43 billion/year. Moreover, developing countries consume only

5% of cytotoxic drugs, while the 90% is sold in developed nations, where 39% of global cancer occurs [32]. This economic impact could be reduced if the pharmaceutical companies had more facilities to find specific treatments for each kind of disease.

99.5 billion US dollars were spent worldwide on pharmaceutical sales in oncologic during 2019 and its revenue is constantly increasing (see Figure 3) [33]. Specifically, cancer medicine spending rose to $133 billion globally in 2017 with USA being the country with the highest spending on this (see Figure 4). In fact, in EU5 (France, Germany, Italy, Spain, UK), biosimilars of erythropoietin's and GM-CSF drugs are already widely available, reducing costs in supportive care, while therapeutic treatments continue to drive growth. As for in the rest of the world, the largest driver of growth was wider use of novel therapies [34].

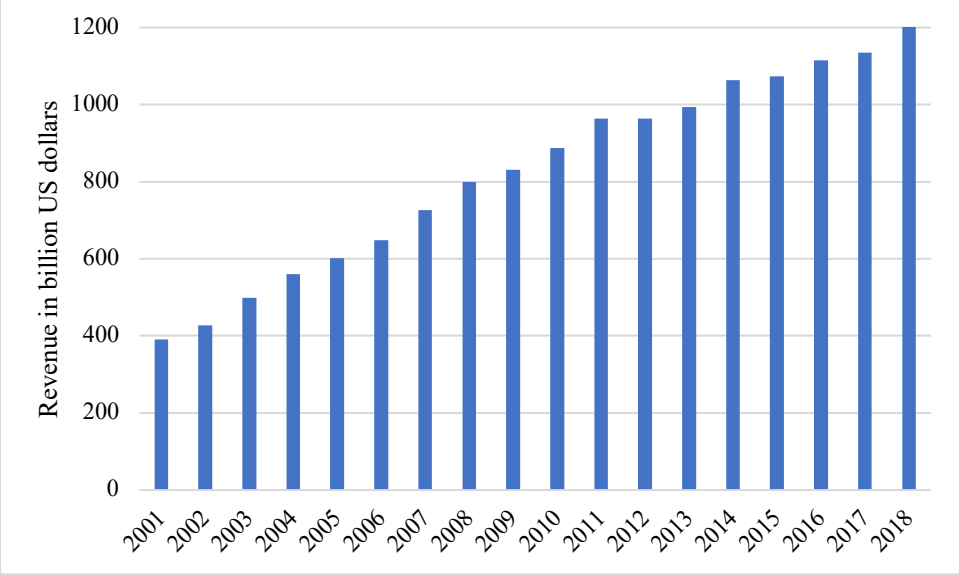


**Figure 3.** Revenue in billion US dollars of the worldwide pharmaceutical market from 2001 to 2018 [33].

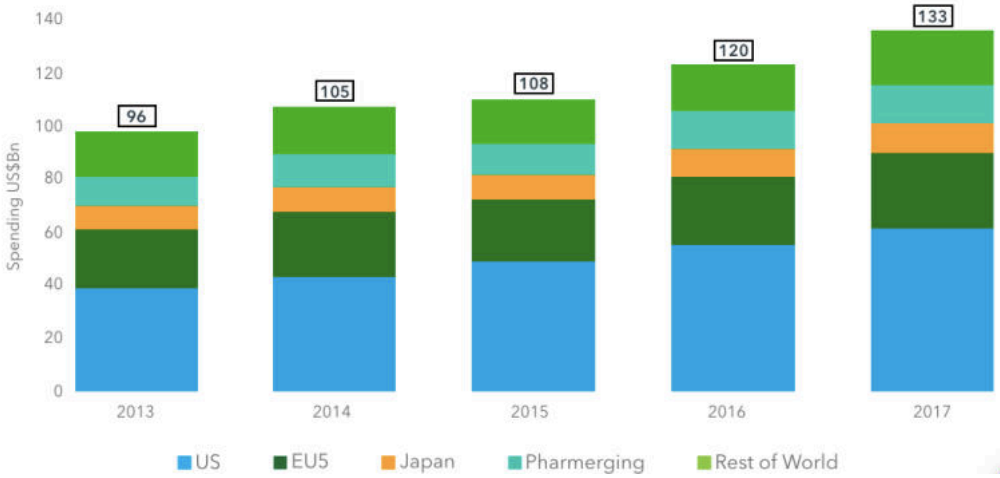


**Figure 4.** Money spent per year for cancer medicine, from 2013 to 2017. Source IQVIA MIDAS; IQVIA Institute, Dec 2017.

Over one-third of clinical trials are using biomarkers to stratify patients, pointing to more personalized cancer treatments in the future. Even though a lot of effort is put in the acceleration of the time it takes to bring a new cancer medicine to patients, drug approvals in 2017 had a median of 14 years, so there is a need of speeding up the whole process and my project might be a help. What is more, 700 organizations, from academic institutions

to large pharmaceutical companies, are active in late stage oncology research (see Figure 5), which is favorable for my target market [34].

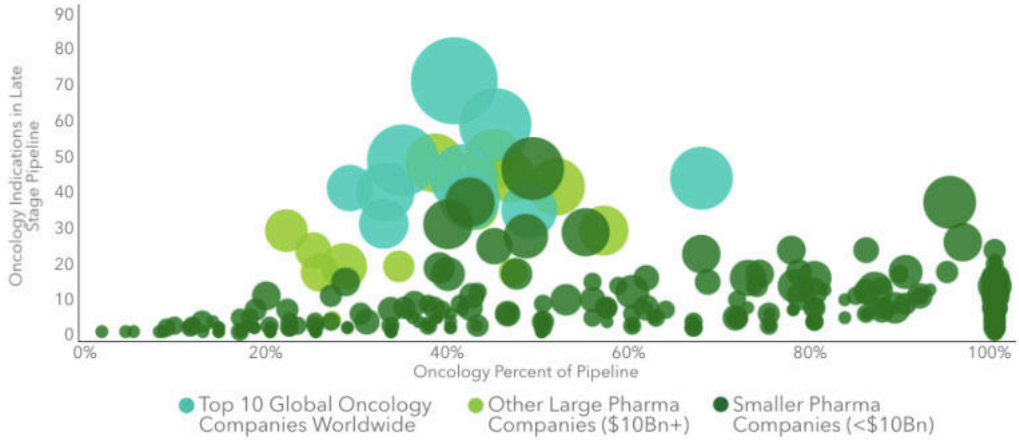


**Figure 5.** Different oncology and pharma companies in active late-stage oncology programs vs the percentage of pipeline. Source: IQVIA R&D Intelligence. Dec 2017; IQVIA Institute, Apr 2018.

The driving forces that will improve oncology treatment and improve costs over the next decade will be advances in technology (drugs and medical devices) and use of information (such as artificial intelligence and mobile apps) [34]. With this, global oncology therapeutic medicines will average 10-13% growth over the next 3 years (see Figure 6).

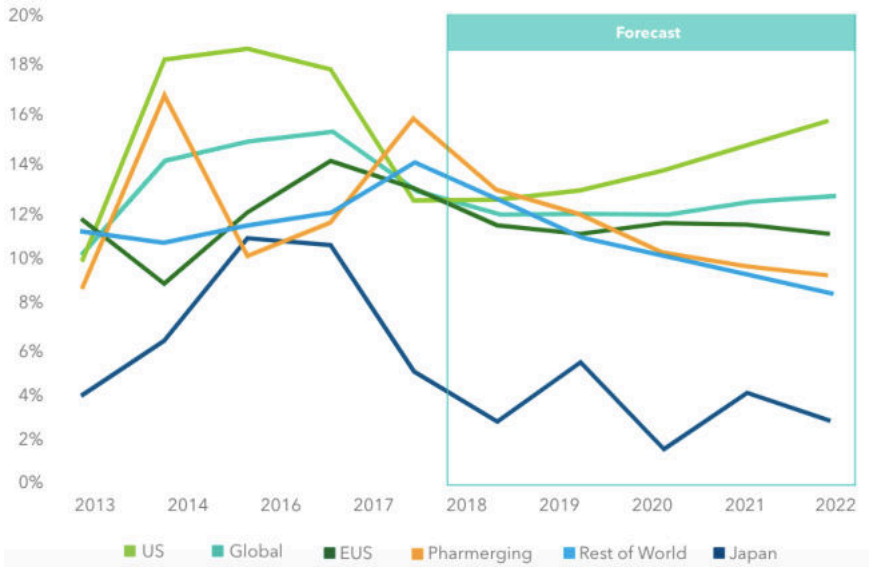


**Figure 6.** Forecast of the oncology therapeutics medicines' growth. Source: IQVIA Institute, Dec 2017.

Throughout the past, small animal imaging techniques have been used in order to study different human diseases. At present, small animal imaging represents an innovative research method able to study a wide variety of pathologies in which animal models of disease are used to clarify the mechanisms underlying the human condition and to allow a translational pharmacological – or other – evaluation of therapeutic tools [35]. Due to advances in US technology, commercially available US systems have the spatial and temporal resolution to obtain accurate images of rat and mouse hearts, kidneys, and other target tissues including tumor masses [36]. The use of US imaging for mouse models in cancer research has been increased during the last few years (see Figure 7). This means that this project might have good market viability as there is currently a demand which is becoming bigger.

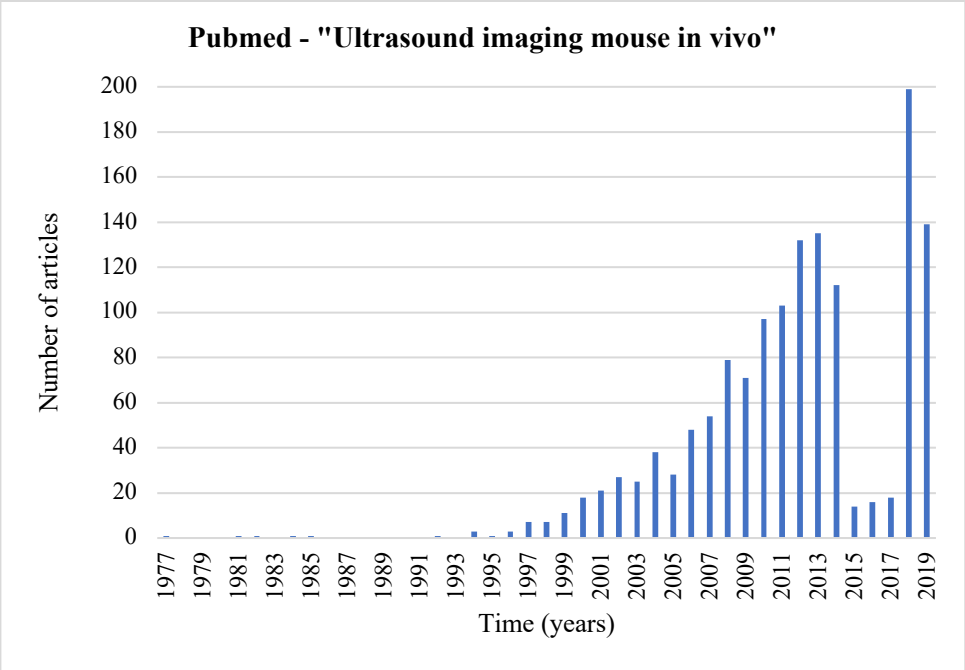


**Figure 7.** Evolution of the number of articles about the topic "ultrasound imaging in mouse in vivo" in PubMed. Source: PubMed.

Academia and pharmaceutical companies are increasingly expressing their interest in small animal molecular imaging because this kind of models constitute established research tools for molecular imaging and the biological validation of new therapies [37]. In this way, the final product of this project is mainly addressed to pharmaceutical companies and research institutes, which can be sold as a total product or as a way of consulting (service).

All of the above data shows that Schallware is in the correct market for the following reasons: number of diseases is increasing, there are more and more pharmaceutical companies that are in the preclinical stage for drug tests and the use of US for small animal imaging research is being a tendency so far.

This section will be further discussed in 2.2.1. and 3.2.1.

# 2. Methodology

The steps carried out in the project are explained in this section. The thesis was divided in two parts – technological and business part.

## 2.1. Technology

The technological part of the project describes the development cycle of the product and follows the same development process as all the products in the company. The process is based on an input (the mouse), which goes through several transformations (transformation process: designing, evaluating, testing, etc.) in order to obtain an output (the determination of 3D tumor volumes). With this, every step is connected so it is an iterative process – that means, repeated cycle of actions so that one can go back to make improvements. The horizontal dimension involves the tangible things (see Figure 8), and the vertical dimension the setup schedule in time (see Figure 9).

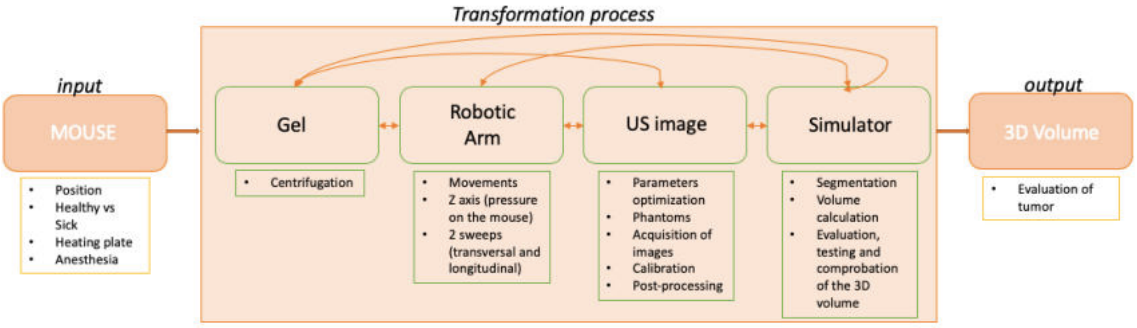


**Figure 8.** Horizontal dimension of the methodic construction for the project development process.

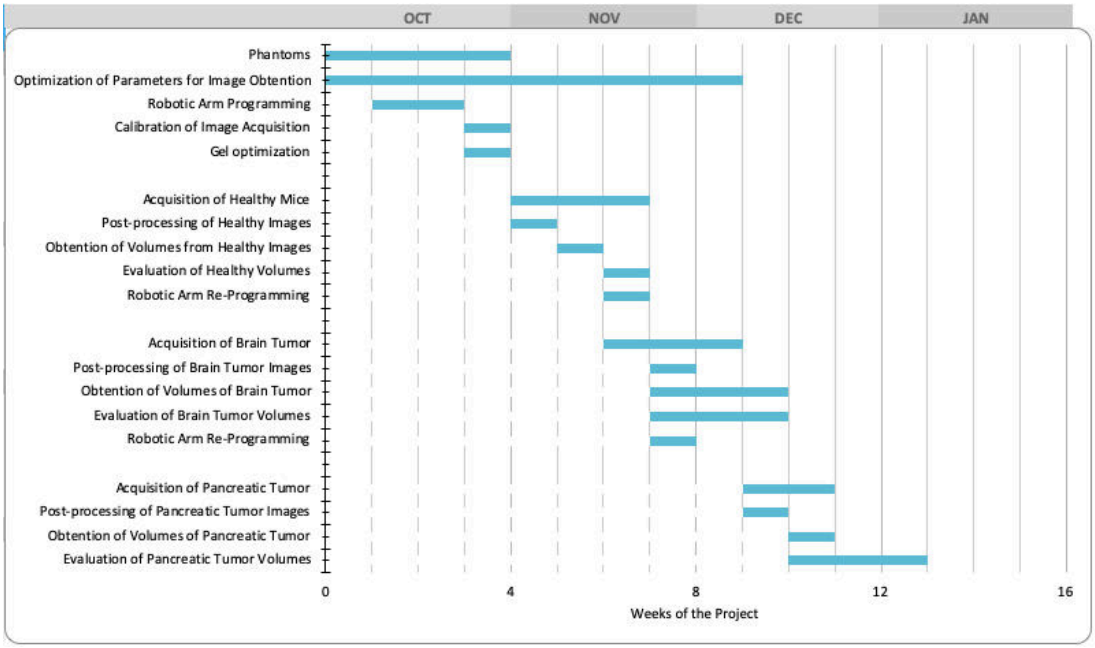


**Figure 9.** Vertical dimension (GANTT chart) of the methodic construction for the project development process.

### 2.1.1. Optimization of US parameters

My colleagues in Schallware already made a first attempt using the robotic arm for the US scanning of healthy mice from EPO, and identified two main problems: they realized it was not possible to see anything below the skull (see left image of Figure 10) and could not differentiate any organ in the abdomen (see right image of Figure 10). Apart from that, they observed that the air bubbles from the US gel disturbed the images.

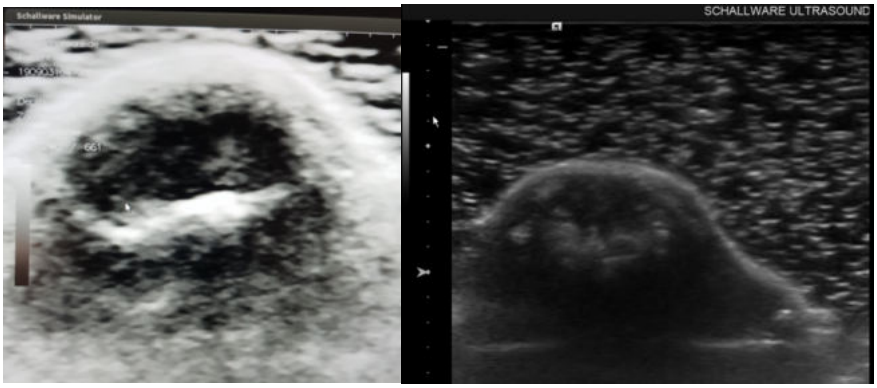


**Figure 10.** *Left:* image of the first attempt of mouse brain (post-processed data with normalized histogram). *Right:* image of the first attempt of mouse abdomen. Source: Schallware's unpublished data.

Having this in mind, the first step was to optimize the parameters for image acquisition in order to recognize the tumor in different organs. In order to do so, I worked with 3 kind of phantoms, where a chicken bone mimicked the mouse skull (see Figure 11).

**Figure 11.** *Left:* phantom made of pigmented silicone (in the left side, a hazelnut mimicked the brain, and in the right side, water did it). *Center:* phantom made of transparent silicone (a grape mimicked the brain). These first two phantoms did not work because the silicone reflected all the US waves. *Right:* final phantom made of a bone screwed on a piece of wood and a grape mimicked the brain.

The second step was to decide which transducer was the best for my approach. We asked SIEMENS for two different linear probes (9L4 and 14L5) and I compared the images obtained from them using the final phantom (see Figure 12 and 13). Both figures contain images after optimizing the parameters for image acquisition. The transducer 9L4 did not work well for my purpose as the minimum possible depth is 3cm, which is too big for the phantom and mouse, and its maximum frequency is 9MHz – not enough for small organs. Nevertheless, transducer 14L5 was able to differentiate the fibers inside the grape even though when it was below the bone, thanks to its maximum frequency of 14MHz; also, it was possible to use a depth of 2cm, which is the adequate for a mouse size.

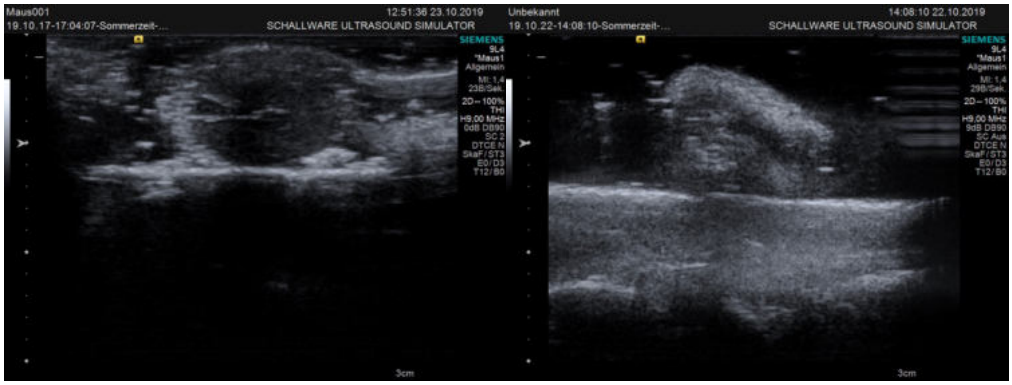


**Figure 12.** *Left:* grape without bone with the transducer 9L4. *Right:* grape below the bone with the transducer 9L4.

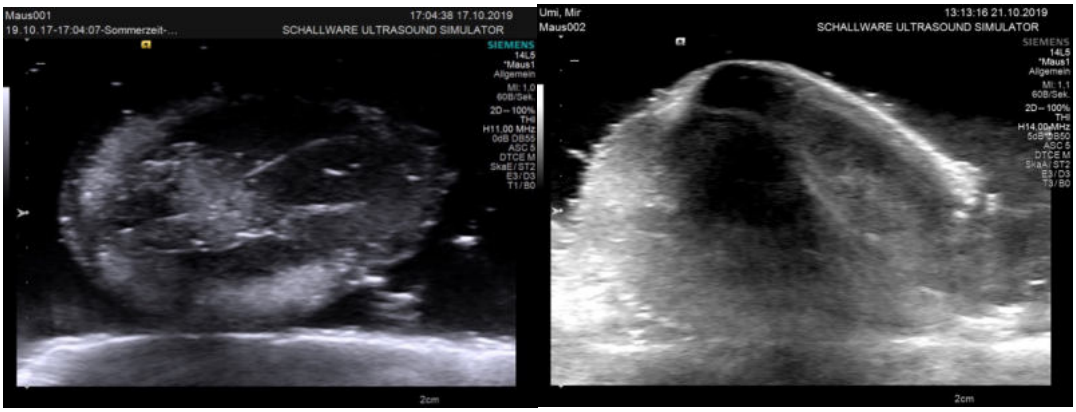


**Figure 13.** *Left:* grape without bone with the transducer 14L5. *Right:* grape with bone with the transducer 14L5. Both after optimizing the parameters for image acquisition.

Once the transducer and the phantom were set up, I had to change the parameters from the US machine in order to improve image resolution. The resolution is the quality of the equipment that allows us to see better the details of the image [38]. I used the SIEMENS ACUSON S2000 US machine, which provided several programs already set up for different human target organs. In the case of small animal imaging, to differentiate all small details is even more important, so I worked with different parameters until I could select the best values for mice imaging. Among all the parameters that can be changed in the device, the ones I used were the following: gain adjustment (for the overall brightness of the image), Time Gain Compensation (TGC, it adjusts the gain in specific areas of the image and is important to get rid of the reflections from skull bone), focal position (it specifies the depth at which I obtain the highest resolution), Tissue Harmonic Imaging (THI, it allows US to identify body tissue and reduce artifacts for a better quality), dynamic range (for the echo intensity displayed as shades of gray), Advanced SieClear (improves contrast resolution and border detection), energy, temperature and color [39]. I chose the B-mode (the brightness of the signal is proportional to the amplitude of the backscattered echo from the transducer) for the mouse scanning as it is the most common one for obstetrics and internal medicine and the image is easier to be interpreted, but M-mode could be a solution as well (it also shows a continuous time ramp applied to the horizontal axis). The results will be shown in section 3.

The last step to take into account was the US gel. As seen in Figure 10, the air bubbles caused by the gel disturbed the image resolution, so I needed to get rid of them. First of all, the gel was warmed up in order to make it more liquid, then it was put in a vacuum machine at 91.5kPa, and finally ethanol was poured on the surface so that the air bubbles could break. Unfortunately, this procedure was useful only for the superficial air bubbles. The total elimination of air bubbles finally came up when a centrifuge machine was used.

### 2.1.2. Image Acquisition

Once imaging parameters were optimized, the next procedure was to obtain all the scans. In order to do so, I needed a robotic arm system – responsible of holding and driving the transducer – and the "acquisition" software from Schallware – which recorded all the images obtained from the transducer's sweeps. (See Figure 18 of section 3.1. to better understand the set out of the devices).

The robotic arm used was the model LBR iiwa 7 R800 CR, from KUKA, and its software language was in java (using an IDE called Sunrise Workbench). It has seven joints and seven degrees of freedom. As it is a medical robotic arm, it is very sensitive to any contact

with the mouse. I developed two software in Java, one for the brain scanning and another for the abdomen (see Annex 1 and 2). Knowing the sizes of the mice and the plate where they lay on, I could calculate the positions the robotic arm's joints must follow. It is worth saying that after the first approach with healthy mice, we saw the importance of making two sweeps (transversal and longitudinal) in order to get as much information as it is possible. With this, I obtained two volumes in each scanning (the transversal and the longitudinal). Moreover, I needed a slow scan in order to avoid artifacts from any movement (breathing, blood flow, muscle reflex, etc.), so the optimum velocity was 2mm/s. As the transducer is in contact with the animal during the scanning, the stiffness of the robotic arm was set to 0, so that it could follow any surface's shape without making any pressure on the mouse. The robotic arm only starts its movements when we press the "start" button on the controller. We also use this controller to answer the programmed questions and to stop the movements when something unusual happens. See Figure 14.

**Figure 14.** *Left:* controller of the robotic arm showing the different programs you can select. *Right:* robotic arm with its controller.

The US device is connected to a computer, which is also connected to the robotic arm. In this way, all the images we can observe in the US device can also be seen and recorded in real time in the computer thanks to the "acquisition" software. An important step before making any scan was the calibration of the software, which allowed me to get real and accurate measurements. What I had to do was to set in the computer the same scale of sizes as in the US device. First of all, I adjusted the window of the "acquisition" software in the computer so that I had exactly the same dimensions as in the US device screen. Then, as the number of pixels in width (968px) and height (668px) in the "acquisition" window are known, I measured the width in cm of the US device screen for every depth (from 1cm until 5cm) and obtained the relation between cm and px (cm/px). In the end, I could obtain the same sizes and dimensions from the mouse in the US device screen and in the set of images recorded, which allowed me to measure volumes of tumors in real sizes.

The last step before calculating the volumes was the post-processing of the package of images obtained after the scan – that is, the volume. This was also made by the "acquisition" software. The post-processing follows three techniques: "remove doubles" (as the volume is made of several images, it is necessary to delete images which have the same information), "normalize grayscale" (to have a normalized histogram of gray values) and line-up of all the slices (it adjusts small movements of the mouse during the scan so that all edges of the objects in every image are aligned).

### 2.1.3. Volumes Determination

Once the images are post-processed, they are ready to be studied to find target organs or tumors. I used the Schallware simulator to observe the whole volume of images from each scan, which also provides the reconstructed images (images that are viewed from the perpendicular view).

For the determination of 3D volumes of tumors, I used the "segmentation" software from the Schallware simulator system. The target tumor must be manually segmented and then, the software provides the total volume, area of the surface, diameters and a 3D figure which you can turn around to observe its shape. "Segmentation" is based on the convex hull algorithm: knowing the coordinates of the slices chosen for the manual segmentation, it constructs the whole volume involving the smallest convex set containing all segmentations (see Figure 15 to better understand the algorithm).

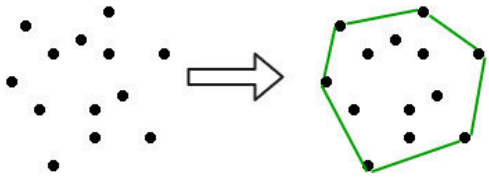

**Figure 15.** Convex hull algorithm for a set of points; it involves determining the smallest convex set ("convex hull") containing a discrete set of points [40].

In order to ensure that the "segmentation" software was calculating accurate measurements, I manually measured the grape from the phantom, scanned it and used the "segmentation" software to compare the measurements (see Table 1). The measurements varied a little bit because of the manual segmentation in the Schallware simulator system, but it was considered accurate.

| | Manual measurements | "Segmentation" measurements | % variation of "segmentation" in relation to manual measurements |
|---|---|---|---|
| Length | 1.9cm | 1.93cm | 1.57% bigger |
| Height | 1.4cm | 1.44cm | 2.86% bigger |
| Width | 1.5cm | 1.59cm | 6% bigger |
| Volume | $2.1cm^3$ | $2.3cm^3$ | 1.9% bigger |

**Table 1.** Comparison between manual and "segmentation" software measurements to check an accurate volume obtention.

### 2.1.4. Scans

When a project is developed in a company with the collaboration of another one, it is crucial to stay in contact and to keep everybody updated, and so I did. During my first month, I had a meeting with Prof. Dr. Wolfgang Walther (CSO of EPO) and Joshua Alcañiz (scientific researcher specialized in orthotopic brain tumor models) so that I could clearly understand their objectives and their current situation and set up our milestones. We decided to evaluate two kind of tumors (glioblastoma and pancreatic cancer, as examples of brain and abdominal tumors, respectively) and make three types of scans:

1. The first attempt was with 4 healthy NMRI nu/nu immunodeficient mice. I scanned the whole bodies so I could obtain images from both, brain and abdomen. We decided to use healthy mice so that afterwards I could compare them with images containing tumors and to make any improvements to the entire procedure. As explained before, thanks to this first approach I saw one scan for each mouse was not enough, so I programmed the robotic arm to make two sweeps each time (transversal and longitudinal). Additionally, I added a function where the robotic arm asks whether the mouse is well positioned before continuing with the scan so that I could ensure the target organs in the center of the images.
   After this first attempt, we realized we needed to fully understand the anatomy of the mouse to correctly locate each organ and future tumors, so we took more time than expected to study the position of all the organs.
2. The second attempt was with 4 mice with brain tumors (glioblastoma). As the age affects the hardness of the skull (the older, the harder) and so the image of the brain below the bone, two of them were female and 12 weeks old and the rest were male and 8 weeks old. The orthotopic tumor transplantation was made through a small incision in the forehead at the same time in all mice (see Figure 16), so when the females were 10 weeks old and males were 6 weeks old. The brain tumor cell line was U87MG. Apart from making normal scans, we also tried to use microbubbles to enhance the contrast of the image and to better see the vessels inside the brain. In order to do so, it was important to scan the brain with the probe in a static position. We injected 50µl of VEVO MicroMarker Contrast Agent VisualSonics to each mouse.
   The mouse must be in prone position for a correct scan (see Figure 17). As we put gel all over the head, we built a small plastic tube adjustable to the mouse's snout so that it could breathe during the scan.

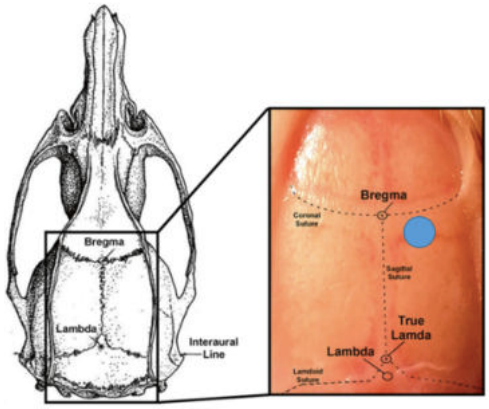


**Figure 16**. The blue circle indicates the tumor injection site [41].

**Figure 17.** Prone position of the mouse for brain tumor scan. Notice the plastic tube for breathing. The green plastic is the heating plate.

3. The third and last attempt was for abdominal scans. Here, the age does not make any difference for the image observation, so I got three 10 weeks old female mice. The transplantation of the pancreatic tumor (human cell line PANC-1) was made through a cut in the abdomen and injection to the pancreatic tail. Mouse must lay on its right side to have an optimal orientation for scanning the pancreatic tail (see Figure 18).

**Figure 18.** Mouse lying on its ride side for pancreatic tumor scan.

It is important to note that we anesthetized the mice before each scan with Hypnomidate 2mg/ml, using a dose of 20mg per kg of body weight. Once the mouse is anesthetized, it does not have muscle reflexes during 10-15min, so this is the time we had to make each scan. Apart from that, we used a heating plate and warmed the gel up before the scans so that the mice would not be cold. All results will be shown in section 3.

### 2.2. Business Plan Study

The last objective of this master thesis project was to assess the market viability of the proposed product. As every decision can be risky for the company, especially if the project is entirely new, it is crucial to make prior studies before taking actions. Most of the time, these studies are economic-related analysis and some of them are explained below.

### 2.2.1. Market Analysis

In every kind of business, the first step to take into account is the market analysis in order to ensure that the product we want to sell is fulfilling the demand. Having a clear idea of the market we are addressing to might save money, time and effort. In order to do so, I followed an analysis known as TAM/SAM/SOM. These three acronyms represent different subgroups of a market: TAM (Total Available Market) is the total market demand for a product or service, SAM (Serviceable Available Market) is the segment of TAM targeted by my product which is within my geographical reach, and SOM (Serviceable Obtainable Market or simply Target Market) is the portion of SAM that one can really capture (see Figure 19). This analysis allowed me to have an initial estimation of the market opportunity of my product, and it is especially important for start-ups, or companies that want to provide a new product to a new market segmentation, because it is useful to investors when assessing an investment opportunity.

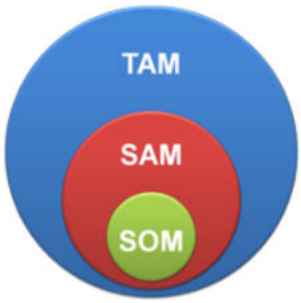


**Figure 19.** Structure of the TAM/SAM/SOM markets segmentation [42].

Among the three kind of markets, the most important for the current project is SOM as it is the short-term target. This decides whether my product is viable in the market or not because if the product does not even succeed in the local market, it will never capture a large part of the global market. SOM must be based on the product, the marketing plan and distribution channels and the strength of the competition [42].

Once SOM is set up and therefore the short-term sales potential, SAM has the purpose of target market share, and TAM enables to assess the upside potential. If these three markets are well defined, the company will work efficiently.

Even though we doubted whether we were going to sell a product or a service, it was easy to assess the target market as our objectives were clear from the beginning.

### 2.2.2. SWOT and CANVAS

To assess the market viability of the new product developed here, a SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis is one of the most useful techniques. This strategic planning technique allowed me to see my advantages and disadvantages, regarding the product, compared to my competition. Normally, it is necessary to gather people from different sections of the company in order to make the SWOT analysis, so I asked my Schallware colleagues the following questions regarding the product for a brainstorm:

- What do we do well?
- What can we improve?
- What trends can we take advantage of?
- What is our competition doing?

The CANVAS business model also helped me to establish the sections I needed to take into account for the business model of the new product. It was a bit difficult to complete all the gaps of the CANVAS as this project provides a totally new product which Schallware has never worked with, but we managed it taking into account that we had as many value propositions as customer segments and they had to fit between them. In this way, before building the CANVAS, we tried to completely understand our customer by asking ourselves how we were helping them (customer jobs, pains and gains) so that we could create value afterwards by studying what we were providing them as a solution (products and services, gain creators and pain relievers). See Figure 20.

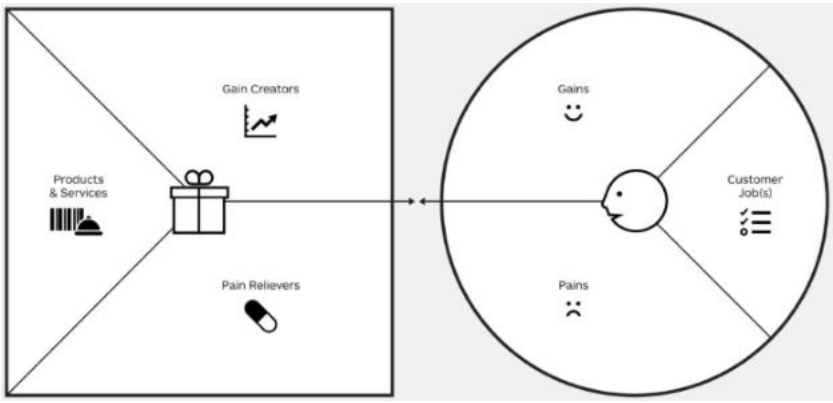


**Figure 20.** The value proposition model. The left side is the value proposition that needs to be fit with the right side, the customer segment [43].

### 2.2.3. COGS and Time Study

Finally, I developed a hypothetic COGS (Cost of Goods Sold) scenario for the first 5 years so that we could have an idea of the gross margin of every year.

We expected to start selling 5 units during the first year and increasing this number during the following years, assuming that the business will be positive. We decided to set the initial price of the developed product to 250000€, which would be increased a 2% every year.

Even though the product is thought to be sold as a unique piece, it is divided in three parts: the robotic arm, the US device (which already includes the transducers) and the acquisition system. Thus, Schallware has three costs, which initially are: 12000€, 50000€ and 3000€, respectively (these prices were estimated by the company itself). The scientific devices, such as the robotic arm and US device, decrease their cost with time because new models with more advanced features appear in the market; that is why their costs in the table are set to be decreased a 3% every year. Moreover, we also have economies of scale and we took it into account as if we are able to get more and more customers, the costs will be able to be reduced throughout the years.

As said in the introduction section, one of the main objectives of this project is to save time for the EPO researchers so that they can evaluate more animals in less time. In the end, this aim is crucial in order to save money, so I also made a time study to compare the time used in the current procedure used in EPO, with the new procedure. However, EPO was not able to provide me some monetary data as some things are private, so the resulting table was not fully detailed.

# 3. Results

This section shows the results of all the project divided in two sections again: technology and study of the business.

## 3.1. Technology

Full product setup is represented in Figure 21.


**Figure 21.** All the components of the product set up for a scan. Notice that both, robotic arm and US device, are connected to the computer for the scan recording.

### 3.1.1. Example of Product Utilization During a Scan Session

Previously to the scan, we need to open the "acquisition" software in the computer and select the new patient's name, the date, the type of transducer, and the image's depth and mode that we are going to use (see Figure 22).

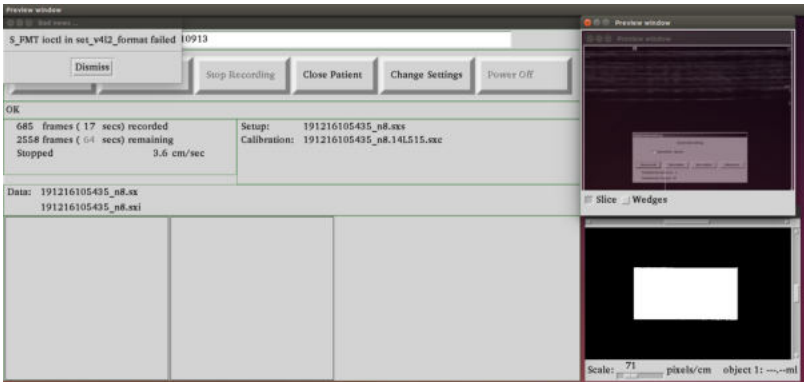


**Figure 22.** Screen of the "acquisition" software for entering patient's details and images recording. Source: Schallware.

Once the mouse is anesthetized and lying on the heating plate, we gently pour warm gel without air bubbles on it and we are ready to start the scan. First of all, we need to select the program we want to use depending on the organ we want to scan – "MausScanningBrain" or "MausScanningAbdomen" –, and then we can click on the "start" button in the controller (see Figure 14). The robotic arm, which holds the 14L5 transducer, starts going down slowly until it reaches the body and makes a transversal movement until it stops in the middle – in the case of brain scan, the center is in the first 1.5cm (total scan of 3cm), while for abdominal scan, the robotic arm stops at 3cm (total

scan of 5cm). At this point, the message “Press OK if everything is in the correct position” appears on the controller, which allows us to ensure the correct position of the mouse so that we can have the target organ in the center of the US image by slightly moving the mouse while we observe the US screen. After pressing “OK”, the robotic arm moves to the initial position and makes the transversal scan in a velocity of 2mm/s. Then, the robotic arm goes up, rotates 90º, goes to the center of the target organ and goes down again to start the longitudinal scan. “Acquisition” program automatically starts and finishes recording all images from the beginning until the end of each scan, so for every mouse we can have at least two volumes (transversal and longitudinal). When the scan finishes, we wash the mouse and put it in its cage again. The next step is to post-process the images (see Figures 23 and 24).

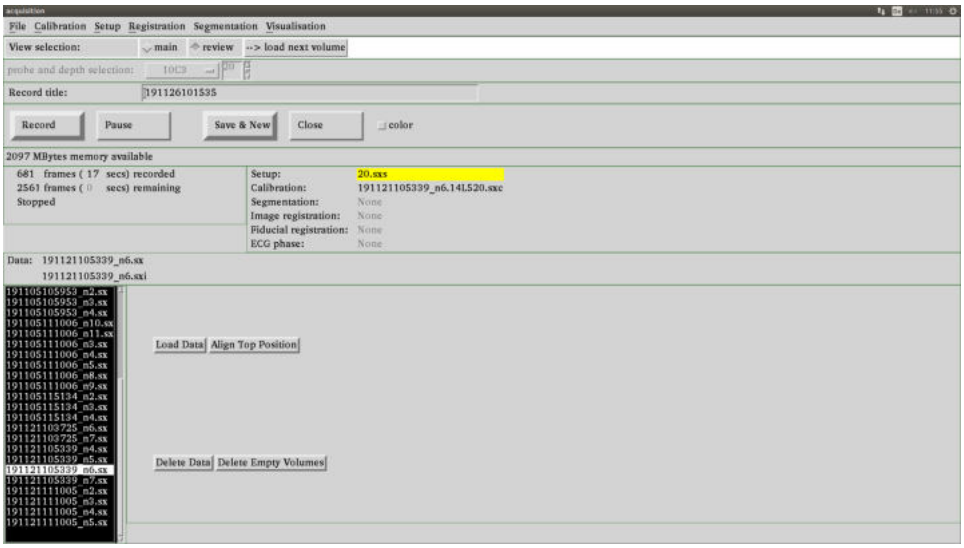


**Figure 23.** Screen of the “acquisition” software for the images post-processing. Source: Schallware.

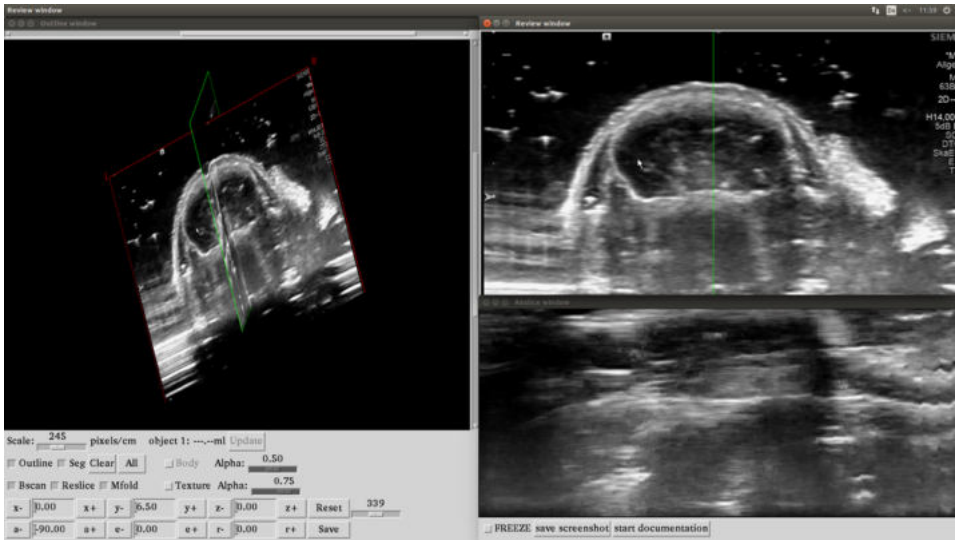


**Figure 24.** Post-processing of brain images. The image at the bottom right corner shows the transversal perspective automatically generated by the “acquisition” program.

Post-processed images must be loaded to the Schallware simulator system so that we can evaluate the whole volume of images obtained and make the segmentation of the regions of interest (see Figure 25). Finally, we obtain the 3D volume with its volume and area values in $cm^3$.

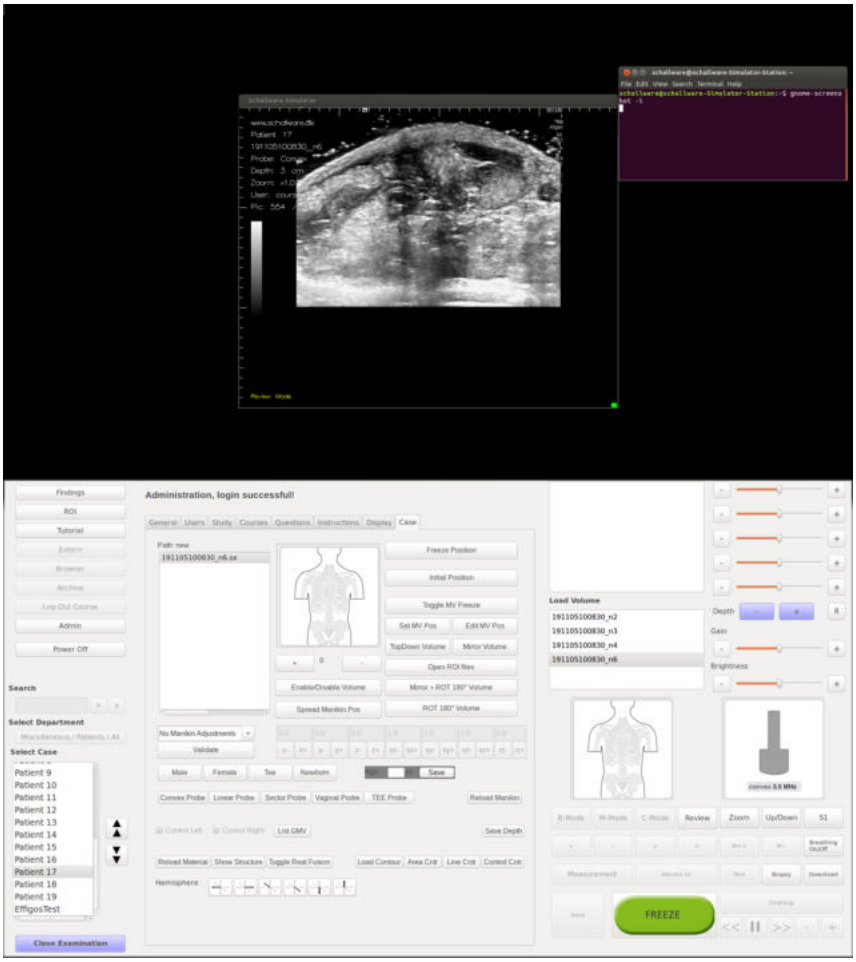


**Figure 25.** Screens of Schallware simulator system. Source: Schallware.

The results obtained from both kind of tumors are explained below.

### 3.1.2. Brain Tumor

The final parameters for brain US images are shown in Figure 26. Main possible artifacts that can appear because of the bone are: shadowing (US wave loses energy through the bone, so everything below gets dark), and reverberation or mirroring (the image is reflected, so everything one sees below the skull is wrong, no information). In order to avoid the first layers of skull reflection, I regulated the time-gain compensation so that I could increase the signal-to-noise (SNR) ratio to the depth of interest. I also set the focus at the depth of the region of interest so that I could get more resolution there. The energy was set to 39% as it was the optimal to differentiate the structures inside the brain. The mechanical index (MI) automatically decreased to 0.8 as it is related with the energy. By decreasing the energy, the crystals inside the transducer oscillate less because the frequency remains the same, so the amplitude of the waves are smaller.

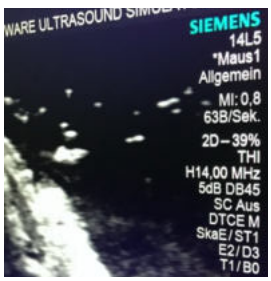


**Figure 26.** Parameters for brain tumor scan.

EPO provided me the cresyl violet stained sections of the previously US scanned brains, showing the position, size and shape of the U87MG glioblastoma within the brain, so that we could compare them with the scan. But brain images turned out to be really difficult to understand and evaluate because of the possibility of artifacts. We even added a new function in the Schallware simulator system which inverted the scale of grays to better differentiate the structures in the brain while the comparison between the scans from brain with tumor and healthy brain, but it did not solve our doubts either (see Figure 27 and 28). Therefore, we asked for help to a few doctors who collaborate with Schallware. They helped us to find the structures inside the brain, but it was not possible to find the tumors. Moreover, we realized the importance of the mice age for brain scan – older mice (12 weeks old) had thicker skull so we could not see anything below it, but with younger mice (8 weeks old) we could see some structures inside.

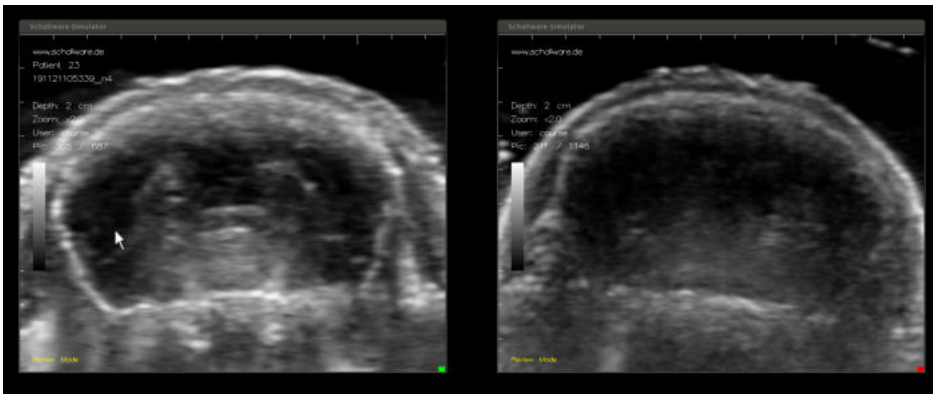

**Figure 27.** Comparison of US images from young brain with tumor (left) vs young healthy brain (right).

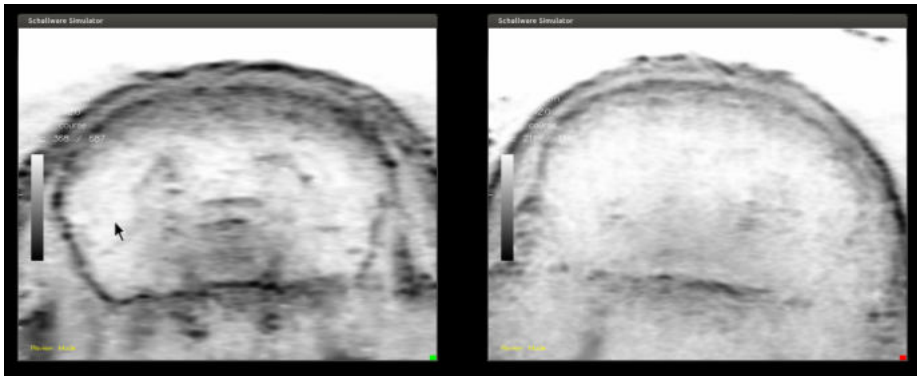

**Figure 28.** Comparison of US images from young brain with tumor (left) vs young healthy brain (right) using the new function of gray-scale inversion.

One of the differences we can detect by evaluating the images in Figure 27 is the difference of brightness of the first brain layer, the cortex. As the glioblastoma is normally growing in the cortex, caudoputamen and in contact with or growing into the right lateral ventricle, we though that a possible explanation could be that it is an artifact provoked by the liquor. The tumor might have changed the composition of that part of the brain.

As we could not see any structure within brains of old female mice (see Figure 29), and mouse male 1 had no tumor engraftment, only results from the second male mouse of 8 weeks old are shown below. Left images from Figure 30 to 34 are from atlas brain map [44]. Images in the center in same figures are unpublished data from EPO [45]; keeping in mind that mice were sacrificed after the study. Volume obtained from stained brain sections was about 0.41mm$^3$, estimated from cones and frustums using all areas and

positions. Notice again that the tumor is growing throught the cortex. This could be a reason why the cortex is seen very bright in the US scan.

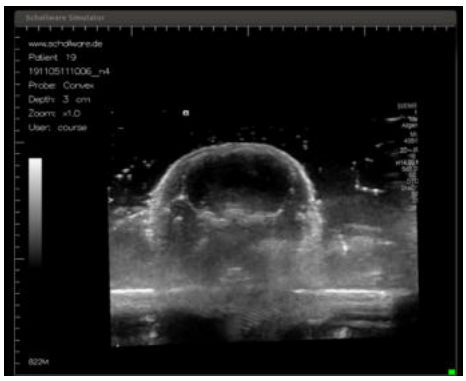

**Figure 29.** US image shown by Schallware simulator system of an old (12 weeks old) mouse brain scanned *in vivo* with our product. Notice that it is not possible to differentiate any structure within the brain because of the skull thickness.

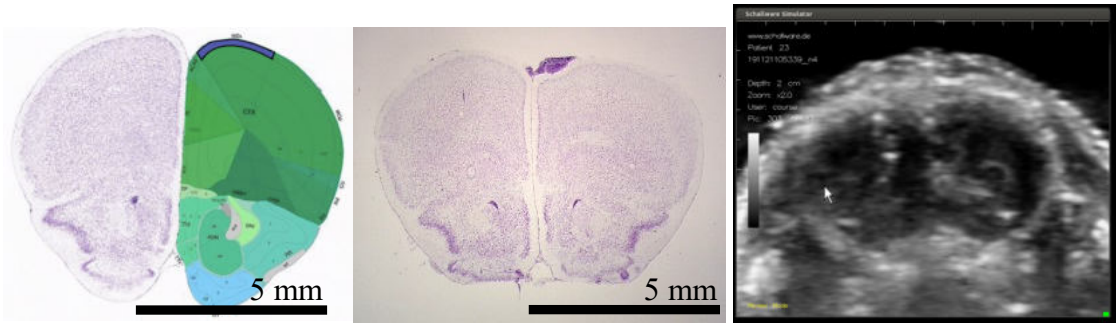


**Figure 30.** *Left:* image 35/132 from mouse brain atlas map; the purple zone is the first layer of the secondary motor are, where the tumor should be. *Center:* first brain slice containing glioblastoma in dark purple made generated at EPO. *Right:* US scan of the mouse brain made with our product, image 303/687 obtained by Schallware simulator system (from top to bottom, it measures 1cm).


**Figure 31.** *Left:* image 43/132 from mouse brain atlas map. *Center:* second brain slice made at EPO. *Right:* US image 337/687 made with our product.

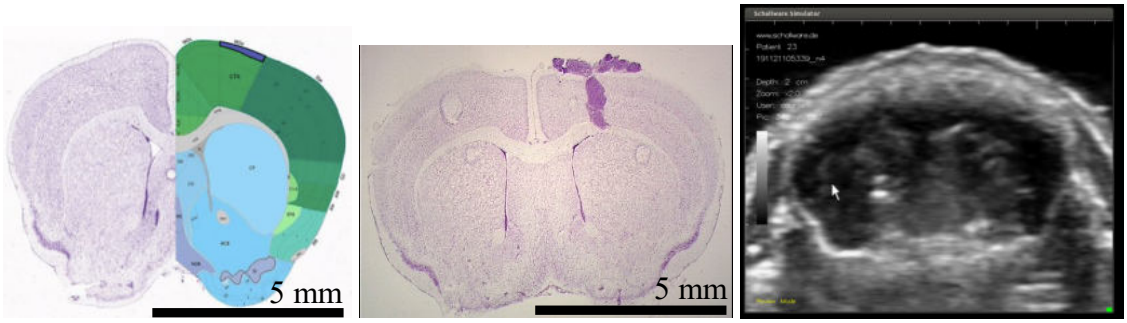


**Figure 32.** *Left:* image 45/132 from mouse brain atlas map. *Center:* third brain slice made at EPO. *Right:* US image 342/687 made with our product.

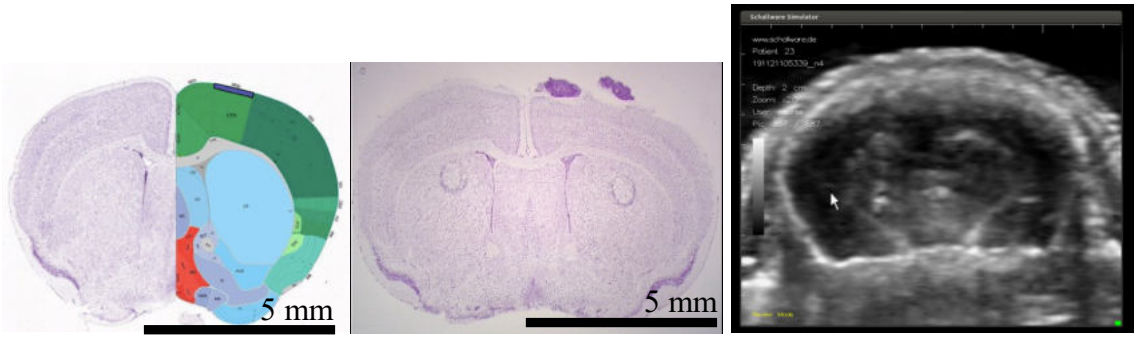


**Figure 33.** *Left:* image 50/132 from mouse brain atlas map. *Center:* forth brain slice made at EPO. *Right:* US image 357/687 made with our product.

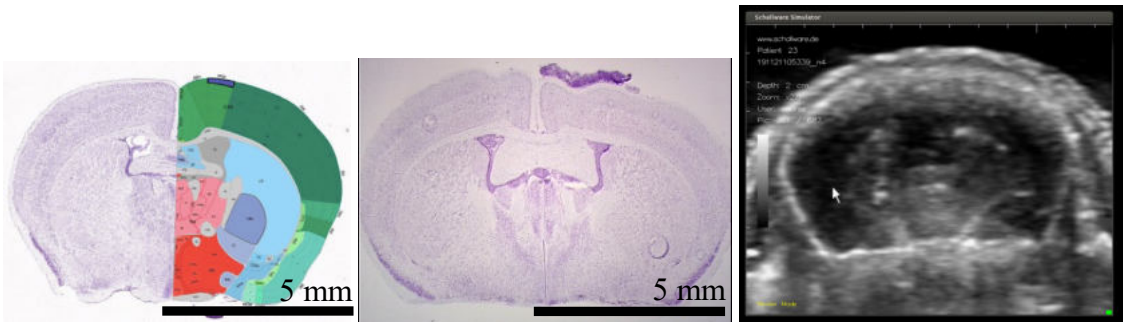


**Figure 34.** *Left:* image 59/132 from mouse brain atlas map. *Center:* last brain slice made at EPO. *Right:* US image 361/687.

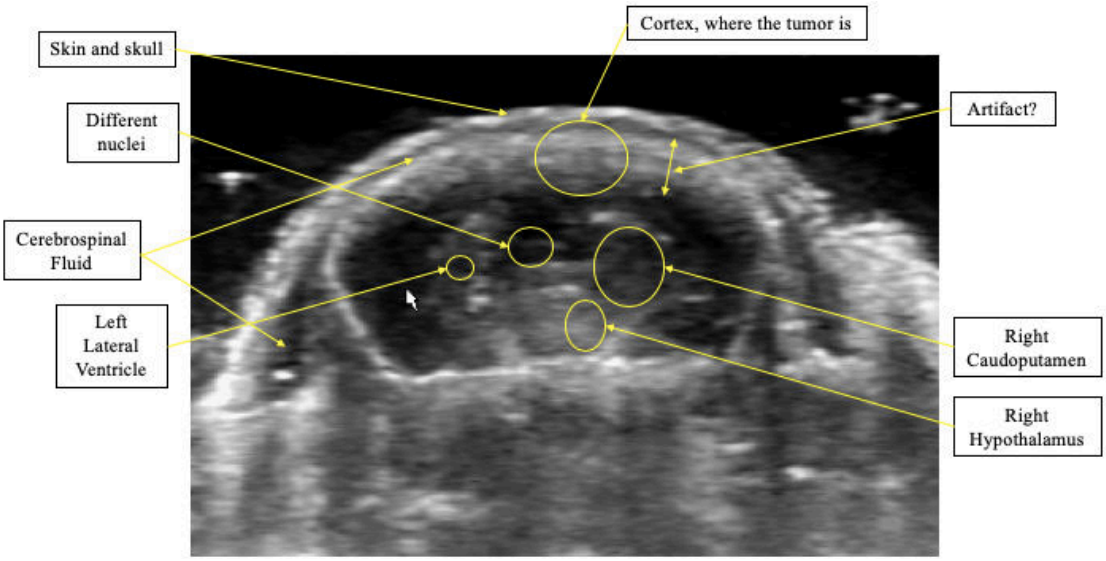


**Figure 35.** Different structures observed inside the mouse brain from US scan *in vivo.*

We could not obtain any volume from brain scans, but we could differentiate several structures in it. This is a good result, but still needs few further improvements.

### 3.1.3. Pancreatic tumor

The final parameters for pancreatic tumor US images are shown in Figure 36. The only difference with parameters for brain scan is that a 100% energy was used with a MI of 1.1.

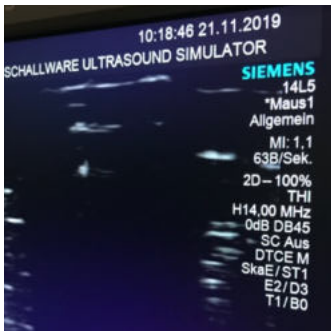


**Figure 36.** Parameters for pancreatic tumor scan.

Before finding the tumors, we needed to understand the anatomy of the mouse in order to find all structures in the images obtained from the scans. See Figures 37 and 38.

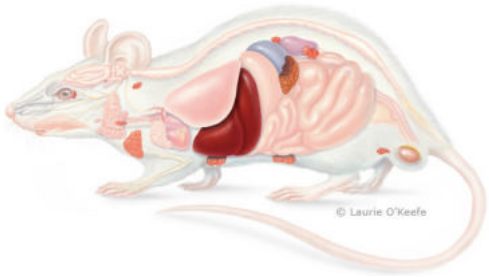


**Figure 37.** Anatomy of the organs of a mouse [46].

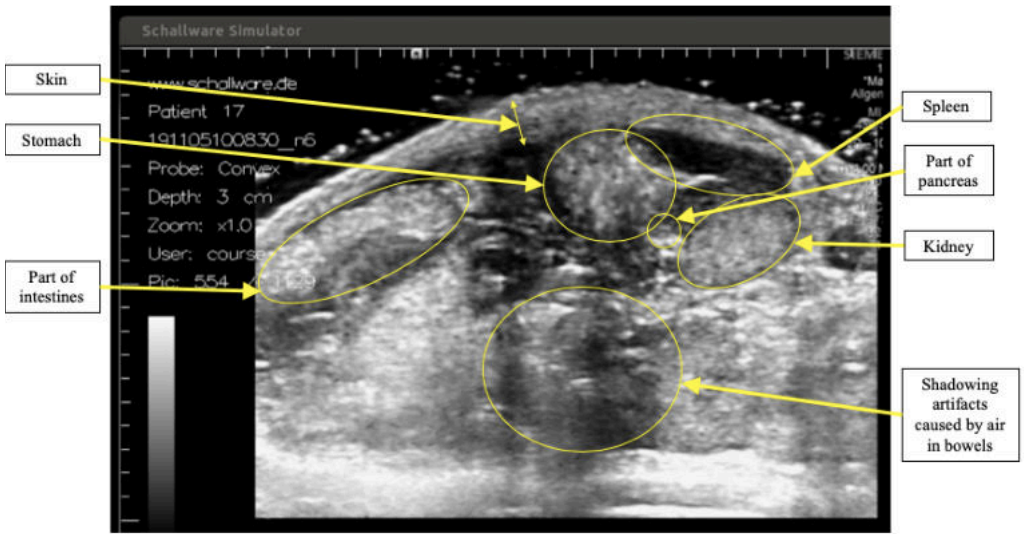


**Figure 38.** Different structures within the healthy mouse abdomen from US scan *in vivo.*

To better find the tumors in the US images we compared them with the images in the same position of scans from healthy mice, as well as with the dissection images provided by EPO when the study finished [47]. See following figures.

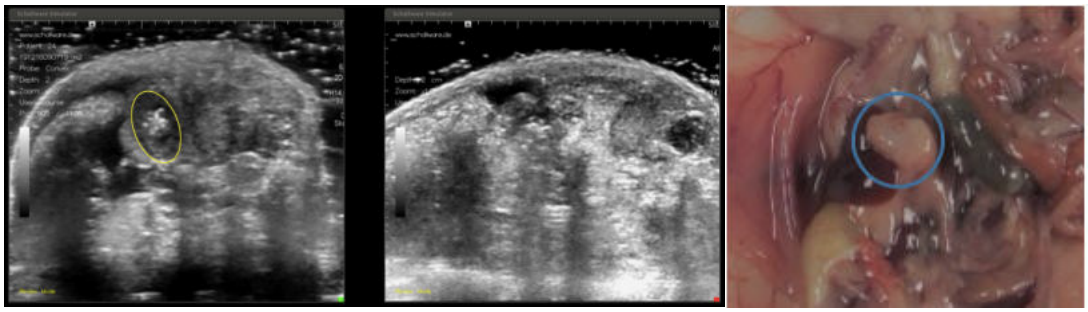

**Figure 39.** *Left:* abdominal scan *in vivo* of the first mouse; the tumor is inside the yellow circle. *Center:* abdominal scan of a healthy mouse *in vivo*. *Right:* dissection of the first mouse; tumor is inside the blue circle, which is in contact to the pancreas, at the ventral end of spleen, in proximity to the left kidney.

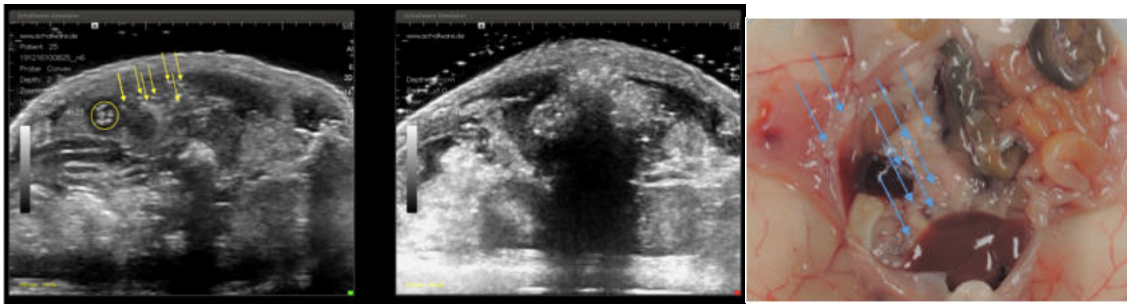

**Figure 40.** *Left:* abdominal scan *in vivo* of the second mouse; tumors are inside the circle and tagged with the yellow arrows. *Center:* abdominal scan of a healthy mouse *in vivo. Right:* dissection of the second mouse; tumors are tagged with the blue arrows. This mouse had various small metastasis of about 1-2mm diameter within the pancreas, on the spleen, the peritoneum and mesentery.

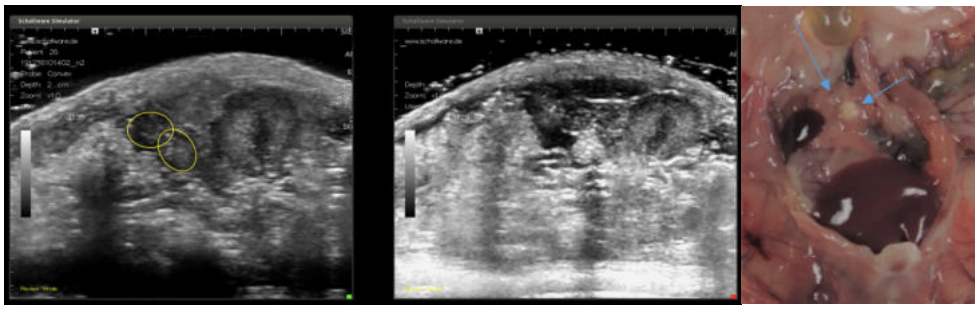

**Figure 41.** *Left:* abdominal scan *in vivo* of the third mouse; tumors are in yellow circles. *Center:* abdominal scan of a healthy mouse *in vivo. Right:* dissection of the third mouse; tumors are tagged with the blue arrows. This mouse had two neighboring tumors within the pancreas; one translucent and one white.

Pancreatic tumor 3D volumes and their information are shown below.

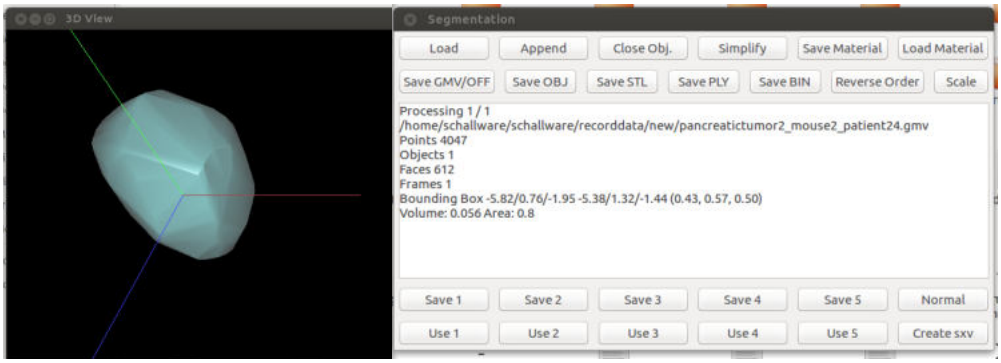


**Figure 42.** *Left:* 3D volume of pancreatic in mouse 1; in the Schallware simulator system it is possible to rotate the volume so that you can evaluate its shape. *Right:* measurements information provided by the "segmentation" software in $cm^3$; bounding box means the diameters of each axis (x, y and z).

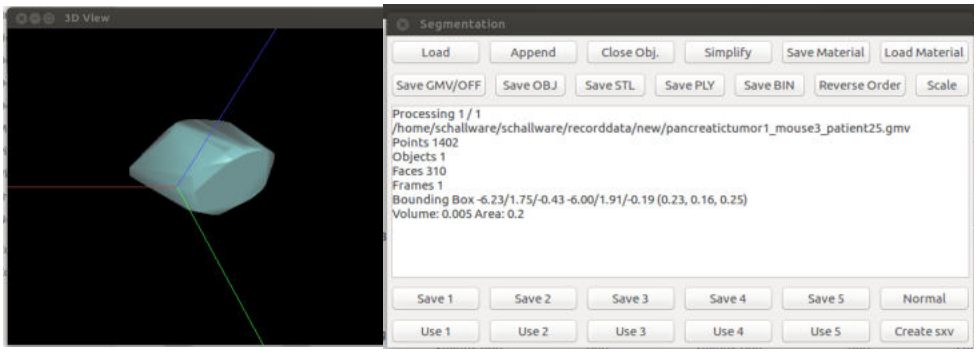


**Figure 43.** *Left:* 3D volume of the biggest pancreatic tumor in mouse 2; it is the one tagged inside the yellow circle in left image of Figure 40. *Right:* measurements information from "segmentation" software in $cm^3$.

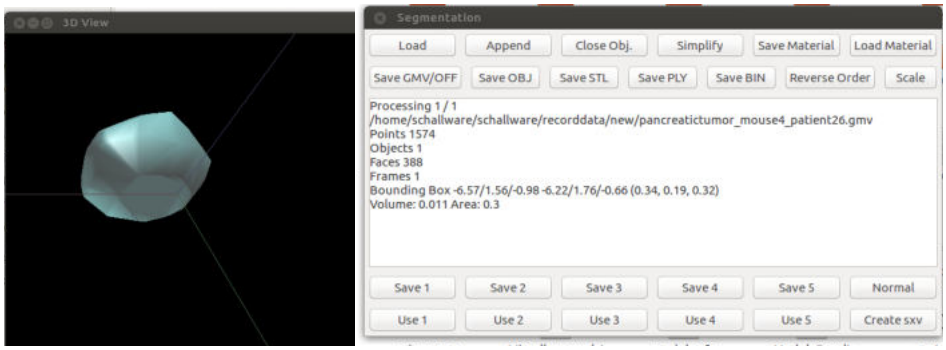


**Figure 44.** *Left:* 3D volume of the lower pancreatic tumor in mouse 3, tagged inside the lower yellow circle in left image of Figure 41. *Right:* measurements information from "segmentation" software in $cm^3$.

Diameters of each tumor measured at EPO with a scale bar after dissection from sacrificed mice are 5mm, 1 to 2mm, about 2mm each one for first, second and third mouse, respectively.

| | Volume obtained with our product | Theoretical volume | % variation of our volume in relation to theoretical calculation |
|---|---|---|---|
| Mouse 1 | $0.056cm^3$ | $0.065cm^3$ | 13.8% smaller |
| Mouse 2 | $0.005cm^3$ | $0.004cm^3$ | 25% bigger |
| Mouse 3 | $0.011cm^3$ | $0.008cm^3$ | 37.5% bigger |

**Table 2.** Volumes of tumors. Theoretical volumes are measured with sphere formula using diameters measured with scale bar.

## 3.2. Business Plan Study

This subsection includes the results obtained from the economic evaluation for the assessment of new product/method's market viability.

### 3.2.1. Market Analysis

The market analysis started deciding who my TAM was. Schallware's potential objective was to sell the product mainly to pharmaceutical companies and private research institutes because they have budget enough to buy the product. Nevertheless, we also thought about universities and clinics as possible customers. Thus, the TAM was the worldwide healthcare research field. Going more into detail, the top 10 worldwide pharmaceutical companies and oncologic research institutes are listed in the tables below:

| # | Pharmaceutical company | Market capital | Growth | Country |
|---|---|---|---|---|
| 1 | Johnson &Johnson | $345.907 billion | -10,7% | USA |
| 2 | Novartis | $226.539 billion | 0,3% | Switzerland |
| 3 | Merk & Co. | $216.409 billion | 11,4% | USA |
| 4 | Roche | $211.513 billion | -1,6% | Switzerland |
| 5 | Pfizer | $205.039 billion | -18.9% | USA |
| 6 | AbbVie | $128.791 billion | -4.4% | USA |
| 7 | AstraZeneca | $124.4 | 21.3% | UK |
| 8 | Sanofi | $114.421 billion | 1.1% | France |
| 9 | GlaxoSmithKline | $110.224 billion | 9% | UK |
| 10 | Eli Lilly | $108.677 billion | -8.6% | USA |

**Table 3.** Top 10 worldwide pharmaceutical companies ranked by their market capital [48].

| # | Research Institute | Country |
|---|---|---|
| 1 | Hospital of the University of PA – Abramson Cancer Center | USA |
| 2 | Roswell Park Cancer Institute | USA |
| 3 | Johns Hopkins Hospital Sidney Kimmel Comprehensive Cancer Center | USA |
| 4 | University of Texas MD Anderson Cancer Center | USA |
| 5 | Memorial Sloan-Kettering Cancer Center | USA |
| 6 | Wake Forest University Baptist Comprehensive Cancer Center | USA |
| 7 | Princess Margaret Cancer Centre, University Health Network | Canada |
| 8 | USC Norris Comprehensive Cancer Center | USA |
| 9 | Christie Hospital NHS Foundation Trust | UK |
| 10 | Spanish National Cancer Research Center | Spain |

**Table 4.** Top 10 most technologically advanced cancer centers in the world [49]. Thus, more likely to buy our innovative product.

Observing the previous information, we saw that our total global market was centralized in USA, Switzerland, UK, Canada and Spain – for us, it might be interesting to expand

our market there (see Annex 4 for more information). But the available market that we could reach (SAM) was smaller. We would rather focus on a few European biotech companies, such as QPS (Austria), Experimentica (Finland), Dreudenberg (Germany), Pfizer CentreOne (Ireland), Recipharm (Sweden) and Quotient Sciences (UK).

As for the SOM, it is based on actual customers of the company or the ones that probably can be our customers soon. Schallware already has AbbVie and Pfizer as pharmaceutic customers, so we could try starting to sell the product to them; and EPO is our collaborator and potential customer.

### 3.2.2. SWOT and CANVAS

The SWOT is composed by two types of features: the internal ones (strengths and weaknesses), which I have some control over and can change; and the external ones (opportunities and threats), which are the things that are going on outside our company, in the larger market [50]. The results of the SWOT analysis are listed below:

Strengths:

- Results: volumes are quickly obtained and accurate and the resulting 3D image can be rotated to see the full shape and the direction of growth of the tumor. It provides high-quality data and easily quantifiable and rich data for a strong statistical power (necessary for pre-clinical studies). It is possible to track the tumor growth in any tissue which is not below a bone, without the need of sacrificing the mice. It allows the researchers to test more drugs in less time because of the fast results.
- Innovative product: it is the only one that can make longitudinal and transversal sweeps and allows the observation of structures inside the brain.
- User-friendly and minimal training.
- Repeatable: the volumes can be obtained as much as it is necessary.
- Non-invasive.
- Cheaper than MRI.
- Experience: Schallware has already been doing business with pharmaceutic companies for almost 20 years.

Weaknesses:

- Product not able to find glioblastoma.
- Unadaptable to all types of tumors and animals.

Opportunities:

- Market trends: the use of US in the research field is increasing year by year, oncologic therapeutic medicines will increase in the next few years and pharmaceutic companies are increasing their sales in oncology.
- Technology: scientific US devices and specific probes are already available in the market, so we can add them to achieve new features.
- We are having some upcoming faire where we can promote the new product.

Threats:

- Competition: the Vevo imaging systems from FujiFilm VisualSonics are similar products already in the market.

- Suppliers might increase the price of my resources (robotic arm, US device).
- Even though the US is cheaper, customers can opt to change to MRI.

As for the CANVAS, as mentioned in the subsection 2.2.2., before completing it, we tried to divide the business model in the following sections in order to clarify our ideas:

a) Customer segment:
    a. Customer jobs: evaluation of carcinogenic tumors volumes in mouse models in vivo, research in new treatments, scientific studies, etc.
    b. Customer pains: volume quantification is not accurate and takes too much time, for brain tumors they need to kill the mouse in order to make slices and obtain the tumor volume, they cannot obtain all the necessary information (OS, TGI, RTV) in one study, etc.
    c. Customer gains: time and money savings, 3D volumes, accurate values, more information in each study.
b) Value proposition:
    a. The product.
    b. Gain creators: time and money savings, tracking the tumor volume and 3D volume obtention, accuracy, reliability by being able to use more mice in each study, obtention of more data.
    c. Pain relievers: obtention of accurate volumes is faster, it is possible to check if there was a tumor before the transplantation (RTV) and to know if the drug is effective by diminishing the tumor size without the need of killing the mice, etc.

And then, we filled the CANVAS. See the following Figure.

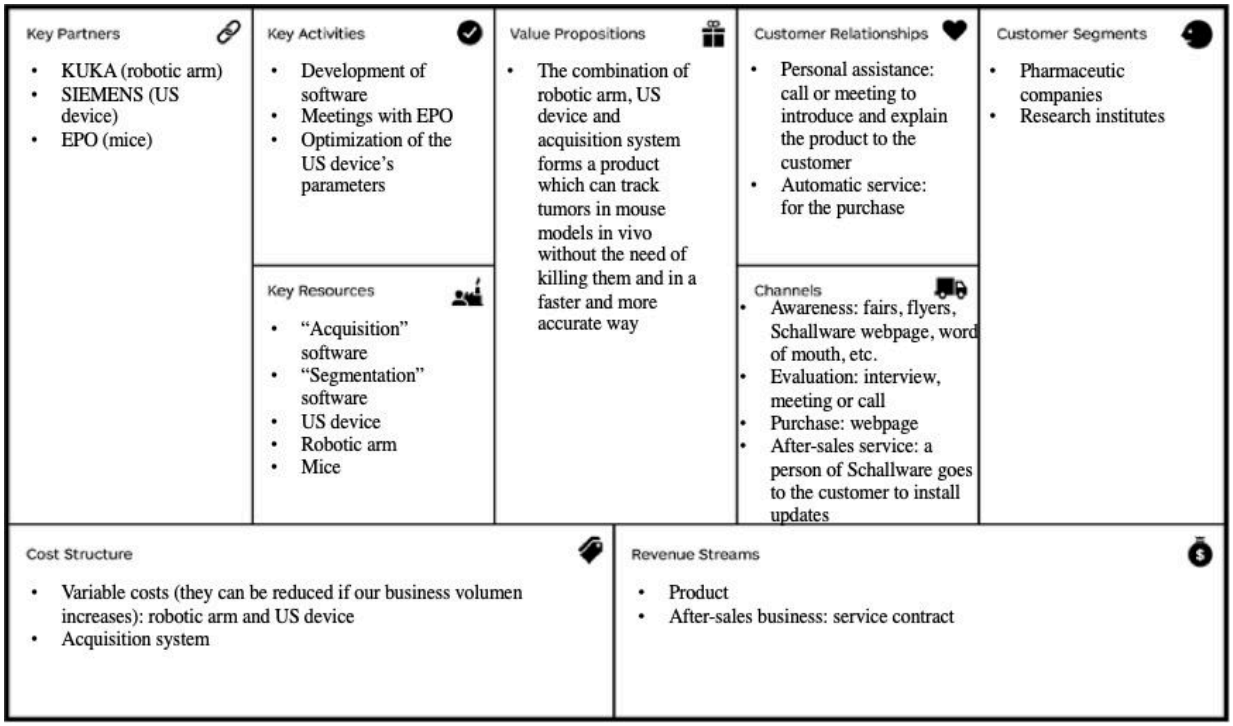


**Figure 45.** CANVAS business model of the new product.

Although the companies in key partners can be changed in the future, I put KUKA, SIEMENS and EPO as they were the ones I worked with. Regarding the types of channels, the way of delivery is not taken into account because it is normally paid by the customer

itself and it is not related with Schallware. Nevertheless, it is established that if the customer cannot directly pay the transportation, it has to pay around 30% more so that Schallware can look for a distributor.

It is worth mention that our cost structure is based on economies of scale, which means that if we get more and more customers, we will be able to reduce the costs (for example, through agreements with the key partners). Finally, in the revenue streams section we have an after-sales business, which is the service contract. Its price is between 30000€ and 60000€ per customer per year and it depends on the level of need of assistance. This service contract is mainly based on personal assistance for future updates of the program, new hardware requirements (special probes, new functions, etc.).

### 3.2.3. COGS and Time Study

The results of COGS scenario analysis are shown in the following table.

| ***Sales Forecast*** | | | | | |
|---|---|---|---|---|---|
| | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
| **Unit Sales** | | | | | |
| Product | 5 | 20 | 30 | 50 | 100 |
| | | | | | |
| **Unit Prices** | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
| Total Product | 250.000,00 € | 255.000,00 € | 260.100,00 € | 265.302,00 € | 270.608,04 € |
| | | | | | |
| **Sales** | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
| ***Total Product Sales*** | ***1.250.000,00 €*** | ***5.100.000,00 €*** | ***7.803.000,00 €*** | ***13.265.100,00 €*** | ***27.060.804,00 €*** |
| | | | | | |
| **Direct Unit Costs** | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
| Robotic Arm | 12.000,00 € | 11.640,00 € | 11.290,80 € | 10.952,08 € | 10.623,51 € |
| Scientific US Device | 50.000,00 € | 48.500,00 € | 47.045,00 € | 45.633,65 € | 44.264,64 € |
| Acquisition System | 3.000,00 € | 3.000,00 € | 3.000,00 € | 3.000,00 € | 3.000,00 € |
| ***Total Product*** | 65.000,00 € | 63.140,00 € | 61.335,80 € | 59.585,73 € | 57.888,15 € |
| | | | | | |
| **Cost of Goods Sold** | | | | | |
| ***Total COGS*** | ***325.000,00 €*** | ***1.262.800,00 €*** | ***1.840.074,00 €*** | ***2.979.286,30 €*** | ***5.788.815,42 €*** |
| | | | | | |
| ***Gross Margin*** | *925.000,00 €* | *3.837.200,00 €* | *5.962.926,00 €* | *10.285.813,70 €* | *21.271.988,58 €* |

**Table 5.** Hypothetic results of COGS and Gross Margin for the next 5 years.

We decided to set an initial price for the product of 250000€ taking into account the price of the competence's machine (VEVO), that we are novice in this specific market, and that it has to be less than the cost of the other current imaging techniques (MRI costs 900000€). Furthermore, if we would get from 2-3 customers, we would need to employ a specialist for this product – Schallware would have more expenses, but also more incomes.

| **Brain Tumor in EPO** | | **Abdominal Tumor in EPO** | | **Our product** | |
|---|---|---|---|---|---|
| Taking the brain out | 4min | Scanning and obtention of volumes with VEVO 2100 | 1h | Setting all materials | 10min |
| Preparation of sections | 40min | | | Waiting for anesthesia effects | 5min |
| Staining | 16h 32min | | | Finding the best position of the mouse | 5min |

| | | | | | |
|---|---|---|---|---|---|
| Taking photos | 12min | | | Scanning | 2min |
| Measuring and sorting data | 7min | | | Post-processing | 2min |
| Evaluation | 4min | | | Volume obtention | 15min |
| **Total** | **17h 33min** | **Total** | **1h** | **Total** | **39min** |

**Table 6.** Comparison of amount of time spent for one mouse for the two tumor types in EPO vs time spent using our product. In mice with brain tumor, taking the brain out consists in 2min/mouse, plus about 2min/mouse more to prepare the workplace and clean it afterwards. The staining step needs 12min for putting the 19 slides in staining solution, 12min for the same but in alcohol solutions, about 3min to glue the cover slides on the slices, 2-3min to let the glue dry, 1-2min to remove air bubbles in the glue and approximately 16h (one night) to let them dry. The last step is the evaluation of results, which is based on copying data into prepared excel sheets, check that all formulas are correct, etc. [All information provided by Joshua Alcañiz, researcher from EPO].

Knowing the amount of money a German average scientific researcher with a doctorate can earn (48000€/year ≈ 0.40€/min, even though it varies depending on the time of experience [51]) and taking into account the time results of Table 6, we can know how much they can save by using our product. In this way, evaluating a brain and abdominal tumor in EPO costs 37.20€ (time of drying overnight is not taken into account as it is out from labor hours) and 24€, respectively, while using our product for any kind of tumor costs 15.60€. Therefore, our product is faster and cheaper.

# 4. Discussion and Conclusion

## 4.1. Technology Discussion and Conclusion

Overall results confirm that we can achieve fast and accurate results with our new product. All the objectives set for this project were achieved, except from the volume obtention of glioblastomas.

It was a big challenge for us to obtain scans with a good resolution giving that many variables were involved – position of the mouse, its age, any movement such as breathing or awakening, US parameters, type of probe, paths of the robotic arm, artifacts, force of the probe on the mouse, etc. In fact, avoiding artifacts is usually very important, but in the case of glioblastoma, if the brightness in the cortex is an artifact, it helped us to detect changes in tissue, which is as good sign to detect the tumor. What is more, doctors usually use this information when examining a clinical image as a first step to injures detection.

We realized that we could not detect the glioblastoma because of reflection of the US wave in skull. Therefore, we asked for advice to the doctors who collaborate with Schallware and they gave us the idea of trying to make the scan through the eye so that we could avoid the skull. We firstly scanned a human eye (see Annex 5) and I made another Java software for the robotic arm to scan a mouse eye. However, for this approach we needed specialized convex probes. Other further improvements could be done in order to detect glioblastomas, but due to technical limitations, these could not be done with our SIEMENS US device. As the next steps must be more specialized, we need a scientific US device with specific settings, like the one that EPO has, in order to improve the brain scans.

Regarding pancreatic tumor, we reached all the objectives we wanted. Differences in volumes in Table 2 are due to the calculation of theoretical volumes as if tumors were a

sphere – therefore, same diameter in all axes –, but this is not the reality. Our segmentations follow the shape of the tumor, which was confirmed by visual inspection, so volumes are more accurate.

Overall, we still need to improve our product. Some of the objectives for next steps are to minimize artifacts and to understand more and more the structures and characteristics of the images. We can also add some functions to the US device in order to find tissue borders to detect tumors, such as elastography (based on change of tissue), color Doppler (to detect tumor vessels), and M-mode (it is only one beam, but more accurate). We could also try special probes for different organs and animals. Another option would be to buy a new US device and reprogram it adding all the features we want for small animal scans – this is called an OEM (Original Equipment Manufacturer) product.

### 4.2. Business Plan Study Discussion and Conclusion

The study of the business plan indicates that, despite it seems that we already have a foot in the door and that we have a correct SOM, our product is not yet prepared for the market. The Gross Margin we can obtain from COGS is not enough, no one would start a business like this because the advantage is not enough to maintain it. The ideal price, and therefore the potential objective, would be to sell the whole product at 500000€ once it is really precise and specialize in each kind of tumors and animals. Thus, we need some years of expertise and once we are more settled, we could easily grow in the market thanks to the economies of scale. In addition, time study demonstrates that we are the fastest solution for EPO so far, and CANVAS model and SWOT results are also favorable for us.

We are in the first step of development and the product will be viable for the market when it is faster and adapted to more situations thanks to the improvements mentioned in the previous subsection.

The use of non-invasive US imaging in research represents both a significant refinement as a potential replacement for more invasive techniques and a significant advancement in research techniques for small animals. This means that we can have many potential customers and that we are placed in the correct market.

All in all, I have learnt that developing a product in a short-time period is such a challenge, especially when having to deal with living animals as there are things you cannot control. My internship in Schallware GmbH has allowed me to first-hand experience the real business world and to prepare myself for the upcoming future. Science has always been built up step by step and, with this, I have tried to make my own small contribution to the scientific-technologic progress for the society. Maybe the product developed in this project will be successful in the market, or maybe it will become stagnant in this first step of development, who knows. What we know for sure is that future is uncertain and any change, even if small, can affect company's future.

## 5. References


[1] *Tierschutzgesetz.* Bundesministerium der Justiz und für Verbraucherschutz. 2006.
[2] *Animal Research*. The Institute of Cancer Research [Online]. 2019. Available at: https://www.icr.ac.uk/
[3] Scott K. Lyons. "Advances in imaging mouse tumor models *in vivo*". *The Journal of Pathology.* 2005 Jan; 205(2): 194-205.
[4] Therasse P, Arbuck SG, Eisenhauer EA, et. al. "New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada". *J Natl Cancer Inst.* 2000 Feb 2; 92(3): 205-16.
[5] JanVier Labs [Online]. Available at: https://www.janvier-labs.com/en/
[6] *Schallware* [Online]. Available at: https://www.schallware.de/
[7] Joel John. "Global portable ultrasound devices market will reach USD 8.06 billion by 2025: Zion Market Research". *Zion Market Research.* 2018 Sep.
[8] James Lamb. "Ultrasound devices market size, share, report, analysis, trends & forecast to 2026". *Stratistics Market Research Consulting (MRC).* 2018 June.
[9] "Global healthcare/Medical simulation market is set to reach USD 4.0 billion by 2024, growing at a CAGR of 14.2% during the forecast period: VynZ Research". *VynZ Research.* 2019 July.
[10] Simon R. Cherry, Sanjiv S. Gambhir. "Use of positron emission tomography in animal research". *ILAR Journal.* 2001; 42(3): 219-232.
[11] Tian Xia, et. al. "Molecular imaging in tracking tumor stem-like cells". *J Biomed Biotechnol.* 2012.
[12] Benjamin L. Franc, et. al. "Small-Animal SPECT and SPECT/T: Important Tools for Preclinical Investigation". *JNM.* 2008; 49(10): 1651-1663.
[13] Alan R. Penheiter, et. al. "Pinhole Micro-SPECT/CT for noninvasive monitoring and quantitation of oncolytic virus dispersion and percent infection in solid tumors". *Gene Ther.* 2012 Mar; 19(3): 279-287.
[14] John D. Olson, et. al. "A gated-7T MRI technique for tracking lung tumor development and progression in mice after exposure to low doses of ionizing radiation". *Radiat Res.* 2012 Oct; 178(4): 321-327.
[15] Paula J Foster, et. al. "Cellular magnetic resonance imaging: in vivo imaging of melanoma cells in lymph nodes of mice". *Neoplasia.* 2008 Mar; 10(3): 207-216.
[16] Rajiv Ramasawmy, et. al. "Monitoring the growth of an orthotopic tumor xenograft model: multi-modal imaging assessment with benchtop MRI (1T), high-field MRI (9.4T), ultrasound and bioluminescence". *Plos One.* 2016; 11(5).
[17] Andrew Webb. "Introduction to Biomedical Imaging". *The Institute of Electrical and Electronics Engineers, Inc.* 2003.
[18] D. P. Clark and C. T. Badea. "Micro-CT of rodents: state-of-the-art and future perspectives". *Phys Med.* 2014 Sep; 30(6): 619-634.
[19] Turnbull DH, et. al. "Ultrasound backscatter microscope analysis of mouse melanoma progression". *Ultrasound in Med. & Biol.* 1996; 22: 845-853.
[20] Jie Ni, et. al. "Monitoring prostate tumor growth in an orthotopic mouse model using three-dimensional ultrasound imaging technique". *Transl Oncol.* 2016 Feb; 9(1): 41-45.
[21] Thomas Deffieux, et. al. "Functional ultrasound neuroimaging. A review of the preclinical and clinical state of the art". *Elsevier*. 2018; 50: 128-135.
[22] Alan Urban, Clara Dussaux, et. Al. "Real-time imaging of brain activity in freely moving rats using functional ultrasound". *Nature Methods.* 2015.

[23] Claudia Errico, et. al. “Transcranial functional ultrasound imaging of the brain using microbubble-enhanced ultrasensitive Doppler”. *NeuroImage.* 2016; 124: 752-761.
[24] Kenichi Funamoto, et. al. “Ultrasound imaging of mouse fetal intracranial hemorrhage due to ischemia/reperfusion”. *Frontiers in Physiology.* 2017; 8:340.
[25] Byung Kook Kwak, et. al. “High-resolution ultrasound in research of mouse orthotopic glioma and ultrasound-guided cell implant”. *Advances in Molecular Imaging.* 2011; 1: 24-32.
[26] Émilie Macé, et. al. “Whole-brain functional ultrasound imaging reveals brain modules for visuomotor integration”. *Neuron.* 2018; 100: 1241-1251.
[27] Sieu L-A, Bergel A, Tiran E, Deffieux T, Pernot M, Gennisson J-L, Tanter M, Cohen I. “EEG and functional ultrasound imaging in mobile rats”. *Nat Methods.* 2015, 12: 831-834.
[28] Robert-Guenther Goetze, et. al. “Utilizing high resolution ultrasound to monitor tumor onset and growth in genetically engineered pancreatic cancer models”. *Journal of Visualized Experiments.* 2018; 134.
[29] Jie Ni, et. al. “Monitoring prostate tumor growth in an orthotopic mouse model using three-dimensional ultrasound imaging technique”. *Translational Oncology.* 2016; 9(1): 41-45.
[30] “Cancer imaging for translation research”. *FujiFilm VisualSonics* [Online webpage]. Available at: https://www.visualsonics.com/application/preclinical/oncology
[31] “Worldwide cancer incidence statistics”. *Cancer Research UK.* [Online]. 2018 Sept. Available at: https://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer/incidence#heading-Zero
[32] Dr. Kristoff Muylle. “A decade of action on cancer control and the way forward. The economic impact of cancer”. *International Atomic Energy Agency Scientific Forum.* 2019 Sept.
[33] Matej Mikulic. “Global pharmaceutical industry – statistics & facts”. *Statista.* 2019 Aug.
[34] “Global Oncology Trends 2018”. *IQVIA*. 2018 May.
[35] Cuccurullo V, et. al. “Small-animal molecular imaging for preclinical cáncer research: .μPET and μ.SPECT”. *Curr Radiopharm.* 2016; 9(2): 102-13.
[36] Coatney RW. “Ultrasound imaging: principles and applications in rodent research”. *ILAR J.* 2001; 42(3): 233-47.
[37] Beom-Su Jang. “MicroSPECT and microPET imaging of small animals for drug development”. *Toxicol Res.* 2013 Mar; 29(1): 1-6.
[38] “28. La calidad de la imagen. Resolución”. *Ecografía fácil* [Blog Online]. 2018 Feb. Available at: https://ecografiafacil.com/2018/02/13/28-la-calidad-de-la-imagen-la-resolucion/
[39] “Ultrasound Imaging Guide”. *Providian Medical Equipment* [Online]. 2019. Available at: https://www.providianmedical.com/ultrasound-imaging-guide/
[40] Dan Sunday. “The convex hull of a planar point set”. *SoftSurfer.* 2012.
[41] Matthew John Benskey and Fredric Manfredsson. “Intraparenchymal stereotaxic delivery of rAAV and special consideration in vector handling”, from the book *Gene therapy for neurological disorders: methods and protocols.* 2016 Jan; 199-215.
[42] “TAM SAM SOM – what it means and why it matters”. *The business plan shop* [Online blog]. Available at: https://www.thebusinessplanshop.com/blog/en/entry/tam_sam_som
[43] Osterwalder et. al. “Value proposition design: how to create products and services customers want”. *Wiley.* 2014 Oct.

[44] Mouse atlas brain map. *Allen Brain Atlas.* [Online]. Available at: http://atlas.brain-map.org/
[45] Joshua Alcañiz. "Orthotopic U87 MG Glioblastoma in NMRI nu/un mice". Unpublished data. Experimental Pharmacology and Oncology GmbH, Berlin, Germany. 2019.
[46] "Carcinogen selector". *Merck.* [Online]. Available at: https://www.sigmaaldrich.com/life-science/cell-biology/cancer-research/learning-center/carcinogen-selector/endocrine-system.html
[47] Joshua Alcañiz. "Dissection of NMRI nu/nu mice with PANC-1 pancreatic tumor". Unpublished data. Experimental Pharmacology and Oncology GmbH, Berlin, Germany. 2019.
[48] Alex Philippidis. "Top 10 pharma companies of 2019. Drug pricing concerns, competition take their toll on market caps of legacy drug developers". *Genetic Engineering & Biotechnology News.* 2019 Nov.
[49] Iris Stone. "30 most technologically advanced cancer centers in the world". *Top Master's in Healthcare Administration.* 2016 Mar.
[50] Noah Parsons. "What is a SWOT analysis, and how to do it right (with examples)". *Live Plan.* 2018 April.
[51] "Tarifvertrag für den öffentlichen Dienst 2019". *Öffentlicher dienst.* [Online]. Available at: https://oeffentlicher-dienst.info/c/t/rechner/tvoed/bund?id=tvoed-bund-2019&matrix=1

## Acknowledgements

I would like to express my gratitude to everybody who has supported me during the development of this master thesis project.

First of all, I want to thank my supervisor and thesis director, Gernot Jehle, who gave me the great opportunity to join the company and always dedicated me his time whenever I needed; and my colleagues from Schallware GmbH. This project would not have been accomplished without them. Since the very moment I joined them, they helped me and treated me as one member more from the Schallware family. Especially, to Genís, my daily desk colleague and friend – I would definitely not be in the company without him –, and Henk for always helping me with IT problems, among others. They all have made, from my first work in a company, such a pleasant experience. I also would like to thank EPO GmbH, Prof. Dr. Wolfgang Walther and Joshua Alcañiz, who provided me the mice and all the information I needed for the project. I really enjoyed learning new things about the scientific research world and about mice.

Secondly, I want to thank Roser Sala, who directly accepted being my thesis' tutor from UB, and kindly supported me from the distance in a very efficient way. When I asked for specific questions, she even introduced me to some of her colleagues so that I could have more specific information.

Finally, I want to thank my family and friends who have always been there for me, believing in me and giving me their moral support, help and new points of view whenever I needed. Everything would be way harder without them.