Project Report: AixSense

Analytical System for Opto-Electronic Monitoring, Control and Detection of Complex Biological and Chemical processes

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1. Abstract

It has become essential to realize miniaturized analytical systems that can service various diagnostics fields and offer attributes such as ease of use, portability, and multi-faceted detection to counter the emergence of new biological threats and their global spread. The ability of the analytical platforms to carry out real-time analysis and their versatile integration capabilities like regulating various biological process conditions such as temperature, flow, pressure etc. have become key technical advantages of real consequence in real-world applications. For example, an analytical platform with smart integration of process control mechanisms aided by the highly configurable fluidics can be deployed for real-time electrical detection of biomolecular binding of complex biological processes such as disease diagnosis or drug detection.

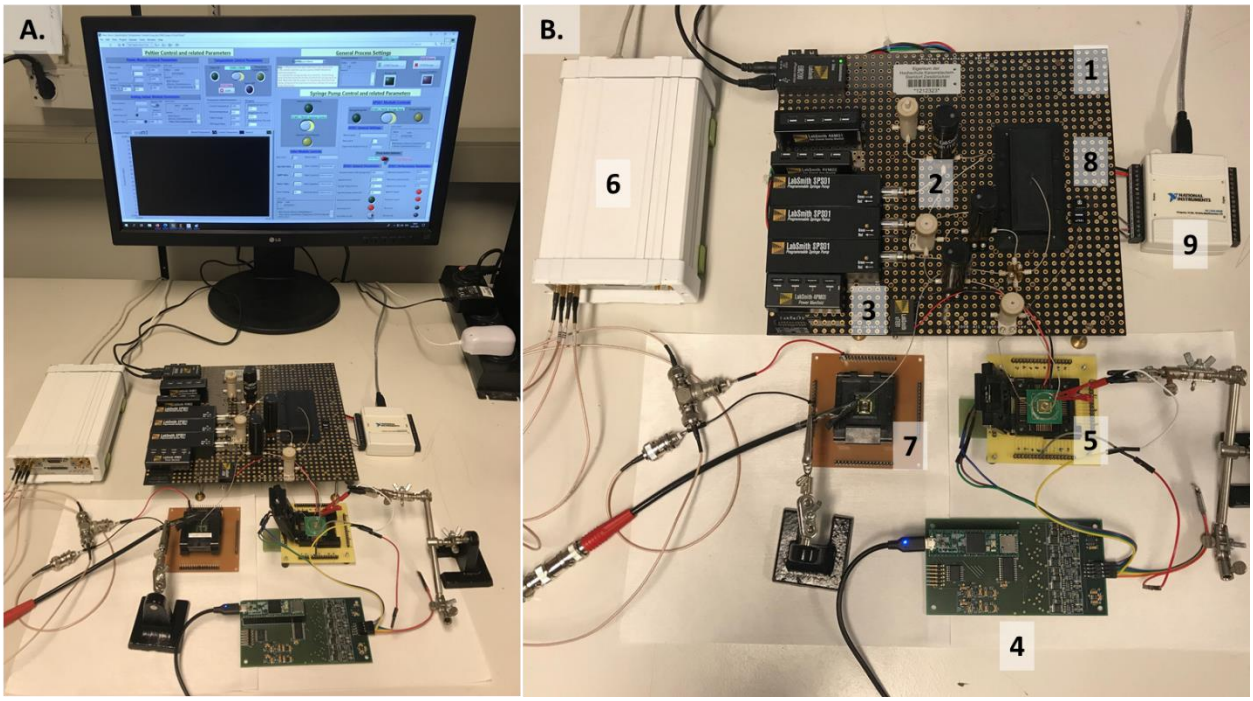

Figure 1: A. The entire analytical system and its accessories. B. A detailed view of the system with its different components, 1. LabSmith platform with 2. Flow control and 3. Temperature control, 4. ISFET sensor Characterization module and 5. Electrical ISFET sensor, 6. Impedance Analyser (Sciospec ISX-3 mini) and 7. Electrical Impedance sensor (IDE), 8. Optical sensor (Photomultiplier Tube) and 9. Optical detector signal processing module (NI USB-6008).

The analytical system developed in this work as demonstrated in the Figure 1, involves flow and temperature control schemes with the provision for parallel electrical and optical characterization of a sample solution. The system comprises a flow scheme consisting of components such as programmable valves and pumps that can be used for fluid sample preparation and injection to the

designated sensor-site, a temperature control scheme consisting of a temperature sensor and a heating/ cooling element (Peltier Element) with a closed-loop control that maintains the temperature of the analysis setup at a particular defined value. The setup also offers possibilities for electrical detection based on Electrochemical Impedance Spectroscopy (EIS) and ion-sensing field-effect transistor (ISFET) characterization techniques as well as an integrated optical sensor for parallel detection of desired analytes in a sample. This entire system is controlled through graphical user interfaces (GUI) from a computer as depicted in Figure 2. This enables straightforward control of process conditions, effortless analysis, and comparison of responses of the detection mechanisms employed.

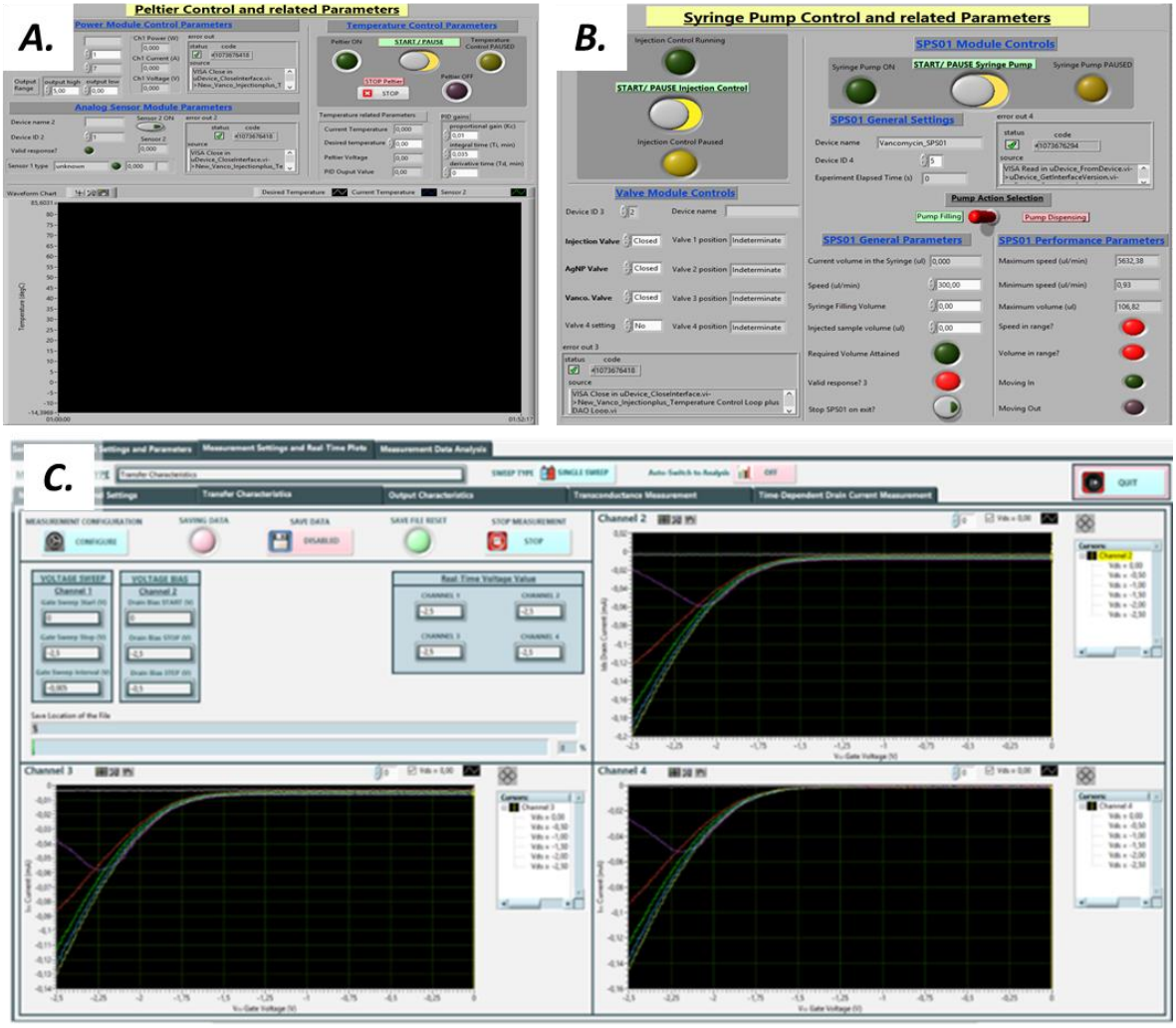

Figure 2: GUI developed for A. Temperature Control Scheme, B. Flow Control Scheme and C. ISFET Sensor Characterization Module.

The automated system thus exhibits a platform capable of performing complex analytical functions at desired process conditions with greater accuracy and efficiency. This will serve as the basis of portable drug analysis and disease diagnostics tools.

2. Innovation

The study of sophisticated biological processes on a chip requires integration of various control mechanisms, small-volume sample handling systems and biosensor readout techniques to monitor the biological interactions involved in these complex processes. The prototype deals with realization of an 'ultra-light three-way readout and automated analytical system' (ULTRAS) for accurate opto-electronic drug-detection and disease diagnostics.

The prototype consists of an automated microfluidic platform with a wide range of control capabilities, realized for implementing various fluidic control mechanisms required for realization of complex biological and biochemical processes. Development of analytical set-ups such as ULTRAS, featuring reconfigurable open and closed-loop microfluidics, with precise temperature and flow controls, is vital to ensure ideal process conditions for accurate measurement of the biological components. Integration of various optical and electrical biosensing mechanisms in ULTRAS are aimed at parallel measurements of biomolecular interactions as well as carrying out fast and accurate analysis of molecule-specific sensor signals. A dedicated user-interface was also developed in LabVIEW for active control of ULTRAS as well as for optical and electrical readout tools.

The measurement and analysis of molecule specific signals on ULTRAS was demonstrated by detection of vancomycin in different media using optical and electrical techniques, such as chemiluminescence and electrochemical impedance spectroscopy, respectively. Such a setup can be easily modified to detect numerous other antigens or molecules such as COVID-19, Influenza virus etc. with immense accuracy and in a just a few minutes. This establishes the multi-faceted use-case as well as flexibility of our prototype along with the possibility of using multiple measurement techniques.

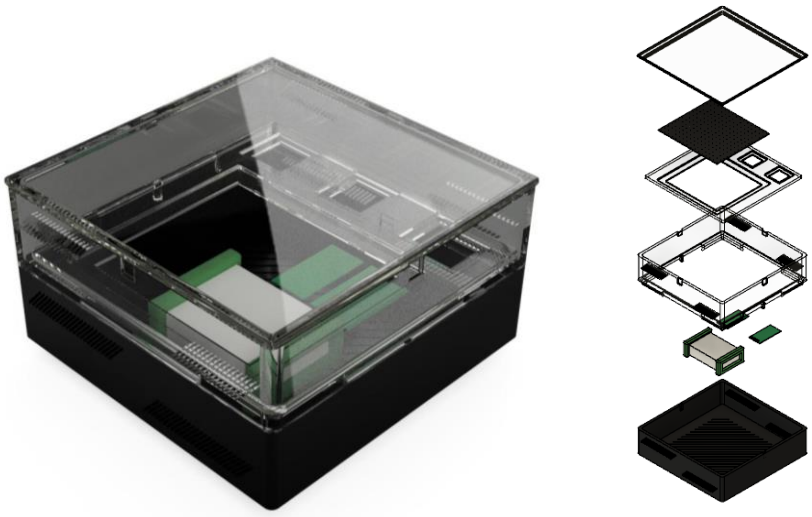

Figure 3: The first design of the enclosure of our system.

3. Performance of the ULTRAS

The performance of the prototype was tested in the detection of a drug called Vancomycin in water media. The dose response of vancomycin was investigated using optical and electrical measurement techniques to establish a relation and reference for accurate indication of the amount of antibiotic present in the sample. The Figure 4 depicts the biomolecular binding interactions that take place in case of such specific measurements. The sensor surface is functionalised with antibodies related to the antigen being detected (in this case, its Vancomycin). The antigen if present in the sample attaches itself to the antibodies and this binding interaction can be detected using several readout strategies.

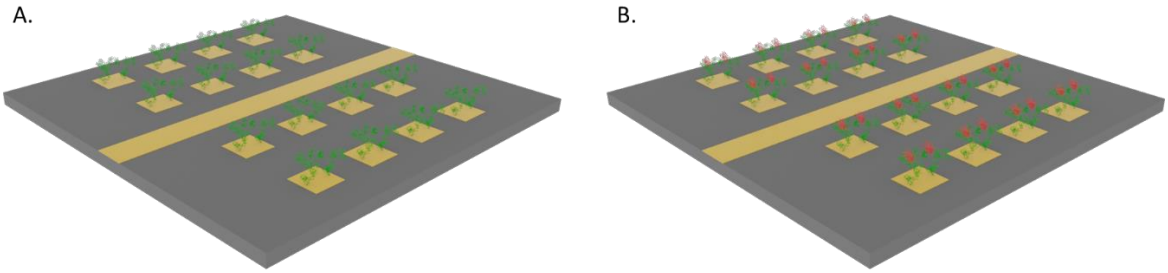

Figure 4: A graphic depicting the electrical sensor having antibodies on its surface, A. without the Antigen and B. with the Antigen attached.

The optical measurement of Vancomycin was carried out by taking advantage of the chemiluminescence (CL) reaction between Luminol and Hydrogen Peroxide which leads to the generation of photons having wavelengths around 425 nm. It has been reported that the CL signal is inhibited by the presence of Vancomycin molecules. Thus, a higher concentration of Vancomycin leads to higher inhibition or reduction of the optical signal intensity giving a measure of Vancomycin concentration in the solution (Figure 5A).

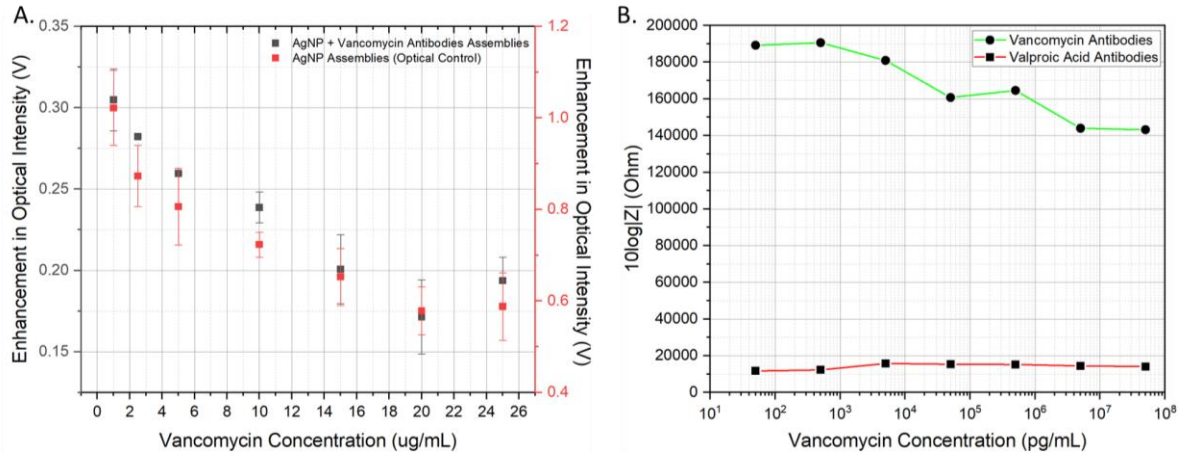

Figure 5: The performance of our prototype in detecting the concentrations of Vancomycin using A. Optical and B. Electrical Measurement techniques.

In a similar manner, vancomycin concentration was measured using ‘Electrochemical Impedance Spectroscopy (EIS)’ as seen in Figure 5B. The vancomycin molecules lead to the reduction of impedance of the electrical sensors. Hence, the impedance of the sensor reduces with increasing concentration of Vancomycin.

The results in aboveFigure 5 showcase a strong potential of the prototype to measure antigens or molecules with multiple measurement strategies in parallel and good accuracy and repeatability.

4. Real-World Applications of the ULTRAS

The development and expansion of the pharmaceutical industry has led to an increase in the production of various drugs to fulfil the needs of the growing population. However, the ease of access and availability of drugs has caused several issues. One of the leading issues that is being studied and researched by various health organizations around the world is 'Antimicrobial Resistance (AMR)'. The World Health Organization (WHO) defines AMR as, 'Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death'. As a result, the current recommended dose-level are not adequate to tackle these diseases. The solutions to these issues are to either increase the dose-levels or to find alternative drugs that are more powerful than the existing ones. The WHO has thus declared AMR as one of the top 10 global public health threats.

Vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$), a branched tricyclic glycosylated peptide is used to treat life-threatening infections due to gram positive bacteria such as methicillin-resistant Staphylococcus aureus. Vancomycin acts as a bactericide by destroying the cell wall of the bacteria. Vancomycin is a commonly available medicine which is listed by the WHO as essential and critically important medicine. Some studies report that the expansive use of vancomycin for treating the bacterial infections resistant to other drugs has led to the development of AMR against vancomycin and that this AMR has necessitated the study of vancomycin administration through measurement of the drug concentration.

It has thus become important to monitor the level of vancomycin in human samples, environmental and water samples to prevent the development of AMR due to low dose levels and side-effects such as loss of hearing ability (Ototoxicity) and deterioration in the kidney functions (nephrotoxicity) due to high dose levels. Some recent studies suggest that the trough levels (of vancomycin concentration) of 10-15 µg/mL or 15-20 µg/mL are appropriate depending on the type of infection. The trough concentrations are important as they can be correlated to the related side-effects. Different detection strategies can be employed to detect the concentration of vancomycin in a particular sample solution due to availability of antibodies specific to vancomycin.

The prototype developed by AixSense can accurately and efficiently measure the levels of vancomycin in microliter volume fluid samples, thus enabling targeted diagnostics and preventing wastage of precious samples while providing a mix of optical and electrical detection methods.

5. ULTRAS: Prototype Setup

The process parameters such as fluid flow and temperature are regulated by means of a system created using LabSmith components such as programmable pumps, automated valves, temperature sensors and heaters etc. All the LabSmith components have been obtained from LabSmith, Inc. (USA).

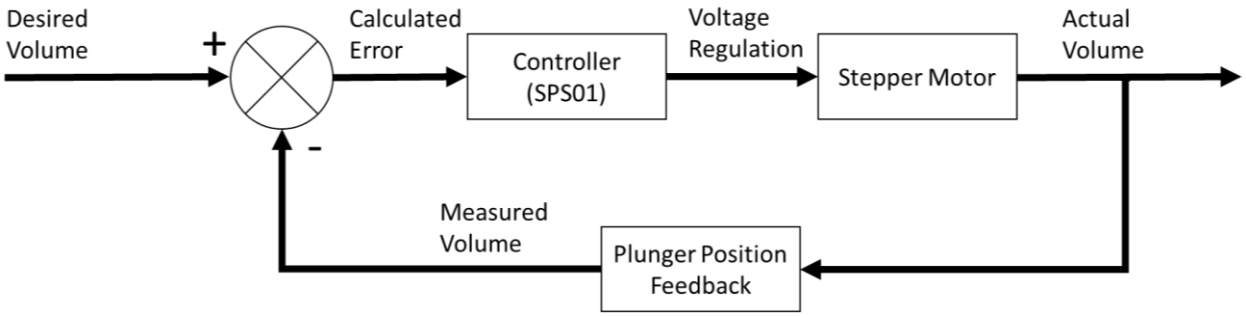

Figure 6: The feedback control scheme for fluid flow control has been illustrated.

The dilution, mixing, circulation and manipulation of various reagents or analytes is extremely significant in analysis of various biological, chemical, or biochemical processes. This is achieved through the precise regulation of fluid flow rate and pressure in the fluidic circuit by means of an intricate web of sensors and actuators operated using control algorithms. The system consists of a controller that constantly monitors an input signal from the user and then based on the pre-defined conditions and correlated mathematical calculations in the algorithm, it applies proportionate output signal to an actuator to manipulate the desired parameter within an acceptable error margin. The sensors then detect the actual value of the manipulated variable and feed this signal back to the controller in the form of a calculated error value (*Figure 6*).

Figure 7: A. Electronic Interface Board (EIB200), B. Programmable Syringe Pump (SPS01), C. Borosilicate glass syringe with a plunger, D. PEEK fittings and tubing, E. Reservoirs for fluids, (F-H) Automated Valves (F. AV201, G. AV202 and H. AV801), and I. Four channel Valve Manifold (4VM02). Operating Temperature of all electronic components is 10-80°C.

The foundation of the configurable fluidic system as shown in Figure 8 is the use of automated valves, programmable syringe pumps and reservoirs connected to each other via a network of PEEK (Polyether ether ketone) tubing and fittings that promote extremely low dead-volumes and ensure a leak-proof fluidic system. The devices are connected to their respective control modules using 6-pin flat-ribbon cables.

The control loop comprises of a programmable syringe pump and automated valves controlled by the user through the GUI. The bi-directional motion of the syringe pump is influenced by the user-specified set-point and the positional feedback is conveyed to the controller constantly. The pump ceases to move as soon as the desired position is attained resulting in accurate sample volumes and reduces wastage and prevents experimental inaccuracies. The fluid flows through the fluidic circuit through the automated valves that control the path of the fluid flow.

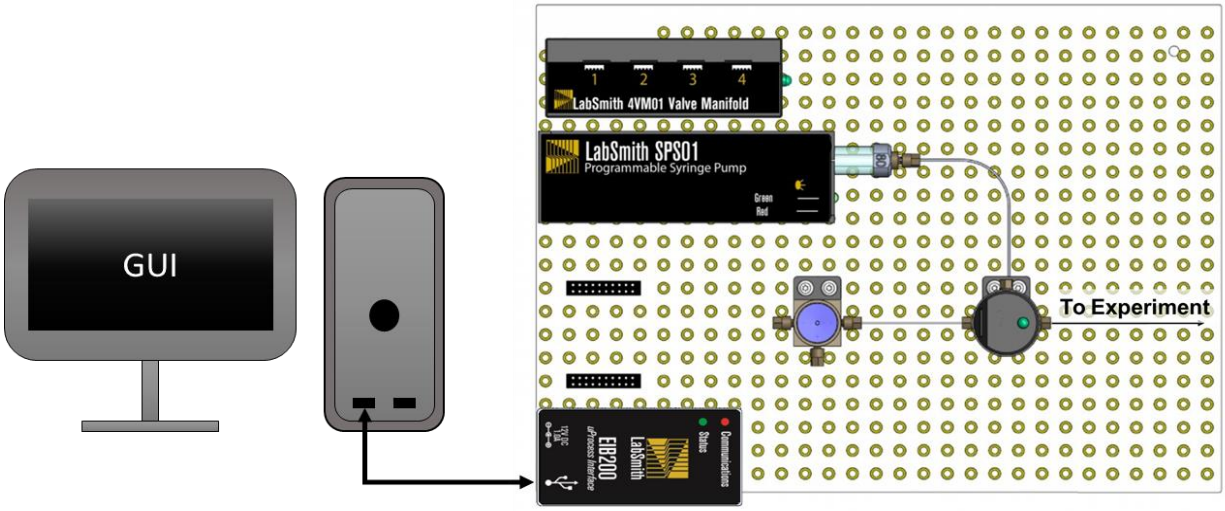

Figure 8: A schematic depicting the installation, connection, and communication within the different components of the flow control setup.

One of the most important consideration in the bioprocesses is maintaining the temperature of the analyte or the entire system at a certain value to promote an ideal milieu for the significant interactions being sensed.

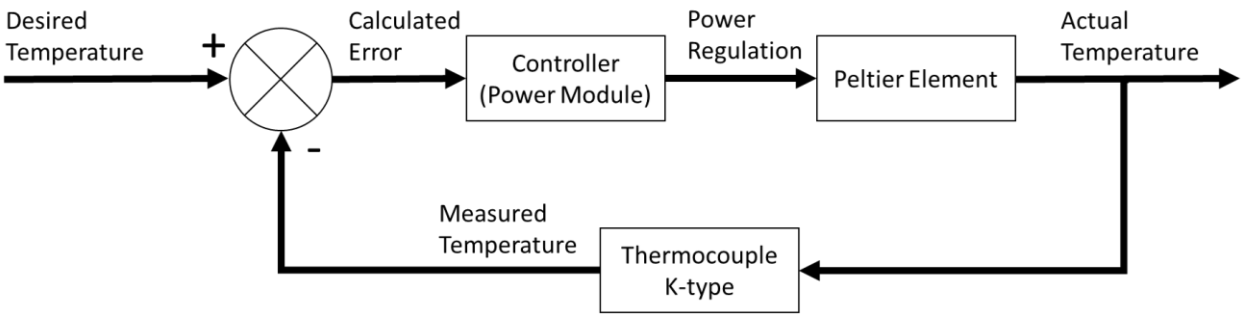

Figure 9: A schematic illustrating the temperature feedback control loop.

A temperature control loop caters to this specific requirement. It comprises of a power module (controller) that takes in the input from the user and regulates the amount of power being provided to the Peltier element (heater). This leads to a change in the temperature which is detected by a temperature sensor (K-type thermocouple). The actual temperature reading is then fed back to the controller as a computed error after being subtracted from the desired temperature input as described in Figure 9.

The control setup in Figure 11 represents a control loop with a temperature sensor (K-type thermocouple) and a Peltier element (uTE) that are connected to the analog sensor module and the power module respectively using flat-ribbon cables (components shown in *Figure 10*). The EIB200 acts as the bridge between the devices and the GUI.

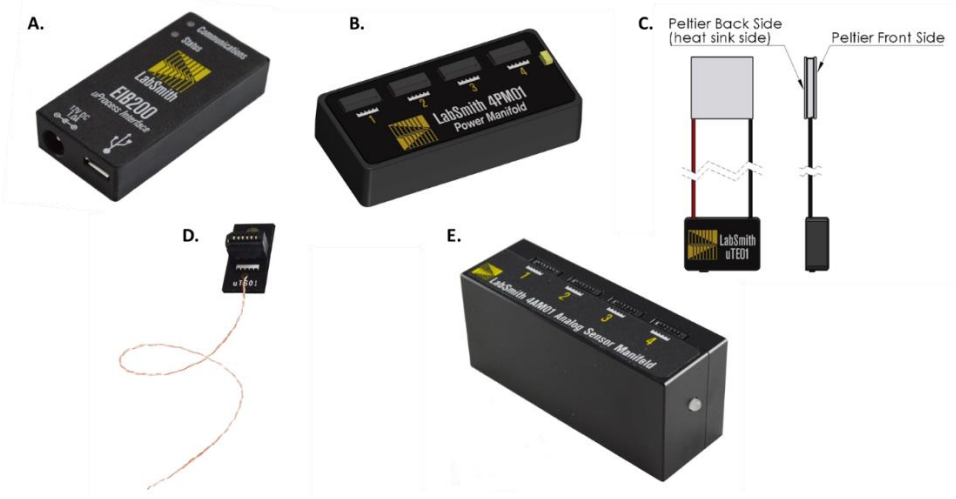

Figure 10: A. Electronic Interface Board (EIB200), B. Power Manifold (4PM01), C. Peltier Element (uTE01), D. K-Type Thermocouple (uTS01), and E. Analog Sensor Manifold (4AM01). Operating Temperature of all electronic components is 10-80°C.

The temperature sensor senses either the temperature on the chip surface or in the fluid itself and feeds it back to the controller through the analog sensor module. The control algorithm then calculates the power required to drive the temperature to the desired level and sends it to the power manifold. The power manifold supplies the required power to the Peltier element that can be used for heating or cooling depending on the user defined temperature set point. The controller computes the error between the desired temperature value and the actual measured temperature value and corrects the power that is being applied to the Peltier element.

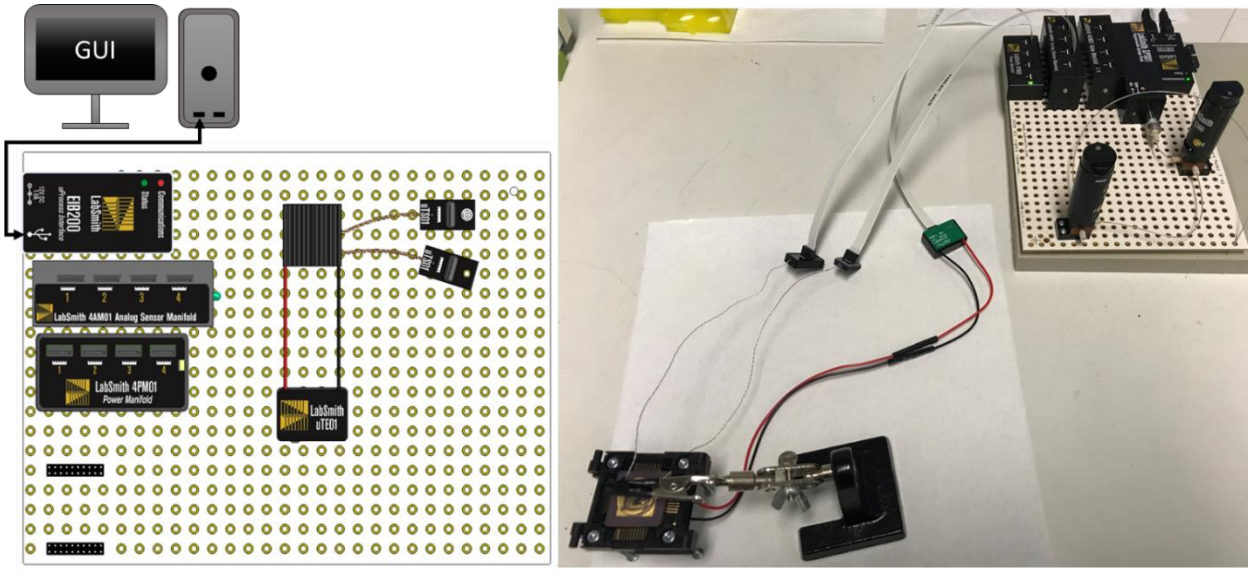

Figure 11: A schematic depicting the installation, connection, and communication within the different components of the temperature control setup. The actual temperature setup is depicted on the right.

The fluid flow and temperature schemes are controlled through a Graphical User Interface (GUI) developed in LabVIEW Application Builder using the LabVIEW Professional (licensed software from National Instruments). The GUI depicted in Figure 12 consists of several controls for the user, to easily operate, monitor and control the various devices in the two control loops consisting of switches, indicators, user input fields, and error troubleshooting windows. It has been designed considering the ease of operability for the user. The important instructions are provided on the front panel, to explain the working of the system to the user and to prevent undesired operation of the devices.

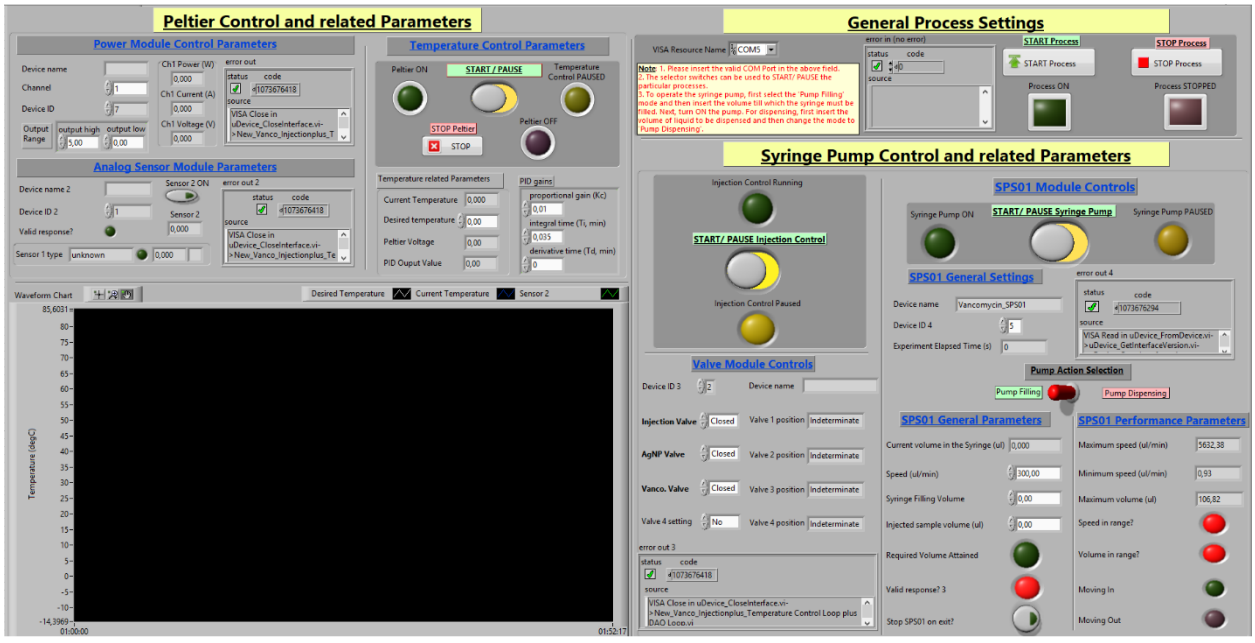

Figure 12: The graphical user interface for temperature and flow control is illustrated.

5.1 Measurement Techniques

5.1.1. Electrical Sensing

Figure 13: Measurement devices for electrical sensing, A. 4-Channel Portable Readout Module, B. Sciospec ISX-3 mini and C. Yamaichi IC-51 adapter.

The electrical detection in the various experiments was performed by employing the field-effect measurement and Electrochemical Impedance Spectroscopy (EIS) principles. The wire-bonded and encapsulated electrical sensors were inserted in the Yamaichi IC-51 sockets (Figure 13C) installed on an interface PCB board which connects the sensor to the measurement devices.

5.1.1.1 Field Effect Device Measurement

Silicon-Nanowire chips and the p-type ISFETs were characterized by gauging their field-effect utilizing the 4-Channel Portable Readout Module. The 4-channel voltage-current readout module (Figure 13A) makes use of high-precision circuitry to aid in characterization of diverse sensors such as traditional transistors, nanowire, and even graphene-based transistors. The module boasts of four channels, each of which supplies voltage input to the sensor as well as measures the voltage response from the sensor at the same time.

The module is configured and controlled for the measurements using the GUI created with the help of the LabVIEW Application Builder. The communication between the module and the GUI is established through serial communication protocol via a USB connection.

The module can be used to perform four types of measurements. The modes allow the user to study the transfer characteristics, output characteristics, transconductance, and time-dependent drain current analysis of a transistor device. The general layout of each mode (Figure 14) consists of a configure, data save, measurement stop buttons and related indicators. The path directory for the save file and the measurement progress in percentage are also provided to the user. The modes also show the real-time values of the voltages supplied by each channel. The voltage bias and voltage sweep ranges are also shown in the front panel.

Additionally, the layout also features three waveform graphs that display the real-time measurement data.

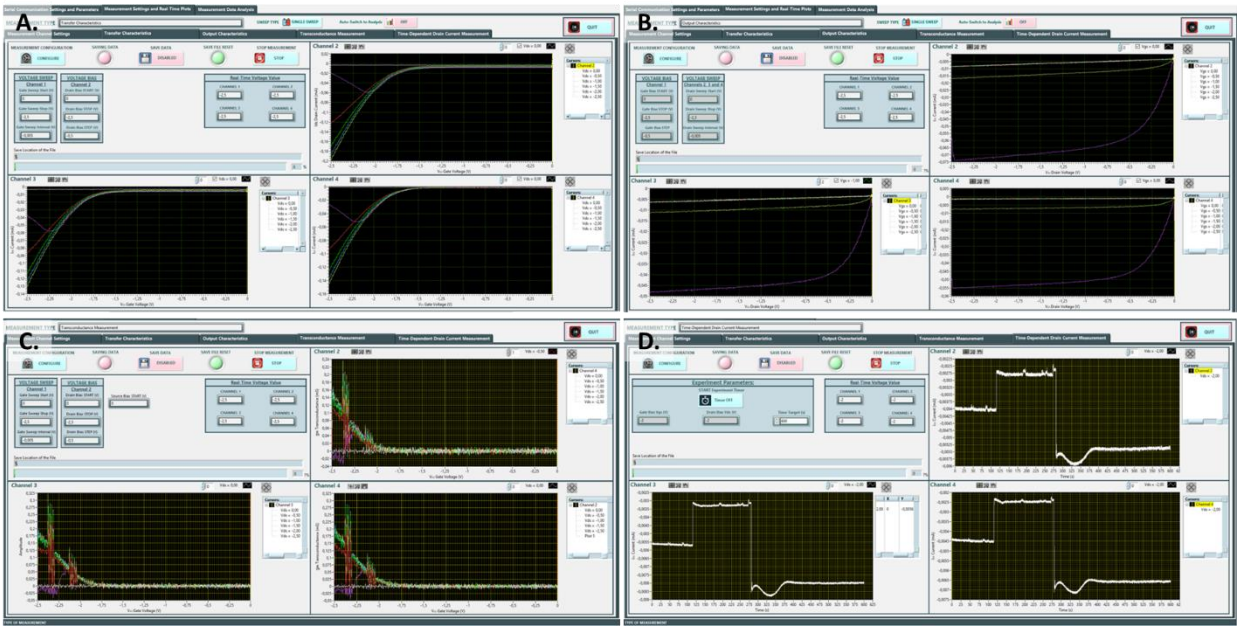

Figure 14: A. Transfer characteristics measurement, B. Output characteristics measurement, C. Transconductance measurement, and D. Time-dependent drain current measurement.

5.1.1.2 Electrochemical Impedance Spectroscopy

The impedance sensors were characterized employing the EIS measurement principle with Sciospec impedance analyser.

The analyzer is calibrated, configured, and operated through the different modes available, such as device calibration, device setup, and real-time measurement. The user can calibrate the analyzer via the calibration mode. The measurement settings such as start frequency, stop frequency, frequency count, amplitude, measurement channel, measurement precision, range of measurement can be selected in the device-setup mode.

The measurement mode of the device can be controlled via the GUI (Figure 15) developed using the LabVIEW application builder. The user can connect the device through this interface and communicate with the device through various controls and buttons provided on the front panel. The measurement can be initialized by toggling the 'Measurement ON/ OFF' button. The program then establishes a communication link to the device and puts up the device ID in the respective indicator and the 'Measurement ON' indicator is illuminated. The GUI consists of interactive waveform graphs that show the measurement results such as the Bode and Nyquist plots, and the real and imaginary impedance values. The user can personalize these fields for ease of use and readability. The software also prompts

the user in case of any connection, communication or power issues and automatically suggests solutions to solve these issues.

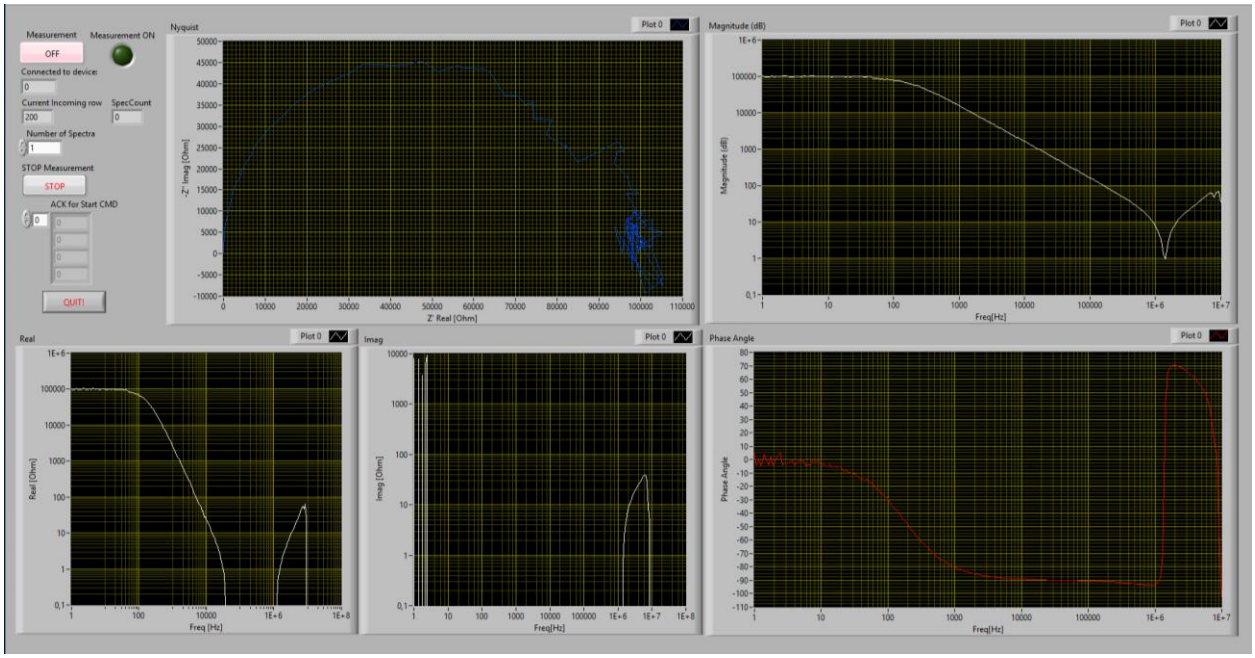

Figure 15: The GUI developed for operating the Sciospec Impedance Analyzer.

5.1.2 Optical Sensing

The main components of the optical sensing system are the photomultiplier tube (PMT) from Hamamatsu Photonics K.K. and NI-DAQ USB-6008 from National Instruments Corporation.

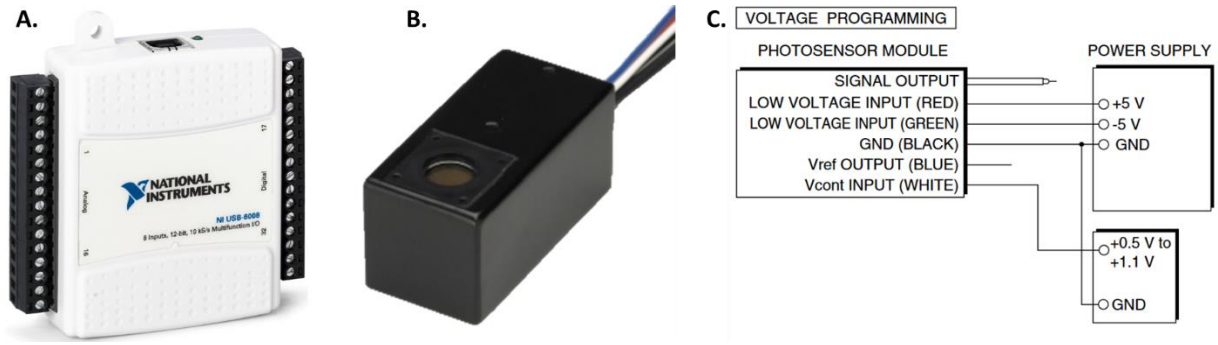

Figure 16: A. NI-DAQ USB-6008. B. PMT (Model: H10723-20) and C. The connection diagram for the PMT.

The optical readout module H10723-20 is a photo-multiplier tube (PMT) packaged in a metal housing and manufactured by Hamamatsu Photonics K.K. The PMT has an optical window size of 8 mm diameter, and it is sensitive to wavelengths varying from 230 nm to 920 nm. The module consists of a low noise amplifier and a high-power supply circuit that can be operated with low power, thus enhancing its portability and ease of use. The amplifier converts the PMT current output into a voltage output signal that can be processed easily. The signal processing of the PMT output is carried out by

the NI USB-6008, a multifunction data acquisition device from National Instruments, USA. The device features 8 analog inputs (AI) or 4 differential inputs and 4 analog outputs (AO). The USB-6008 I/O are addressed and can be configured and controlled using a computer connected via the USB interface.

The optical sensor is operated using a GUI developed with the LabVIEW Application Builder. The GUI allows the user to control the NI DAQ device. The values for the positive input supply voltage and the control voltage can be set within the advised limits according to the instructions on the front panel. The other parameters such as the sampling rate, sampling mode and the voltage output readout channel can be selected. The GUI also has control switches for switching the digital I/O channel values. The optical detection can be commenced by pressing the 'START' button and then by selecting to turn on the PMT. The PMT starts measuring the optical signal and transmits a proportionate output voltage. The PMT can be paused or stopped, if necessary, with the control button on the front panel. The output voltage can be monitored real-time in the indicator and is also displayed graphically on the waveform chart that shows the change in PMT output voltage with time. The optical detection data can be saved, and the directory of the file is displayed in an indicator.

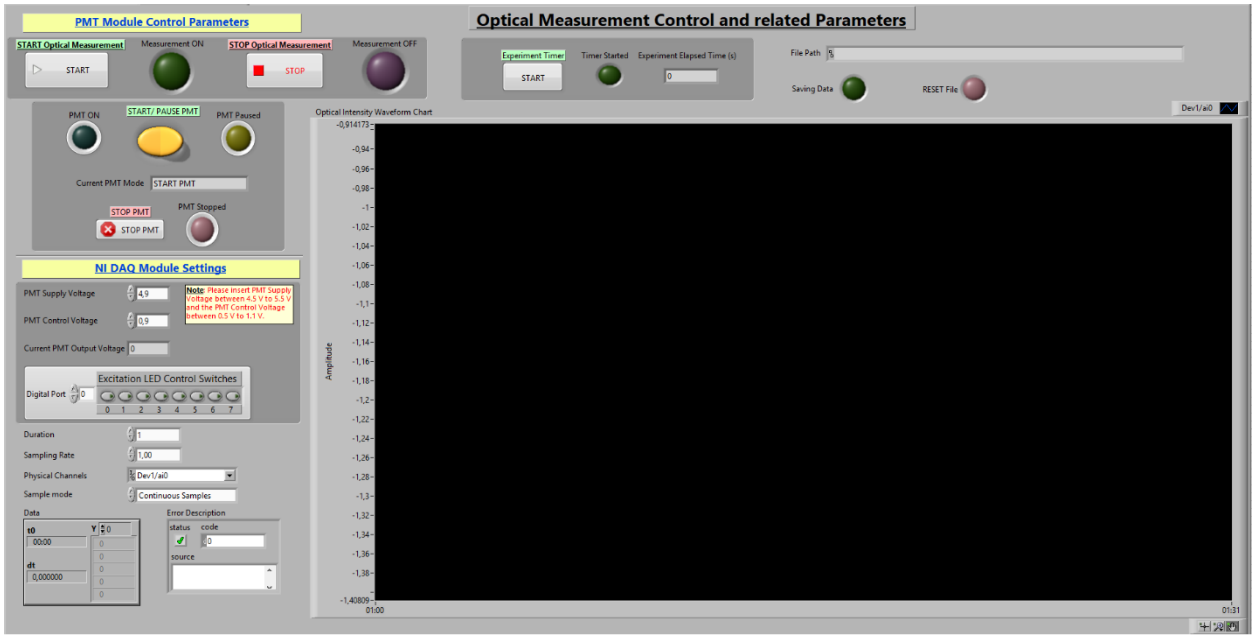

Figure 17: The GUI for operating the optical detection setup.

The optical sensing was very sensitive to the ambient or stray light. Thus, it was very important to carry out these measurements in an enclosure that offers a dark area eliminating the interference due to other light sources. An enclosure was designed using the Inventor software from Autodesk, Inc. and then was 3D-printed with ABS Plastic as shown in Figure *18* respectively. The enclosure is made of four parts: a housing for the optical sensor, a lock plate to prevent the lateral motion of the sensor, a carrier to hold the optical devices, and a top cover to close the housing and create a dark environment.

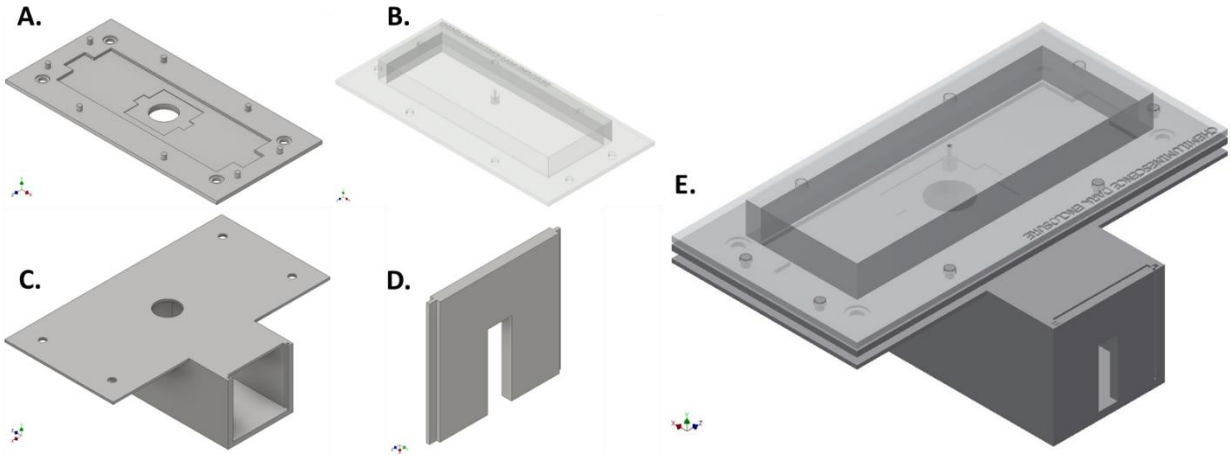

Figure 18: The 3D designs of the E. optical enclosure assembly made up of A. optical chip-holder, B. top-cover, C. optical sensor housing and D. a locking plate are shown.

The optical devices used for measurements contain a PDMS cut-out (10 mm x 10 mm) glued onto a glass substrate i.e., a glass cover slip (18 mm x 18 mm) or a microscope slide (76 mm x 24 mm) as illustrated in Figure *19*D.

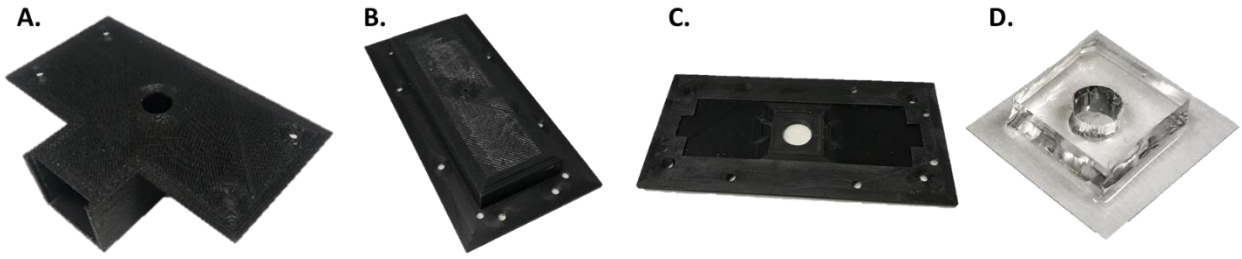

Figure 19: The 3D-Printed parts: A. optical sensor housing, B. top-cover, and C. optical chip-holder. D. Glass chip reservoir.

An enclosure was designed to fit all the sensor and components into a compact and portable 3D-model as shown in Figure 20.

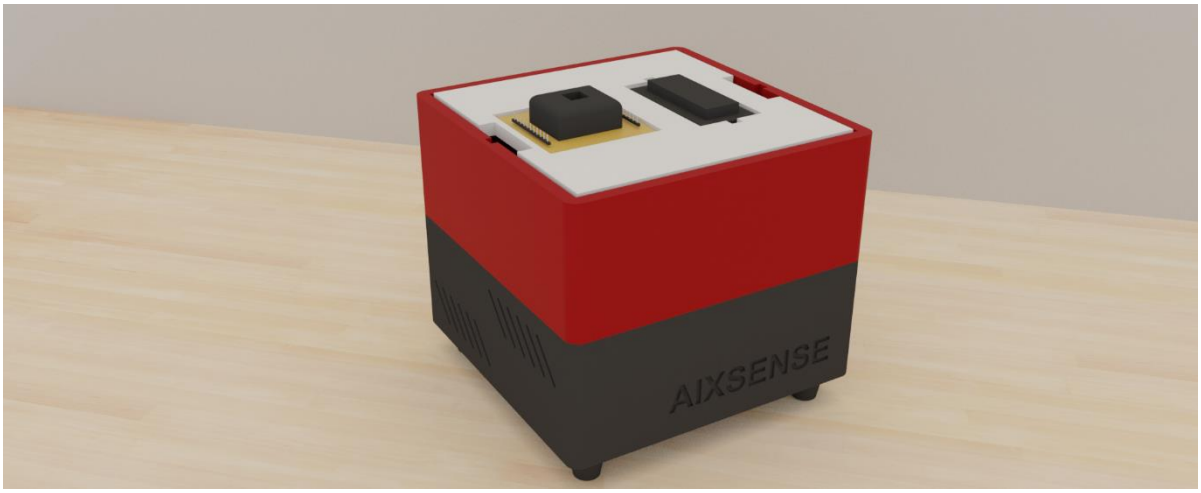

Figure 20: A rendered image of the 3D-model of the prototype designed and built by AixSense.

6. Market Analysis

Introduction of improved technology, such as digital microfluidics, which enables on-chip biochemical analysis and handles low-volume sample devices, is anticipated to boost the growth. The global biosensors market size is expected to reach USD 24.2 billion by 2021. It is anticipated to register a compound annual growth rate (CAGR) of 18.07% during the forecast period. Optoelectronic Sensing Market is expected to reach a worth of $77.9 Billion by 2027, Growing at a CAGR of 9.6% From 2020.

The electrochemical segment dominated the market for biosensors and accounted for the largest revenue share of 71.9% in 2021 owing to its widespread applications for quantification and analysis in biochemical and biological processes. Electrochemical transduction presents considerable advantages over thermal, piezoelectric, or optical detection, thereby leading to high consumption and greater market penetration.

The use of optical sensors for biosensing applications in the healthcare industry allows monitoring of drug delivery and functions of other vital organs in the human body. Thus, the use of non-intrusive, inexpensive sensors across advanced healthcare applications offers a paradigm change in terms of consumer wellness. Thus, market entities have already begun to integrate microfluidics technology into their existing portfolio of diagnostic products. The ULTRAS setup can be of special interest for microfluidics market, with the focus on therapeutics, lab analytics and in-vitro diagnostics and Point-of-care testing, to name a few.

The large and growing biomarker market has attracted a lot of attention, and new players are entering the market. To be able to enter the expanding market, we plan to have Germany-based companies as our sponsors in the near future. One of our target companies is Dynamic Biosensors, a provider of instruments, consumables, and services in the field of analytical systems for the characterization of biomolecules and molecular interactions.

7. Outlook

The aim of the work following this project could be concentrated around addition of supplementary readout strategies and control loops such as fluorescence detection. This fluorescence optical sensing method can be used in conjunction with the electrical setup assembled during this thesis to carry out parallel detection of neurodegenerative disease biomarkers such as microRNAs, Proteins etc.

We have already started work on the use of our prototype in the realization and characterization of a vital optical technique called 'Surface Plasmon Resonance (SPR)' analysis. We have interfaced our ULTRAS with the SPR system to enable a faster, controlled and precise delivery of sample solution. We would extend the usability of our prototype to cater to the next generation of SPR sensor that we are currently developing in parallel to this work.

We are also focussed on securing funding for this next phase of this project, in addition to mentorship from leading business development personnel as part of various initiatives from RWTH University and the German government. Our goals also encompass publishing the performance analysis of our system, advertisement of our platform and securing deals with potential customers.

8. Our Team

AixSense: Best friends as teammates!

We are students from RWTH Aachen University, working in the Institute of Material in Electrical Engineering (IWE1) since October 2019. Although having studied different subjects, we were involved in interdisciplinary projects in IWE1. Having different competences and experiences, from material sciences to electrical engineering, helped us to develop practical ideas to tackle real world problems.

From Right to Left:

- **Mohit**: A Master's student in Electrical Engineering with a specialization in micro and nanoelectronics from RWTH Aachen University and is currently carrying out an internship. Interested in mechatronics, sensors, automating various systems and designing innovative solutions.
- **Daniele:** A PhD student in IWE1 institute at RWTH Aachen University, currently focused on plasmonics and 2D materials bio nano surfaces. A creative material scientist who is very good at thinking out of the box. Spends most of his time in the labs!
- **Niloofar:** A master thesis student, soon to be graduated in the field of biomedical systems engineering track in Electrical Engineering from RWTH Aachen University. A fast learner, goal-oriented person and the programming guru of the team. Gathered experience in the field of wearable sensors, from fabrication to signal readout.