BiosensUM

17th of August 2021

Team Results Document

From
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BiosensUM

Table of contents

1 - Summary

This year, BiosensUM was faced with the challenge of measuring the concentration of hemagglutinin 1 (H1), an Influenza A H1N1's surface protein to diagnose rapidly an infection using only a small saliva aliquot for a minimally invasive test. Our final optical fiber sensor based on localized surface plasmon resonance (LSPR) will require only 50 µL of saliva inserted in a disposable cartridge to detect an H1 concentration within a range of 1-10,000 pg/mL in 5 minutes. Influenza viruses, although closely watched by health institutions, have been the cause of many epidemics and pandemics. Current influenza detection technologies fail to combine accuracy, inexpensiveness, and rapidity to offer a simple point-of-care (POC) biosensor to the public. The need to quickly detect viral infections before being contagious has motivated BiosensUM to optimize current biosensor technologies and develop a very competitive system that aims to surpass antigen-based tests' rapidity and cost-efficiency while being as accurate and specific as molecular-based tests. BiosensUM's goals during this competition were to promote our team values, such as scientific innovation and being environmentally friendly, during the entire experience. We are eager to share our ideas for the future of our simple POC device with the world.

2 - Biosensor system and assay

Hemagglutinin subtype 1 (H1) is one of many markers that can be used to identify an influenza A H1N1 infection [1]. This protein is essential as it binds the virus to the host's cells, which is the most important step for virus reproduction and infection. It is also what the host's immune system targets to eliminate the virus from its body [2]. Our biosensor can rapidly measure the hemagglutinin concentration in a saliva sample in order to detect an infection. Furthermore, since it gives a precise measurement of the concentration, it could be used to give an indication on when a patient was infected [3]. To measure the H1 concentration, we have designed a biosensor (see figure 1

) that uses an (LSPR) immunoassay for H1 detection in saliva using U-bent fibers [4].

As shown below, the device uses three white LEDs controlled by a microcontroller to analyse a saliva sample which is contained in the cartridge. The light is directed through optical fibers towards the optical alignment plate. It aligns the optical fibers with with the U-bent fibers that are coated with anti-H1 antibodies. The signal is then brought back to the biosensor and analysed by a spectrometer which is controlled by the microcontroller. If H1 proteins are present in the sample, the absorbance peak due to plasmon resonance will shift proportionally to the concentration of H1. The concentration of H1 is determined through the analysis of the spectrum of the returned light and is displayed to the user on the device organic light-emitting diode (OLED) display.

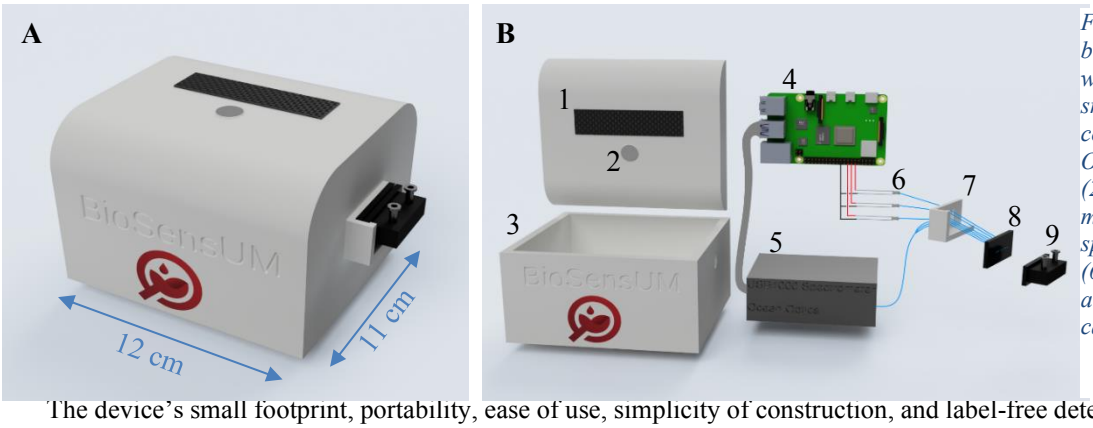

Figure 1: Design of the biosensor. A) The biosensor with its simple aesthetic and small footprint. B) Main components of the sensor: OLED display (1), press button (2), HDPE casing (3), microcontroller (4), compact spectrometer (5), white LEDs (6), cartridge holder (7), optical alignment plate (8), disposable cartridge (9).

The device's small footprint, portability, ease of use, simplicity of construction, and label-free detection makes it a competitive biosensor that could be readily deployed in the case of a future pandemic as well as being an accessible influenza test in normal times. More detailed information on the physical dimensions of the biosensor can be found in appendix 1. Further below, we delve into more details on the molecular recognition method, the physical transduction, the cartridge design, and the reader/user technology and usability of our design.

2.1 Molecular recognition and assay reagents

The first step in the detection of H1 is to bind the protein unto our optical fibers. H1's binding was accomplished using monoclonal anti-influenza A H1N1 hemagglutinin antibodies (ab128412, Abcam) immobilized on gold nanoparticle-coated U-bent optical fibers. The 400 nm in core diameter optical fibers were bent by heat using torch lighters. These U-bent shaped fibers greatly increase LSPR signal [4]–[6]. It does so by promoting interaction between the light and the gold nanoparticle coated surface. Additionally, the cylindrical geometry of the fibers increases the surface area to volume ratio when compared to a traditional SPR prism. The bent fibers are coated with homemade 60 nm gold nanoparticles [7] (AuNPs). The nanoparticle-coated surfaces are prepared by dipping the cleaned fibers in a polystyrene (PS) and poly(4-vinylpyridine) (P4VP) polymer (see appendix 2). Then, the citrate capped AuNPs form an electrostatic bond with the positively charged pyridine group of the P4VP. Once the AuNPs are on the fibers, they were soaked overnight in Afficoat (Affinité Instruments), a peptide solution to provide a protective self-assembled monolayer for metallic materials that improves molecular recognition by minimizing non-specific adsorption. The terminal carboxylic group of the peptides allows it to react with a mix of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDC) and N-Hydroxysuccinimide (NHS) prior to immobilizing the antibodies.

Inspired by previous work on antibodies conjugation and nanoparticles [8], we explored the possibility of conjugating anti-H1N1 antibodies with 15 nm gold nanoparticles [9] by electrostatic interactions /covalent coupling to increase our biosensor's sensitivity. In doing so, we have an optional secondary detection step akin to a labelled assay to increase sensitivity for low concentration samples. The antibody-nanoparticles solution is added to the cartridge after rinsing the residual sample with a small portion of milli-Q water. By binding nanoparticle@anti-H1 antibody complexes to the H1 already bound onto the surface, the two plasmonic particles are at a distance below one diameter's length and plasmon coupling occurs. The resonance of individual particles starts to hybridize, and their resonance spectrum peak wavelength will shift (either blueshift or redshift), depending on how surface charge density distributes over the coupled particles which massively enhances the LSPR signal.

2.2 Physical transduction

The LSPR method relies on a simple optical system that offers great reproducibility and easy mass production[8]. A white source lights the nanoparticle-coated fibers to stimulate the electrons of the metal particles, thus creating a localized plasmon resonance. This generates an absorbance peak in a frequency that depends on multiple features of the binding surface, notably the refractive index around the surface and the mass bound to it. When H1 proteins bind to their corresponding antibodies, the size and shape of the surface change, causing a shift on the absorbance peak.

This shift can be detected by comparing the spectrum of the returning light after sample injection to the one prior to sample injection. First, to find the absorption peak at a given time, the spectrum taken is subtracted to a reference spectrum for each of the three LEDs used in the sensor. To build this reference, 20 spectra were taken and the average was kept, reducing the noise. During the measurement, the ratio between the reference and the measurement spectra gives the absorbance peak [10]. A polynomial fit can then be applied to find the maximum which is the absorption wavelength. By taking multiple data points at multiple times, the concentration dependant binding dynamics can be studied, and thus the concentration of a sample can be determined by comparing with a calibration curve. The calibration gives a correlation between the shift rate and the final value of the absorbance wavelength, and the sample H1 concentration [11], [12].

2.3 Cartridge technology

We designed a novel cartridge to house a triplicate of U-bent fibers and direct a small aliquot of sample toward them all the while being fully enclosed and disposable to avoid the spread of diseases (see figure 2 and appendix 1**Erreur ! Source du renvoi introuvable.**). At its core, it provides a place for 3 coated U-bent fibers to interact with the saliva sample. To make this possible, we use perforated HDPE plates identical to the optical alignment plate of the sensor

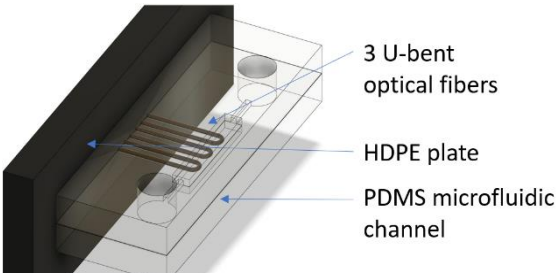

Figure 2: 3D model of our cartridge without cover

to hold the 3 U-bent fibers in place and align them with the biosensor optical system. The U-bent are coated with the gold nanoparticles before they are sandwiched between both halves of our microfluidic PDMS chip [13]. To limit ambient stray light entering the system and to solidly hold the PDMS in place, a 3D printed box surrounds the PDMS and is glued to the HDPE plate (not shown in figure 2**Erreur ! Source du renvoi introuvable.**). An entry and an exit reservoir that are held in place by the tight holes in the PDMS are used to hold the fluid before it enters the U-bent chamber. A saliva sample added to the entry reservoir won't spontaneously penetrate the U-bent chamber as the Laplace pressure is greater than the hydrostatic pressure [14]. Once the user is ready for analysis, the sample can be pushed further in the chamber by adding positive pressure to the entry port with a modified syringe.

2.4 Reader instrument and user interaction

Our reader instrument contains four main components. The first part is the user interaction part of the sensor. It contains a screen and buttons located on top of the biosensor with which the user interacts to perform the test. This part also contains the holder localized on the side of the sensor consisting of a 3D printed piece where the user simply slides in the cartridge. A tensioning mechanism will eventually be added to make sure that the optical alignment plates are well aligned. The current solution is an elastic band which works satisfyingly.

The second part is the optical system that emits light for LSPR analysis and that decomposes the spectrum of the to-be-analysed light. Three LEDs are driven by the microcontroller to be turned on only one at a time in a quick succession to take as many time wise data points as possible. The light is directed inside the reader instrument using optical fibers (see appendix 3). At first, we designed and built a Czerny-Turner spectrometer [15]–[17] to analyse our data, but for reasons detailed further below in 3.2.1**Erreur ! Source du renvoi introuvable.**, we resorted to using a commercial portable spectrometer from Ocean Optics, although we are still working on our Czerny-Turner set up.

The third part is the electronics of the biosensor. We currently use a Raspberry Pi to drive the python code that controls the sensor, reads the USB4000 spectrometer, and analyses the data. A microcontroller this powerful is not an optimal solution for such a simple application. In future versions of the biosensor, the microcontroller would be reduced to a less powerful one to save on cost and reduce the environmental footprint of the device.

Finally, the last part is the Python program that drives the biosensor, the data, and outputs a result for the test. To achieve this, it uses the spectrum returned by the spectrometer as described in 2.22.2. The python code is available in appendix 4.

3 - Technological feasibility

This section presents the results of the most important tests along the remaining challenges and future upgrades and plans for the coming development of a fully market-ready biosensor.

3.1 Molecular recognition

First, the binding between antibodies and H1 proteins was verified by SPR with an Affinité Instruments P4SPR. The tests showed that they bind undoubtedly well (see figure 4). Then, the experiment was repeated with H1 proteins in artificial saliva and compared to BSA to verify the specificity (see figure 3). After that, the gold nanoparticles (AuNPs) were synthesized according to Ghosh et al.'s protocol [18], [19]. The 60 nm size was confirmed by UV-Vis Spectrometry (530 nm) (See results in appendix 5).

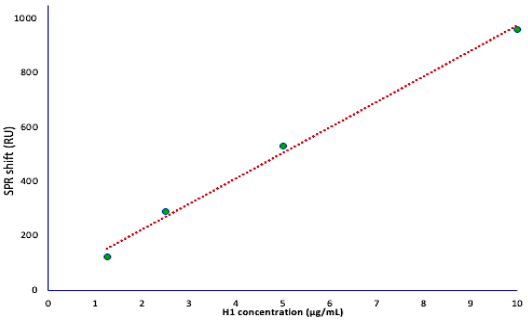

Figure 4: SPR shift of antibody-H1 protein binding in running buffer (RB). R2 = 0,9944

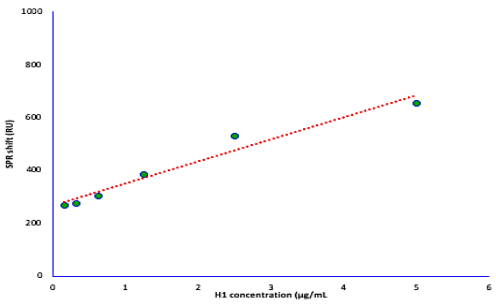

Figure 4: SPR shift of antibody-H1 protein binding compared to BSA in artificial saliva. R2 = 0,9599

Along with the 60 nm AuNPs, 15 nm ones were also synthesized for secondary detection according to Luo et al.'s protocol [20]. In their article, they were making core molecule shell (CMS), we simply synthesized the Au cores (15 ± 3 nm). The UV-Vis measurement showed a plasmon peak at 511 nm, which confirms the size of the AuNPs, since the size of the NP influences the peak position (see appendix 5). The conjugation between antibodies and 15 nm gold nanoparticles was done and tested while making the calibration curve presented in section 3.2.2.

3.2 Physical transduction

The physical transduction was laced with multiple challenges to overcome along the way. Namely, there were issues with the spectrometer we built, the communication between hardware and microcontroller, and the precision of the signal.

3.2.1 The spectrometer

The goal of building our own spectrometer was to tune it precisely to the spectral range required to do LSPR. By reducing the wavelength range hitting the CCD, each pixel would be associated to a smaller Δλ, giving us a better resolution. Following this, to detect smaller concentration of H1, we conceived the compact Czerny-Turner spectrometer presented in appendix 3. However, using a suboptimal order of diffraction ($m = -1$) and spreading the spectrum on a longer length comes to a cost in terms of signal intensity. This loss in signal, our lack of expertise in optics alignment, and a PCB driver for the CCD that was not delivered on time rendered our spectrometer unsuitable for scientific measurements. Even though we were able to record a white light spectrum for the spectral range of interest for our measurements (as seen on figure 5.A), we choose to use the Ocean Optics USB4000 spectrometer as it gave much better LSPR results than our own spectrometer as a temporary alternative (as seen on figure 5.B). Further development of our own spectrometer would be required to use it at its full potential.

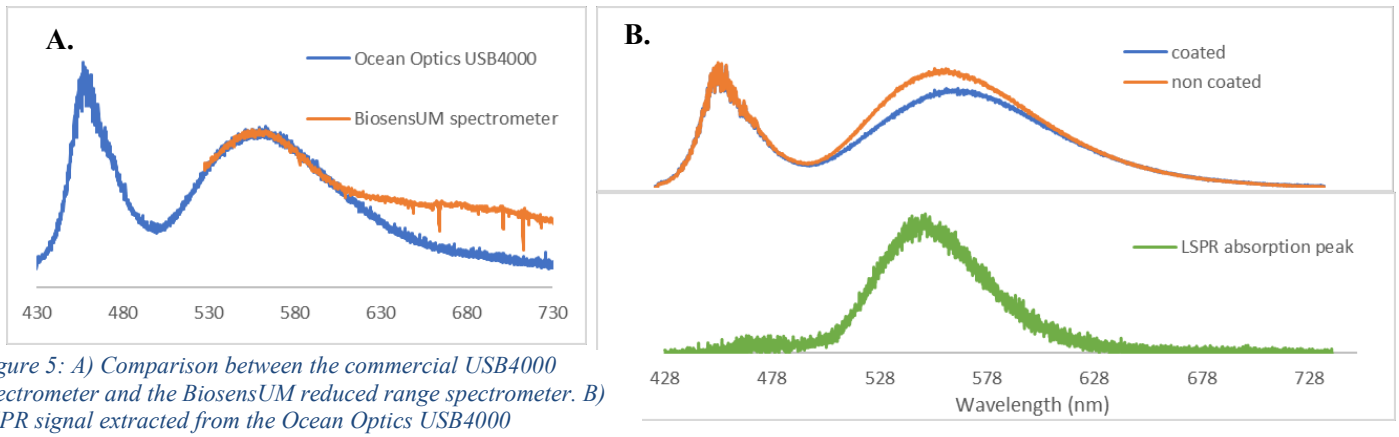

Figure 5: A) Comparison between the commercial USB4000 spectrometer and the BiosensUM reduced range spectrometer. B) LSPR signal extracted from the Ocean Optics USB4000

3.2.2 LSPR on U-bent fibres and calibration

Calibration establishes the link between the analytical response and the concentration of analyte present in solution by testing the device using standards whose exact value is known. Since the microfluidic cartridge were not ready for the calibration, we used an alternative method where the U-bent fibers are directly dipped in a 70 µl PDMS pool containing the sample. While testing we discovered major issues that are not yet solved. Mainly, the curvature radius of the U-bent fibers is too tight to account for higher refractive index of the saliva compared to air and following Snell's law, the light does not undergo total intern reflection. There is not enough difference in the reflective indexes of the fiber core and the saliva sample. This could easily be fixed by calculating an adequate radius and making new U-bent fibers. To be able to continue testing, we removed the fibers from the sample before taking the spectrum. This way, the fibers are in air so there is a signal, and since the fibers were let in the sample for a few minutes, there should still be detectable H1 bound to the surface.

Another issue was that with only normal LSPR, we could not detect a significant signal after 4 min (the measurement time we are aiming for) i.e., there was no difference between the shift measured for a sample with no H1 and a sample with 10000 pg/ml. There still was a detectable shift in both cases, but it was almost identical. Therefore, our hypothesis is that it was due to residual saliva on the fibers. There is a need for further exploration of the technique to either refine it or fully demonstrate that it is not suitable for such measurements. To have a detectable signal, we implemented the secondary detection discussed previously.

Unfortunately, no calibration curve will be presented in this document, due to a lack of time. On the other hand, we will be testing our samples with our conjugated nanoparticles to see if there is any improvement. Calibration will be completed before the Testing Event.

3.3 Fluidic cartridge

We still have issues with the plasma adhesion of the two layers of PDMS [13], [21]–[24]. The 3D printed mold either will not let the PDMS cure (not enough post-printing cleaning with isopropanol) or does not have a smooth enough surface to make a strong bond (to much post-printing processing). We almost finished the optimization of this step, but since time was lacking, we opted for an alternative solution to make the calibration curve in time for the team result document submission. We currently directly dip the U-bent fibers in the sample using a small PDMS pool and a 3D printed alignment device (see appendix 1). This method is simpler to make and robust, but it is not a final design since it is slightly more complex to operate for the end-user.

Another aspect that greatly interested our team is the reusability of the cartridges. We documented the various possible ways to achieve this. The way that seemed the most suitable to us was to inject Glycine, a low pH-buffer, to wash the surface of the nanoparticles [25]. This works because most proteins become partly unfolded and positively charged at low pH which will cause the protein binding sites to repel each other. Although it looks like a great advantage, reusing the cartridges has its disadvantages. Since users are handling biological samples, throwing the cartridge leaves less room to cross contamination and will cost less in terms of shipping. Users could, indeed, inject glycine themselves, however, that requires specific skills and time that users might not have. Future development would be required to make this a fully viable and implemented solution.

3.4 Reader instrument

One of the biggest challenges we faced was the programming of the microcontroller since we had no team members with prior experience in this domain. We were able to find multiple solutions along the way to accommodate our different needs in microcontroller, especially to drive the CCD detector while we were working on our spectrometer. Since our current design iteration uses the USB4000 spectrometer that can be driven from a computer, we currently use a Raspberry Pi microcontroller that is essentially a full fledge computer. However, this microcontroller is unnecessarily powerful for our device. Future iterations would have a more striped down microcontroller driven by custom software to lower its economic and environmental cost.

The alignment of the optical alignment plate on the device and the one on the cartridges was also challenging. Currently we ensure the alignment by drilling the holes in both plates with a jig and using an elastic band to reference both plates together. A simple latch would be designed instead of the elastic band for a final product. The optical alignment was verified by ensuring that the spectrum intensity was maximal when the plates were passively aligned with the elastic band.

3.5 Detection of whole influenza virus

With our detection method, LSPR, the detection of the whole influenza virus should be even better than H1 proteins. The reason for that being the bigger and heavier a molecule is, the more it creates an absorbance shift, the easier it is to detect. Since our antibodies bind H1 proteins, they would easily bind the whole virus because there are hundreds of hemagglutinin on their surface [26] and H1 proteins have 5 possible epitopes (structure that binds with the antibody) [27].

4 - Originality

4.1 Team

The detection of viruses, especially influenza A H1N1, has been researched extensively during the last few years, which is natural considering the number of past pandemics that threatened the world [28]. The team perused literature to find multiple promising detection technologies to finally choose SPR, our supervisor's expertise. However, we found that LSPR had interesting features compared to SPR. Although there were a few publications on LSPR-based biosensors, it was not as common as other detection methods. We therefore chose to use LSPR because of the simplicity of the optical setup, the potential of a better sensitivity and the lower cost of the cartridges for this technology. A part that is especially novel is the use of U-bent optical fibers instead of a prism. To the best of our knowledge, few have attempted this method and even less have explored the use of U-bent optical fibers for an H1 biosensor. Apart from validating our choices with our supervisor and our coach, we found the ideas by ourselves based on literature search.

The team completely designed the cartridges geometry and the junction between the internal optics of the reader and the U--bent fibers in the cartridges. Our supervisor did, however, propose to use some sort of alignment-based junction instead of using traditional optical fibers connector.

Team members had the idea of aiming for a stand-alone device that could work on battery packs without the use of an external computer. This would allow the device to be deployed in more rural areas where access to power or technologies might be restricted. This guided our choice of electronics and internal workings for the reader.

Designing our own spectrometer (see appendix 3) is a choice the team made as an alternative to purchasing one to have a spectrometer better suited to the needs of LSPR. A prototype was built, but we were not able to fully optimize it due to delay in shipping and other issues discussed above. With more time, we would optimize our spectrometer to have a better biosensor and a less costly alternative during production. Our supervisor guided us towards a Czerny-Turner configuration for the spectrometer.

After our supervisor suggested a secondary detection to have a better detection of low concentrations, we explored the advantages of a sandwich immunoassay. We found in the literature a protocol for the conjugation of antibodies with 15 nm gold nanoparticles [29]. When an H1 protein binds to an antibody on the surface, a conjugated antibody-nanoparticle can subsequently bind to it, hence increasing its size and mass for optimal detection.

4.2 Supervisor

I confirm the statement from the students. The ideas presented in their document comes from their own work and resourcefulness. The main innovations in their project are in the engineering of the cartridge and reader, which are fully of their own and unique. I do not have students or researchers working on these elements, as such, they did not draw these ideas from my lab and it is their innovation. The use of U-bent fibers is indeed found in the literature, but albeit seldom used. The (bio)chemical detection scheme is more standard, and they made use of some of the methods and SPR instrumentation from my labs. Among them, they validated their protein selection by using our SPR instrument and they have used Affinité's surface chemistry in their design. However, I do not have anyone at the moment working on LSPR detection, as such, this element is also from their own readings and initiative. I consider that the level of innovation of the instruments and reader makes them significantly different from any commercial sources of SPR.

My involvement with the team was limited to steering meetings on a monthly basis and the few suggestions they mentioned in the text above. Due to the pandemic and current regulations on campus and in Canada, nearly all interactions with them were online. As such, they worked remotely for the most part of their project, limiting the interactions with my team members, further strengthening their independence from my own work.

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Lucas Aubé

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5 - Translation potential

5.1 Business model canvas

<table>
<tr>
<td rowspan="2">Problem(s)
1. No medical or scientific expertise to interpret data such as viral loads → Need to work with experimented scientists/analysts or need to work with a pre-designed viral load scale (not available with current epidemiological data)

2. Current time of detection (TOD) takes too long for precise results (RIDTs are faster but less precise) and need to detect before the patient becomes contagious

3. There is no real need to detect a very specific strain (H1N1) of a very specific virus (Influenza A) in a diagnostic setting (e.g: during triage at emergency)

Existing alternatives
Other teams' biosensors, (RT-)PCR or RIDT tests.</td>
<td>Solution(s)
1. Give P/N results and if P, give option to see viral load.

2. TOD in 5 mins or less and LOD of very small viral loads.

3. Biosensor may be polyvalent and allow for the detection of other viruses by changing the chip/cartridge.</td>
<td rowspan="2">Unique value proposition
· Fast, precise POCT device for detection of H1N1's hemagglutinin surface protein.

· Detection of small viral loads allowing for detection before symptoms appear and before contagiosity

·Simple biosensor and interface that anyone can use anytime and anywhere, even at home.</td>
<td rowspan="2">Unfair advantage
BiosensUM's university experts on the technology of detection used (LSPR).

Readily accessible SPR biosensors and SPR experts to help us understand the basics of the detection technology.

Early access to labs for the conception of the biosensor.</td>
<td rowspan="2">Customer Segments
Hospitals, pharmacies, clinics. (target customer)

General public and other institutions (non-target customers; may be redirected towards our product by our target customers)
Other customer segments to explore in the biosensor's future
· FluWatch Canada
· CDC's FluView
· INSPQ(CIQ)/MSSS
· Scientists and epidemiologists analysing infectious diseases
· More specialized industries such as research laboratories, healthcare suppliers, etc.
Early adopters
Health professionals e.g. physicians and clinicians, pharmacists, nurses andbeneficiary attendants</td>
</tr>
<tr>
<td>Key Metrics
Instagram audience:
· 55.7% from Montreal
· 8.5% from the Netherlands
· 6.6% from Spain
· 4.7% from the US
· 4.7% from Sweden

· 47.2% aged 18-24
· 43.4% aged 24-34
· 4.7% aged 35-44
· 4.6% aged 45 and above

· 56.1% are women
· 43.9% are men</td>
<td>Channels
· Social media and advertisements

· Word of mouth to family, friends, and colleagues.

·Hospitals affiliated with Université de Montréal (CHUMs)

· Social media and advertisements</td>
</tr>
<tr>
<td colspan="3">Cost Structure
<u>Fixed costs</u>
· Certifications (ISO 15189 and/or ISO 22870 for Canada approved POCT and IP/trademark rights)
· Services: website/platform/app, customer service, infrastructure, personnel/employees, etc.

<u>Variable costs</u>
· R&D
· Production (according to demand)
· Marketing and sales administration (according to demand)
· Clinical study for proof of concept
· One-time costs</td>
<td colspan="3">Revenue Streams
· Direct biosensor sales (to pharmacies, hospitals and clinics)
· Indirect biosensor sales (eg: pharmacies selling to patients, to at-home nurses, to beneficiary attendants, etc)
· Chips/cartridge sales (to direct and indirect buyers)
· Biosensor rentals (to health professionals or industries)
· Advertisements in the biosensor's platform/app
· Initial Public Offering (stock market and exchange)</td>
</tr>
</table>

5.2 Stakeholder desirability

Influenza viruses are one of the most common viruses circulating and its types A and B are one of the main causes of seasonal flu whose annual cases of severe illness are around 3 to 6 million cases worldwide [30]. Influenza type A is one of the most infectious strains to humans and optimizing its detection and diagnosis is crucial to avoid endemic or pandemic cases, especially amidst the SARS-CoV-2 pandemic [26]. Current technologies allow for the detection of influenza, but they never seem to fully combine the accuracy and precision of molecular based tests and the rapidity of an inexpensive antigen-based test [31], [32]; especially considering that RIDTs' negative test results could be false negatives and require to be double-checked with a molecular test [33]. Also, most tests require difficult sampling, such as nasopharyngeal swabs, or difficult manipulations requiring mixing reagents and, therefore, do not quite meet the requirements for POC technologies that could allow for a personal use, without requiring some scientific background [33], [34]. Our history with epidemics and other health crises has shown us that fast detection of contagious diseases is primordial to contain contagion and technologies that detect infected individuals before symptoms appear are needed [34], [35].

BiosensUM offers a POC, antigen-based, LSPR-based biosensor using U-bent optical fibers and AuNPs that effectively combines the accuracy and sensitivity of molecular-based detection technologies and the rapidity and inexpensiveness of antigen-based detection technologies. A device such as ours has great potential for stakeholders as it shows incredible improvement in the biosensing field both economically, as our technology offers a cheaper alternative to equally accurate alternatives on the market, and technologically, as our sensor includes major improvements in the technologies integrated such as the optimization of traditional

LSPR sensors. Our main customer segment is composed of healthcare professionals and providers[1] that will, in turn, sell or rent the biosensor or may also keep it for on-site testing. More specialized[2] industries also constitute a customer segment of their own and will be further discussed in the business feasibility and financial viability sections.

The proposed biosensor allows healthcare providers to test a patient's sample for Influenza A H1N1 infections on-site and give an accurate result in 5 minutes or less. The sample required for the viral detection test is also non-invasive as it requires only 100 µL of saliva or less, without any pre-treatments. Additionally, as a comparison, current rapid antigen-based tests on the market (e.g., RIDTs) can give results in 10-15 minutes, which is 2 to 3 times longer than what our solution offers, while most clinical tests (including molecular-based tests) are able to give results in 15-45 minutes [36]. Rapid antigen-based diagnostic tests for Influenza on the market are also only able to give moderate sensitivity (ranging from 50-80%) [37] and often cannot provide information about Influenza virus subtypes. Not only will our biosensor achieve results faster and more accurately than its competitors (RIDTs), but it should also allow to give quantitative results allowing a limit of detection between 1-10,000 pg/mL, while RIDTs only give positive/negative results [38]. The easy-to-use interface and testing method will allow anyone to be able to run the test and therefore, alleviate the burden of requiring scientific expertise to run lab-quality tests. Although anyone could use the biosensor, it is an especially great tool for health professionals (mainly pharmacists, doctors, and clinicians), who will be able to give a fast diagnosis and course of treatment; thus, reducing healthcare workload and rush hours at pharmacies, hospitals and walk-in clinics. In Québec, for example, current laws allow pharmacists to prescribe medication for minor conditions, for conditions that do not require diagnosis and laboratory analyses and tests [39]. Pharmacists are also often first in line to guide the patient towards medical consultation, as they are very accessible in Québec; and interviews we conducted with professionals showed us that they also offer a variety of biosensing services, ranging from taking and interpreting blood pressure, blood glucose and PT/INR[3] tests.

Although it is a very promising technology that BiosensUM offers, during many interviews we conducted with our target customers, they have expressed the limited need for a biosensor limited to detecting a very specific strain of a virus. However, our technology is relatively easy to transpose for other viruses or even bacterial infections thanks to its lab-on-a-chip design: our biosensor's cartridges consist of U-bent optical fibers coated in antibody-conjugated AuNPs that will detect the presence of a wanted protein in a sample delivered by microfluidic components. This model is therefore reproducible for different cartridges conjugated with different antibodies for the detection of different infections, making the biosensor a very polyvalent tool with only a cartridge switch. A portable device such as this will also be revolutionary for healthcare in developing countries, for our non-targeted customers (general population; see 5.1. Business Model Canvas), where doctors are not always an option. This biosensor therefore has the potential to sell around the world and could very well be the first step to revolutionizing the healthcare industry with its innovations.

5.3 Business feasibility

BiosensUM's start-up will be divided in 3 main teams: science, technology and business/marketing and will be developing itself and its ideas under our university's R&D incubator (BRDV) which offers help in fundraising and project setup, funding administration and the valuation of research [40]. Université de Montréal is known to be very resourceful in the research field thanks to its affiliated universities and labs and that will allow our start-up to work directly with our community's experts [41]. The science and technology team will be working with UdeM's specialists in biosensing and SPR/LSPR [42] and with Polytechnique Montréal's specialists in microfluidics and engineering. The business/marketing team will be working with HEC's specialists and BRDV's affiliated committees and will refer to the CIPO's 5-year business strategy in order to adequately revise and further build our business plan, our marketing strategy [43]–[45] and our IP/patents/certification strategy [46]. Not only will the BRDV's committees help in our technology transfer from the university's lab to the market and industry in general, but BiosensUM also plans on securing partnerships with key leaders in the industry such as Thor Labs, who will provide our optic fibers; Ocean Insights is also a great resource to help us manufacture our own optical design: we planned to build it ourselves during this competition but we had problems with the delivery of our base components and have decided to use a premade optical system provided by Ocean Insights. Since BiosensUM will be working with BRDV and UdeM's affiliated research laboratories, we are confident that our project will attract many more partners in the future.

The commercialization strategy for the biosensor will initially depend on direct biosensor sales to our target main customer segment and main adopters: health professionals. After conducting many interviews and surveys with health professionals, we have

[1] Includes clinicians such as family doctors, nurses and first responders, and other healthcare professionals/providers such as pharmacists, beneficiary attendants or school nurses and workplaces where fast diagnosis may be required.
[2] Such as pharmaceutical industries, SensUs organization and partners, research labs, etc.
[3] Prothrombin Time (PT) test / International Normalized Ratio (INR) – Tests conducted to see if one's blood clotting is normal [53].

confirmed the need and want of our biosensor on the market. In Canada, pharmacists and clinicians are one of the main resources for the general public to be updated with diagnostic tests and technologies. Pharmacists are especially essential on the North American market as they are also a reference for physicians, nurses and other health professionals since their job also requires them to be constantly up-to-date with the newest technologies and medication, especially in biosensing. Indirect sales will then follow thanks to smart marketing and the use of our device by health professionals, which will help the general public put its trust in our product. BiosensUM has also started contacting pharmaceutical companies involved in medical equipment sales such as Abbott Laboratories to widen its product's distribution and to maybe plan a B2B2C marketing model [47] if direct sales present too many financial step backs for our start-up. As shown in the business model canvas as well, one of the aspects that makes our biosensor competitive is the many optimization ideas we have (see section 7 – Final remarks) that allow us to also analyze another customer segment in the future: research-oriented and government industries; this makes it even more interesting for pharmaceuticals to partner up for a B2B2C marketing model in order to secure long lasting relations with the scientific community.

5.4 Financial feasibility

Our projected financial viability is based on interviews with health professionals as well as help from an experienced business accountant from Bowker Capital Inc. In the province of Quebec only, with the number of Influenza A H1N1 tests reduced because of COVID-19 tests, there were 103 710 influenza tests done in the year 2019-2020 [48]. The number of tests done in Canada is then estimated by ratio at 460 933 tests for the year 2019-2020. Since there are 1265 hospitals, 13 305 pharmacies and 91 205 physician offices in Canada [49], the number of potential customers is as high as 105 775.

In our five-year projection (Fig. 5), the first two years will be dedicated to research and development, finding funds (appendix 7), get a patent as well as an *Investigational testing study permission* from Health Canada (appendix 8) [50], so there will be no sales. We will be in deficit for the first two years, however during 2024, we expect to reach about 2.5% of the Canadian market by selling 2 644 biosensors and 11 523 cartridges. From this, we anticipate growing to 5% of the market in 2025 and 10% in 2026. Since the cartridge production costs in mass production about $2 CAD and the biosensor about $1000 before optimization, we would sell them at $4 CAD and $2500 CAD respectively. This would give us a 50% profit on the cartridges and avoid being too costly for hospitals that would need an enormous amount. In this projection, we would start to make profit in the third year and be viable until the next influenza A H1N1 pandemic that is sure to come [51], in which case our profit would be off the chart. In the event of an influenza A H1N1 pandemic, we would reduce the cost of the chips at $3 CAD if hospitals order in mass to enable them affordable tests without losing profits.

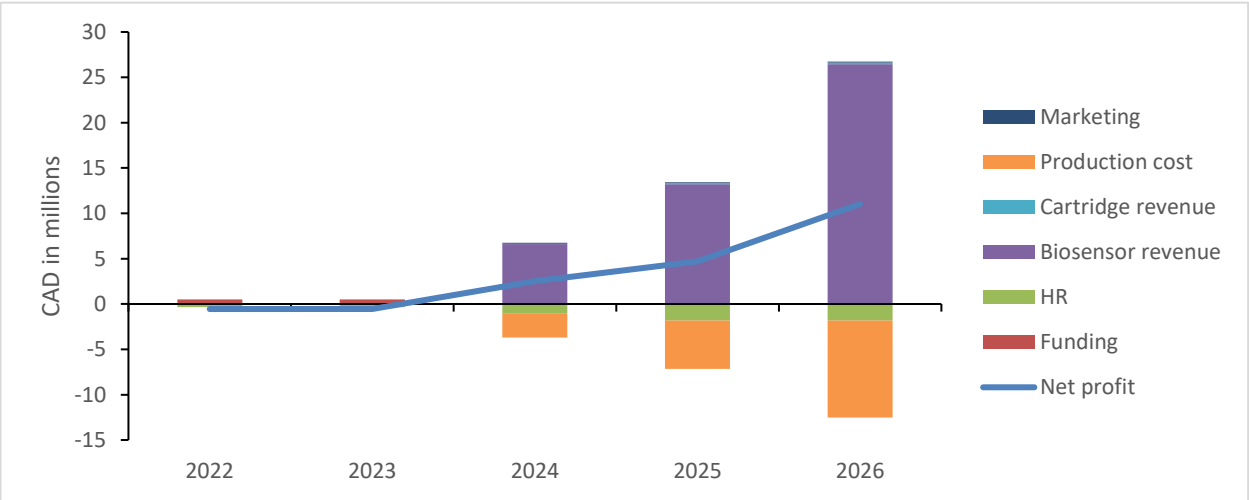

Figure 6: Five-year projection of revenues, costs and net profit in millions of Canadian dollars (CAD)

6 - Team and support

6.1 Contribution of the Team Members

· Lucas Aubé (team co-captain) is also a member of the technological subunit and has worked on the microfluidics and optics designs, and the integration of our biosensor. He is also a member of the entrepreneurial subunit although his role both as team captain and in the technological subunit did not allow him to be as present as he wanted to.

· Alexandrine Frappier (team co-captain) is also a member of the scientific subunit and has been one of the main members to work in the lab to realize our assays. She is also a member of the entrepreneurial subunit and has helped a lot to the completion of our entrepreneurial goals and deadlines.

· Ryma Boudries is the head of the scientific subunit and is the main actor of it, providing protocols and late-minute solutions. She is also a member of the entrepreneurial subunit and has also helped with our presence on social media.

· Katia Cherifi is the head of the entrepreneurial subunit and by far main actor of it. She is also a member of the scientific subunit and is the team promotion person who led our presence on social media.

· Pierre-Alexandre Aubé is the head of the technological subunit and has worked mostly on our software programming and electronics, as well as our 3D printing expert. His role as head of the technological subunit has, however, precluded him from participating in the entrepreneurial subunit.

· Marie-Ève Ippersiel is a member of the technological subunit who supported the effort of the technological subunit and worked on the electronics and software.

· Nadine Padillo is a member of the entrepreneurial subunit and has helped a lot with late-minute completion of documents and English. She is also a member of the scientific subunit and has always offered to help in the lab. She was also always present during our team bonding activities.

· Kiran Shewbaran is a member of the scientific subunit who participated most notably in the planning of our online event.

· Jiayi Chen is a member of the scientific subunit and helped with literature research at the beginning of the project.

· Rachel Pryce is a member of the scientific subunit that was present at meetings and helped at the beginning with literature research.

· Victoria George is a member of the technological subunit who helped at the beginning of the competition with research and by providing ideas for the biosensor.

· Jeanne Péloquin is a member of the technological subunit who was only present at the bonding activities and some meetings.

6.2 People who have given their support

· Jean-François Masson (team supervisor) introduced the currently available biosensor concepts on the market. He was readily available to answer our questions and evaluate our suggested prototype ideas.

· Arnaud Laramée (team mentor) was present at every team meeting to guide us in prioritizing our tasks within our given timeframe. He gave us realistic goals to be achieved for each checkpoint during this project.

· Frédéric Fournelle (BiosensUM 2018 and BiosensUM 2019) gave us suggestions on what to expect every step of the way and feasible objectives for this project based on his previous experience.

· Dominic Pelletier (Family Medicine resident doctor): gave us insight on the need of point-of-care biosensors in the healthcare workspace. He also confirmed the convenience and practicality of our design in a clinical setting.

· Team of Shopper's Drug Mart Ruth Boachie: both pharmacists and lab technicians have given us a lot of information on how the pharmaceutical market works and helped us plan our business proposals consequently.

· Gregory Quentin Wallace: shared his knowledge on LSPR and surface characterization using gold nanoparticles.

Claude Frappier, a business accountant from Bowker Capital Inc. gave us information needed to complete the financial plan and helped create our 5-year projection.

6.3 Sponsors and partners

· Université de Montréal (incl. Polytechnique Montréal, ASEQ, FAECUM and all departments we have contacted): It is safe to say that without the generous help of our universities, both through funding and having access to various labs and equipment, this project would not have been possible. UdeM has also provided us with a safe and clean environment to work in the COVID-19 pandemic.

· Medtronic (SensUs partner) provided us with professional insight on sample preparation and binding element selection. They were happily willing to help us find ways to make our cartridges as sustainable as possible.

· The MUFO lab (Pr. Thomas Gervais): https://mufolab.ca/fr/ provided materials, equipment, and lab space for the microfluidic portion of this project.

· The SPR & Plasmonic Biosensors research group (Pr. Jean-François Masson): http://www.sprbiosensors.com/ provided a lot of information on the technologies explored, materials, equipment and lab space for biological and chemical assays.

7 - Final Remarks

We would like to wholeheartedly thank our supervisor Jean-François Masson and our coach Arnaud Laramée for their advice and guidance throughout the project; our partners for providing us with good options and solutions to the many aspects involved in the conception of our biosensor; SensUs organization for having a very interesting and impactful project that allowed us to expand our knowledge; our sponsors for believing and supporting us; the microfluidics for oncology lab for supplying us with materials and a workspace; resident doctor, Dominic Pelletier, for helping us understand the needs in healthcare associated with respiratory viruses; Frédéric Fournelle for giving us great advice since he participated in the SensUs competition twice before and finally Gregory Quentin Wallace, a member of Pr. Masson's research group, that lent us a helping hand when needed. Although COVID-19 came with its fair share of restrictions such as having a limited number of people in the labs or having to do most of our meetings online, we got to collaborate wonderfully, and have a memorable experience. This project has inspired a few of us to want to further explore the domain of biosensors in our future endeavors. After the competition, some team members would be really interested in further developing our biosensor.

In the technological aspect of our biosensor, we would like to switch our LCD display for an LED display, which would be safer both for our users as LED displays have smaller backlighting and are much safer for the eyes, and for the environment as LCD displays require mercury for their production [52].

One of our ideas is more research-oriented and would consist of the development of a database on our biosensor's platform, for research purposes. This feature would be presented as a pop-up window while turning on the biosensor or while putting a new chip in requesting the patient's consent to save his/her viral load (keeping the result anonymous could also be an option). This could allow our customer segment to directly participate in national health databases and could also allow virologists to draw more accurate infectiousness curves with, eventually, precise viral loads tested with biosensors. This could very well revolutionize epidemiology as well since it'll be providing very precise information on viral shedding and viral tendencies during a certain period. This is even more revolutionary when considering the fact that our biosensor is polyvalent and could be modified to detect other viruses by changing the cartridge for another one that's conjugated with different antibodies to detect different microorganisms. If the biosensor does end up on the market, our team wishes to introduce one of the first reusable cartridges in the biosensing industry. We have not only found protocols allowing us to fully "clean" and reconjugate our cartridges a number of times without affecting the detection's precision [7] but one of our team member's research has also proved that fact with their own separate results. It would also be an interesting benefit for our stakeholders to consign our cartridges or to recycle them in order to generate a more reliable long-term profit.

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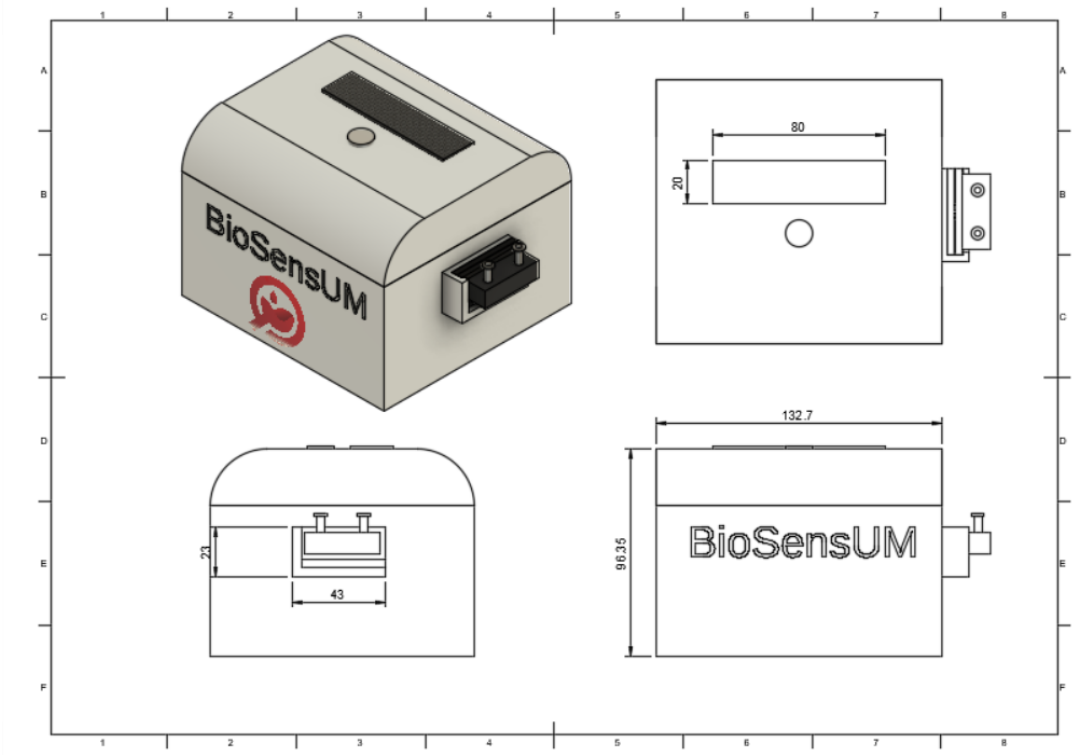
Appendix

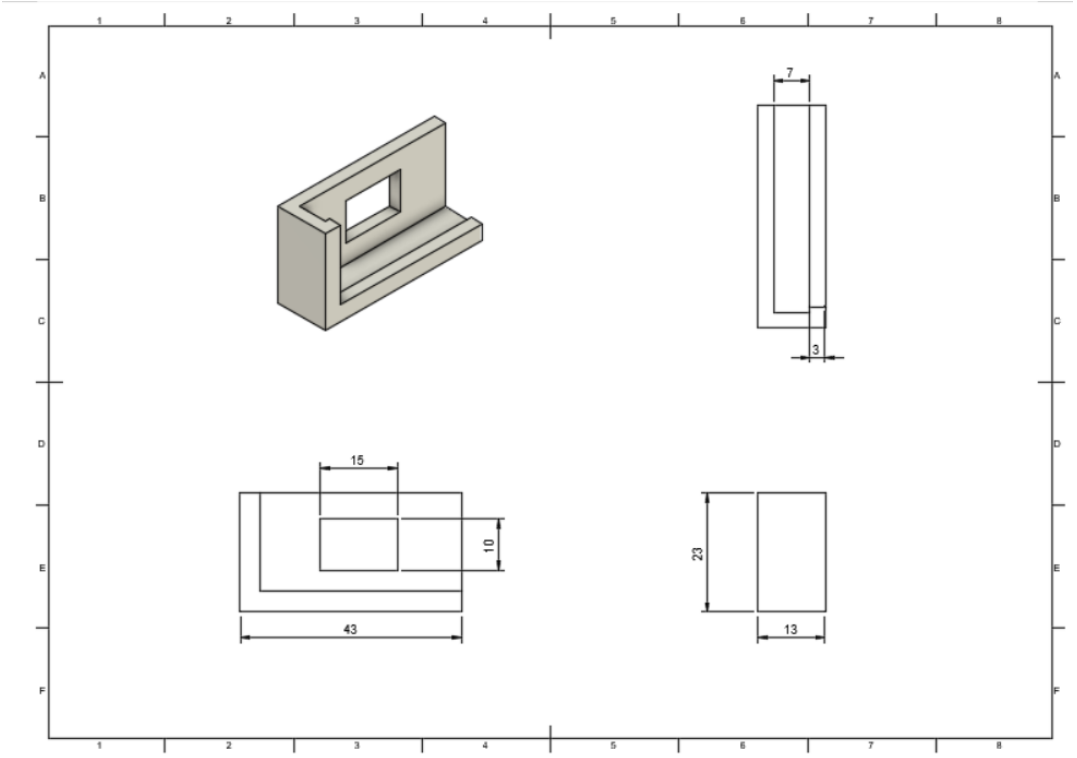
Appendix 1: Dimensions of the biosensor

Below are the technical drawings of the main parts of our biosensor with their measurements.

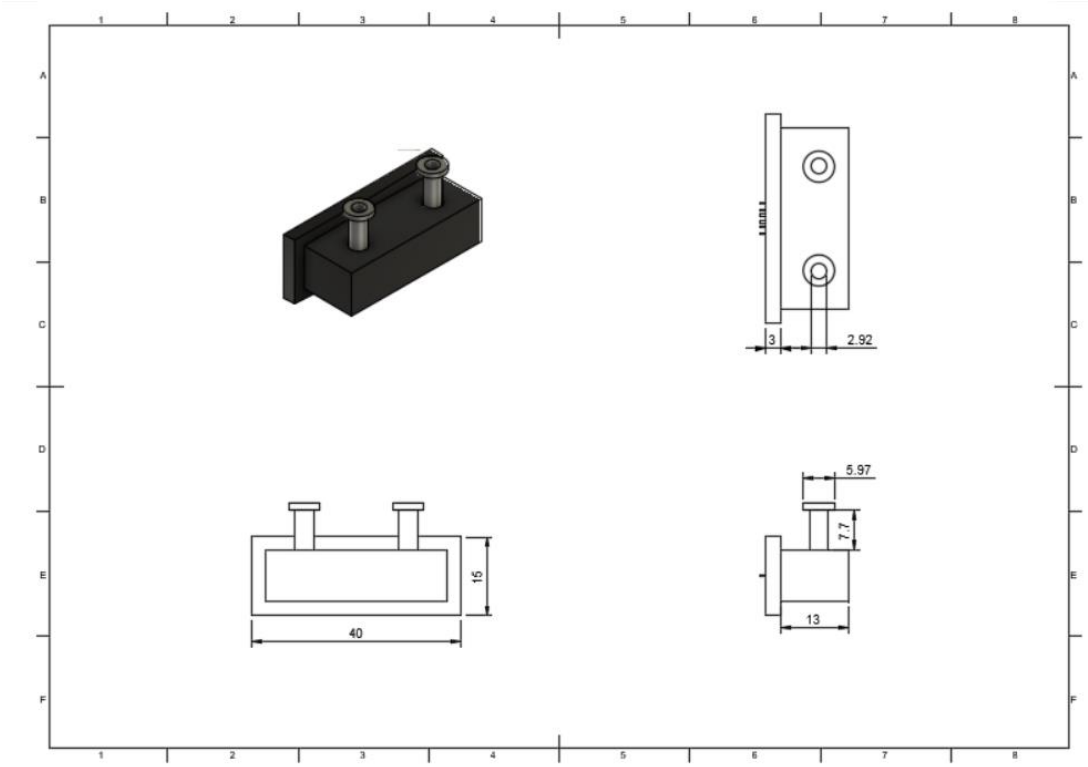
The first drawings are the entire biosensor box with its dimensions.



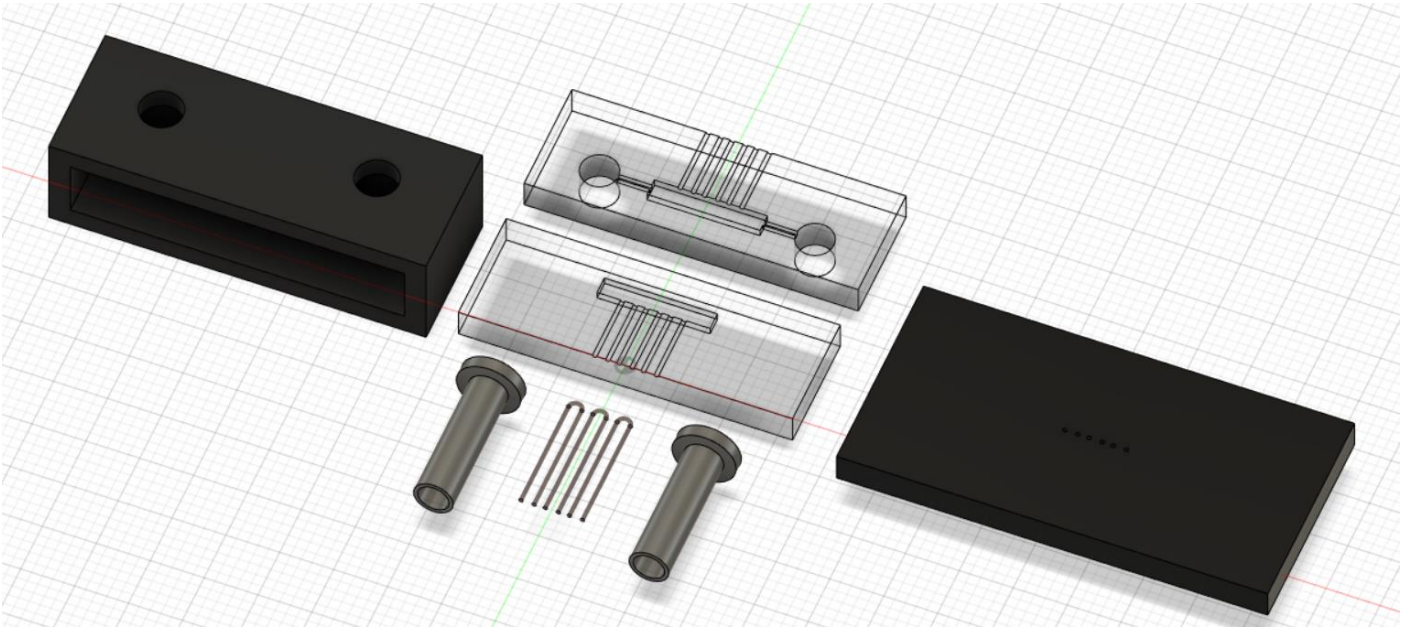
The second set of drawings is the chip alignment system which hold our chips in place during the analysis.



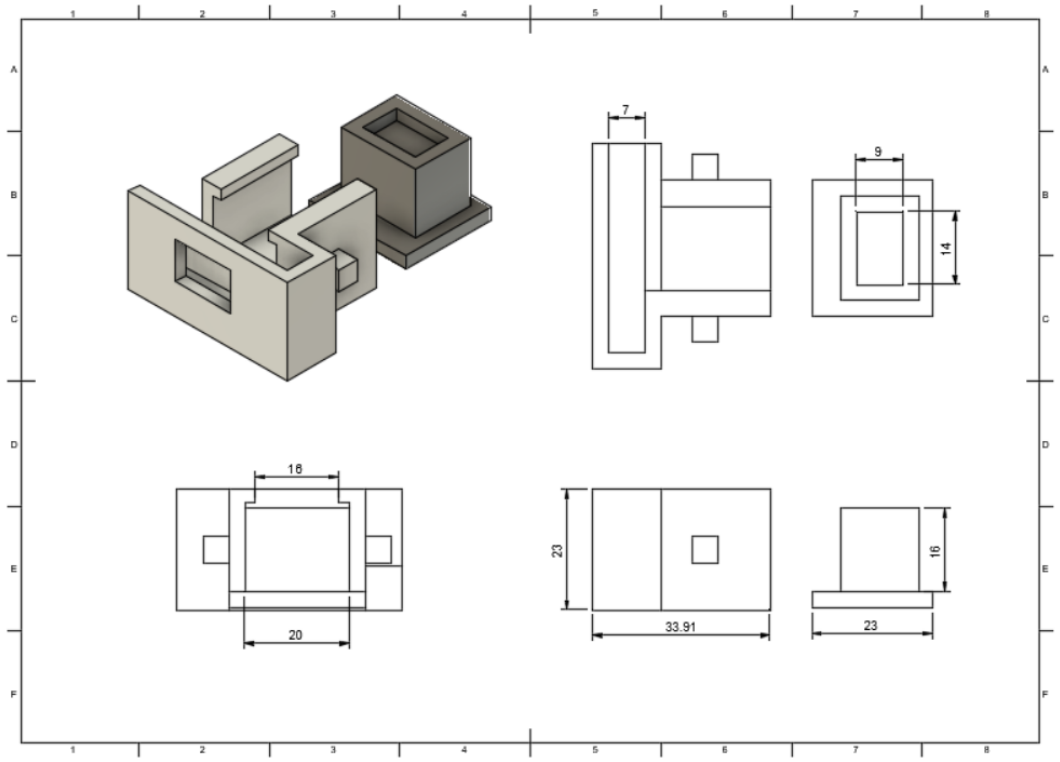
The third set of drawings show our microfluidic chip's outer casing and tubes in which the saliva samples are inserted.



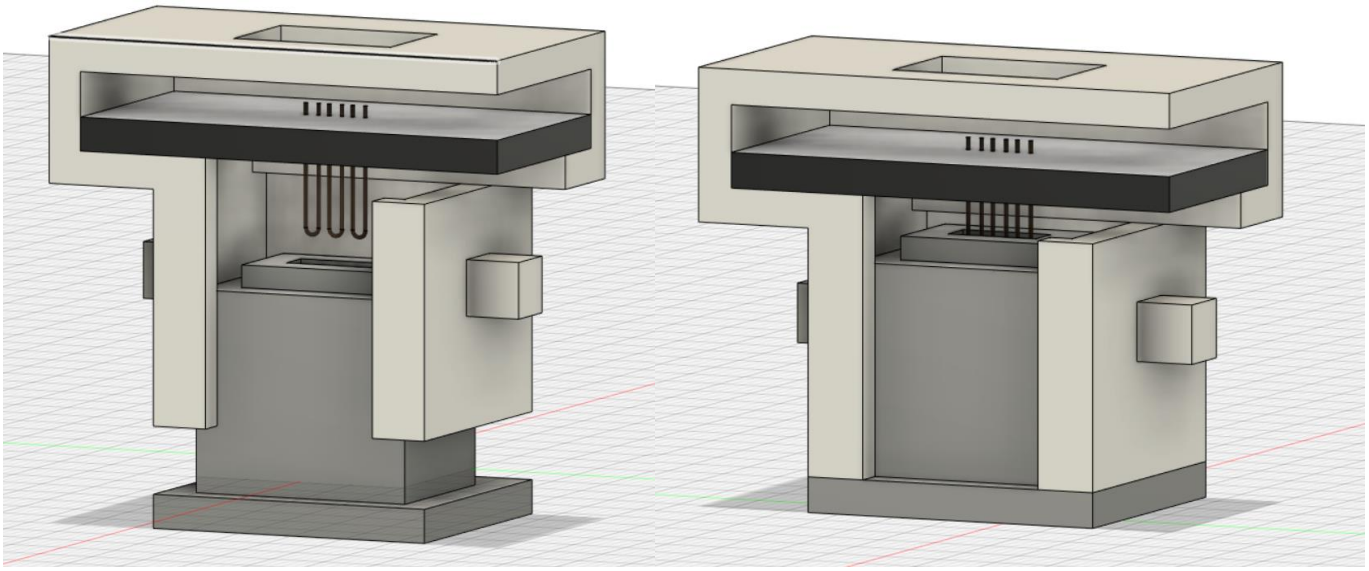
Here are 3D models of all the cartridge pieces.



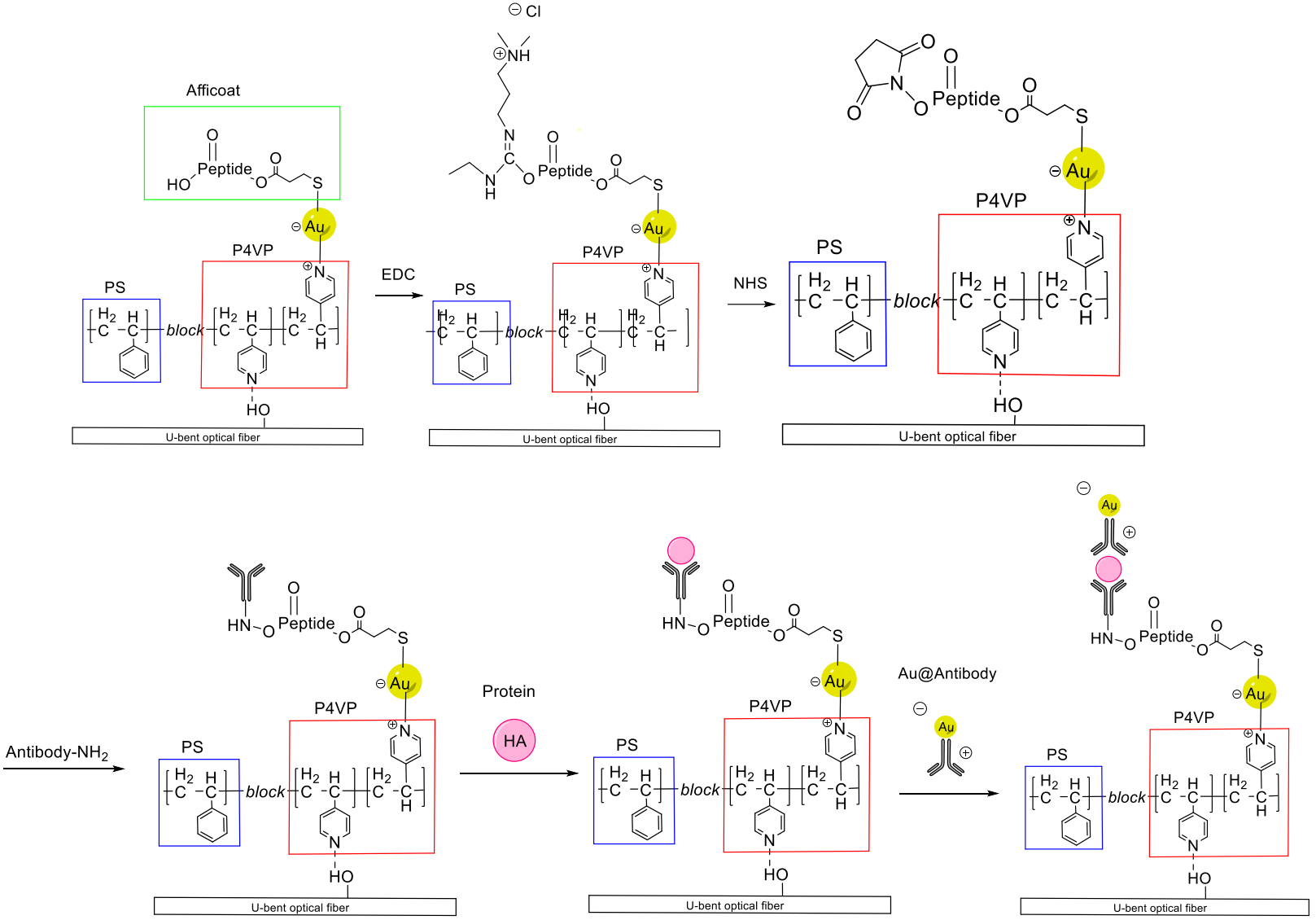
The fourth set of drawings show the system that uses no microfluidics at all that we used to do some tests. It involves a pusher and the alignment system for the chips.



Here is the new plate holder with the PDMS sample pool aligner.

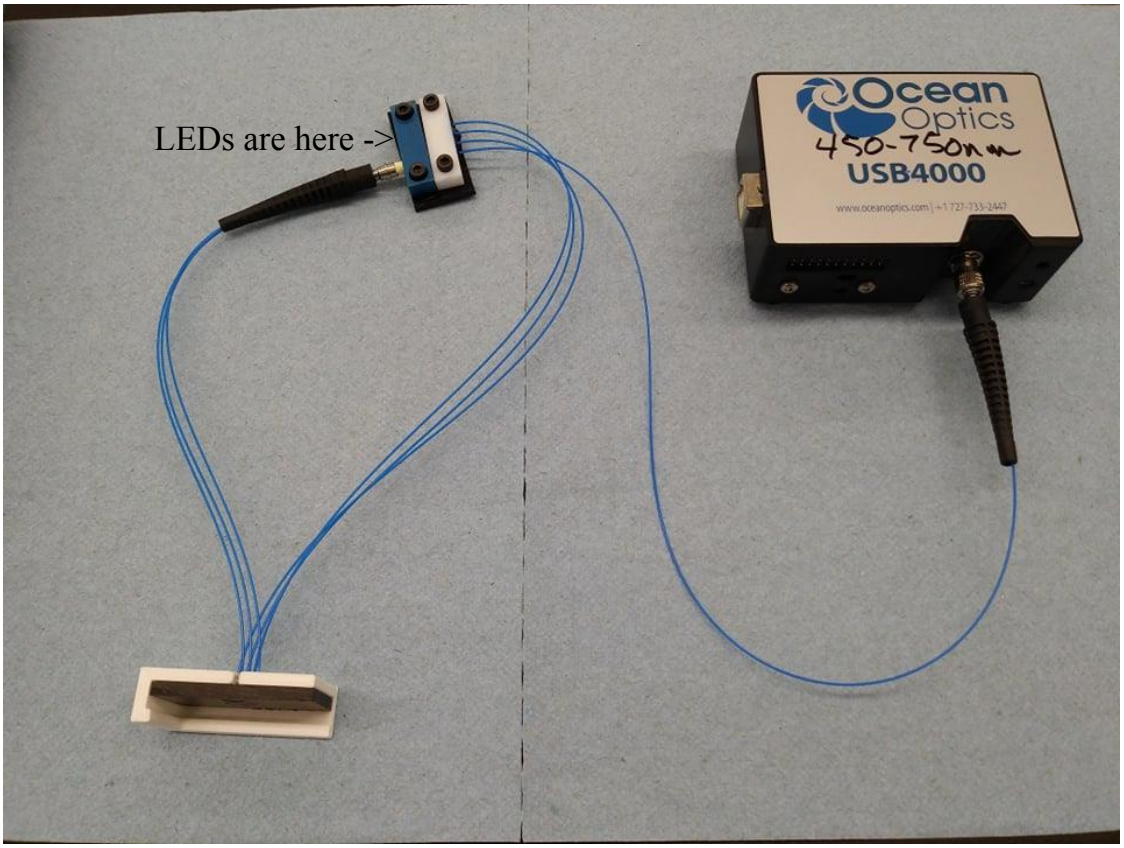


Appendix 2: Reaction dynamics to coat the fibers

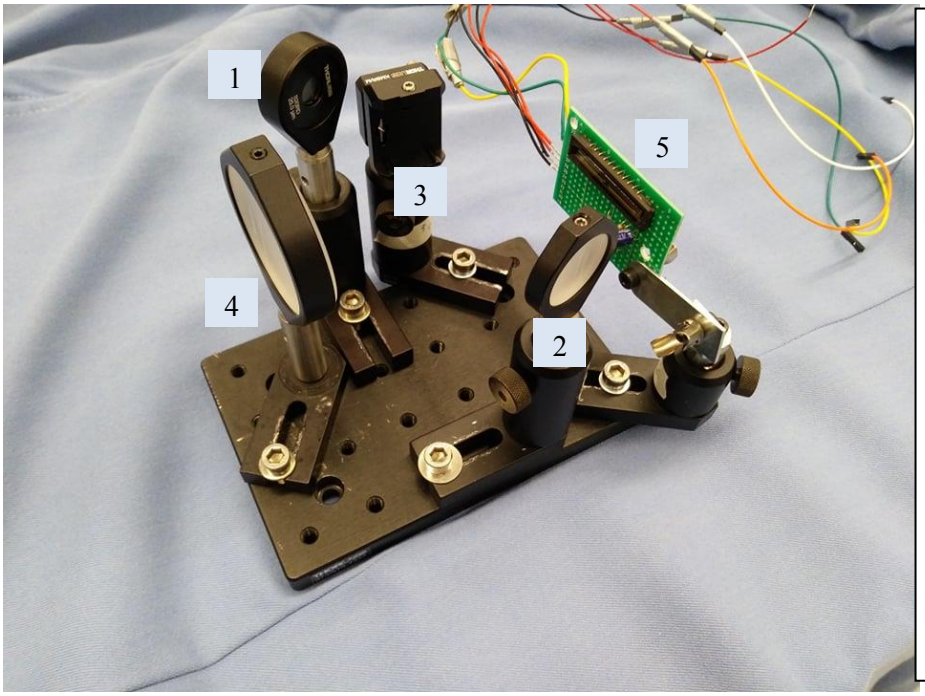


Appendix 3: Optical system

Optical fibers routing the light inside the detector



Our own compact Czerny-Turner spectrometer. Obviously, measurements were taken with the spectrometer encased in a blacked-out box.



The component of our compact Czerny-Turner spectrometer are in optical order :

1) A 20-micron slit
2) A one inch collimating mirror (f = 100 mm). Surface reflective
3) A reflective holographic diffraction grating with 1200 groove per mm. The incoming light hit the grating at 12° to maximize the diffraction in the m = -1 order.
4) A two inch focusing mirror (f = 100 mm)
5) A TCD1304DG linear CCD detector with 3648 active pixels

Appendix 4: Python code that drives the biosensor

```
#Librairies import
import numpy as np
from scipy.optimize import curve_fit
import pandas as pd
import matplotlib.pyplot as plt
import csv
import json
import RPi.GPIO as GPIO
import time
import datetime
from gpiozero import Button
from seabreeze.spectrometers import Spectrometer

def GetData():
    """Get the data from the OceanOptics"""

    # Connect from first available spectrometer
    spec = Spectrometer.from_first_available()
    # Set integration time
    spec.integration_time_micros(20000)
    intensities = spec.intensities()

    return intensities

def GetDivision(datapoint, led_number):
    """Get the difference between the datapoin and the reference spectrum"""

    # Load the reference spectrum from the reference file
    reference = pd.read_csv('led_calibration/leds123.csv', header = None)
    reference = reference.to_numpy()
    reference = reference[led_number-1]

    division = np.zeros(len(reference))
    index = 0
    # Get the difference by normalizing the data first
    for ref in reference:
        ratio = ref / datapoint[index]
        division[index] = ratio
        index += 1

    return division

def GetMax(dataset):
    """Get the position in pixels of the maximum intensity value"""

    # Select only the portion of interest in the dataset
    y_data = dataset[529:2208] # 529 - 2208
    x_data = np.array(range(len(y_data)))
    # Define an approximite maximum
    approx_max = np.argmax(y_data)

    # Define the gaussian fonction that will fit the data
    def gauss(x, a, b, c, d, e):
        return a*x**4 + b*x**3 + c*x**2 + d*x + e

    coeff, var_matrix = curve_fit(gauss, x_data[approx_max-200: approx_max+200], y_data[approx_max-200: approx_max+200])

    a, b, c, d, e = coeff[0], coeff[1], coeff[2], coeff[3], coeff[4]

    y_answer = []
    x_answer = []
    for i in range((approx_max-200)*100, (approx_max+200)*100):
        x_answer.append(i/100)

    for x in x_answer:
        fx = a*x**4 + b*x**3 + c*x**2 + d*x + e
        y_answer.append(fx)
    # Get the maximum of the points generated above
    maximum = y_answer.index(max(y_answer))/100 + x_answer[0]

    return maximum
```

```
def BiosensUM(led):
    """Drive the LEDs and the analysis using previous functions"""

    if led == 1:
        pin = 17
    elif led == 2:
        pin = 27
    elif led == 3:
        pin = 22
    GPIO.setmode(GPIO.BCM)
    GPIO.setwarnings(False)
    GPIO.setup(pin, GPIO.OUT)

    GPIO.output(pin, GPIO.HIGH)
    time.sleep(0.2)

    data = GetData()
    time_now = datetime.datetime.now()
    div = GetDivision(data, led)
    maximum = GetMax(div)


    GPIO.output(pin, GPIO.LOW)

    return maximum, time_now

maxima1 = []
maxima2 = []
maxima3 = []
time_now1 = []
time_now2 = []
time_now3 = []

button = Button(23)

while True:
    led1 = BiosensUM(1)
    print(led1)
    maxima1.append(led1[0])
    time_now1.append(led1[1])

    led2 = BiosensUM(2)
    print(led2)
    maxima2.append(led2[0])
    time_now2.append(led2[1])

    led3 = BiosensUM(3)
    print(led3)
    maxima3.append(led3[0])
    time_now3.append(led3[1])

    plt.plot(time_now1, maxima1, label = 'Serie 1')
    plt.plot(time_now2, maxima2, label = 'Serie 2')
    plt.plot(time_now3, maxima3, label = 'Serie 3')
    plt.pause(0.05)

    if button.is_pressed:
        break

plt.show()

concentration = '1pgml'

with open('calibration_data/' + concentration + '.csv', 'a') as f:
    csv_writer = csv.writer(f)
    csv_writer.writerow(maxima1)
    csv_writer.writerow(time_now1)
    csv_writer.writerow(maxima2)
    csv_writer.writerow(time_now2)
    csv_writer.writerow(maxima3)
    csv_writer.writerow(time_now3)
```

Appendix 5: Confirmation of gold nanoparticles' size

After gold nanoparticles' synthesis, their size was confirmed by UV-Vis Spectrometry. The results of the tests below were compared to the graphs provided by Sigma-Aldrich on their website (https://www.sigmaaldrich.com/CA/en/technical-documents/technical-article/materials-science-and-engineering/biosensors-and-imaging/gold-nanoparticles).

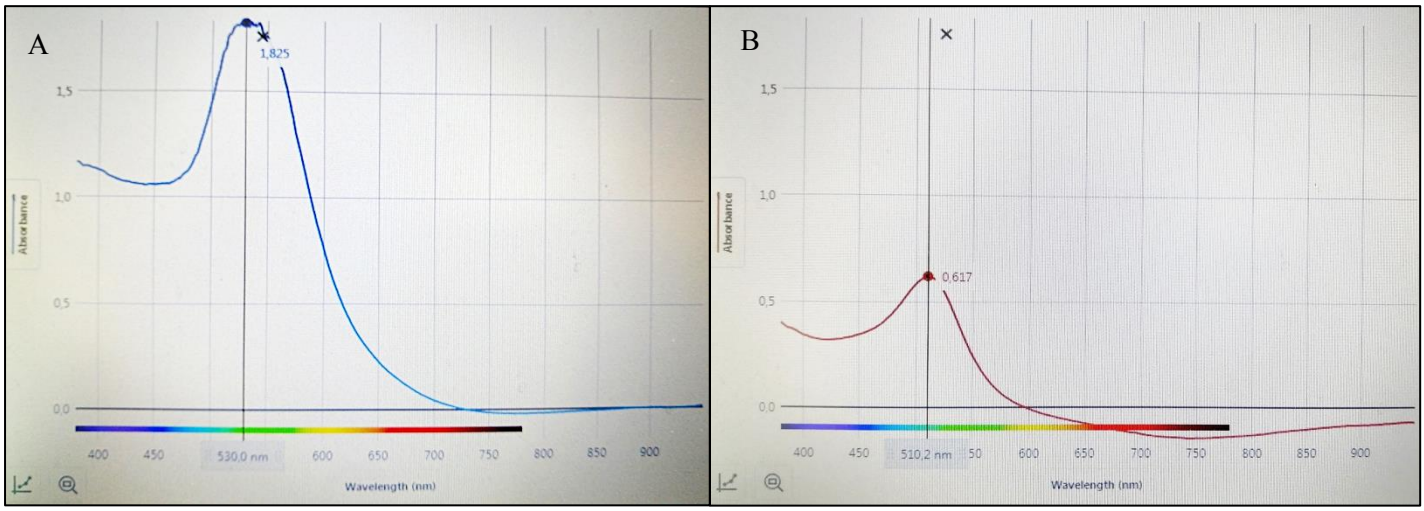


Figure x : UV-Vis spectra of our synthesized A) 60 nm AuNPs B) 15 nm AuNPs.

Appendix 6: A few of current rapid influenza detection tests and nucleic acid detection-based tests available on the market

Technology	Manufacturer	Product	Approved specimen	Test time
Antigen-based detection tests (RIDTs)	BiosensUM	N/A	Saliva	<5 min
	Abbott	Binax Now Influenza A & B Card 2	NPS, NS direct	10-15 min
	Becton Dickinson & Co.	BD Veritor™ Flu A + B	NPS, NS direct	10-15 min
	Quidel Corp.	Sofia® Influenza A + B FIA	NS, NPS, NPA, NPW direct, NP, NPA, NPW in VTM	10-15 min
	Remel-Thermo Fischer	XPECT™ Flu A & B	NW	10-15 min
Nucleic acid-based detection tests	Abbott	ID NOW™ Influenza A & B 2	NPS, NS direct, or NPS and NS in VTM	<15 min
	BioFire, Inc. BioMerieux	FilmArray® Respiratory Panel EZ	NPS in VTM	1-2 hr
	Cepheid	Xpert Xpress Flu	NPS, NS in VTM	30-60 min
	Mesa Biotech. Inc	Accula Flu A/Flu B	NS direct	<30 min
	Roche Molecular Diagnostics	Cobas® Influenza A/B Assay	NPS in VTM	<30 min

A = aspirate

N = nasal

NP = nasopharyngeal

S = swab

VTM = viral transport media

W = wash

Appendix 7: Financing in Canada and Quebec

Here are grants and loan opportunities in Canada or Quebec for our start-up:

- SADC and CAE (youth strategy fund): organization offering up to 50 000$ to entrepreneurs between 18-39 years old
- Industrial Research Assistance Program (IRAP): federal program giving a grant up to 60%-80% of internal technical labour and subcontractor expenses.
- Bank for Canadian entrepreneurs (BDC): loan up to 100 000$.
- Idea to Innovation Grants: federal program funding up to 50% of R&D.
- PME MTL-Young Business: offers up to 15 000$ to help start-up.
- Investissement Québec: up to 50 000$ to help small businesses.
- Canada Small Business Financing Program: up to 350 000 loan with 3% interest.

Appendix 8: Health Canada regulation

Health Canada regulates every medical product available on the Canadian market. This means our biosensor must be reviewed by this organization to sell it in Canada. Our biosensor belongs to the Class III medical device for a near patient in vitro diagnostic which costs 16 032$ for a license then we must pay a fee of 381$ per year to keep the license active (https://www.canada.ca/en/health-canada/services/drugs-health-products/funding-fees/fees-respect-human-drugs-medical-devices/medical-device-licence-application-review-funding-fees-drugs-health-products.html). This investigation takes about 60 calendar days before reaching a first decision which could be either requesting additional information, approving the application or refuse the application.