SenseNC

AccuFRET
North Carolina State University

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Summary

We are SenseNC, the SensUs team representing North Carolina State University and the United States. We are an interdisciplinary team of engineers, designers and entrepreneurs working together to design, fabricate, and market a biosensor to detect adalimumab.

The AccuFRET uses the affinity and form of the analyte, adalimumab, to detect its concentration using fluorescence. The sample is added to a UV cuvette containing fluorescently labeled proteins in solution. The labeled proteins interact specifically with adalimumab and cause a change in fluorescence. Once the sample has been incubated in the solution, an adalimumab concentration dependent fluorescent response is detected by a photodiode placed behind an optical filter. In-system processing of the generated signal is used to determine the concentration of adalimumab and display that reading on the integrated touchscreen display. With this technology backing it, the AccuFRET system is designed to be quick, reliable and user-friendly to support both hospital and at-home use.

Biosensor System and Assay

Molecular Recognition and Assay Reagents

The molecular recognition is accomplished by interaction between adalimumab, TNF-alpha, and Protein L **(Fig 1)** . Protein L binds specifically to the variable light chain (V_L) region of adalimumab. Adalimumab will selectively bind TNF-alpha as it's target analyte. Both TNF alpha and protein L are labeled with fluorescent dyes that have an overlapping emission and absorption profiles. Once both binding events have occured, the dyes will be within FRET range enabling detection of adalimumab.

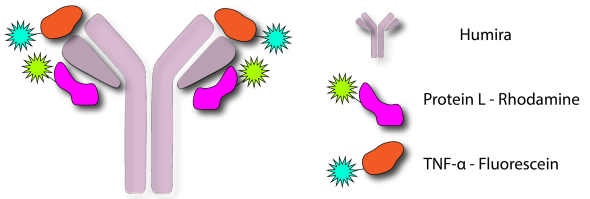

Figure 1: A representation of where each labeled protein will bind to the adalimumab antibody.

The binding of protein L does not interfere with the antigen binding site present on each arm of the antibody structure [1]. It should be noted that protein L will bind to a majority of the antibodies present in normal human serum. Therefore, an excess of protein L was added to compensate for this factor. We plan to investigate appropriate excess levels needed for quantification, but have decided on using a factor of 10 excess to maintain cost effectiveness.

N-Hydroxysuccuninimide (NHS) chemistry was used to label the TNF-alpha and protein L at free amine groups with fluorescein and rhodamine, respectively. The protein concentration in the final reaction solution was quantified using a Braford assay and performed using manufacturer specifications. The following formula was then applied to calculate the extent of labeling of each protein.

$$\text{Moles dye per mole protein} = \frac{A_{max} \text{ of the labeled protein}}{\varepsilon' \times \text{protein concentration (M)}} \times \text{dilution factor}$$

As shown in the below (**Table 1**), TNF-alpha - Fluorescein and protein L - Rhodamine were labeled at 1.34 and 7.69 moles of dye per mole of protein, respectively. We chose this ratio based on literature to provide an excess of rhodamine relative to fluorescein [2-3]. Rhodamine acts as the acceptor of the FRET pair with fluorescein acting as the donor

Table 1: Quantitative Results of Labeling Including Protein Concentration, Extent of Labeling, and Acceptor to Donor Ratio

Protein	Fluorophore	Concentration (µg/mL)	Fluorophore/ Protein Ratio	Acceptor/ Donor
Protein-L	NHS-Rhodamine	338.22	7.69	5.75
TNF-α	NHS-Fluorescein	406.59	1.34	

Physical Transduction

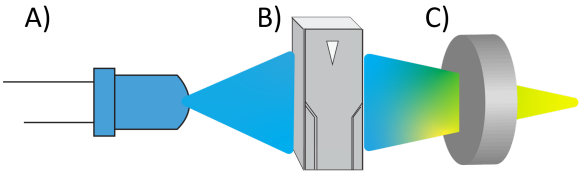

Figure 2: An illustration of the optical sensing method to excite and detect interactions between fluorescent proteins. (A) 470 nm LED shining excitation light onto the testing solution in the (B) cuvette. Emission and excitation light passes through an (C) optical long-pass filter removing excitation light.

As previously mentioned, the signal is produced after the molecular binding between adalimumab and the fluorescently labeled TNF-alpha and protein L. Once the complex is formed, the fluorescently labeled proteins were brought into close proximity where Förster resonance energy transfer (FRET) can occur. The strength of the FRET signal directly corresponds with the concentration of adalimumab present in the sample.

The FRET signal is generated according to the illustration shown above (**Fig 2**). A high-power

LED emitting a peak frequency of 470 nm (**Fig 2, A**) is used to excite the sample within the cuvette (**Fig 2, B**). The signal excites fluorescein molecules in solution which emit at peak 520 nm. If the fluorescein is within 10 nm of a rhodamine molecule, the rhodamine will accept the energy from fluorescein and emit at peak 575 nm. The generated signal then passes through a long-pass filter (**Fig 2, C**) with a cutoff of 550nm.

The resulting signal is measured using the Texas Instruments OPT301 Photosensor. The photodiode component of the sensor converts the fluorescent energy into electrical current. This current is then transformed into a readable voltage by an integrated transimpedance amplifier. The raw signal from the OPT301 has a significant amount of induced noise so a single-stage RC low pass filter with an approximate cutoff frequency of 100Hz is used to smooth the signal. The filter output is converted to a digital 16-bit unsigned format using one of the analog inputs of the KL25Z microcontroller. Using this level of precision, the device is capable of measuring voltage differences of 50.3μV although due to inherent system noise the actual measurement step size is larger.

Cartridge technology

Our device utilizes a simple and easy to use and manufacture cartridge cuvette, no complex microfluidics are required. The UV micro cuvette is loaded with the testing reagent and unknown sample, mixed via pipette, and measured in the same cuvette. The target sample volume depth is maximized along the viewing path of the sensing diode to allow for maximum FRET light emissions to be captured by the sensing photodiode.

Reader Instrument and User Interaction

The AccuFRET is built on top of a NXP KL25Z microprocessor (ARM M0+ core) running custom C code which has been optimized for ultra low power operation. Combined with a custom printed circuit board, the KL25Z provides all necessary control for the sensor peripherals as well as the user interface features. The sensor hardware consists of an excitation LED, a Texas Instruments OPT-301 photosensor, and a single-stage, passive low pass filter. The TI OPT-301 combines a high sensitivity photodiode with a built-in, configurable transimpedance amplifier.

The measurements from the OPT-301 are used to determine the concentration of adalimumab by using a fitted linearly-approximated curve determined empirically. This curve allows us to take in the excited wavelength from our FRET technology, filter it, convert it to a voltage via the photosensor mentioned above, and accurately convert it into a readable value for the user to understand.

The user interface is comprised primarily of the integrated touch screen. In order to better serve our target population, interaction with the device was kept to a minimum. The user need only push the start button on the screen once the cartridge has been inserted and the test will give immediate feedback on the progress of the test, and the result once the test has concluded. Results are given in both a numeric and visual form for easy consumption.

The prototype device casing and sensing element black out box were 3D printed with a Formlab stereolithography 3D printer which allowed for between 25 to 100 micron precision on dimensions which was necessary to align the 2mm x 2mm sensor with the 1.3mm by 1.7mm sample cross section size. A light blocking resin was chosen for the part of the box that enclosed the sensing diode, test sample, light filter and emitting diode. The outer casing of the prototype box was kept as small as possible in a prototype design with dimensions of 120mm tall, 110mm wide and 70mm in depth.

Novelty and Creativity

Already Available

Molecular Recognition

Molecular recognition of proteins commonly incorporates fluorescent labeling. One way to label proteins is using NHS ester labeling. NHS esters are reactive compounds able to modify amino groups. Since proteins commonly have amino acids, NHS esters are commonly used to adapt biomolecules. This is done using click chemistry. The proteins used for our biosensor were protein L and TNF-alpha. Protein L was chosen for the unique ability to bind to kappa light chains without interfering with an antibody's antigen-binding site. TNF-alpha is a signaling protein involved with inflammation. These proteins were labeled for our system using NHS labeling chemistry. The labeling is important in order to be able to detect the proteins using FRET. TNF-alpha and protein L are also ideal proteins to bind to adalimumab. With a large amount of information known about both proteins, our molecular recognition strategy has combined the proteins in a system with adalimumab. With both proteins binding to adalimumab, they become close enough to detect FRET, making our system easy, convenient, and accurate.

Physical Transduction

Proteins involved in molecular recognition were labeled with fluorescent particles using pre-existing NHS chemistry techniques.

When two fluorophores are located in close proximity of each other and the emission spectrum of one overlaps with the excitation of the other there is a dipole-dipole interaction that occurs. This interaction is commonly known as fluorescence resonance energy transfer (FRET), which drove the innovative idea for this sensor. FRET is inherently sensitive and has a high selectivity making it a good way to detect low concentrations of antibody. A favorable FRET environment can be created by using antibodies to pull two fluorophores within close proximity. The more antibody there is, the more FRET that will occur. Upon the addition of adalimumab, the two fluorophores used, fluorescein and rhodamine, both bind to an arm of the antibody, bringing them close enough to FRET. This signal is then analyzed in order to quantify the total antibody in the solution **(Fig 3)**.

The premise for our sensor was based on fluorescence resonance energy transfer (FRET). FRET occurs when the electronic excited state of a donor dye molecule is transferred to an acceptor dye molecule without emission of a photon. We then utilized conjugation chemistry to label TNF-alpha with a donor dye and Protein L with an acceptor dye. It is known that for FRET to occur the molecules need to be between 1-10 nm [3]. Our system combined the concept of conjugation chemistry and FRET by creating a FRET responsive solution that is dependent on the concentration of adalimumab antibody. Once adalimumab is added, the labeled proteins are brought into close enough proximity to show a FRET response. In order to detect the binding of the molecules we used a UV micro cuvette to load into a spectrophotometer, where the fluorescent signal of solution can be measured. There measurements are done by converting the light into an electric current by using a photodiode, much like a microplate reader does.

Fluidic Cartridge

In order to keep our biosensor system simple and cost effective, we chose to design our detection system around a commercially available UV cuvette. Fluid handling is kept to a minimum and can all be done with a pipette in the cuvette. Further, we chose to work with a plastic cuvette that could be disposed of after a single use rather than a multi-use cuvette made of another material (e.g. quartz). In a way, the simplicity of the whole cartridge could be credited as the novelty of the system, as none of the components were needed to be specially made for this design.

Sensor Device

The sensor device can be considered a custom made device consisting of stock parts

readily available. Of particular interest is the OPT301 photodiode, which combines both a photodiode and transimpedance amplifier into a single package. Without this core component, this design would have needed to be much more complex and would have required significantly more development time. However, by using a part with the proper functionality included, our team was able to focus on rapidly developing the sensing system to begin testing and verification.

New Developments

Molecular Recognition

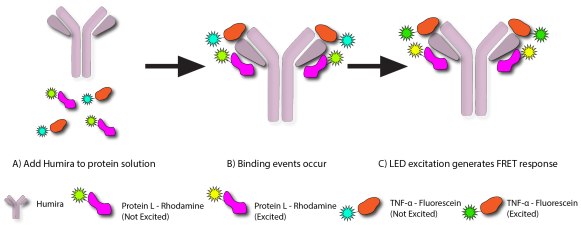

Figure 3. The experimental schematic of the proteins binding to adalimumab then being excited by an LED to emit a FRET response.

The novelty of this idea is in the simplicity of the measurement. There is potential for a non-invasive sensor to monitor dynamic changes of antibody concentrations in small sample volumes. The sensor provides a fast, simple system to measure the amount of adalimumab in serum using FRET - a concept not previously used in this manner.

Physical Transduction

Once the adalimumab is mixed with the TNF-alpha and protein L, binding occurs over an incubation period of 5 minutes. After binding has occurred the signal is then produced. The signal comes from the fluorophores being brought within 10 nm of each other. The FRET signal is then translated into the concentration of adalimumab present within the sample. The system does not require treatment of the sample making it ideal for at home use. Once the sample is loaded the signal is produced directly after the 5 minute incubation time.

Innovating a way to quickly, easily, and accurately quantify the levels of adalimumab in a sample drove the idea behind this biosensor. Keeping these goals as a priority has driven this project to a state where testing can be done hassle free in any environment.

Fluidic Cartridge

The simple design of the cuvette and the fluid handling process was purposeful and necessary in order to reach a large user base. Fluid handling can be done with only a pipette and the cuvette and the user need only insert the cuvette into the device in order to take a measurement. In the future, we envision taking this a step further by integrating the testing reagents into the cuvette package where the user would only need to rehydrate the proteins or otherwise open an included package containing the testing materials. This would fully fulfill our vision of a more user-friendly device.

Sensor Device

The sensor device is comprised of a custom printed circuit board the cuvette enclosure and device casing **(Fig 4)**. Each piece was designed in-house to guarantee the functionality and operability of the device.

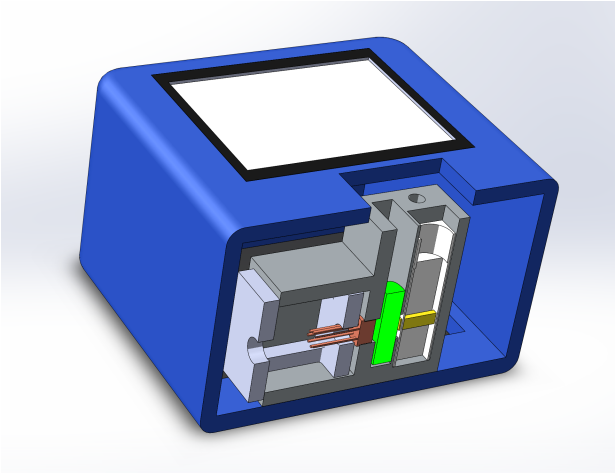

Figure 4. CAD rendering of the device casing and cuvette assembly.

Analytical Performance

Our sensor requires 20 uL of sample that will be mixed in a cuvette with 50 uL containing pre-labeled proteins. The output result can be obtained within 5 minutes.

The performance of the biosensor was first determined using a well plate based experiment. A 96-well plate, loaded with different concentrations of proteins and antibodies, were measured in a plate reader (Biotek Synergy H1) for the fluorescent spectrum of each well. The device excited at a wavelength of 480 nm, starting to read the emission spectrum at 510 nm and sweeping until 700 nm. Figure 5 shows the generated dose response curves examining the ratio of TRITC to FITC max emissions at 0.3x, 0.6x, 1x, and 1.3x labeled protein concentrations (with respect to the maximum dose possible in the competition) with increasing antibody concentration.

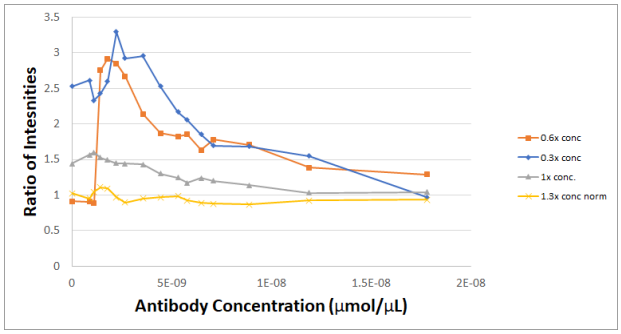

Figure 5. Trend lines were generated using the ratio of the max emission intensities for TRITC and FITC at various labeled protein concentration

The biosensor developed by our group was designed to excite the fluorescent proteins at 470 nm, beginning to read the emission at 550 nm. To determine the performance of the biosensor, replicate solutions were made to the 96-well plate and the data was analyzed in the device. Figure 6 shows the preliminary dose response results determined by the biosensor. For each test in Figure 6, the same samples were run 4 separate times and then an average of the curves was generated. The proteins in this experiment correlated to the 0.6x concentration shown in Figure 5.

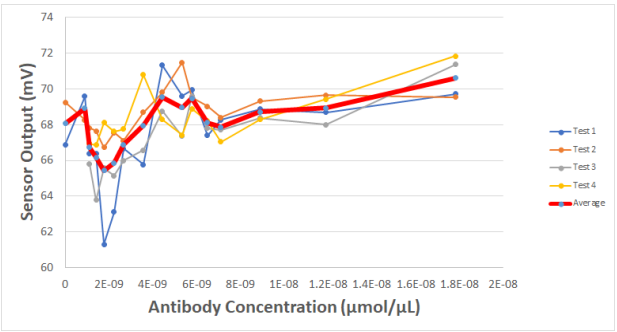

Figure 6. Sensor output (mV) for a standard curve using the 0.6x concentration of labeled proteins with varied antibody concentrations. Average is shown by thick red line.

The well plate experiment was repeated using dosed human plasma. In summary, solutions containing the same protein concentrations above were created by mixing protein solutions with 20 uL of dosed human plasma at the same adalimumab concentrations tested above. The wells were excited at 480 nm and fluorescent spectra obtained.

The same chemical recognition system was tested again using our custom device. Performance and measurements of the custom electronics were confirmed by using external high performance benchtop power supplies and oscilloscopes.

Current Test Plan

The biosensor design is being optimized to account for optical irregularities seen in pror results **(Fig 6)**. New dose response curves and iterations will be obtained before the competition day, but were unable to be generated at the time this document was written.

We plan to use the same pre-labeled proteins at concentrations greater than those used in the microplate experiments to generate dose-responses that we can pick up using the photodiode. From these curves we will obtain the performance metrics of the device for the competition day.

Translation Potential

Business Model Canvas

See **Appendix Figure 1** for the Business Model Canvas.

Stakeholder Desirability

Adalimumab is currently administered via a single standard dose and unmonitored in patients. Due to this lack of monitoring, patients and providers are unaware as to whether the standard 40 mg injections result in excess or deficiency of effective dosing. Using a higher dose than what is required to elicit a therapeutic effect could lead to negative adverse side effects, in addition to increased costs for patients and insurance companies. Significant cost savings could be achieved through reduction of excessive dosing. In contrast, if the standard doses remain shy of effectiveness, serious economic implications could be mitigated due to high levels of disability among patients with inadequate treatment.

AccuFRET will be marketed to both patients and providers. During an interview, a patient taking adalimumab for treatment of RA expressed significant concerns regarding “more frequent and dangerous side effects for anyone who takes these biologic medications” and knowing when they need to take another dose to prevent symptoms. Because adalimumab is dosed once every two weeks, patients report difficulty in remembering to take the next dose and tend to rely on the emergence of physical symptoms as a signal to take another dose. One patient, who is a pharmacist, recounted that the ability to “test the level of adalimumab in [their] bloodstream…would be hugely beneficial” because they often lapsed in giving injections when scheduled and suffered pain as a result. The AccuFRET monitoring system will ensure that patients remain effectively dosed without depending on these painful indications. AccuFRET empowers patients to personalize their RA treatment and reduce their dosing if too much adalimumab is present in their bloodstream, reducing the risk of negative side effects and saving money to all stakeholders. The quantitative results provided by AccuFRET will inform patients on the effectiveness of adalimumab treatments, remove the guesswork involved with injection frequency and dose, and advise patients and physicians on future treatment decisions. Additionally, AccuFRET creates supplemental value by enabling at-home monitoring, which in turn reduces costs associated with physician visits and grants rheumatologists time to diagnose new patients. According to a 2018 study in Rheumatology Advances in Practice, the mean annual cost of all-cause outpatient visits for RA patients on US Medicare was $9,022 USD with another study determining that 2.9 million physician visits annually are due to RA [4,5]. Implementing our device for adalimumab dose management has the potential to significantly reduce these costs, benefiting patients, clinicians, and healthcare organizations. Additionally, according to studies in Poland and the US, lag time for Polish patients to see a rheumatologist can be as long as 35 weeks from onset of symptoms despite recommendation to begin treatment within 12 weeks, with 25% of US patients waiting 4 months or longer to see a rheumatologist. [6,7]. By reducing unnecessary follow up visit with home monitoring, AccuFRET will not only create convenience for current patients but will also help providers diagnose and begin treatment for new patients.

SenseNC has been able to develop an AccuFRET prototype that is able to monitor adalimumab treatment levels in a portable, user-friendly setting. Patients and providers will be able to easily use AccuFRET to run tests rapidly and receive results within 15 seconds. The device is sized to 120 mm x 110 mm x 70 mm with a large display screen that prompts users to insert a sample and displays numerical results with a scale to indicate whether current adalimumab levels are appropriate. By displaying numerical values and graphical representations, people with both an abundance of medical knowledge and a basic

understanding will be able to achieve functional results.

Financial viability

Based on the materials for hardware components of the device, manufacturing expenses, and desired profit margin, the estimated cost of a single AccuFRET would be $440 USD, with a cost breakdown shown in **Appendix Table 1**. Each disposable cartridge consisting of a UV micro cuvette with the FRET assay reagents ready for sample application has a projected cost of $145 USD.

Table 3. Assay Cartridge Cost Breakdown

Cartridge Components	Unit Price ($)
Disposable UV Cuvettes	$0.4000
FITC-Labelled Recombinant Protein L	$0.0054
NHS Rhodamine (TRITC)	$0.0001
Recombinant TNF-alpha	$0.0180
Materials Total	$0.4234
Development and Approval Costs	$100
Total w/ Additional Costs	$101
Expected Revenue per Cartridge	$110.93

The device will be marketed to both clinicians and patients to maximize exposure and target different patient populations. Because matching the cost of the device and cartridges out of pocket may be difficult for certain patients, recommendation by physicians will enable insurance approval and broaden the impact of the device. On the other hand, patient populations for severe chronic conditions tend to be proactive in researching and seeking out new treatment options; equally marketing directly to patients will ensure these individuals are reached. According to the American College of Rheumatology and National Rheumatoid Arthritis Society, 1.3 and 2.3 million adults have been diagnosed with Rheumatoid Arthritis in the US and EU respectively [8,9]. Because adalimumab has several indications outside of RA and biosimilars have recently been launched in the EU, the prospective market is significantly larger. Assuming 2% of patients purchase the device and use a cartridge every 2 weeks, the annual cost per patient can be projected at $4,210 USD with a net annual revenue of 329 million USD and profit of 32.9 million USD.

Business Feasibility

In order to fully develop the AccuFRET we foresee needing a considerable amount of engineering work on both the chemical assay as well as the sensor device. The current prototype encapsulates most of the functionality that would be present in the final design. The primary areas of expertise that would be required to finalize the design would be UI/UX design and packaging of the cartridge. Given that we see the AccuFRET being used by patients or at home caregivers, ease of use and durability are of utmost concern. We expect that a small team of 5-10 engineers could shrink the device to the size of a handheld phone, redesign the UI and design a single-use cartridge for the assay. These changes do not impact the operation of the device, which will reduce the amount of additional testing that must be performed.

Scaling up production of the AccuFRET would be done in two branches. We would first scale up production of the assay materials. This would include partnering with a chemical distributor as well as a company specializing in medical packaging. The final product of this pipeline would be individual test packets that would contain both the assay and cartridge. The second branch, which would happen simultaneously, would involve finding a few partners that would manufacture, collectively, the sensing devices. We see outsourcing construction of the circuit boards and packaging as the more efficient option, rather than developing our own in-house processes. These components would then be sent to a third manufacturer that would assemble each unit.

Team and Support

Team Members

Austin Cronin - B.S. Biomedical Engineering - Electrical Team - Austin was only able to participate until July, but he contributed heavily to the success of the electrical team on the hardware side. He worked to develop the circuit schematics and layout for the PCBs.

Chris Fesmire - Ph.D. Biomedical Engineering - Electrical and Design Team - Chris assisted in the electrical system initial design and validation. Chris also constructed a device casing capable of measuring low intensity light signals.

Katie Kilgour - Ph.D. Chemical Engineering - Chemistry Team - Katie participated in performing and analyzing experiments to confirm the detection of proteins linked to fluorescent dyes in the presence of antibodies. She worked to verify that the detection would be accurate and reliable at small concentrations similar to those given at the competition.

Alexander Kyu - B.S. Biomedical Engineering - Electrical Team - Alex led the development of the device's display and microcontroller programming. He developed all of the algorithms used to measure and analyze the samples.

Sarah Michalets - B.S. Chemical Engineering - Chemistry Team/Business Team - As a member of the chemistry team, Sarah helped with the early stage assay development and proof of concept. She also worked on the business team and conducted interviews for market research.

Matthew Sabo - M.S. Computer Engineering - Team Lead - Mathew was in charge of leading SenseNC this year and coordinated the three teams, chemical, electrical and business. He specifically led both the electrical and business teams through the biosensor development process utilizing his experience from the previous two SensUs competitions.

Matthew Traenkle - B.S. Biomedical Engineering - Business Team - Matthew led delivery of the business team through the entrepreneurship assignments. He utilized interviews conducted by other members in addition to market research to develop the business plan and use case validation for the device. He is also responsible for the translational pitch.

Brendan Turner - Ph.D. Biomedical Engineering - Chemistry Team Lead - Brendan lead the development of the molecular recognition system for the team. He planned out the necessary conjugation chemistry to attach fluorescent dyes to system proteins and validation experiments for confirmation of molecular recognition. Additionally, Brendan analyzed and prepared the data resulting from these experiments and helped to synthesize the components into a functional device.

Michael Wilkins - Ph.D. Electrical Engineering - Team Advisor - Mike assisted with technical advice, equipment access and training, and logistical support.

Faculty Advisors

Dr. Michael Daniele - Team Advisor - In addition to providing advice and expert analysis, Dr. Daniele navigated all financial issues associated with the project. Without his support we would not have made it across the ocean.

Dr. Stefano Menegatti - Co-Advisor - Dr. Menegatti supported the team with regular meetings to discuss results. His valuable insight helped the team surpass the challenges associated with the project.

Sponsors

NCSU ASSIST Center - provided funding and admin support across the project.

NCSU College of Engineering - generously funded our undergraduate student's travel expenses.

Advanced Circuits - provided consulting services and free circuit board manufacturing services for our hardware team.

ThorLabs - provided consulting services and optical filters for our device. (They also provided some awesome snacks for the team.

Final Remarks

Nothing worth doing is ever easy. After accounting for personnel, technical and organizational mishaps, SenseNC 2019 has been no exception. Even with a combined total of 7 years of SensUs experience entering the 2018-2019 cycle, we still struggled in many areas. However, if one were to ask if those struggles were worth the end result, every single member of SenseNC would answer “absolutely”. The lessons learned, friendships formed and challenges overcome far outweigh the rough patches experienced during development of the AccuFRET. Our mission is not only to build a biosensor each year, but to also cultivate newer members so that they can carry the team forward in futures years and achieve greater and greater success. All of the difficulties faced will serve as lessons for those continuing members and strengthen SenseNC as it continues to grow and thrive.

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Appendix

Figure 1. Business Model Canvas

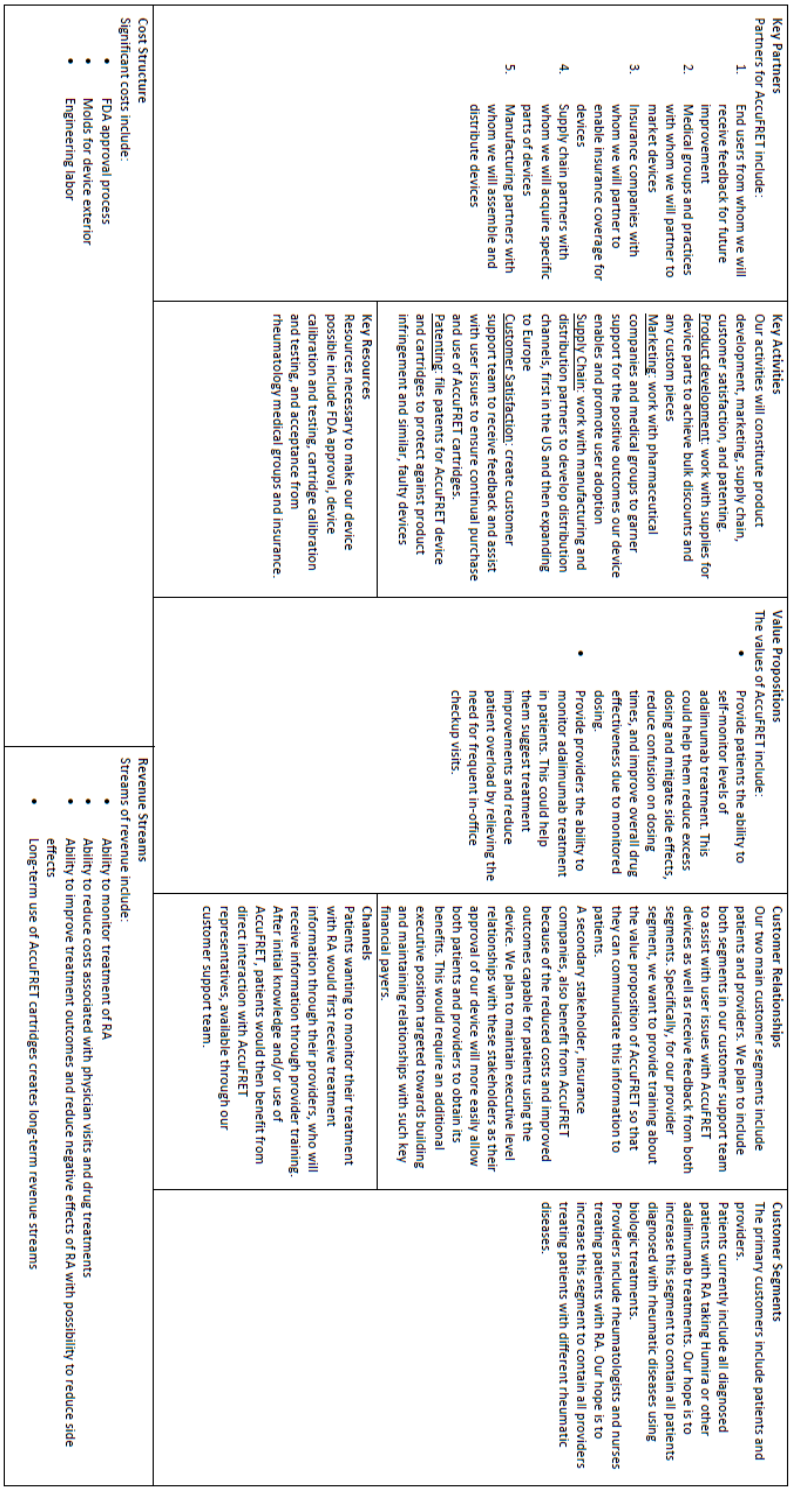

Table 1. Cost Calculations for Biosensor Device Based on Materials, Labor, and Profit Margin

Components	Unit Price ($)
CAP CER 0.1UF 50V X7R 0805	$0.0201
LED GREEN CLEAR 2SMD	$0.1569
LED YELLOW CLEAR 2SMD	$0.1008
IC CONTROLLR LI-ION 4.2V SOT23-5	$0.4326
MOSFET N-CH 60V 0.38A SOT23-3	$0.0407
CONN HDR 20POS 0.1 GOLD PCB	$0.7007
IC REG BUCK 3.3V 1.5A SOT23-5	$0.5408
RES SMD 100K OHM 1% 1/8W 0805	$0.0076
CAP CER 10UF 10V X5R 0805	$0.0221
CAP CER 10UF 10V X5R 0805	$0.0272
CAP CER 4.7UF 16V X5R 0805	$0.0315
RES SMD 4.7K OHM 0.1% 1/8W 0805	$0.0495
CONN RCPT USB2.0 MICRO B SMD R/A	$0.4536
FIXED IND 1UH 800MA 100 MOHM SMD	$0.0664
IC REG CHARGE PUMP INV 8SOIC	$0.7045
CAP CER 22UF 6.3V X5R 0805	$0.0341
RES SMD 2.2K OHM 5% 1/2W 0805	$0.0197
RES SMD 330 OHM 1% 1/8W 0805	$0.0076
IC MCU 32BIT 128KB FLASH 32QFN	$4.2413
470NM TO-18 METAL CAN DOMED	$5.6030
3.5" EVE2 TFT STD. MODULE CAP TO	$64.6800
Circuit Board Mfg. and Assembly	$1.0000
Materials Total	$78.94
5 Engineers @ $30/hr	$6,000/week
100 Devices/Week	$60/device
Total w/ Labor	$138.94
Development, Approval, Marketing Costs	$250.00
Total Cost Per Device	$388.94
Expected Revenue per Device	$427.83