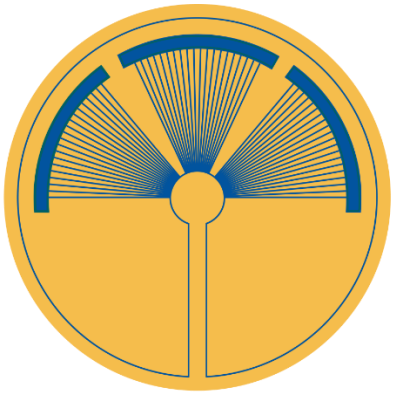

AixSense

AixSense

RWTH Aachen University

Website: www.aixsense.net

Team Members: Apurva Roy, Gayatri Vasudevan Rajeswari, Arka Dipta Das, Dibyendu Khan, Ruijun Yao, Aparna Sai Malisetty, Chenxu Zhu, Felix Burkhard, Sushil Jacob, Aby Valiyaveettil Ajith

Supervisor: Prof. Sven Ingebrandt

Coach: Dr. Vivek Pachauri

Date of Submission: 18 August 2019

AixSense

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1. Summary for the SensUs Website

AixSense plans to use a new system-integrated platform for optical and electrical detection of the drug Adalimumab (AM) in blood plasma. For Electrical readout, we immobilized the surface of the Graphene based transducers with anti-Adalimumab (Anti-AM) and then made the drug flow over the transducer area. Changes in the impedance before and after the flow, are measured using Impedance Analyzer. AM specific transducers show increase of impedance against different concentrations AM molecular binding within clinical ranges (0.1µg/ml to 10 µg/ml).

To further strengthen the sensor platform, an optical readout strategy is integrated on the same sensor interface. A miniature optical (fluorescence) microscopy set-up was developed for high-sensitivity biomolecular imaging in fluidic circuit by using a nanomaterial-based optical label. Silver nanoparticles (Ag NPs) are used here as an optical label bound with TNF-α. TNF-α shows specific interactions with the surface bound AM which is therefore imaged with the help of NP tags. The strategy can also be replicated in an all solution label free assay format with optical imaging of NPs bound with biomolecules. In addition, optical readout has been further enhanced by utilising microscale thermophoresis, leading to accumulation of drug molecules and therefore enhanced sensor signal.

2. Biosensor system and Assay

2.1 Molecular recognition and Assay Reagents

In our sensor platform, we have used the following molecules for the following reasons:

a) **Anti-Adalimumab Type 1 Antibody**- The Type 1 anti-adalimumab (Anti-AM) antibodies inhibit the binding of the drug adalimumab(AM) to its target, TNF-α, and therefore detect free drug. We have used this protein to bio-immobilize our Graphene based transducers, over which the blood sample containing AM flows.

b) **TNF-α** - Tumour Necrosis Factor alpha (TNF-α), is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signalling events within cells, leading to necrosis or apoptosis. Here, we have used the affinity of TNF-α to AM, by tagging it with optically active silver nanoparticles (Ag NPs) and then flowing it over the Graphene based transducers containing Anti-AM-AM complex.

c) **Silver Nanoparticles-** Silver Nanoparticles were used as the optical tags with receptor proteins specific to AM. The Ag NPs and other nanomaterials were synthesized and provided by the Nanomaterials and Bio-Nano Interfaces Group at IWE1, RWTH Aachen University

2.2 Physical Transduction

The sensor platform which is multimodal in nature involves: (i) graphene based transducers for electrical readout for the specific binding of AM, whereas (ii) optical readout is realized on the same interface for the recording of optical emissions from labelled secondary antibodies of TNF-alpha(labelled with Ag NPs) which specifically binds to the Anti-AM AM complex bio immobilized over Graphene transducers. Moreover, in order to enhance the optical signal, and improve the bioassay readout, dedicated microfluidic circuits are planned – which in effect increase the local concentrations of the analytes at the transducer thereby increasing the efficiency of the sensor platform by several folds.

While the electrical readout does give out the concentration, it is not a very accurate measurement technique. A common cause of problems in Electro-Impedance Spectroscopy (EIS) measurements and analysis is drift in the system being measured. This can happen due to transducer material not being stable or the interface getting changed due to various interactions at the solid-liquid interface. Normally, drift is present on an ideal platform, just that its much lower than the measurable changes for the different analyte concentrations that you record. Therefore, in order to cross check the results from EIS measurements, we have integrated a parallel detection platform, which in our case is an optical based detection. The Optical based results are slow to appear but are very accurate. On the other hand, EIS measurements are rather quick but result in reduced accuracy. Through our sensor platform, we tried to achieve a trade-off between the two methods. With the optical detection, we have also utilised the technology of Microscale Thermophoresis (MST). Directed movement of particles in a microscopic temperature gradient leads to accumulation of analyte molecules and thus leads to an enhanced sensor signal due to the increased local fluorescence. In our sensor platform, we have proposed a novel design for realizing this technology.
In Fig.1, we can see an application of the principle of EIS. We are immobilizing the surface of the graphene electrodes with Anti-AM Type 1 antibodies (the Y shaped structures in the figure). The Anti-AM antibodies inhibit the binding of the drug AM to its target, TNFα, and therefore detect free

drug. We utilised this binding property of AM in our detection. We then pass the blood sample containing AM over the top of the electrodes. The AM is bound to the Anti-AM protein, thus leading to a change in the impedance which can be observed in the analyser. At a certain frequency the impedance variation of the electrodes is maximum, and this variation directly correlates to the concentration of the drug. To verify the results of EIS, we employed fluorescence measurement techniques.

The sample already flowing through the transducers flows through the capillaries. In addition, optically labelled TNFα was made to flow through. TNFα binds to the Anti-AM AM complex already formed before and fluorescence is indicative of the binding sites. To realize MST; heat is supplied to the capillaries resulting in a temperature gradient and therefore enhanced fluorescence. Extra optically labelled TNFα is washed away and only the fluorescence due to the bound sites is recorded.

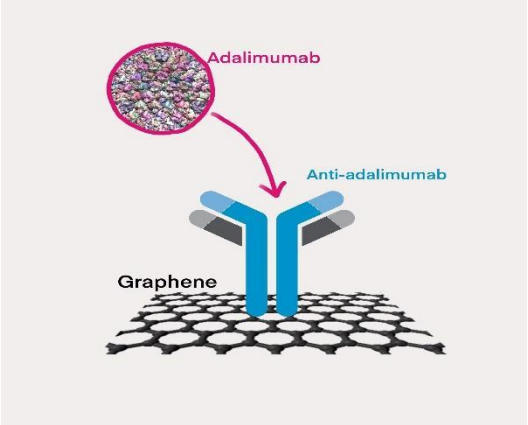

Fig.1

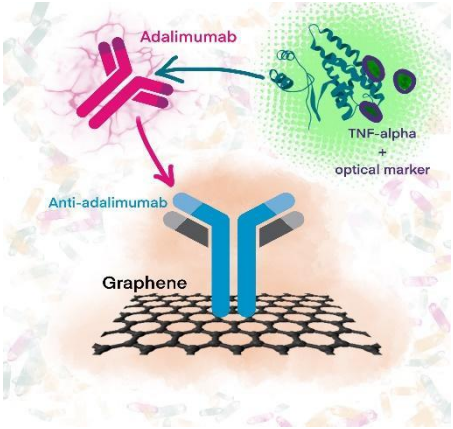

Fig.2

Fig.1 Depiction of readout using Electrochemical Impedance Spectroscopy
Fig.2 Depiction of readout using Fluorescence measurements

2.3 Cartridge Technology

For measuring the concentration of AM, we plan to inject blood samples containing the drug into the disposable chips (having graphene electrodes functionalized with Anti-AM) and then the optically labelled TNFα is made to flow through it. Once the fluid is stationary the electrical and optical measurements are made in the respective platforms and the chips are disposed after each measurement.

2.4 Reader instrument and user interaction

We have an integrated optoelectronic read out platform in order to improve the accuracy and speed of measurement. The entire setup is 20x20x40 cm^3 in dimensions. The sample is initially put in the disposable chips, as described in the previous section. The user must administer the blood sample and TNFα in the chip and put the chip in the measurement system. The electrical readout is measured using an Impedance Analyzer, where the impedance change obtained at a particular frequency will indicate the concentrations. The optical readout is basically an image of the fluorescence captured by the camera. This image is processed with python using OpenCV and NumPy and the corresponding results, after correlating the fluorescence and the calibration curve of concentrations, are displayed on a laptop. The user must adjust the focus of the camera to capture the image.

3. Novelty and Creativity

3.1 Already Available

Most widely used methods available in the market today use an antibody-antigen sandwich with AM and TNF-α in ELISA based assays to detect AM. The antibody-antigen binding is made visible using some form of pigmentation (for example-marked colloidal gold nanoparticles used in RIDA QUICK ADM Monitoring and Quantum Blue AM, a blue coloured chromogenic solution containing tetramethylbenzidine and hydrogen peroxide used in apDia AM ELISA) These methods are generally quite time consuming(requiring from 15 minutes to 2 hours of incubation timing) and a very high dilution ratios (from 1:20 to 1:2000) for some assays.

3.2 New Developments

In our sensor we have used a combination of electrical and optical readouts which helps deliver accurate and faster result (See Appendix for the pictures of our apparatus). The sensor currently available in the market doesn't work on the combination of these two technologies. The fluidic design is unique (Fig.3). It is symmetrically designed, with a common fluidic source at the centre and the capillaries distributed radially outward, so that the excess unreacted sample can be taken out of the chip. The capillaries are all of the same dimensions, between the source and drain points of the fluid, thus yielding consistent results. Due to the increased number of capillaries, we also have the advantage of obtaining more number of data points of measurements, thus enhancing the transduction signal(both electrical, in terms of more number of sensing electrodes and optical, in terms of more fluorescence) and giving more accurate results. We have also used the concept of MST to separate heavy and light molecules under a heat gradient, so that we have enhanced fluorescence from only a particular section of the capillary and not luminescing the entire capillary. Imaging set-up was 3D printed and micromachined in house to capture the fluorescence of the fluid using a T2 mount CMOS sensor. The images are then processed using python using OpenCV and NumPy and the results obtained from the electrical impedance measurements are cross verified. The time to readout for our platform is under 10 minutes, without any dilution required. The chips that we use for measurements are very cheap and essentially used once per measurement. In addition to this, our sensor can sense in a wider clinical range (0.1µg/ml to 15 µg/ml). Therefore, our sensor can also be used to detect AM that has been administered to cure diseases other than Rheumatoid Arthritis (RA), like Psoriasis [7] where the clinical range is higher than for RA.

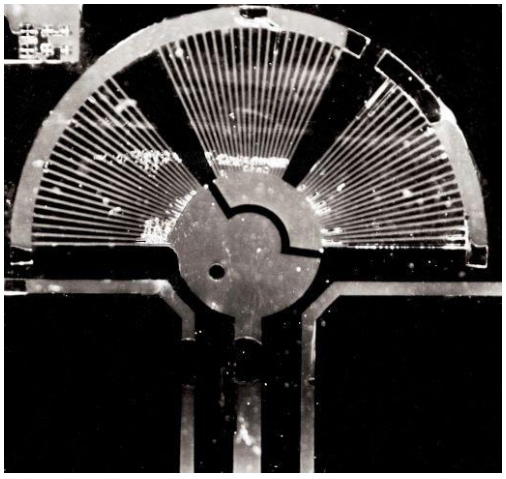

Fig.3 Image of the Fluidic Capillaries

4. Analytical Performance

a) **Electrical Readout:** We carried out EIS measurements on our chips with different concentrations of AM. Our intention was to relate the impedance changes with different rates of binding due to different concentrations. In our results, we found out that the impedance increases slightly immediately after adding the solution and then gradually decreases and if left for long enough, saturates. In the following instance, AM was added at 5 time-instants and the gradual decrease after each interval was studied. It was concluded that the surge at the time-instants (between 1200s and 1700s) was due to artefacts. The region with gradual decrease were accounted to the molecular bindings, as shown in the figure. The reading shown below is for the frequency of 857,145 Hz.

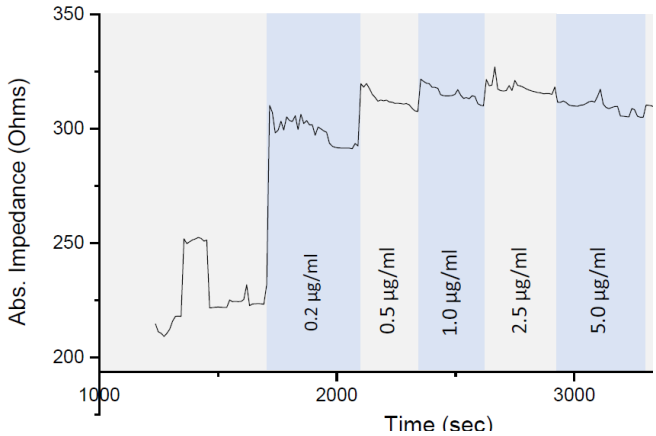

Fig. 4 Electrical Readout Results from Impedance Analyzer

b) Optical Readout: Using optical readout, our intention was to bind the TNF α (which is already bound to the Ag NPs) with AM. First, we carried out fluorescence spectroscopy to find the optimum concentration of the TNF α and nanoparticles solution, at which all the TNF α molecules are bound to the nanoparticles and there are no excess unbound free nanoparticles or excess unbound TNF-α. Then this solution is being progressively used with different concentration of AM to record the fluorescence intensity (excess unbound TNF α and nanoparticles, are flushed out) from our chips. We found this optimum concentration to be 3.8 g/ml TNF α solution with Ag NP stock solution. Exact calibration curve for recorded fluorescence intensity with different concentrations of AM will be presented in our technical pitch.

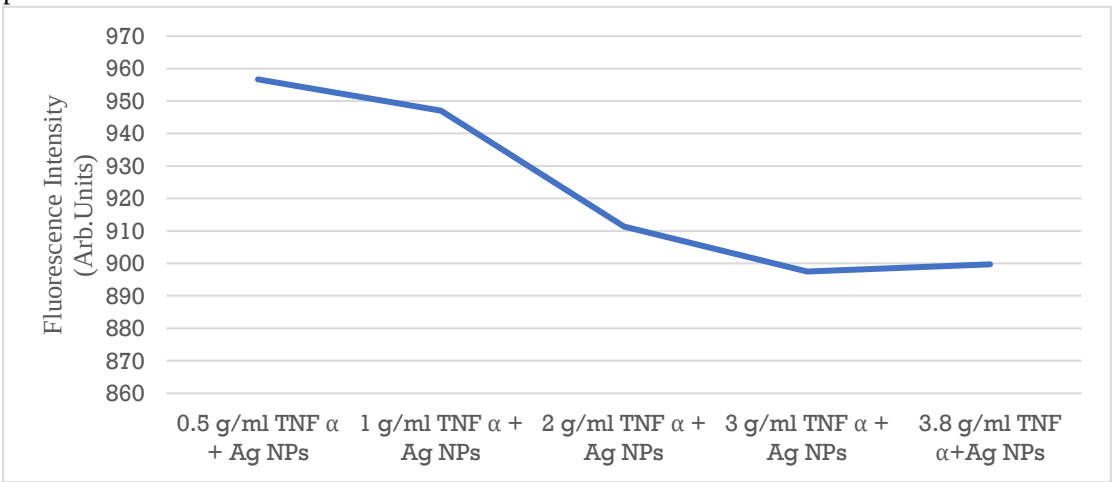

Fig. 5 Optical characterization of Silver Nanoparticles tagged with TNF- α solution

5. Translational Potential

5.1 Business Model Canvas

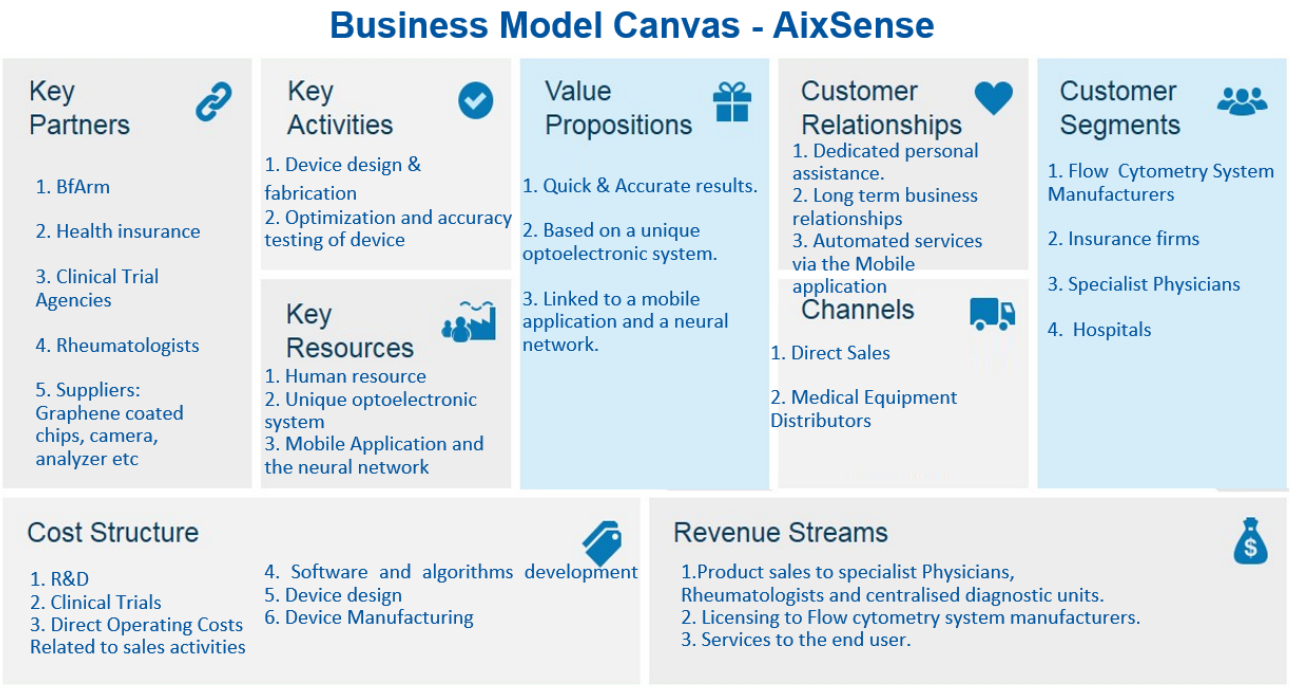

Fig.6 Business Model Canvas

5.2 Stakeholder Desirability

The AixSense team defined the problem to be addressed as the time, accuracy and ease of use of existing systems. Considering the trade-offs between these three aspects, we identified accuracy as the focal driver. The unique multimodal measuring system provides the user a double-checked measurement by two independent measurement principles to provide the most accurate and validated measurement of AM concentration.

The key growth driver for the AM biosensor market is the increase in the prevalence of Rheumatoid Arthritis and other diseases associated with the administration of AM, such as Psoriasis and Crohn's disease due to increase in life expectancy and environmental factors(American College of Rheumatology,2016). This leads to biological pharmaceuticals such as TNF-α blockers, which have been proven to be effective in treatment, to be used widely in its treatment. However, more than one third of the patients do not respond to the treatment or lose initial response within a few years (Bartelds, 2011). There is increasing evidence of the role of Anti-Drug Antibodies (ADAbs) in the decreased clinical efficacy and the association with low serum drug concentration. (Kneepkens, 2015) (Menting, 2014) (Garcês S,2013)

Having identified the rationale behind the need for concentration measurement, we set about identifying the roles of the various stakeholders in the ecosystem within Germany.

Based on some interviews conducted with patients as well as doctors within this context we were able to determine that patients would be interested in the proposition considering that many would like to better understand their condition and personally assess the efficacy of their treatment to rule out any other causes of alleviation.

University Hospitals do not necessarily see a need to invest in such an apparatus as the diagnostics within the hospital are focussed on tests which are conducted at high frequency. The tests which are conducted at a lower frequency such as a test for the concentration of AM, are routed to central diagnostic centres, which have been set up either at a state or at the federal level.

These diagnostic centres are supplied with flow cytometry systems, which are basically automated systems which combine a set of diagnostic tests into one single package. Central diagnostic centres tend to follow one homogenous system and therefore will not be able to acquire the biosensor directly until and unless it is integrated into their existing system.

Manufacturers of these systems could be potential customers for the biosensor but tend to follow buyout strategies when confronted with a breakthrough technology.

Finally, we have identified the specialists or the Rheumatologists within Germany to be out primary and potential customer segment. Commission for Health Care, 2008 estimated that 1 rheumatologist is currently available for every 50,000 people in the population in Germany. Therefore, we can conservatively estimate a total market of 1,640 individuals.

Insurance companies will ideally show interest when they see the potential cost savings in drug administration and therefore will be influential in the diffusion of the solution in the market.

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Current Market **Business Model**

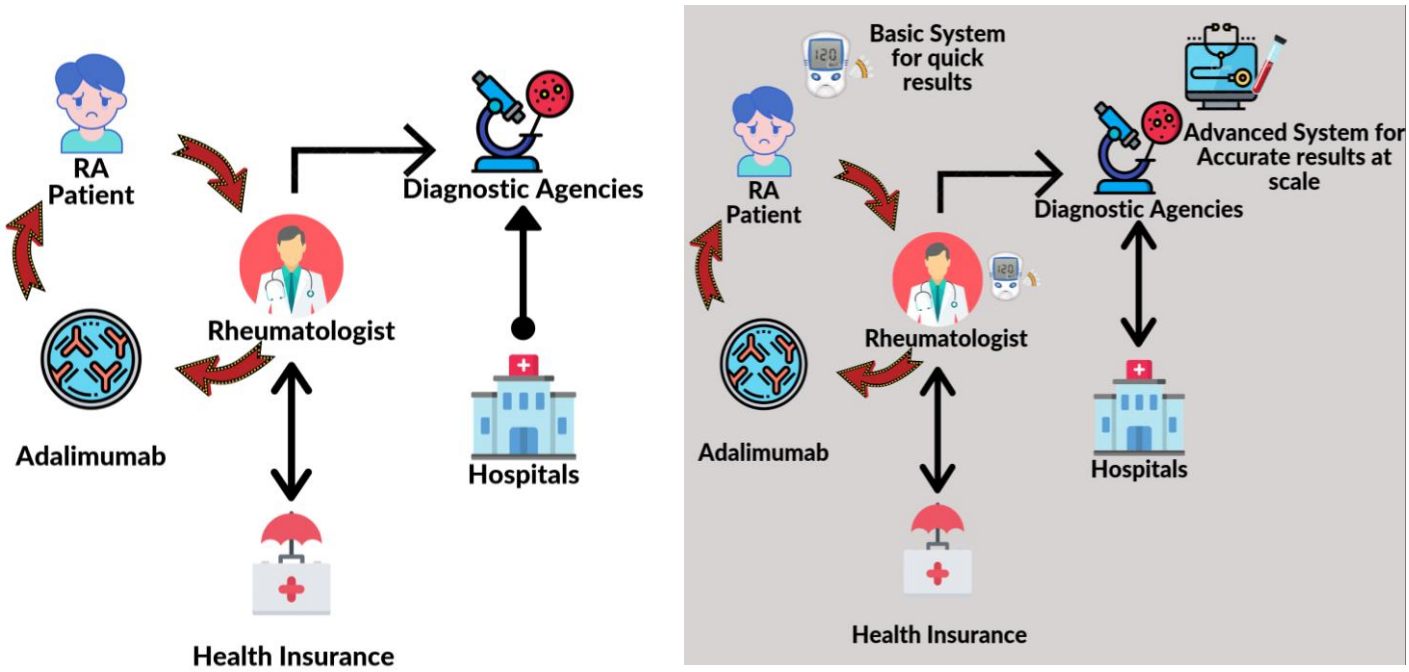

Fig.7 Market Scenario of an Adalimumab Measurement System

5.3 Financial viability

An average of 11% of adults in Germany are suffering from autoimmune diseases [Kantar Health. (2018)]. Going with a plan of targeting 500,000 RA patients in a market which doesn't yet have such a biosensor suitable for the patient's home, we expect revenues upwards of 70 million with a value-based pricing plan for the basic system. The 1,640 Rheumatology specialists and the diagnostic centres can drive in more revenues with the purchase of the advanced system.
Additional recurring revenues are expected from the consumables such as the chips for the basic and advanced systems.

5.4 Business Feasibility

The total cost of the prototype was approximately 1000 EUR. This included both the electrical and the optical readouts. The product offering will have three separate product lines with a modular system which allows the assembly of either the electrical and optical systems or both based on the final requirements. This will allow us to provide customers with multiple options but at the same time incentivise them through pricing to select the complete package. For example, the cost of the device containing only the electrical readout system can be brought down to less than 100 EUR. This would make the device viable for an RA patient to have one in their homes. Additionally, negotiations with suppliers and system integrators could considerably drive costs down with the help of economies of scale. The cost saving in terms of the optimal administration of the drug will therefore be the basis of our value-based pricing model. It will be vital to ensure that pharmaceutical companies also provide their customers with varying dosage options. Alternatively, we will be required to enable a complimentary business of AM storage which will allow users to store AM for later use.

6. Team and Support

6.1 Contributions of the team members

a. Apurva Roy

Apurva is a Masters student in Micro and Nano Electronics at RWTH Aachen University and the captain of the AixSense Team. Apart from being the primary contact person and a motivator, he has taken care of the Electrical based Measurements.

b. Gayatri Vasudevan Rajeswari

She is a Masters student in Micro and Nano Electronics and the second team captain of the AixSense team. In this project her contributions have been the Fluidic Design Verification and chip fabrication.

c. Arka Dipta Das

Originally hailing from a Power Engineering background, he is currently a Masters student in Micro and Nano Electronics. He is our chip technology developer cum banner designer cum travel manager.

d. Dibyendu Khan

Dibyendu is an ardent Masters student of Micro and Nano Electronics and has contributed with his valuable skills with Chip Design, Mask Designing for our chips and PCB Design for chip carrier. He is also involved with the design and assembly of the 3D structure for our final measuring apparatus.

e. Ruijun Yao

He is a master's Student in Micro and Nanoelectronics and is contributing to the team through the development of the Optical platform of our biosensor. He has also come in handy with some stress busting techniques in the last few strenuous working sessions.

f. Aparna Sai Malisetty

Aparna is a master's Student of Biomedical Engineering at RWTH Aachen. Apart from meticulously functionalized proteins on our biosensor, she has also been an active part of the Electrical sensing team.

g. Chenxu Zhu

Chenxu is pursuing her master's in biomedical engineering. Being quite a mathematician, she has contributed to the bio-functionalization team by accurately calculating the Dilution ratios and the clinical range of our biosensor.

h. Felix Burkhard

Felix is undertaking his master's degree in computer engineering and is our IT Man! He is our website designer and has made the algorithms to classify optical measurements.

i. Sushil Jacob

Coming from an Engineering background, he is currently enrolled at the RWTH Business School and undertaking specialization in Technology Marketing and Innovation Management. He is involved in the translational aspects of the project.

j. Aby Valiyaveettil Ajith

Pursuing his MS in Technology and Innovation Management, Aby is in charge of business development for AixSense.

6.2 People who have given support

This project has been an extensive work of 1 year, with a lot of people other than the team members chipping in at various stages of the project. First, we would like to thank our coach and mentor Dr. Vivek Pachauri, head of Nanomaterial and Bio-Nano Interfaces Group at the Institute of Materials in Electrical Engineering 1 (IWE1) for being always there to guide us newbies through the development of the biosensor. We are also grateful to our supervisor Prof. Sven Ingebrandt, Director of the IWE1 for opening the lab facilities to us and giving an overall ground support and direction to the project. We would like to specially mention Ms. Yuriko Maruichi, Visiting Researcher from the Masahiko Hara group of Earth and Life Science Institute, Tokyo Institute of Technology, Japan for her help with the optical sensing platform and characterizations. We express heartfelt gratitude to medical doctors- Dr. Sravya Muddu of ESI Hospital, Bangalore, India; Dr. Akshay Raju of Medical College Kottayam, India; Dr. Meera G Koottummel of TD Medical College, Allepy, India; Dr. Arathi Anil Azeezia Medical College Hospital Kollam India, Dr. Deepa Thomas of MOSC Medical College, India and other doctors from University Hospital Aachen for helping us understand the nuances of the behaviour of the antibodies and biomolecules involved in our project and also giving a sneak peek of the procedures of biomedical equipment acquisitions and clinical trials of the biosensors. Last but not the least, we would like to thank our family members, friends and colleagues of IWE1 for being supportive of the work we accomplished over the past year.

6.3 Sponsors

AMO GmbH has been our main sponsor. We are indebted to Prof. Dr.-Ing. Max Christian Lemme, CEO AMO GmbH and Dr. Thorsten Wahlbrink, Senior Researcher at AMO for providing all the necessary technical and material help for the project.

7. Final Remarks

Working on this project was a dream come true for all the team members and we got to learn a lot about the world of antibodies, how the biochemical reactions work and what are the different stages involved in the development of a biosensor.

We already have thought of an extension plan to this project, which goes much beyond the competition. Different diseases have different clinical ranges of administration of AM and therefore we want to further widen our clinical range to have more diseases in the ambits of our measurement. Next, using the concept of our radially outward flowing capillaries from a single source, we want to improve upon the existing lateral flow assays that are used to detect some target analyte in a liquid sample. We want to implement this concept on our capillary design and multiplex our capillaries further to increase the number of biomarkers that can be detected, in addition to AM, using a single chip. We can then create radial diagrams with different biomarkers and use the information to essentially detect (or even predict) any existing ailment in a patient, that has not been detected till now.

In addition to this, we also want to include a transponder unit in our measurement apparatus, that can send measurement data to a mobile application, thereby helping the doctor and the patient to track the results obtained.

AixSense

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[14]Electrical Impedance Analyzer image: https://www.sciospec.de/wp-content/uploads/2017/07/Sciospec-IsX-3mini.jpg

[15] Sensus logo: https://www.sensus.org/

9. Appendix

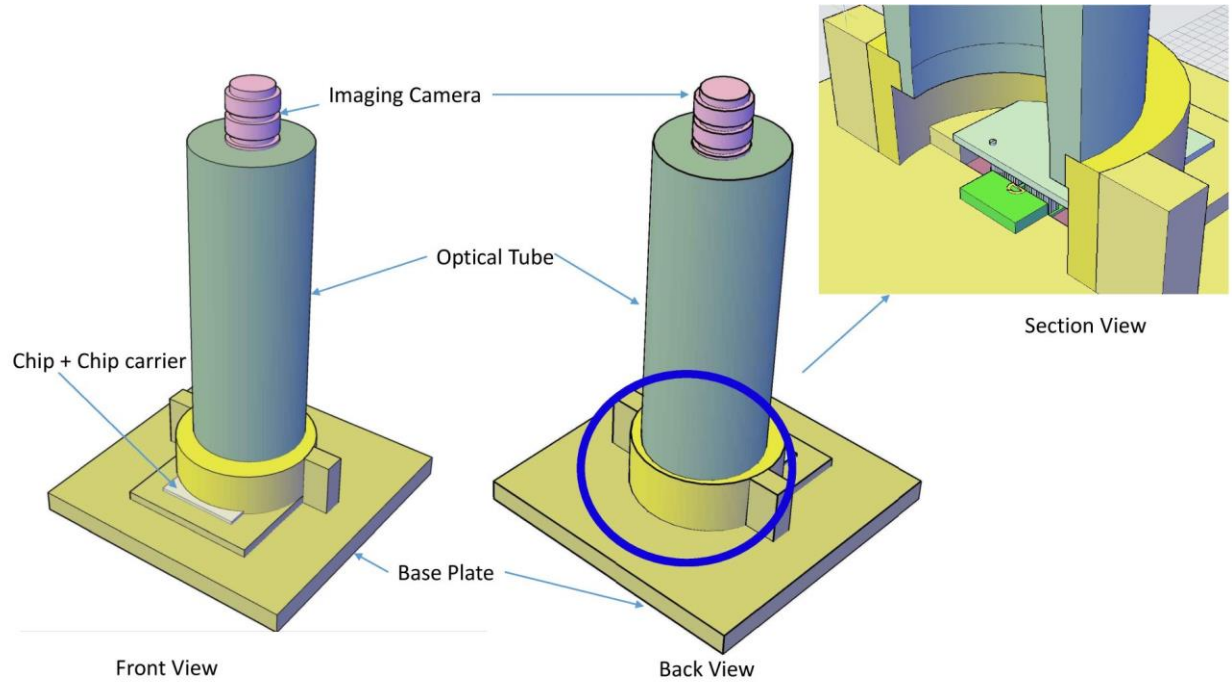

Fig.8 Optical Readout Apparatus

Fig.9 Electrical Impedance Analyzer for EIS measurements
(Courtesy: Sciospec Scientific Instruments GmbH)