
TECHNISCHE UNIVERSITÄT DARMSTADT

# Team Results Document

## TUcanSense

**Technical University of Darmstadt**

**Team members:**

Sarah Ameer
Jana Beier
Evgenia Borisova
Johan Friman
Hana Kim
Jannis Kuhmann
Maren Krupka
Jacqueline Mohr
Michael Petek
Daniel Piro
Fiona Scannell
Theresa Schmid
Tobias Schwenk
Catalina Staver
Seska Zimmermann

**09.08.2024**

**Supervisor:**

Prof. Dr.-Ing. Andreas Blaeser

**Coaches:**

Leonie Maria Holderbach
Tim Eike Weber

SensUs

**SensUs 2024**
**Acute Kidney Injury**

## 1. Abstract

We have developed a biosensor for the detection of creatinine, a critical marker of Acute Kidney Injury (AKI), utilizing a specifically engineered DNA aptamer. This aptamer selectively binds to creatinine and is immobilized on a gold surface of a custom-manufactured, miniaturized screen-printed electrode. Upon exposure to a sample containing creatinine, the aptamer on the sensor binds to the creatinine molecules. To enable continuous monitoring of creatinine levels, the sensor can be briefly heated to 80 °C, causing the aptamer to denature and release the bound creatinine. Subsequently, the aptamer reverts to its native conformation, available to bind creatinine again. We use Electrochemical Impedance Spectroscopy (EIS) to quantify the creatinine concentration. This technique applies a small electrical signal and measures the system's response, providing detailed information on proportional creatinine levels. A custom Python program processes the data, ensuring precise readings even with repeated sensor usage. The biosensor is integrated into a compact microfluidic system, which facilitates precise control of sample flow and measurement conditions. This integration enhances the sensor's reliability and repeatability for creatinine monitoring. In summary, our biosensor presents a promising tool for healthcare applications, especially for patients with kidney conditions, offering a reliable method for continuous creatinine monitoring.

## 2. Biosensor

### Molecular Recognition

For the detection of creatinine, the ssDNA aptamer with the following sequence was chosen as molecular recognition unit of the biosensor: 5'-CGACGGTGGCCTATTAAATAGCTTTAGTTTAAGAAAAGTAAAGGGGGTGTCG-3' (Ganguly, Gunda, & Prasad, 2024). The aptamer was ordered with a 5' thiol group modification via a C6 spacer (Figure 1). A terminal amine functionality was attached at the 3'-end. The thiol group enabled the immobilization of the aptamer by self-assembly on the gold surface of the screen-printed electrode (SPE) (Aliakbarinodehi et al., 2017; Lyalina, 2023). The amine group at the 3'-end enabled further modification with functional groups such as the redox indicator methylene blue, potentially enhancing signaling sensitivity.

The aptamer was manufactured by Sigma Aldrich, carrying a protecting group at the thiol group. For deprotection, the aptamer was incubated with Tris(2-carboxyethyl)phosphat (TCEP) (Sigma-Aldrich, CN C4706) for 1h at room temperature (RT) (Tapio et al., 2021).

The electrode surface was electropolished using 0.5 M $H_2SO_4$ and cyclic voltammetry (CV), following the instructions from PalmSens®. A volume of 8µL of deprotected aptamer (c= 10 µM ) was distributed on the working electrode and incubated for 1 h at RT. After washing with MilliQ water, the gold surface was blocked with 6-Mercapto-1-hexanol (MCH) (Sigma-Aldrich, CN 451088). For this purpose, the complete electrode was incubated with 80 µL (c = 2 mM) of MCH solution for 1 h at RT. After washing again with MilliQ water, the electrode was ready for detection of the analyte (Figure 2A) (Ferapontova & Gothelf, 2009).

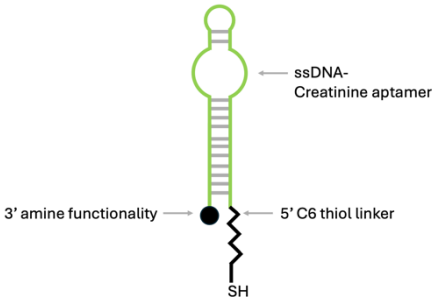


*Figure 1: Structure of the anti-creatinine aptamer. The ssDNA aptamer was modified with 3'-end amine functionality and 5'-end C6-thiol-linker.*

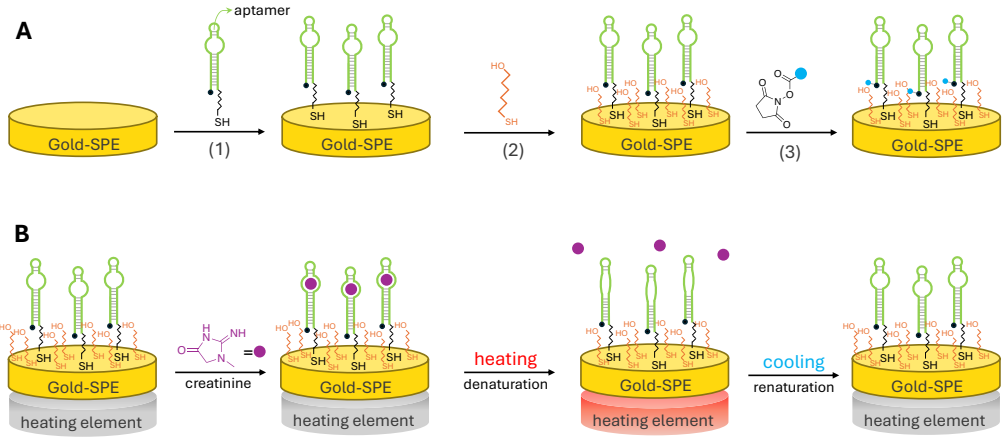


*Figure 2: A) Immobilization procedure. (1) Deprotected aptamer with 5' thiol modification was immobilized on gold working electrode. (2) Remaining gold surface was blocked with 6-Mercapto-1-hexanol (MCH). (3) Optional coupling of methylene blue as redox indicator at 3'-end amine functionality. B) Measurement cycle for continuous biosensing. Creatinine molecules are captured in the big loop of the ssDNA anti-creatinine aptamer, which is immobilized via a thiol bond on the gold screen-printed electrode (SPE). Heating of the electrode denatures the aptamer structure, thereby releasing creatinine. Regeneration of the sensor is achieved by cooling down, which renatures the aptamer structure.*

To release the bound creatinine molecules, the electrode was heated up via the heating element integrated in the microfluidic platform, as further described in the Feasibility section in Physical Transduction. The temperature leads to denaturation of the capturing aptamer, which results in the release of creatinine (Figure 2B). Renaturation and thereby regeneration of the aptamer structure was achieved by cooling down the electrode to RT. This setup enables repetitive measurement cycles, as needed for continuous monitoring of biomarkers like creatinine.

## Physical Transduction

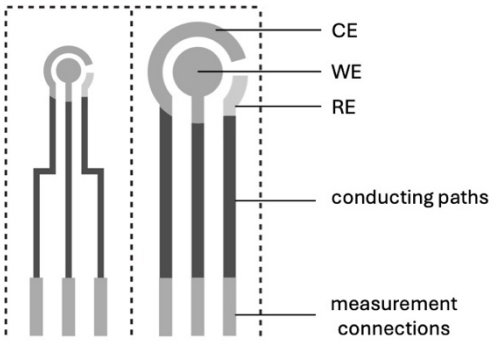


*Figure 3: SPE design. Left: miniaturized electrode with 2mm WE diameter. Right: SPE with 4mm WE diameter.*

For transducing and evaluating our electrochemical signal, in-house produced screen-printed electrodes (SPE) were used. Therefore, the conductive paths as well as the working (WE) and counter electrode (CE) were screen-printed with a conductive silver ink (Loctite, EDAG, PF 050, Henkel) onto a polycarbonate substrate. The reference electrode (RE) was printed separately out of silver/silver chloride ink (4375, DuPont) to keep a constant and well-known reference potential. The rest of the conducting paths were coated with an encapsulant paste (7165, DuPont) for isolation. To enable better conductivity, adhesion and successful aptamer immobilization, the measurement connections, the WE and the CE were coated with chrome (layer thickness = 5 nm) and gold (layer thickness = 50 nm) by vaporization. To receive different current intensities, we developed two different SPE-designs (Figure 3). During our development process we tested several electrochemical measurement methods: Electrochemical Impedance Spectroscopy (EIS), Chronoamperometry (CA) and Cyclic Voltammetry (CV). For the final evaluation we chose EIS, since it is a powerful technology which measures the impedance of an electrochemical cell over a range of frequencies. By applying a small AC voltage to the system and measuring the resulting current, EIS provides insight into the resistive and capacitive properties of the interface (Yadav & Dhar, 2021).

## Reader Instrument & User Interaction

To obtain a measurement resulting from the electrochemical signal response we used the potentiostat Sensit Smart from PalmSens®. In the data analysis, a Python program was developed to determine the concentration of a sample from the measured data. The model takes various variables into account, particularly the concentration based on a specific measurement point that shifts proportional to the amount of aptamer binding, indicating the creatinine concentration. The imaginary part at 5 kHz has proven to be the most suitable measuring point. Since the temperature control cannot completely restore the initial state, the decrease in sensor sensitivity over 24 measurements is accounted for. A mixed model estimates the current concentration considering various effects and the sensor sensitivity decrease. The model evaluates the change compared to the previous measurement to establish a robust correlation between the measured values, the number of measurements, and the concentration. A Python-based responsive graphical user interface (GUI) has been designed to be user-friendly and to enhance data analysis. It presents the serum creatinine concentration in a heat map format, thus facilitating pattern recognition and long-term monitoring. The current value, percentual increasement and trend are depicted underneath the graph. Additionally, daily changes in serum creatinine are visualized using a bar chart (see Appendix, Figure 9).

## Cartridge and Device

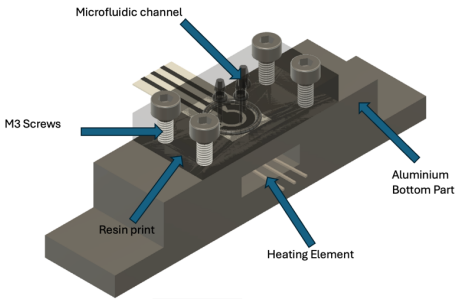


*Figure 4: DLP-printed microfluidic platform with an aluminium bottom part and an integrated heating element.*

The microfluidic system is defined by the electrode size and a sample volume of 100 µL. The sample chamber volume is less than 50 mm³ to dissociate the sample using heat and rinse it with a portion of the next sample. The lower part of the microfluidics is made of aluminum and a Peltier element is placed beneath the electrode (Figure 4). A sealing ring is integrated to address capillary effects. Fluidic channels are aligned with the working electrode (WE) surface, with three channels in total: two for sample inflow and outflow, and one for removing air bubbles to minimize the influence on the measurements. The components are attached with four M3 screws. The removable top enables the electrode to be replaced.

## 3. Technological feasibility

### Molecular Recognition

To prove a successfully immobilized anti-creatinine aptamer, the electrode was prepared using the same aptamer as previously described but modified with Cyanine 5 at the 3'-end (manufactured by Merck KGaA). The aptamer was immobilized on gold electrodes, which were polished beforehand according to the settings described above. The fluorescence was measured with a plate reader at an excitation wavelength of 630 nm. Emission was measured in the range between 660 nm and 720 nm. The results are shown in Figure 5. The positive control shows higher fluorescence intensities than the regularly prepared electrode, whereas the negative control does not show any measurable fluorescence. Thereby it can be concluded that the aptamer was successfully immobilized on the electrode surface.

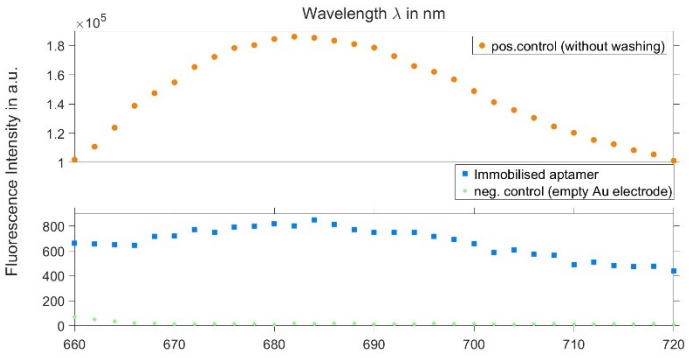


*Figure 5: Aptamer immobilization control by fluorescence with a plate reader. Fluorescence emission spectrum of the Cyanine 5 modified aptamer immobilized on gold screen-printed electrodes (SPE). Figure 5B represents a zoomed section of Figure 5A. Comparison of empty electrode (neg. control), electrode with specific aptamer binding and electrode with aptamer excess (pos. control).*

### Physical Transduction

We compared different electrode materials and different electrode sizes to find the optimal configuration for our measurement set up. The aptamer immobilization on the platinum surface was challenging, and it was not easy to prove a successful outcome. Since we had to deal with current overloads by using our self-printed gold SPE, we designed two electrodes with different WE diameters (2 mm and 4 mm) to evaluate the best size for our material choice. In the end we chose the bigger electrodes as our final configuration. The signals were more stable and the creatinine concentrations easier to distinguish.

The graph in Figure 6 illustrates the phase angles a function of the logarithm of frequency for an immobilized gold electrode subjected to different concentrations of creatinine. The measurements were conducted in Phosphate Buffered Saline (PBS) solution as a control and with creatinine concentrations in PBS of 30 µM, 100 µM, 300 µM and 30mM. The distinct separation between lines corresponding to different concentrations suggests a clear concentration-dependent response of the electrode. However, the sensitivity between the concentrations of 30 µM and 100 µM is less, indicating a need for further optimization to enhance the resolution within this range.

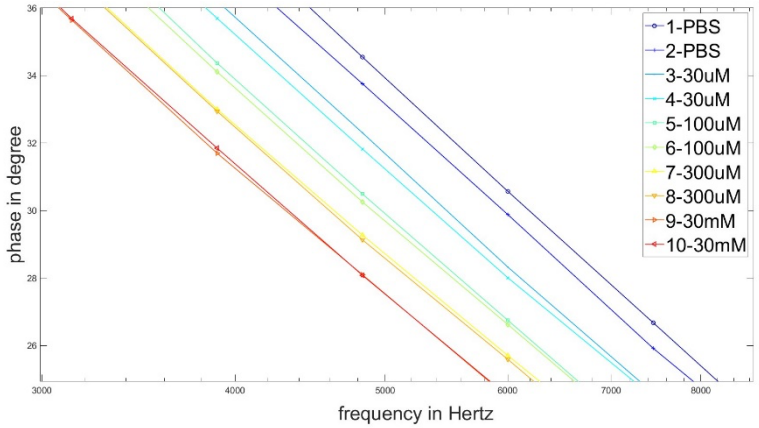


*Figure 6: EIS measurement of an immobilized Au Electrode with varying concentrations of creatinine.*

To be able to implement reversibility of the sensor without a rinsing process, the selected aptamer can enable denaturation of the bound creatinine with the aid of heat (see Appendix, Figure 10). Therefore, we implemented a temperature control system which heats the electrode to 80° and cools it down back to room temperature (24 °C). This enables a controlled measure of temperature.

## Reader Instrument

Through analysis of the measurement data, we identified a specific frequency (5 kHz) for EIS that allows for targeted measurement at a singular point rather than across a broad bandwidth of frequencies. This approach significantly reduces the measurement time. The specific measured impedance value at the measurement frequency can then be transformed back into the concentration of creatinine in the sample.
Due to the inherent challenges in predicting the exact amount of creatinine bound to the aptamers during a given measurement and the subsequent release of creatinine following the heating process, the reduction in sensor sensitivity associated with previously bound creatinine remains non-deterministic.

## Cartridge and Device

The main aim of the cartridge is a continuous flow of the ISF sample. For that, the total fill volume of the device is limited according to the sample volume of 100 µL. By reducing the height of the channels, the volume amounts to approximately 12 µL. A crucial aspect is the predictable flow of the samples without mutual mixing. Moreover, for the liquid protection of the potentiometer a reliable sealing is indispensable. These are competing aspects. The leakage is caused by capillary force between the surfaces (Ramsden, 2016). Therefore, a seal is realized with an O-ring which is pressed between the component surfaces. The closed curve shape prevents first-hand openings. The O-ring does not have an optimal elasticity to adapt to abrupt transitions of component surfaces. Therefore, the ring is smaller than the width of the even electrode surface. The additional advantage of the O-ring is its temperature resistance up to 125 °C. However, the cross section of the O-ring prevents a predictable flow. A certain amount of liquid remains between the ring and the component surfaces due to wetting effects (Oertel Jr Hrsg, 2022). Moreover, mixing with the previous sample during pumping the next one is unavoidable because of the channel positioning. A possibility to solve these problems is to use rings with quadratic cross sections which can be formed more easily according to a linearly directed flow channel. In addition, instead of using screws magnets are an option to reduce the effort for disassembling. But before that the influence of a magnetic field must be checked.

## 4. Originality

**From the Team**

Our team initially conceptualized the core design of the biosensor, focusing on label-free molecular recognition methods. After evaluating various options, we selected aptamers due to their cost-effectiveness and suitability for our objectives. Although aptamers were mentioned in preliminary research, we specifically chose a modified version that best met our requirements. Based on external advice and a review of relevant research papers, we decided to immobilize aptamers on electrodes. Furthermore, we designed and optimized the measurement and evaluation algorithms, along with the graphical user interface (GUI) for real-time creatinine concentration monitoring, using MATLAB and Python. Extensive testing was conducted to determine the most effective electrochemical measurement techniques. We evaluated Electrochemical Impedance Spectroscopy (EIS), Chronoamperometry (CA), and Cyclic Voltammetry (CV), ultimately selecting EIS based on iterative testing and analysis. We made critical adjustments to the size of the self-printed electrodes and the temperature control system through hands-on experimentation and team discussions. The microfluidic cartridge was designed by our team, incorporating and adapting models from various sources. This design underwent multiple iterations based on testing and feedback to ensure it met our specific requirements. External advisors provided valuable input on the use of aptamers and their immobilization on the electrodes, which was instrumental in refining our approach. External contribution was crucial for the electrochemical analysis and provided a foundation upon which our team built and customized our specific measurement protocols.

**From the Supervisor**

The TUcanSense team has successfully engineered a continuous electrochemical aptamer-based biosensor. This achievement originated from extensive literature research exploring various methodologies, ultimately leading to the identification of two promising approaches: an aptamer and a molecularly imprinted polymer (MIP) strategy. Although theoretically appealing, the MIP approach was discontinued due to the practical challenges of working with highly toxic monomers and producing chemically stable polymers. Consequently, the team concentrated on the aptamer method, investigating three distinct electrochemical measurement techniques: EIS, CV and CA.

Initially, platinum electrodes were employed for these measurements, but the team switched to self-fabricated gold-coated screen-printed electrodes later, as the platinum electrodes have not yielded the expected results regarding aptamer binding affinity. For CV, the team modified the aptamers by adding methylene blue as a redox group. Each technique was meticulously evaluated, with optimizations including modifications to the environment, electrode design and size, regeneration temperature, evaporation control, incorporation of microfluidics, and diverse strategies for aptamer binding on the electrode surface. Following extensive testing, EIS was selected as the optimal measurement method due to its superior performance.

A significant objective for this year was to develop a continuous sensor that operates without washing steps. To achieve this, the team implemented a novel approach for aptamer regeneration within the microfluidic sensor via temperature-based denaturation. This regeneration method is a non-washing biosensor technique, marks a significant advancement for our university.

While the use of screen-printed electrodes is not entirely unprecedented, the TUcanSense team's innovative approach differentiates their biosensor. They integrated microfluidics with a heating-based regeneration system and a sophisticated, sustainable design that ensures user-friendly handling. The microfluidic system, heating circuit, micropump system, cartridge, and electrode were all self-designed and manufactured by the team. The resulting biosensor, capable of continuously measuring creatinine without the need for washing steps and with a continuous flow, stands out for its unique composition and holds significant potential for versatile applications in the clinical field.

*Team Captain (J.Kuhmann)* *Team Captain (J.Friman)* *Supervisor (Prof. Dr. A. Blaeser)*

# 5. Translation potential

## 5.1 Introduction and Business Model Canvas

Our biosensor is a minimally invasive, easy-to-use device for continuous monitoring of creatinine levels. Designed as a point-of-care (POC) device, it allows patients at elevated risk of Acute Kidney Injury (AKI) to monitor their condition from home, with data transmitted directly to their hospital or clinician. This solution combines convenience, cost-effectiveness, and sustainability, ensuring early detection of kidney problems and improved patient outcomes.

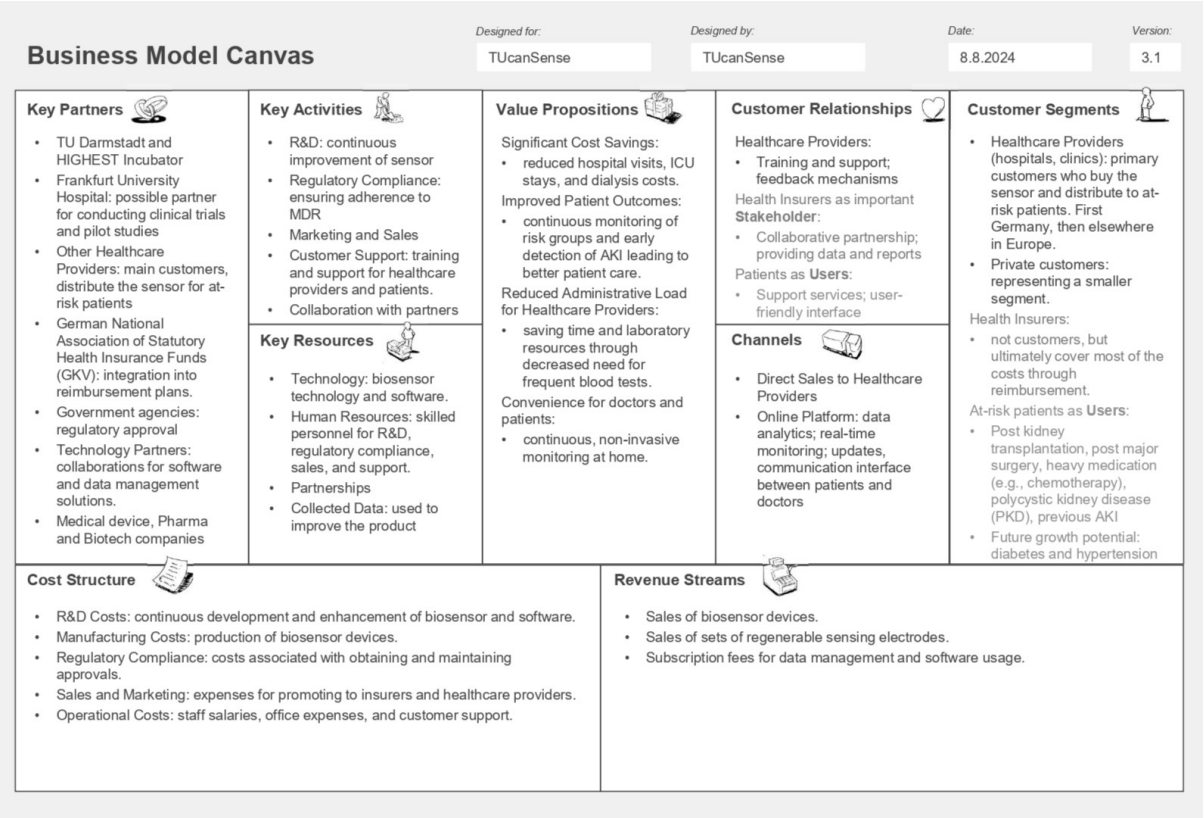


*Figure 7: Business Model Canvas Biosensor TUcanSense*

## 5.2 Stakeholder Desirability

Our key stakeholders include healthcare providers, health insurers and patients. Healthcare providers, such as hospitals and clinics, are our primary customers. They will distribute our POC sensor to patients in high-risk groups, including those who have received kidney transplants, undergone major surgeries (e.g., heart, abdominal and vascular surgery) (Rodriguez et al., 2024), or are receiving heavy medication like chemotherapeutics (Rosner & Perazella, 2019). Other high-risk groups include patients with hereditary polycystic kidney disease (PKD) ("Autosomal Dominant Polycystic Kidney Disease," n.d.). These groups were identified through literature research and validated through interviews with a nephrologist from the Frankfurt University Hospital. Future expansion may include patients with diabetes (Kaur, Sharma, & Kumbala, 2023) and hypertension (National Kidney Foundation, 2024).

Health insurers play a crucial role in market adoption, as reimbursement is key for widespread use. Collaborating with the German National Association of Statutory Health Insurance Funds (GKV) is essential to include our product in their reimbursement plans (AiM GmbH, 2020).

Our primary value proposition is continuous health monitoring for at-risk patients, enabling early detection and improved patient care. This reduces hospital visits and laboratory tests, shortens intensive care unit (ICU) stays,

and avoids continuous dialysis, resulting in significant long-term cost savings for healthcare providers and insurers.

For example, post-transplant patients can reduce hospital visits through remote monitoring, and early AKI detection improves prognosis and shortens ICU stays, which cost approximately €1,265 per day (Martin, Neurohr, Bauer, Weiß, & Schleppers, 2008). Acute kidney failure leading to permanent dialysis incurs even larger expenses. In Germany, a dialysis patient costs the statutory health insurance approximately €44,000 per year (Gandjour, Armsen, Wehmeyer, Multmeier, & Tschulena, 2020). By preventing AKI from progressing to severe chronic kidney disease (CKD) and the associated need for dialysis, our sensor can save the healthcare system several hundred thousand euros in lifetime costs for a single patient.

Our product also benefits healthcare providers by improving patient compliance and reducing administrative burdens. Traditional creatinine testing involves drawing blood and laboratory analysis, consuming time and resources. Our device simplifies this with remote monitoring, direct data transmission to doctors, and an intuitive user interface for easy handling.

The only comparable POC creatinine sensor we found is Nova Biomedical's StatSensor®, which measures creatinine from a whole blood sample intended for in-hospital use, primarily in emergency departments (Nova Biomedical, n.d.). Our sensor uses interstitial skin fluid (ISF), easier to access than blood, making it suitable for remote monitoring and home use. Additionally, while StatSensor® uses single-use test strips, our electrode regeneration technique allows multiple measurements with the same electrode.

Our current prototype already demonstrates key features: measuring creatinine concentration from simulated ISF samples, an intuitive user interface displaying creatinine levels, calculated eGFR values, and trend lines. The thermal regeneration technique of the electrode surface is promising but requires further testing. We are still exploring methods for ISF extraction from patients and the software for direct data transmission to doctors and critical threshold alarms needs to be developed.

## 5.3 Business Feasibility

To develop and scale our biosensor, we need advanced technology, intellectual property protection and a skilled team specializing in biosensor technology, data analytics and regulatory compliance. The technology must ensure accurate creatinine measurement, real-time monitoring and continuous R&D for improvements. Our human resources team, which includes researchers and engineers, can be scaled through academic and industry collaborations. Partnerships with healthcare providers, research institutions and technology partners are crucial for recommending the biosensor, covering costs and conducting clinical trials. Our established resources and partnerships are scalable through ongoing investments and further collaboration.

Our multi-channel marketing and sales strategy targets healthcare providers, insurance companies and patients with elevated risk of AKI and CKD, using methods like medical conferences, webinars and social media to build awareness and engage audiences. Key activities include enhancing biosensor technology through R&D, ensuring regulatory compliance, providing customer support and securing patents for innovations like the regenerable electrode to protect and position our technology strategically.

Our business model depends on strategic partnerships with healthcare providers, insurance companies, research institutions, government agencies and medical device companies to develop, deploy and scale our biosensor technology. These collaborations ensure effective integration, cost coverage, clinical validation, regulatory compliance and production scaling, creating mutually beneficial relationships that drive the success and broad adoption of our biosensor.

Our business model integrates sustainability by using a regenerable electrode to minimize waste and reduce the environmental impact of disposable sensors. We prioritize eco-friendly practices across procurement, production and waste management, including energy-efficient manufacturing, responsible material sourcing and robust recycling initiatives.

Our business model ensures that resources, key activities and partners align with our value proposition of delivering accurate, reliable kidney monitoring. Advanced biosensor technology, skilled teams and strategic partnerships with healthcare providers, insurers and research institutions support continuous R&D, regulatory

compliance and effective integration into care protocols. Our marketing and sales strategies, along with clearly defined distribution channels, facilitate efficient delivery to end customers and enhance patient care while addressing cost reduction.
To support our biosensor technology, we conducted expert interviews and literature reviews, gathering insights from specialists like Ashley de Bie Dekker (intensivist at Catharina Hospital, Eindhoven) on continuous dialysis monitoring and Jeannine Lang (nephrologist at Frankfurt University Hospital) on the sensor's potential for home and clinical use. Feedback from these experts, along with market insights from Prof. Dr.-Ing Röse and the HIGHEST innovation center, guided our design and strategy, ensuring our biosensor meets critical clinical needs and market demands. This thorough approach has reinforced the credibility and relevance of our biosensor technology.

Our biosensor demonstrates strong business feasibility through its innovative regenerable electrode, substantial market potential for early AKI/CKD detection, diverse revenue streams and a strategically aligned model that leverages key activities, partnerships and distribution channels for effective market reach and long-term sustainability.

## 5.4 Financial Viability

The cost of our current sensor prototype is approximately €500. However, through a strategic partnership with Palmsens® and by using their EmStat Pico Core potentiostat (PalmSens®, n.d.) chip instead of their ready-to-use Sensit Smart readout device we expect to reduce the material and production costs to €300 per device. For the regenerable electrodes, including the SPE itself and the chemicals and aptamers for immobilization, the costs are estimated at €5 per electrode. Initial development and implementation costs required for market-entry are projected as follows: R&D: €250,000; Clinical Trials: €500,000; Regulatory Compliance: €200,000; Manufacturing Setup: €150,000; and Marketing and Distribution: €200,000. This results in a total initial investment of €1,300,000. This comprehensive investment will ensure the successful development, validation, and market-entry of our innovative creatinine biosensor system.
Our creatinine biosensor system consists of a readout device and regenerable electrodes, each capable of up to 30 measurements. Depending on the frequency of daily measurements, one electrode lasts approximately 1-2 weeks. We offer electrodes in packages of 3 and 10 units to accommodate the varying needs of patients. Our creatinine sensor for continuous kidney monitoring shares similarities with a continuous glucose monitor (CGM), with CGM sensors typically costing between €50 and €100 per unit and total monthly expenses ranging from €200 to €400 (Dexcom kontinuierliche Gewebeglukosemessung (CGM), n.d.). Our pricing aligns with these costs to provide an affordable solution for continuous kidney monitoring. We have set the following sales prices: device: €500; package of 3 electrodes: €45; package of 10 electrodes: €120. Our pricing strategy is designed to have a higher gross profit margin on electrodes (200% and 140% respectively) than on the device itself (67%).
The market for wearable health sensors is rapidly growing, driven by increasing demand for advanced health monitoring solutions. ISF sensors are gaining prominence for their role in non-invasive glucose monitoring. The global market for wearable medical devices, including ISF sensors, is projected to reach $26.5 billion by 2026 (Expert Market Research, n.d.). Currently, CGMs like the Dexcom G6 and Abbott FreeStyle Libre set the standard for wearable health sensors, offering high accuracy and user-friendliness (Market Research Company - Mordor IntelligenceTM, n.d.).
Our wearable creatinine sensor aims to meet the high standards of CGMs, providing continuous, non-invasive monitoring of kidney function. This requires advanced technology and integration with health data platforms. We are developing a software solution modeled after the principles of Kaiku Health to analyze data and improve communication with healthcare providers, potentially reducing hospitalizations through timely interventions (Market Research Reports & Consulting | Grand View Research, Inc., n.d.). The market potential for wearable creatinine sensors is substantial. The high prevalence of kidney diseases and demand for continuous monitoring support the development of these technologies. Studies indicate that the market potential justifies the

development costs and can generate significant revenue, making investment in these technologies viable (Expert Market Research, n.d.).

Our company aims to generate revenue through multiple channels. While the costs are ultimately reimbursed by the health insurers, it is health care providers as the primary customers who pay for the sensor and its maintenance. The sensor is integrated into their treatment plans, and significant revenue comes from the sale of the regenerable sensing electrodes, as well as subscription fees for data management and software usage. Furthermore, private customers present a minor additional customer base.

In our financial outlook, we analyze both the break-even point and the cumulative break-even point. The break-even point occurs when total revenue first exceeds total costs, resulting in a positive net income. The cumulative break-even point accounts for cumulative revenues, costs, and the significant initial investment of €1,300,000, which includes essential expenses such as clinical trials and regulatory approval. These large upfront investments are typical in the medical device sector and necessary for market entry.

The break-even analysis is based on projected sales of 50 devices in Year 1, 200 in Year 2, 600 in Year 3, 900 in Year 4, and 1,200 in Year 5, with corresponding sales of electrodes and subscriptions. Figure 8 illustrates the financial outlook for the first five years post-market entry. Revenue is divided into devices, electrodes, and subscriptions, while expenses are categorized into variable costs (COGS), fixed costs (e.g., sales & distribution, rent, personnel, and R&D), and the initial investment. The analysis was conducted using conservative sales figures that target only a small fraction of the addressable market.

We remain committed to significant investment in R&D to ensure market leadership and maintain our technical excellence. This dedication is reflected in our expense structure and contributes to the long-term potential of the company. The analysis shows a near-linear increase in net income, with break-even projected in Year 3. Due to the substantial initial investment required for market entry, cumulative break-even is expected between Years 6 and 7. This timeline allows for strategic adjustments and scaling to ensure long-term financial viability and sustainability. Several tables are included in the Appendix (see Figure 11, Figure 12, Figure 13, Figure 14, Figure 15) to provide more context and insight into the figures used for the financial outlook.

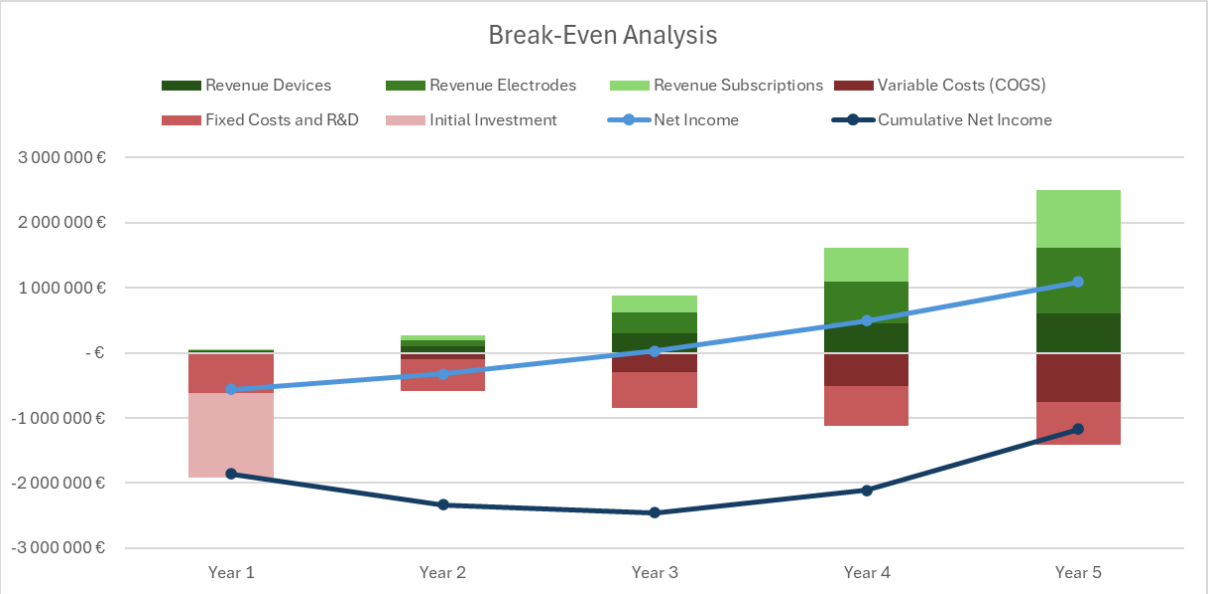


*Figure 8: A financial outlook for the first five years after market entry. The break-even point is reached in year three, where net income becomes positive. Due to the significant initial investments required, the cumulative break-even point is expected between*

## 6. Team and Support

Our team consists of 15 team members from various backgrounds. We have students from chemistry, biotechnology, mechatronics, biomedical, mechanical and electrical engineering. Therefore, we experienced a highly interdisciplinary workforce. We also had the opportunity to use the Institute for Printing Technologies of Prof. Andreas Blaeser and the biochemistry laboratories of Prof. Harald Kolmar at our university. Our supervisors are a PhD candidate in the field of printing technology for electronic components and biosensors, and our second supervisor is a master's student in Chemistry and a former participant in the SensUs competition. Both Professor Thomas Burg, who specializes in Integrated Micro-Nano Systems and Professor Süß, who specializes in Synthetic RNA Biology were very helpful. We also consulted several medical professionals to develop our business idea.

| Team Member | Subgroup | Tasks |
|---|---|---|
| Sarah Ameer | reader instrument | coding, conceptual development, graphic design |
| Jana Beier | physical transduction | measurements, analytics, interviews, documentation |
| Evgenia Borisova | molecular recognition | immobilization and lab work, project management |
| Johan Friman | physical transduction | measurements, business model, interviews |
| Hana Kim | cartridge and device | design of microfluidic system, project management |
| Jannis Kuhmann | physical transduction | measurements, electrode printing, update meetings |
| Maren Krupka | physical transduction | measurements, project management, update meetings |
| Jacqueline Mohr | molecular recognition | immobilization and lab work, project management |
| Michael Petek | reader instrument | heating and cooling unit, coding |
| Daniel Piro | cartridge and device | electrode vaporization, business model |
| Fiona Scannell | molecular recognition | immobilization and lab work, social media |
| Theresa Schmid | physical transduction | electrodes, measurements, interviews, documentation |
| Tobias Schwenk | cartridge and device | design of microfluidic system, micropump |
| Catalina Staver | molecular recognition | business model, lab work, social media |
| Seska Zimmermann | reader instrument | coding, analytics, evaluation, measurements |

## Sponsors

A special thank you goes to our sponsors who supported us with their expertise and broad knowledge in multiple disciplines.

## 7. Final Remarks

We believe our biosensor represents a significant advancement in the field of creatinine detection and continuous monitoring. This project would not have been possible without the support of our university, especially Prof. Andreas Blaeser, Prof. Beatrix Süß, Prof. Harald Kolmar, Prof. Thomas Burg and Prof. Edgar Dörsam. We extend our heartfelt thanks to our coaches Tim Weber and Leonie Maria Holderbach and sponsors for their invaluable guidance and resources.

Looking ahead, we plan to refine our biosensor to enhance its sensitivity and robustness further. Our goal is to develop a commercial product that can be used in hospitals and at home care settings, providing real-time monitoring for patients with Acute Kidney Injury.

We also intend to explore the potential of our biosensor platform for detecting other biomarkers, leveraging the adaptability of aptamer technology. This could open new ways of diagnosing and monitoring a wide range of health conditions and contribute to personalized and real-time health interventions.

## 8. References


[1]. AiM GmbH. (2020). *Reimbusement of Medical Devices*.

[2]. Aliakbarinodehi, N., Jolly, P., Bhalla, N., Miodek, A., De Micheli, G., Estrela, P., & Carrara, S. (2017). Aptamer-based field-effect biosensor for tenofovir detection. *Scientific Reports*, *7*. https://doi.org/10.1038/srep44409

[3]. Autosomal Dominant Polycystic Kidney Disease. (n.d.).

[4]. Dexcom kontinuierliche Gewebeglukosemessung (CGM). (n.d.). Retrieved July 29, 2024, from https://www.dexcom.com/de-DE

[5]. Expert Market Research. (n.d.). Retrieved July 29, 2024, from https://www.expertmarketresearch.com/

[6]. Ferapontova, E. E., & Gothelf, K. V. (2009). Optimization of the Electrochemical RNA-Aptamer Based Biosensor for Theophylline by Using a Methylene Blue Redox Label. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, *21*(11), 1261–1266.

[7]. Gandjour, A., Armsen, W., Wehmeyer, W., Multmeier, J., & Tschulena, U. (2020). Costs of patients with chronic kidney disease in Germany. *PLOS ONE*, *15*(4), e0231375. https://doi.org/10.1371/journal.pone.0231375

[8]. Ganguly, A., Gunda, V., & Prasad, S. (2024). CreCENT: Creatinine and Chloride based Electrochemical Non-faradaic renal health mapping Technology. *Urine*, *6*, 1–7. https://doi.org/10.1016/j.urine.2023.11.001

[9]. Kaur, A., Sharma, G. S., & Kumbala, D. R. (2023). Acute kidney injury in diabetic patients: A narrative review. *Medicine*, *102*(21), e33888. https://doi.org/10.1097/MD.0000000000033888

[10]. Lyalina, T. (2023). Novel electrochemical aptamer-based sensing mechanism inspired by selection strategies. Retrieved from https://papyrus.bib.umontreal.ca/xmlui/handle/1866/27865

[11]. Market Research Company - Mordor Intelligence™. (n.d.). Retrieved July 29, 2024, from https://www.mordorintelligence.com/

[12]. Market Research Reports & Consulting | Grand View Research, Inc. (n.d.). Retrieved July 29, 2024, from https://www.grandviewresearch.com/

[13]. Martin, J., Neurohr, C., Bauer, M., Weiß, M., & Schleppers, A. (2008). Kosten der intensivmedizinischen Versorgung in einem deutschen Krankenhaus. *Der Anaesthesist*, *57*(5), 505–512. https://doi.org/10.1007/s00101-008-1353-7

[14]. National Kidney Foundation. (2024). High Blood Pressure and Chronic Kidney Disease. Retrieved August 2, 2024, from https://www.kidney.org/news/newsroom/factsheets/High-Blood-Pressure-and-CKD

[15]. Nova Biomedical. (n.d.). StatSensor® and StatSensor Xpress™ Creatinine and eGFR Meters. Retrieved August 2, 2024, from https://www.novabiomedical.com/statstrip-creatinine/

[16]. Oertel Jr Hrsg, H. (2022). *Prandtl - Führer durch die Strömungslehre*. (H. Oertel, Ed.). Wiesbaden: Springer Fachmedien Wiesbaden. https://doi.org/10.1007/978-3-658-27894-6

[17]. PalmSens. (n.d.). EmStat Pico Core.

[18]. Ramsden, J. J. (2016). *Nanotechnology*. Elsevier. https://doi.org/10.1016/C2014-0-03912-3

[19]. Rodriguez, J. D., Hashmi, M. F., & Hithe C. C. (2024, January). Perioperative Acute Kidney Injury.

[20]. Rosner, M. H., & Perazella, M. A. (2019). Acute kidney injury in the patient with cancer. *Kidney Research and Clinical Practice*, *38*(3), 295–308. https://doi.org/10.23876/j.krcp.19.042

[21]. Tapio, K., Mostafa, A., Kanehira, Y., Suma, A., Dutta, A., & Bald, I. (2021). A Versatile DNA Origami-Based Plasmonic Nanoantenna for Label-Free Single-Molecule Surface-Enhanced Raman Spectroscopy. https://doi.org/10.1021/acsnano.1c00188

[22]. Yadav, N., & Dhar, R. (2021). Impedance Spectroscopy. *Progress in Optical Science and Photonics*, *13*(3), 515–540. https://doi.org/10.1007/978-981-33-6084-6_19

# 9. Appendix

## GUI Module Description

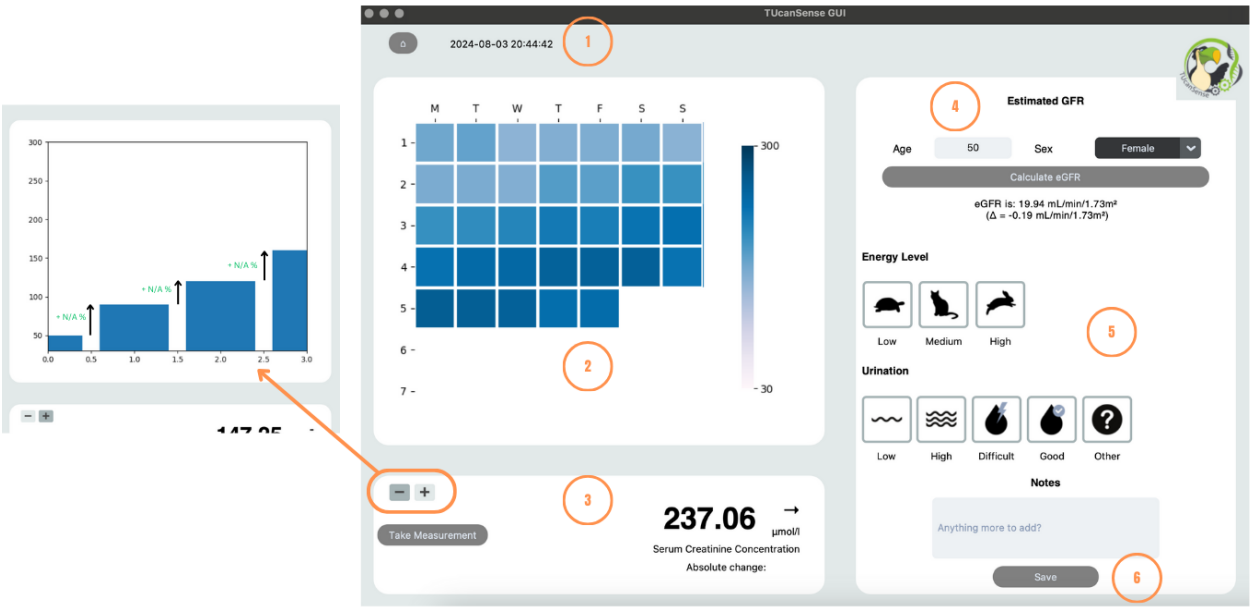


*Figure 9: Graphical User Interface which was developed during competition time*

**1. Navigation Bar:** Shows the current date and time; Navigates back to the Main Page via Home Button

**2. Data Visualization:** Displays serum creatinine concentration on a calendar, with saturation levels indicating the concentration.

**3. Control Panel:** Data representation Modes can be selected:

- *Long-term Visualization ("-"): Displays data as a heat map for an extended period.*
- *Close-up Visualization ("+"): Focuses on data from the past few days and shows change in percentage and an arrow. Rising values are mapped as green colored, falling as red. (draft, still in progress)*

**Measurement Button:** Takes a new measurement and updates both graphs.

**Data Display:** Shows the current serum creatinine value and its change, with a trend arrow indicating long-term change (moving average over 5 measurements).

**4. Estimated GFR Panel:** Allows input of Age and Sex for estimating the Glomerular Filtration Rate (eGFR).

**5. Personalized Entries Panel:** Symptom Icons: Allows selection of common kidney disease symptoms using icons.

**Notes Section:** Provides space to add personal notes.

**6. Save Button:** Saves all entered data and measurements

## Temperature regulation

To be able to implement reversibility of the sensor without a rinsing process, the selected aptamer can enable denaturation of the bound creatinine with the aid of heat. For this purpose, a heating element (heating foil, Peltier element) is controlled via an electronic circuit consisting of a temperature sensor, MOSFET, resistors and a microcontroller. According to research, the design of a reversible sensor without a rinsing process based on the denaturation of aptamers by heat is considered an innovation. The control circuit in Figure 1 for controlling the heating element consists of common components to ensure a certain degree of robustness in the electronics under different conditions and the ability to quickly change control circuit components.

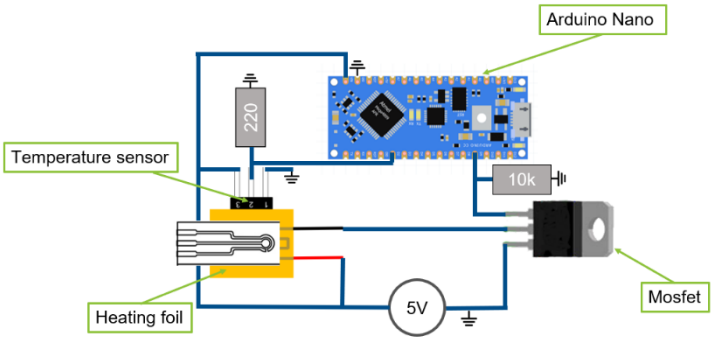


*Figure 10: Temperature regulation circuit with Arduino Nano*

## Financial Feasibility

| Cost estimations, sales price, and profit margins | | | |
|---|---|---|---|
| **Product** | **Material and Production Cost** | **Sales Price** | **Gross Profit Margin** |
| Sensor Device | 300,00 € | 500,00 € | 67 % |
| Set of 10 Electrodes | 50,00 € | 120,00 € | 140 % |
| Set of 3 Electrodes | 15,00 € | 45,00 € | 200 % |
| Software Subscription (per month) | | 25,00 € | |

*Figure 11: Cost estimations, sales price, and profit margins*

| Development and Implementation Costs | |
|---|---|
| **Cost Category** | **Cost (EUR)** |
| R&D | 250 000 € |
| Clinical Trials | 500 000 € |
| Regulatory Compliance | 200 000 € |
| Manufacturing Setup | 150 000 € |
| Marketing and Distribution | 200 000 € |
| Initial Investment | **1 300 000 €** |

*Figure 12: Developement ane implementation costs*

| Revenue Projections | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Year | Devices Sold | 10-Electrode-Sets Sold | 3-Electrode-sets Sold | Revenue from Devices | Revenue from Electrodes | Revenue from subscriptions | Total Revenue | Cumulative Revenue |
| 1 | 50 | 100 | 150 | 25 000 € | 18 750 € | 15 000 € | 58 750 € | 58 750 € |
| 2 | 200 | 500 | 750 | 100 000 € | 93 750 € | 75 000 € | 268 750 € | 327 500 € |
| 3 | 600 | 1700 | 2550 | 300 000 € | 318 750 € | 255 000 € | 873 750 € | 1 201 250 € |
| 4 | 900 | 3400 | 5100 | 450 000 € | 637 500 € | 525 000 € | 1 612 500 € | 2 813 750 € |
| 5 | 1200 | 5400 | 8100 | 600 000 € | 1 012 500 € | 885 000 € | 2 497 500 € | 5 311 250 € |

*Figure 13: Revenue Projections*

| Cost projections | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Year | Devices Sold | 10-Electrode-Sets Sold | 3-Electrode-sets Sold | Variable Costs (COGS) | R&D | Fixed Costs (sales&distribution, rent, personnel) | Total Costs | Cumulative Costs |
| 1 | 50 | 100 | 150 | 22 250 € | 150 000 € | 450 000 € | 622 250 € | 622 250 € |
| 2 | 200 | 500 | 750 | 96 250 € | 150 000 € | 495 000 € | 741 250 € | 1 363 500 € |
| 3 | 600 | 1700 | 2550 | 303 250 € | 150 000 € | 544 500 € | 997 750 € | 2 361 250 € |
| 4 | 900 | 3400 | 5100 | 516 500 € | 150 000 € | 598 950 € | 1 265 450 € | 3 626 700 € |
| 5 | 1200 | 5400 | 8100 | 751 500 € | 150 000 € | 658 845 € | 1 560 345 € | 5 187 045 € |

*Figure 14: Cost projections*

| Break-Even Analysis | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Revenue Devices | Revenue Electrodes | Revenue Subscriptions | Variable Costs (COGS) | Fixed Costs and R&D | Initial Investment | Net Income | Cumulative Net Income |
| Year 1 | 25 000 € | 18 750 € | 15 000 € | - 22 250 € | - 600 000 € | - 1 300 000 € | - 563 500 € | - 1 863 500 € |
| Year 2 | 100 000 € | 93 750 € | 75 000 € | - 96 250 € | - 495 000 € | | - 322 500 € | - 2 336 000 € |
| Year 3 | 300 000 € | 318 750 € | 255 000 € | - 303 250 € | - 544 500 € | | 26 000 € | - 2 460 000 € |
| Year 4 | 450 000 € | 637 500 € | 525 000 € | - 516 500 € | - 598 950 € | | 497 050 € | - 2 112 950 € |
| Year 5 | 600 000 € | 1 012 500 € | 885 000 € | - 751 500 € | - 658 845 € | | 1 087 155 € | - 1 175 795 € |

*Figure 15: Break-Even Analysis*